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Virtopsy investigations of stranded cetaceans in Hong Kong waters (2017-2020)

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INTRODUCTION

With a rapidly expanding economy and population, Hong Kong (HK) has been experiencing an inevitable conflict between development and the environment. The constant growing need for space in HK drives land reclamation projects and the creation of artificial coastlines, thus removing the natural habitats for marine animals and has tremendously altered the ecological and biological health status of marine environment, which is essential for the survival of the endangered local cetaceans and their preys.

As the consequence, a significant reduction in the populations of both endangered residential cetacean species, namely the Indo-Pacific humpback dolphin *Sousa chinensis* (SC), and the Indo-Pacific finless porpoise *Neophocaena phocaenoides* (NP), is expected. Over the past decade, the annual abundance estimates of SC in HK waters were constantly below 100 and reached a historical low of 32 in 2018 (Hung, 2019). Huang et al. (2012) estimated that a loss of 74% of the local SC population is anticipated within three generations, while the rate of decline may accelerate with rapid development in the region. For the more seasonal NP population in HK, abundance estimate remains less certain, however a recent study estimated there to be 176 NPs in 2019 during the peak seasons (winter/spring; Jefferson & Moore, 2020). As the local cetacean species in HK are subjected to various direct and indirect anthropogenic threats (e.g., vessel collision, fishery interaction, pollution), unless decisive and effective conservation measures are implemented to halt the declining trend, the long-term survival of these 2 species in HK waters is unlikely (Karczmarski et al., 2017). This has also been reflected by an increasing trend in stranding numbers in HK waters over the past decade and reaching historical highs of 55 and 52 in 2019 and 2020.

In HK, vast resources have been unequally allocated to the monitoring of local cetaceans for the assessment of abundance and population dynamics through vessel or other forms of cetacean surveys. While these facilitated the development of spatial and population modelling tools to assess the population health (e.g., Huang et al., 2012; Karczmarski et al., 2017; Hung, 2019; Jefferson & Moore, 2020), direct studies focusing on the biological health of local cetaceans remain scarce. To date, there is limited understanding on the aforementioned anthropogenic threats in HK and the responses from local cetaceans to these threats. Currently available analyses of the postmortem (PM) investigations conducted on SC and NP stranded in HK waters are outdated and scattered (Parsons & Jefferson, 2000; Jefferson et al., 2002; 2006; Mauroo, 2017). These past records suggested that cetaceans in HK waters are prone to

respiratory diseases and parasitic infections, whereas human-related mortality typically involves vessel collisions, as well as entanglement or entrapment by fishery interactions.

A pioneering virtopsy project was initiated in March 2014 to advance the cetacean stranding response programme in HK (Kot et al., 2015; Kot et al., 2016; Tsui et al., 2020). Applying postmortem imaging techniques on stranded cetaceans, the role of virtopsy has become more pivotal as veterinarians and other personnel involved in the stranding response became more aware of its strengths (Tsui & Kot, 2015). Over the past years, Kot and his team have developed standardised virtopsy protocols, as well as techniques to diagnose pathologies and causes of deaths (CODs) in stranded cetaceans (Kot et al., 2016; Chan et al., 2017; Yuen et al., 2017; Kot et al., 2018a; 2018b; 2019; 2020a; 2020b; 2020c). Recently, pitfalls encountered during the Cetacean Virtopsy Stranding Response Programme and relevant practical management measurements were reviewed in hope to facilitate the implementation of virtopsy in stranding response worldwide (Tsui et al., 2020). To address the critical knowledge gap of cetacean biological health assessment through PM examination, this paper summarises and discusses the findings from virtopsy investigations of stranded cetaceans in HK waters from July 2017 to November 2020.

MATERIALS AND METHODS

This study included stranded cetaceans found in HK waters during the period of July 2017 to November 2020, which encompassed carcasses ranging from decomposition condition code 1 to 5 (Geraci & Lounsbury, 2005). The study was conducted jointly with the Agriculture, Fisheries and Conservation Department (AFCD) and the Ocean Park Corporation (OPC), licensed by AFCD [AF GR CON 09/68 PT.15]. Upon retrieval, cetacean carcasses underwent postmortem computed tomography (PMCT) and postmortem magnetic resonance imaging (PMMRI), if suitable, using the standardised virtopsy protocols and parameters (Kot et al., 2018b; Kot et al., 2019; Tsui et al., 2020). Afterwards, the carcasses were transported to the necropsy facility for 3D surface scanning (3DSS) and conventional necropsy. Prior to necropsy, 2D and 3D images from virtopsy (PMCT, PMMRI, and 3DSS) were examined and reported. Conventional necropsies were carried out and reported by qualified veterinarians following standard protocols (Geraci & Lounsbury, 2005). Additional samples were collected from selected organs, tissues, or lesions for subsequent analyses (toxicology, histopathology,

microbiology, parasitology, etc.). Primary findings obtained from necropsy were routinely compared to findings obtained from virtopsy assessment.

RESULTS

Between 1 July 2017 to 30 November 2020, virtopsy was conducted in 132 out of 158 (84%) cetaceans stranded in HK waters, including 24 SCs, 92 NPs, and 16 of other species (OT) (Table 1). The remaining 26 stranded cetaceans (16%) were determined to be non-transportable and inappropriate for virtopsy by the field stranding response team of the Ocean Park Conservation Foundation Hong Kong (OPCFHK). For these carcasses, gross necropsy were performed onsite by the response team.

In these 132 cases, the virtopsy findings of 39 stranded cetaceans (30%) (6 SCs, 32 NPs, and 1 OT) were sufficient to assign the CODs to be human-related, namely fishery interaction (e.g., evidence of fishing gear entanglement or underwater entrapment) and vessel interaction (e.g., sharp or blunt force trauma evidential of vessel collision), to varying degrees of confidence (confirmed, probable, or suspected) (Figures 1 & 2). The remaining 93 stranded cetaceans (70%) (18 SCs, 61 NPs, and 14 OTs) were associated with various non-human related CODs such as respiratory diseases, microbial or parasitic infections, and undetermined natural deaths (Figures 3 & 4).

Table 1. Statistics of stranded cetaceans in HK waters between 1 July 2017 to 30 November 2020 (# of stranding cases, # of cases with virtopsy, CODs determined).

	SC	NP	OT	<u>Total</u>
Total stranding cases	29	113	16	158
Cases with virtopsy	24	92	16	132
Determined with human-related CODs (vessel or fishery)	6	32	1	39
Other CODs (diseases and undetermined natural deaths)	18	60	15	93

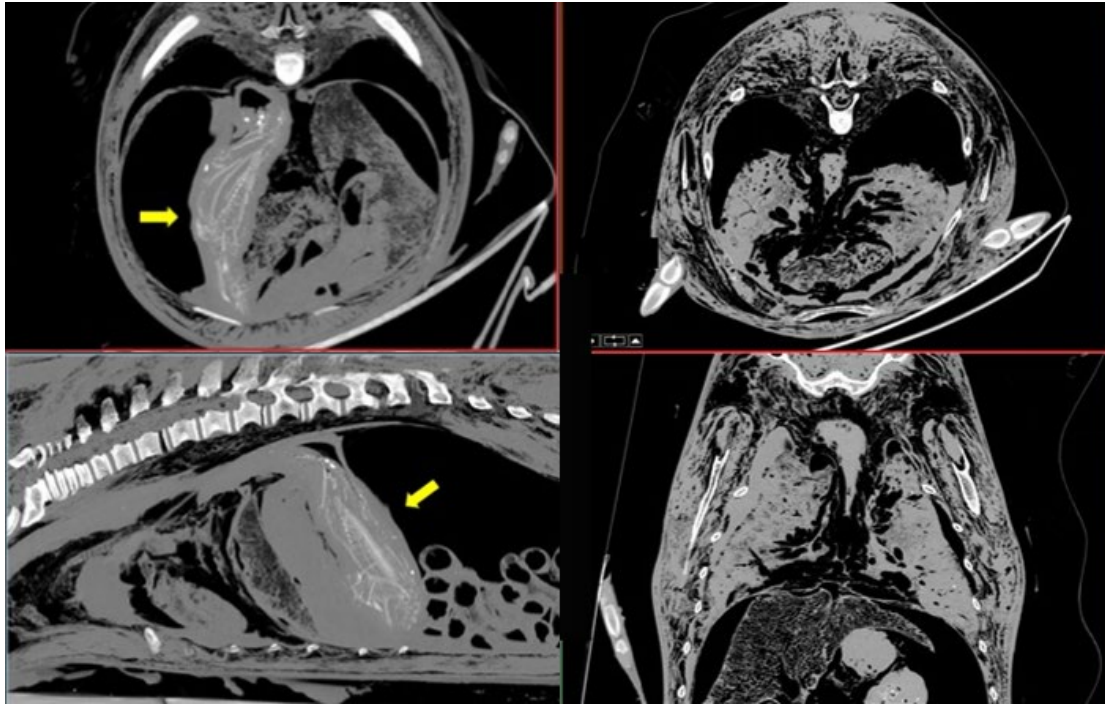


Figure 1. Virtopsy findings using PMCT in a stranded cetacean in HK waters. Presence of food (fish, yellow arrows) in the stomach is suggestive of sudden death, while lung patterns (right) are suggestive of drowning. With the absence of other major pathologies, the COD was determined to be human-related (suspected fishery interaction).

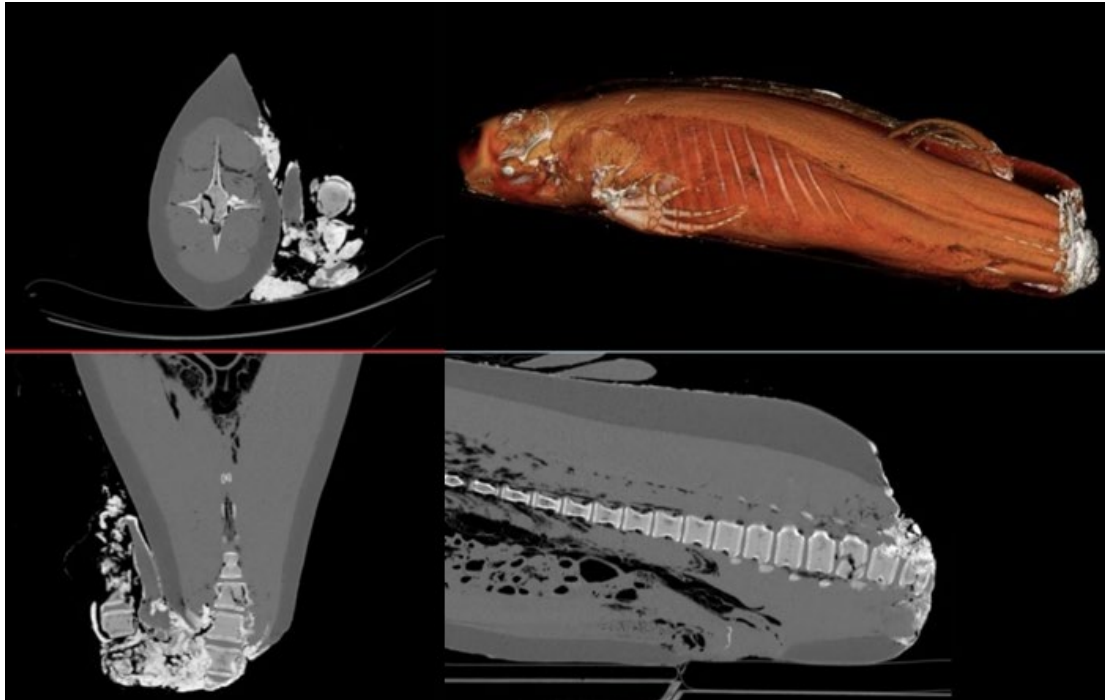


Figure 2. Virtopsy findings using PMCT in a stranded cetacean in HK waters. Amputated tail from caudal peduncle indicates sharp force trauma likely from propeller strike. The COD was determined to be human-related (confirmed vessel interaction).

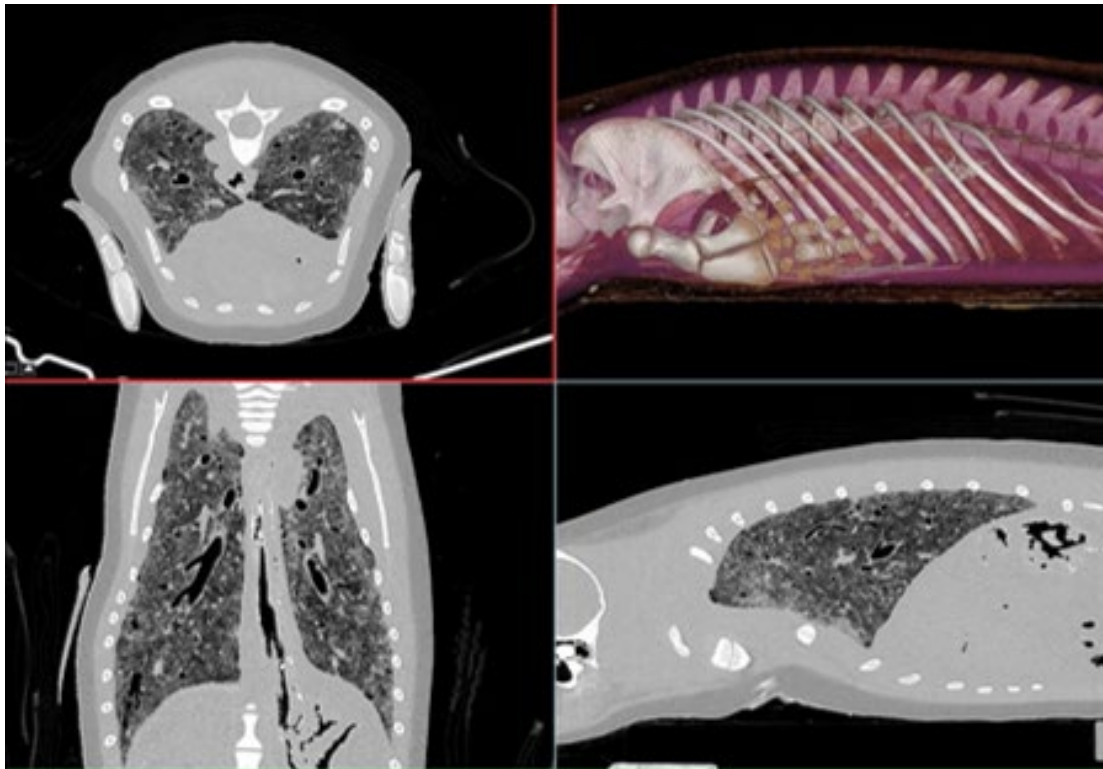


Figure 3. Virtopsy findings using PMCT in a stranded cetacean in HK waters. Multiple hyperattenuated nodules are seen in the lungs and suggestive of parasitic infection. The COD was determined to be non-human related and possibly due to parasitic pneumonia.

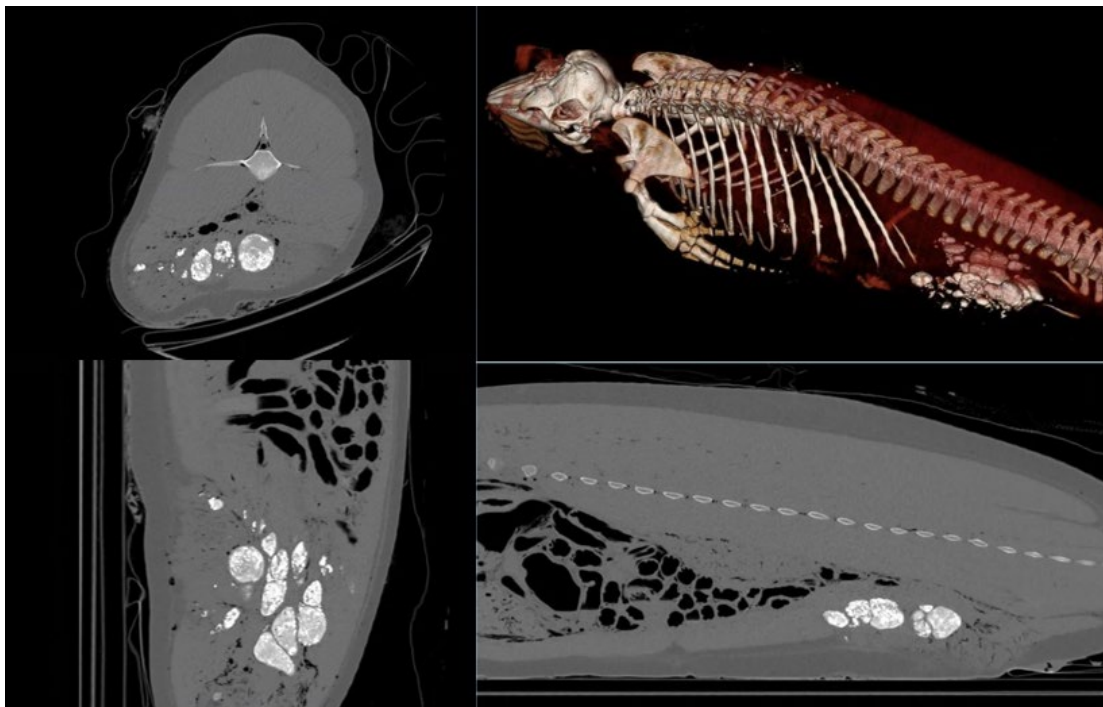


Figure 4. Virtopsy findings using PMCT in a stranded cetacean in HK waters. Multiple hyperattenuated masses in mammary glands suggest parasitic granulomatous mastitis.

In addition to PMCT and PMMRI, 2 handheld 3D scanners were used by the current project to conduct 3DSS for the documentation of external injuries of the carcasses, with reference to the 3DSS standardised techniques established by our team (Kot et al., 2018b). These two scanners were used for the 3D geometrical documentation of external surface in stranded cetaceans, which allowed further measurements, modelling and creation of 3D simulations. By 30 November 2020, a total of 20 cetacean carcasses and skeleton specimens (5 SCs, 12 NPs, and 3 OTs) have been scanned and successfully reconstructed into high-quality 3D models (Figure 5).



Figure 5. Three-dimensional surface scanning (3DSS) of stranded cetaceans in HK waters. Top: operation of the handheld scanner on a NP carcass and the reconstructed 3D model showing the bisecting chop wound and exposed viscera. Bottom: Comparison between photo and 3D model of a SC carcass. All external features (skin lesions, detached skin, and exposed dermis) were accurately depicted in the model.

DISCUSSION

Over the past 7 years, the routine integration of virtopsy into the local cetacean stranding response programme in HK has proven to not only pose little problem to the experienced stranding response team, but also added values to the PM examinations by facilitating better planning and diagnosis through a combination of virtopsy and conventional necropsy. A thoughtful management plan is essential for integrating virtopsy into stranding responses, and would yield valuable information to supplement conventional necropsy for PM investigation of cetaceans (Kot et al., 2015; Kot et al., 2016; Tsui et al., 2020).

Among virtopsy techniques, PMCT was superior to conventional necropsy in revealing skeletal trauma and pathological gas accumulation, whereas PMMRI was superior in detecting soft tissue anomalies (e.g., CNS, abdominal organs, blubber, and musculatures) (Kot et al., 2016; Kot et al., 2018a; Kot et al., 2020a; 2020c). 3DSS was effective in complementing PMCT and PMMRI to document external appearance of carcasses (Kot et al., 2018b), especially in cases where traumatic wounds from vessel collision or fishery entanglement were present. The documentation and analysis of virtopsy findings are time efficient, objective, and non-invasive, which have improved the assessment to the biological profiles and causes of death of stranded cetaceans in HK waters and such findings could eventually assist in generating effective conservation measures.

A review of past and current data on the CODs of stranded cetaceans in HK revealed that anthropogenic impacts, namely vessel and fishery interactions, remain to be major causes of mortality for cetaceans stranded in HK (Table 2), despite ongoing conservation efforts by the local agencies. In addition, environment pollution was also suspected to affect the fitness of residential cetaceans (Jefferson et al., 2002; 2006). In non-human related deaths where CODs were confidently determined, bacterial infection appeared to be one of the major pathologies and was suggested to relate with the high level of sewage contamination in HK waters (Parsons & Jefferson, 2000). Parasitic infections, notably the lungworm *Halocercus pingi*, was a common finding especially in NP (Parsons & Jefferson, 2000; Parsons et al., 2001). Other parasites and pathogens including the nematodes of the genus *Crassicauda* and the bacteria *Brucella ceti* were occasionally reported in case-oriented manner in HK waters (Mauroo et al., 2020a; 2020b). Parasitological findings were also noted by virtopsy (Figures 3 & 4), and further studies are taking place to document and characterise these parasitic infections (Leung et al., 2020).

Table 2. Past and current statistics on the CODs of stranded cetaceans in HK waters.

Study	Species	Vessel-related	Fishery-related	Other*
Parsons & Jefferson (2000) (1993-1998)	SC	3/28 (11%)	6/28 (21%)	19/28 (68%)
	NP	3/32 (9%)	2/32 (6%)	27/32 (85%)
	OT	-	1/4 (25%)	3/4 (75%)
Jefferson et al. (2002) (1995-2000)	NP	3/54 (5%)	8/54 (15%)	43/54 (80%)
Jefferson et al. (2006) (1995-2004)	SC	4/89 (5%)	4/89 (5%)	81/89 (90%)
Mauroo (2017) (2007-2014)	SC & NP	28/258 (11%)**	9/258 (3%)**	220/258 (85%)
This study*** (2017-2020)	SC	5/24 (21%)	1/24 (4%)	18/24 (75%)
	NP	21/92 (23%)	11/92 (12%)	60/92 (65%)
	OT	1/16 (6%)	-	15/16 (94%)

* Other includes non-human related CODs such as diseases and undetermined natural deaths.

** Mauroo (2017) reported an animal with trauma evidential of both vessel and fishery interactions.

*** Only including the 132 cases where virtopsy assessment was performed.

The results of the present study serve as a preliminary update and summary of the causes of mortality of stranded cetaceans in HK, especially with a focus on the major anthropogenic threats (vessel and fishery interactions), based on the findings from virtopsy investigations from July 2017 to November 2020. Up-to-date and reliable PM data are essential to evaluate whether the population and biological health of the two endangered local cetacean species are deteriorating. Virtopsy has demonstrated to be a powerful tool that provides an effective and efficient assessment of the stranded cetaceans to supplement conventional necropsy during PM investigations. Findings from virtopsy enables precise image-guided examination and sample collection by veterinarians during necropsy examination. Proper implementation of virtopsy in cetacean stranding response could significantly improve the ability to diagnose pathological findings and CODs, thereby facilitate better management measures for cetacean conservation.

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