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CASE REPORT

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Interstellar encounter: Postmortem imaging and virtopsy on a preserved anencephalic Indo-Pacific bottlenose dolphin specimen after 30 years

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Abstract

Background Anencephaly is a deadly type of cephalic axial skeletal and neural disorder with a multifactorial aetiology that causes the failure of the rostral neuropore closure, compromising the formation of the neural folds, basicranium, and neurocranium. In cetaceans, there is only one report of this fetal abnormality, dated in late 1991, in a male stillborn Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) and diagnosed using transabdominal ultrasonography on its mother that was kept under human care in the Ocean Park Corporation. After birth, physical and general radiographic examination showed kyphosis of the cervical and lordosis of the thoracic vertebrae, narrowed triangular skull base, thickening of an undersized maxilla, cranial vault defect including the absence of the bones constituting the roof of the cranial cavity. The stillborn was then fixed in formalin for future research.

Case presentation The preserved dolphin specimen was further investigated using different advanced radiological and imaging techniques postmortem, including 3D surface scanning (3DSS), computed tomography (CT), and magnetic resonance imaging (MRI). The scans were then used to conduct virtual necropsy (virtopsy) for detailed gross morphological analyses of the malformation. CT confirmed the total absence of the interparietal bone but also revealed the presence of greatly reduced other bones. The reduction of the parietal and occipital bone resulted in a large cranial vault defect instead of the interfrontal and fronto-occipital sutures. MRI showed the remaining of the optic and vestibulocochlear nerve which suggests the condition of human meroanencephaly.

Conclusions In summary, this study reported the importance of the use of advanced radiological and imaging tools in rare and complex malformations such as anencephaly in a stillborn cetacean. Although the malformation was diagnosed using prenatal transabdominal ultrasonography, it was later confirmed revealing new insights using virtopsy. Although ultrasound is an established method to monitor pregnancy, fetal growth and wellbeing,

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virtopsy provided a more accurate characterization of the bone and neural malformations postmortem. This example highlighted the importance of using virtopsy as a postmortem technique to understand the nature and characteristics of pathologies in cetaceans.

Keywords Anencephaly, Virtopsy, CT, 3DSS, MRI, *Tursiops aduncus*

Background

Among the congenital neural tube disorders, anencephaly is a disease that compromises the formation of the neural folds, basicranium, and neurocranium. The skeletal, and consequently the neural, formation are affected by the lack of paraxial mesodermal closure and rostral neuronal folds during embryonic skeletal-neural axis formation [1–3] although reopening of the neural tube after closure has been hypothesized [4]. The aetiology of anencephaly is multifactorial and not fully understood, including environmental and mechanical insults, drugs [4], vitamin B9 (or folic acid) deficiency and infections [1, 5]. However, recent genetic analysis discovered some genes related to this defect such as PDGFRA involved in cell proliferation and embryonic development, MTHFR and GLDC involved in the folate metabolism [6], VANGL and other genes of planar cell polarity pathway responsible for normal tube elongation and closure [3, 6].

In humans, anencephaly appears differently based on the world region, for example in USA the frequency is 1:1000 births (with North and South Carolina 1:500) or in China 1:200 births, with more frequency in females than males [3].

In veterinary medicine, anencephaly has been reported in lambs [7, 8] and dogs [2, 9]. In cetaceans, certain congenital malformations have been reported such as vertebral column malformations [10–13], cranial malformations [14–16], umbilical cord [17, 18]. The specimen analysed in our study has been previously reported by Brook [1] who described that early diagnosis of the anencephalic live fetus was made by prenatal transabdominal ultrasonography. Gross and radiographic examination of the delivered term stillborn (after 359-day of gestation) were conducted and multiple malformations were observed such as kyphosis of the cervical and lordosis of the thoracic vertebrae, narrowed triangular skull base, thickening of an undersized maxilla, cranial vault defect and absence of the frontal, interparietal and parietal bones [1].

The aims of our study were to revisit, supplement and describe the findings in a preserved anencephalic Indo-Pacific bottlenose dolphin specimen after 30 years, using postmortem imaging and virtopsy techniques, developed in human forensic medicine by Thali and his colleagues [19, 20]. This multimodal approach integrates different radiological and imaging techniques such as 3D surface scanning (3DSS), computed tomography (CT) and magnetic resonance imaging (MRI).

Case presentation

In summary, Brook [1] reported a case study involving a juvenile female Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), kept under human care the Ocean Park Corporation, approximately 6 years old, that presented an acute episode of bacterial pneumonia caused by *Staphylococcus aureus* in February 1991. The day after the detection of the disease, abdominal ultrasonography and chest radiography were performed and no abnormality was found. However, repeated ultrasonographic examination at day 7 revealed an hypoechoic lesion attributable to an abscess on the ventrolateral aspect of the right lung, which gradually reduced to a hyperechoic fibrotic focus over the next 2 years. After a possible episode of copulation in July, the animal presented signs of withdrawal, lethargy and inappetence in September. The suspect of pregnancy was confirmed by ultrasonography that showed a fetus of 6.1 cm long in the left uterine horn and also by elevated progesterone level in the serum (6.2 ng/ml). The ultrasonographic exam of the dam also proved the absence of any acute pulmonary disease. The progesterone level doubled its values two weeks later and an additional ultrasonographic exam was performed, showing an increase of length to 7.8 cm, heart motion but absence of cranium, narrowing of the skull base and prominent orbits. This cranial defects was more visible at week 26, and the fetus was identified as male. The dam maintained good health and weight gain during gestation. Fetal development was normal until week 42 when a kyphosis of the cervical vertebrae was noticed. The fetus remained in a fixed position from then on. By week 50, the spinalmalformation worsened significantly. After 357 days of pregnancy, the dam lost its appetite and 2 days later a male stillborn Indo-Pacific bottlenose dolphin was delivered in February 1992. As reported by Brook [1], the diet of the dam was based on fish (40% of capelins, 20% of sardines, 20% of herrings and 20% of smelts) with the dietary supplements such as calcium, phospholipids and vitamins (C, E and B group).

Gross and radiographic examination that showed narrowed triangular skull base, cranial vault defect and absence of the frontal, interparietal and parietal bones, thickening of an undersized maxilla, kyphosis of the cervical and lordosis of the thoracic vertebrae. The stillborn was immersed and fixed in 10% neutral buffered formalin for about 30 years until recent analyses, in 2023 and 2024.

Whole body 3DSS was conducted with a handheld Artec Leo scanner (Artec 3D, Luxembourg) that was manually operated 360° around the specimen positioned in three orientations (supine, left lateral recumbent, and right lateral recumbent) from a distance between 40–80 cm to allow maximum access of surface geometry and texture to the scanner's sensors.

Whole body postmortem CT was conducted with a Philips 16-slice Brilliance Big Bore CT scanner (Philips Healthcare, Amsterdam, Netherlands). The scan was operated at 120 kV, 47 mA, and 0.8 mm slice thickness. Scan field of view (FOV) was 600 mm.

Whole body postmortem MRI was conducted with a Philips Ingenia Elition X 3.0 Tesla MR scanner (Philips Healthcare, Amsterdam, Netherlands) with head neck coil and dS base coil for the analyses of the head and the cranial part of the thorax up to the first seven thoracic vertebrae, and dS spine coil for the analysis of the rest of the body. In both cases, a fast T_1-weighted 3D sequence was used with the following parameters: FOV = 359 × 640 × 640 mm; repetition time/echo time (TR/TE) = 600/31 ms; voxel size = 0.44 × 0.48 × 0. 48 mm; number of slides = 362.

The acquired 3DSS data were processed in Artec Studio 17 (Artec 3D, Luxembourg) to form a single 3D model in PLY format, which was viewed using a standard 3D viewer. CT images were analysed with Aquarius iNtuition workstation 4.4.13.P7 (TeraRecon, San Mateo, CA, United States), whereas MRI images were processed with Aquarius iNtuition workstation, ITK-SNAP 3.8.0 (www.itksnap.org) and MRtrix3 (https://www.mrtrix.org/). Diagnosis was made by a diagnostic radiographer and imaging researcher (BCWK) who were certificated and had more than 10 years of experienced in postmortem imaging and virtopsy interpretation. Evaluation of anatomical structures was performed by a veterinary anatomist (TG).

3DSS

3DSS provided an enhanced visualization of the true-to-scale surface geometry and photorealistic texture of the specimen over conventional photography. The countershading colouration of the specimen was preserved after 30 years in formalin and documented on the 3D model. The calibrated 3D model allowed rotation, zooming, and measurements of anatomical features, which was useful for comparison with other 3D datasets. All the significant features, including the curvature and depth of the skin defect immediately behind the blowhole reported by Brook [1], were accurately visualized in 3D (Fig. 1).

Postmortem CT

Postmortem CT allowed a detailed visualization of the skeletal features of the specimen. Main findings included absence of the majority of the brain case, reduction of the maxillary, temporal and parietal bones, absence of the interparietal bone, kyphosis of the cervical vertebrae and lordosis of the cranial thoracic vertebrae (Fig. 2). We also observed the two mandibular bones curving inwards to the median plane. Remnants of the frontal bones articulated with the remnants of the parietal bones caudally. The parietal bones, in turn, articulated caudally with the remaining squamous part of the occipital bone caudally (Fig. 2e). In addition, the lack of the formation of the fronto-occipital suture caused the formation of a dorsal opening and therefore absence of the cranial vault (Fig. 2d). The external occipital crest was also absent.

The vertebral, costal and phalangeal formulas were normal and no further skeletal abnormalities were observed.

Postmortem MRI

Postmortem MRI showed certain characteristics of soft tissues. Notably, the skin and the blubber did not develop at the level of the frontal bones and squamous part of the occipital bone ant the crest (Fig. 3). At the usual place of the cranial vault a spongy tissue compatible with the area cerebrovasculosa or cranial venous sinuses were detected which were already visible externally by the 3DSS (Fig. 1). In the caudal part of the remaining brain case, only the brainstem, possibly composed by the medulla oblongata

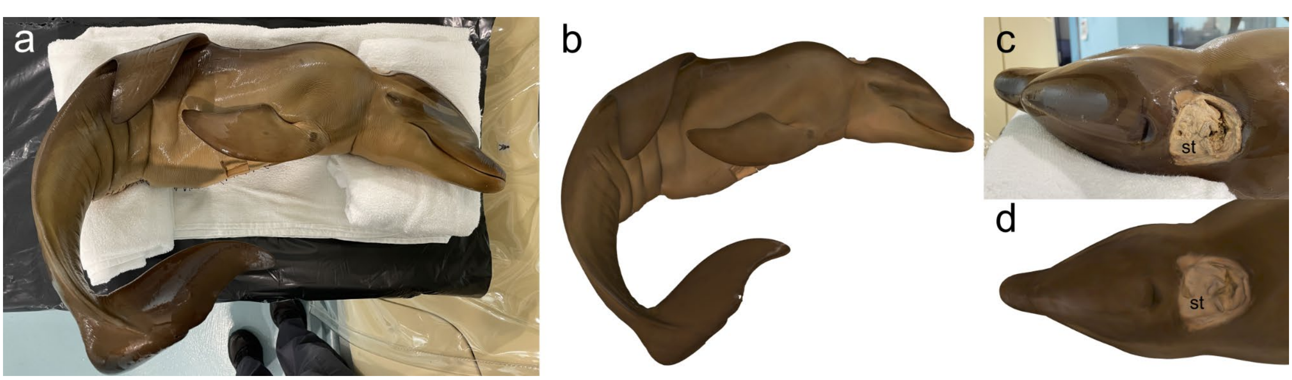

Fig. 1 Photographs (**a** and **c**) and 3D models (**b** and **d**) from 3DSS of the stillborn Indo-Pacific bottlenose dolphin. **a** and **b**, right lateral view of the body; *c* and *d*, dorsal view of the head with focus on the spongy tissue (st) which could represent the cranial venous sinuses or area cerebrovasculosa

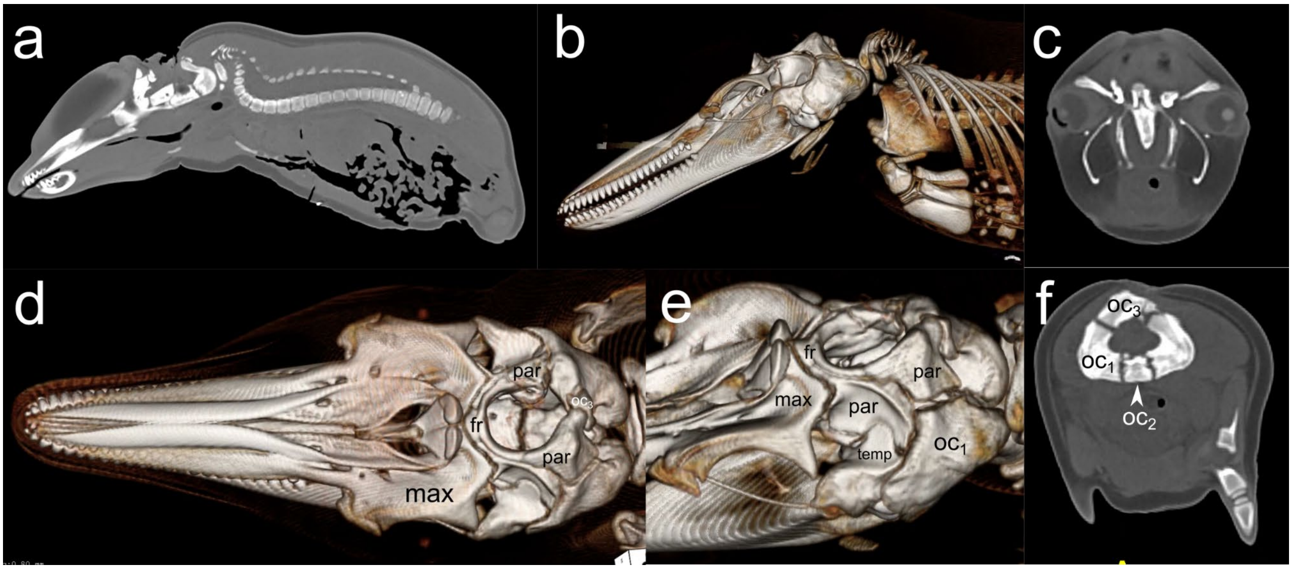

Fig. 2 Postmortem CT images of the stillborn Indo-Pacific bottlenose dolphin. **a**, sagittal view of the whole body showing the absence of the cranial vault, kyphosis of the cervical vertebrae and lordosis of the thoracic vertebrae; **b**, 3D reconstruction of the skeleton including the head and the cranial part of the thorax; **c**, transversal plane showing the decreased thickening of the mandibles and the severe hypotelorism; **d**, dorsal view of the skeletal 3D reconstruction showing the reduced diameter between the lateral margins of the left and right maxillary bones (max), the presence of undeveloped squamous part of the occipital bone (oc_3), the remnants of the frontal bones (fr) and its incomplete fusion with the parietal bone (par); **e**, left lateral oblique view of the skeletal 3D reconstruction showing the existence of the remnants of parietal (par) and temporal (temp) bones; **f**, transversal view of the integrity of the occipital bone in its three components: lateral part (oc_1), basoccipital part (oc_2) and squamous part (oc_3)

and pons (less likely the midbrain) were identified surrounded by the cerebrospinal fluid. Also the cerebellum was not identified. The brainstem then headed dorsocranially and abruptly stopped at the level of the cranial vault defect (Fig. 3a-c). The optic (cranial nerve II) and vestibulo-cochlear (cranial nerve VIII) nerves were visible from their terminations (i.e. retina and cochlea) to then lose them on their way up at the level of the postorbital process of the frontal bone for the cranial nerve II and the sphenoid bone for cranial nerve VIII (Fig. 3d-f). The spinal cord, on the other hand, was present throughout its course along the vertebral column up to the 8th lumbar vertebra.

No other malformations of the skeletal, nervous systems or internal organs of the thoracic and abdominal cavities were identified.

Discussion and conclusions

Postmortem imaging and virtopsy techniques based on 3DSS, CT and MRI have been widely used in human forensic medicine [19–22] and recently adopted also in marine mammal medicine [23–29]. This non-invasive method allows for a detailed analyses of the structures and position of organs prior to any alteration due to conventional necropsy [23, 24, 30–35]. Beyond documenting health profile and finding the cause of death during routine necropsies, virtopsy also has research applications, including the study of fetal malformations in humans [36].

To the best of our knowledge, this is the first report that described a case of anencephaly using 3 advanced postmortem imaging and virtopsy techniques in a cetacean specimen. While the original report published 3 decades ago by Brook [1] found ultrasonography and conventional radiography were extremely helpful for early diagnosis *in utero* and observing skeletal disorders postmortem, virtopsy applied in this report was essential for visualizing all the characteristics of this disease.

In contrast to Brook's [1] description of a total absence of the frontal, interparietal, and parietal bones, postmortem CT confirmed the absence of the interparietal but, on the other hand, showed reduced frontal and parietal bones with the absence of vertex between frontal and occipital bones [37]. No thickening of the mandible was observed on postmortem CT, but rather a convergence in the medial plane, especially in the caudal part, as also reported by Brook [1]. This could explain the radiopacity on the radiograph (see top right radiograph of Fig.4 in Brook [1]) and the severe hypotelorism described. Postmortem CT also confirmed the kyphosis of the cervical vertebrae and lordosis of the cranial thoracic vertebrae, the absence of spina bifida, and other skeletal disorders.

Postmortem MRI provided more detailed analysis and description of internal organs and other soft tissues. Notably, the presence of cranial and neural rudiments, namely the medulla oblongata, the pons, the optic and vestibulocochlear nerves [38], without the involvement of the foramen magnum indicated the development of

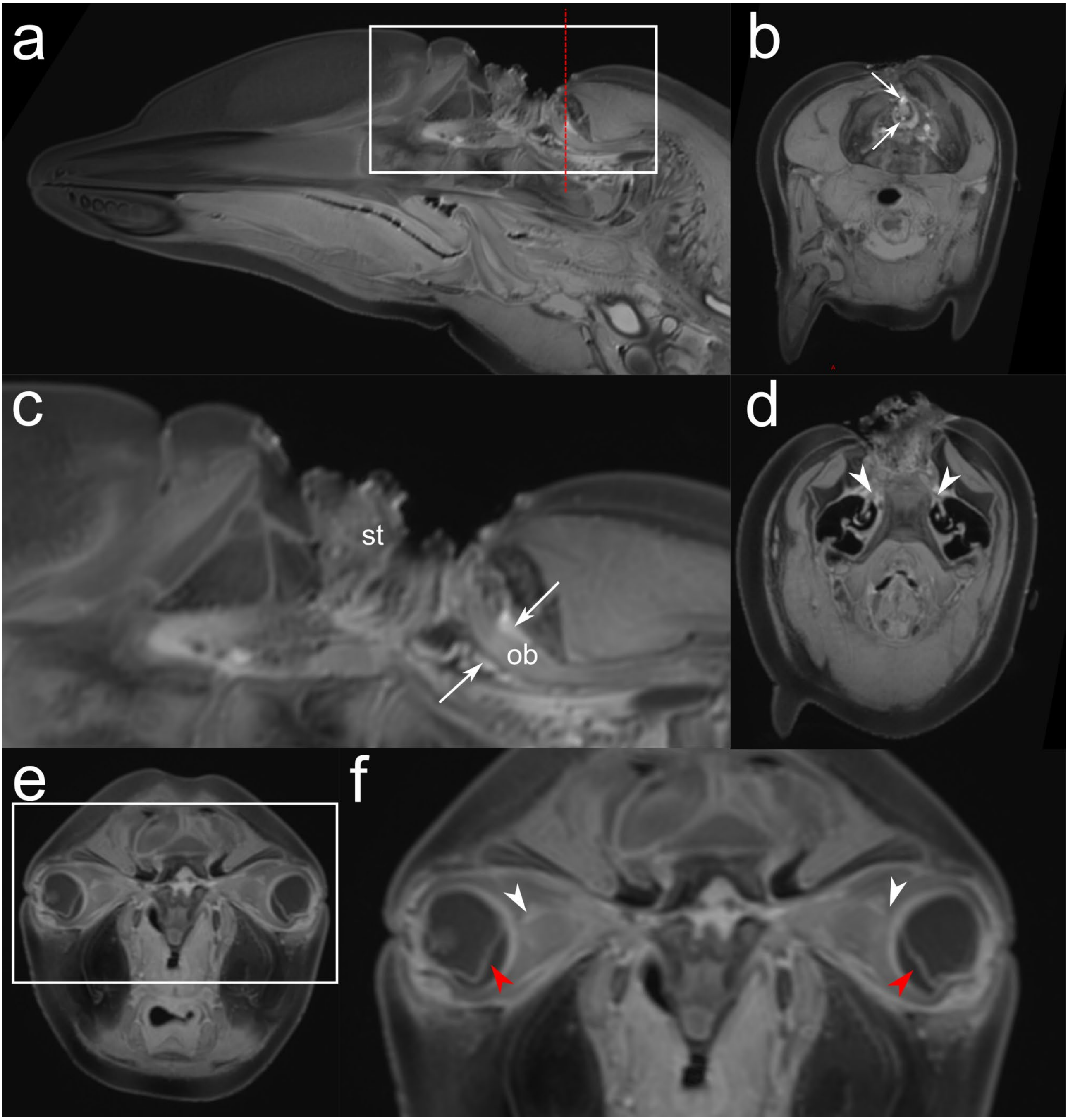

Fig. 3 T1-weighted postmortem MRI images of the head of the stillborn Indo-Pacific bottlenose dolphin. **a**, general sagittal plane; **b**, coronal plane at the level of the red dotted line of *a* with the white arrows indicating the cerebrospinal fluid; **c**, higher magnification of showing the spongy tissue (st) with the white arrows indicating the medulla oblongata; **d**, transverse plane at the level of the middle and inner ear with the arrowheads pointing at the vestibulocochlear nerve; **e**, transverse plane at the level of the eyes and optic nerves; **f**, higher magnification of **e** with white arrowheads pointing at the optic nerves and the red arrowheads pointing at the retina. ac, area cerebrovasculosa; ob, medulla oblongata

a condition known as *meroanencephaly* seen in humans [4, 39]. It was also possible to observe the course of the spinal cord up to the 8th lumbar vertebra. Although, to the best of our knowledge, the literature is devoid of any reference on cetaceans regarding the termination of the spinal cord in the neonatal age, this could be explained by the presence of the spinal cord up to the 5th lumbar vertebra in adult bottlenose dolphins (*Tursiops truncatus* [40, 41]). This phenomenon occurs because, in mammals, the cauda equina is formed due to the faster growth of the bony system compared to the nervous system [42, 43]. In both human and veterinary medicine, MRI and CT are helpful in the differential diagnosis of this disease, particular to similar imaging findings like meningoencephalocele and exencephaly [4, 44]. In cetaceans, according to current information, there are no other reports or studies of neural tube closure malformation, most probably due to the rarity of this disease and the challenges in findings these kind of specimens in nature [44].

It is generally accepted that there are multiple causes of anencephaly, with no specific aetiology [1, 5, 38, 44]. However, early ultrasonographic diagnosis during gestation [1] and information gathered from postmortem imaging and virtopsy led to the conclusion that, the post-infection period or a subclinical infection of the mother [1] was most likely the cause of the non-closure of the neural tube of the fetus rather than vitamin deficiency, considering that the mother was well-fed under human care. Our results were also in agreement with sporadic cases of human anencephaly associated with spinal malformations [45]. Nevertheless, the exact cause of the latter is still difficult to determine, especially in cetaceans. Our findings may shed light on the timing of the defect initiation, i.e. happened between *Stage 2* and *Stage 3* of the embryonic development (adapted from [46], in turn adapted to the Carnegie system of human embryos of [47]). Even though data for *Stage 1* and *Stage 2* is insufficient for a definitive conclusion, the defect in this case was believed to initiate earlier than it does in humans [4], and is not a consequence of exencephaly as seen in mice [48].

In summary, this study reported new findings using advanced radiological and imaging tools on a preserved anencephalic Indo-Pacific bottlenose dolphin specimen after 30 years. Virtopsy offers a more comprehensive understandingof both the external and internal body characteristics compared to findings acquired by gross necropsy on the bone and neural malformations in this case, null congenital malformations in cetaceans.

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Author contributions
TG and BCWK conceptualized the study and interpreted the virtopsy data. TG wrote the first draft of the manuscript. BCWK, TYTC, and HCLT acquired 3DSS and PMCT data and reviewed the drafts of the manuscript. WCWC, CKW, PKS and TG were involved in the PMMRI scan acquisition. WCWC, CKW and PKS also reviewed the manuscript. PM provided the PMCT equipment and was involved in the dolphin specimen management.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The specimen used for this study is a stillborn in the Ocean Park Corporation, as reported in the manuscript, and waspreserved in formalin for further analyses. Therefore, for the purpose of this study the animal was not deliberately sacrificed.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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