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INTRODUCTION

With its rapidly expanding economy and population, Hong Kong (HK) has experienced an acute conflict between development and the environment. The constant growing need for space drives land reclamation, the creation of artificial coastlines, and the removal of natural habitats for marine megafauna, and has tremendously altered the ecological and biological health characteristics and attributes essential for the survival of local endangered cetaceans and their prey.

A significant decrease in the populations of both endangered local residential cetacean species, namely Indo-Pacific humpbacked dolphins, *Sousa chinensis* (SC), and Indo-Pacific finless porpoises, *Neophocaena phocaenoides* (NP), is expected. Annual abundance estimates of SC in HK waters in line-transect surveys are constantly below 100 (Hung, 2019). One report stated an estimated loss of 74% of the population is anticipated within three generations (Huang et al., 2012), which is by far the greatest decline ever in the cetacean population's generational history. Abundance estimates remain uncertain for the NP population in HK waters. Previous line-transect surveys estimated a peak of 152 animals in spring, but only 55 in late autumn. Unless decisive and effective conservation measures halt the current trend, the long-term survival of these two populations is unlikely (Karczmarski et al., 2017).

In HK, vast resources have been unequally allocated to the monitoring of local cetaceans since 1995 for the assessment of abundance and population dynamics. While spatial and population modelling tools have been developed (Jefferson et al., 2009; Hung, 2019), studies on the biological health and profiles of local cetaceans is scarce and lacks detail. There is only limited to moderate understanding of anthropogenic impacts and cetacean responses to these impacts. Postmortem investigations of both SC and NP in HK waters, though scattered and limited to old records, revealed that they are prone to various respiratory concerns and multiple organ parasitic infections, and with deaths likely associated with human interactions, such as vessel and fishery entanglement (Jefferson, 2018; Chan et al., 2017; Kot et al., 2018a).

A pioneer virtopsy project was implemented in March 2014 to advance the Cetacean Stranding Response Programme in Hong Kong (HK) waters (Kot et al., 2015). The role of virtopsy has become pivotal as veterinarians and personnel of the stranding response team became more aware of its strengths (Tsui and Kot, 2015). Standardised virtopsy protocols and techniques for PM pathological diagnosis were adhered to (Chan et al., 2017; Yuen et al., 2017; Kot et al., 2018a, 2019, 2020a,b,c). Pitfalls encountered were addressed with corrective measures to ensure the structural and practical management of the first Cetacean Virtopsy Stranding Response Programme (CVSRP) worldwide (Tsui et al., 2020). This paper aims to summarize and discuss the results of virtopsy investigations of stranded cetaceans in HK waters during the period July 2017 to March 2021.

MATERIALS AND METHODS

The study included stranded cetacean carcasses with Smithsonian condition code 1-5 (Geraci & Lounsbury, 1993) found in HK waters during the period July 2017 to November 2020. The study was conducted jointly with the Agriculture, Fisheries and Conservation Department (AFCD) and Ocean Park Corporation (OPC), and licensed

by AFCD [AF GR CON 09/68 PT.15]. The carcasses were transported to postmortem computed tomography (PMCT) and postmortem magnetic resonance imaging (PMMRI) examination rooms for the virtopsy procedures, using standardized protocols and technical parameters established by the Kot team (Kot et al., 2018; Kot et al., 2019; Tsui et al., 2020). After virtopsy scanning, the carcasses were transported to the necropsy facility for 3-D surface scanning and conventional necropsy, and full resolution 2-D and 3-D images will be produced and reviewed (Chan et al., 2017). Conventional necropsy will be undertaken by qualified veterinarians, according to standard protocol and methodology (Geraci & Lounsbury, 1993). Sampling of various body structures, including blubber from the dorsal thoracic region and major organs in the head, neck, thorax and abdomen, will be collected for chemical analyses and toxicological studies. Gross pathology will be noted during necropsy, and tissue samples will be examined histopathologically. The main findings in the necropsy summary report relevant to biological health will be compared to the results of the radiological evaluation.

RESULTS

During the period 1 July 2017 to 30 November 2020, 132 out of 158 HK stranded cetaceans (83.5%) were performed virtopsy, including 24 Indo-Pacific humpbacked dolphins (SC), 92 Indo-Pacific finless porpoises (NP), 16 other species. All the virtopsy findings were verified by subsequent necropsy, with supplemented information given for the analysis of human interaction related injury and death of stranded cetaceans. The remaining 26 stranded cetaceans (16.5%) were recognized to be non-transportable and inappropriate for virtopsy by cetacean stranding response team of the Ocean Park Conservation Foundation Hong Kong (OPCFHK). Onsite necropsy and sample collection were directly performed on these cetacean carcasses by the team of OPCFHK.

In these 132 cases, findings of 38 stranded cetaceans (6 SCs, 31 NPs and 1 other species) were sufficient to assign the cause of death with confirmed, probable or suspect category associated with human interactions (28.8%), which included evidence of fishery (e.g. fishing gear entanglement/digestion), and vessel interactions (e.g. sharp chopped wound). The rest 94 stranded cetaceans (18 SCs, 61 NPs, and 15 other species) were likely associated with different causes of death, e.g. respiratory diseases, drowning and natural death.

Two handheld 3-D scanners were used in the current project for the documentation of external injuries of the carcass, with reference to the 3DSS standardised techniques established by our team (Kot et al., 2018b). These 2 scanners were used for the production of geometry of the carcass external surface in 3-D, true to scale and with a high-resolution. Exact geometrical reconstructions of the carcass external surface were created, which allowed further measurement, modelling and subsequent 3-D simulations. By 30 November 2020, 20 cetacean carcasses and skeleton specimens (5 SCs, 12 NPs and 3 OTs) in HK were scanned with the 2 handheld scanners and reconstructed into 3-D models.

DISCUSSION

Routine integration of virtopsy into cetacean stranding response program was proven not only posed little problem for the experienced stranding response team, but also added value for the conventional necropsy results from improved planning and better diagnostics. The initial management of stranded carcass and the death investigation should be equally well performed, which often follows accurate and reliable virtopsy examinations. A thoughtful management in a cetacean stranding response program with virtopsy as an integral part would yield valuable supplementary information prior to the conventional necropsy for the death investigation of stranded cetaceans.

PMCT was superior to necropsy in revealing skeletal trauma, whereas PMMRI was superior to PMCT and CN in detecting soft tissue anomalies of central nervous system, abdominal organs, blubber and musculatures. The documentation and analysis of virtopsy findings were investigator independent, objective, and non-invasive, which provided better understanding to the causes of death of stranded cetaceans, and could eventually help generate effective conservation measures.

Parsons and Jefferson (2000) had conducted postmortem (PM) examination and health assessment on stranded cetaceans in HK from 1993 to 1998. Infectious pathogens, namely parasites and bacteria were detected in 29% of the mortalities, with causes of death associated with human interaction. Endoparasitic helminth (EH) (i.e. Halocercu pingi) induced infections were proposed as direct cause of death, but bacterial infections (mainly by Escherichia coli, Streptococcus faecium, and Vibrio cholera) were considered as debilitating factors that could lead to secondary fatalities. Mauroo (2017) studied the neonatology and gynecology of stranded cetaceans in HK waters from 2007 to 2014. Two pathogens of the female reproductive system were recorded and the reproductive health was addressed, i.e. gamma-herpesvirus (3/31) in SC and B. ceti (2/31) in NP (Mauroo et al., 2020). The study also suggested that EH (i.e. Crassicauda spp.) induced mastitis suppressed the lactation with maternal immunity. Insufficient nutrition intake of neonate might lead to hepatic lipidosis due to overload of fat mobilization, and subcutaneous icterus could be observed as a result. Results of the present study updated the causes of mortality and morbidity in stranded cetaceans in HK waters, after almost 2 decades of time. Up-to-date reliable postmortem baseline data are essential to evaluate whether the health of the 2 endangered local cetacean species is deteriorating, which is crucial for the development of robust conservation plans. Collecting virtopsy data as an integral to the postmortem data consistently across 2 local cetacean species could provide a gradient of stressor exposures for cross-sectional or correlational studies.

This study demonstrated virtopsy is a potentially powerful tool, providing non-invasive and objective measurements to supplement the necroscopic findings for cetacean death and biological health and profile investigation. Virtopsy could also facilitate veterinary personnel to perform image-guided target specimen necropsy and sampling for histology and toxicology. The calibrated 3-D documentation and analysis of virtopsy findings would lead to qualitative improvements in conventional necropsy.

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