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SPECIALTY SECTION

This article was submitted to
Marine Megafauna,
a section of the journal
Frontiers in Marine Science

RECEIVED 30 September 2022

ACCEPTED 30 December 2022

PUBLISHED 19 January 2023

CITATION

Romani-Cremaschi U, Zoppi S, Mattioda V,
Audino T, Marsili L, Varello K, Iulini B,
Marra C, Zoccola R, Battistini R, Dondo A,
Garibaldi F, Berio E, Pautasso A, Rosso M,
Ascheri D, Casalone C, Grattarola C and
Giorda F (2023) *Morganella morganii*
septicemia and concurrent renal
crassicaudiasis in a Cuvier's beaked whale
(*Ziphius cavirostris*) stranded in Italy.
Front. Mar. Sci. 9:1058724.
doi: 10.3389/fmars.2022.1058724

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Audino, Marsili, Varello, Iulini, Marra, Zoccola,
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Rosso, Ascheri, Casalone, Grattarola and
Giorda. This is an open-access article
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Morganella morganii septicemia and concurrent renal crassicaudiasis in a Cuvier's beaked whale (*Ziphius cavirostris*) stranded in Italy

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Information regarding bacterial diseases in Cuvier's beaked whale (CBW, *Ziphius cavirostris*) is scattered and mostly incomplete. This report describes a case of septicemia by *Morganella morganii* in a juvenile male CBW with concurrent renal crassicaudiasis. The animal stranded along the Ligurian coastline (Italy) and underwent a systematic post-mortem examination to determine the cause of death. Histopathology showed lesions consistent with a septicemic infection, severe meningoencephalitis, and renal crassicaudiasis. An *M. morganii* alpha-hemolytic strain was isolated in pure culture from liver, lung, prescapular lymph node, spleen, hepatic and renal abscesses, and central nervous system (CNS). The antimicrobial susceptibility profile of the strain was evaluated with the minimum inhibitory concentrations (MICs) method and reduced susceptibility to Trimethoprim-Sulfamethoxazole is reported. Crassicauda sp. nematodes were retrieved from both kidneys. No other pathogens were detected by immunohistochemistry, serology, or biomolecular analyses. Toxicological investigations detected high concentrations of immunosuppressant pollutants in the blubber. The chronic parasitic infestation and the toxic effects of xenobiotics

likely compromised the animal's health, predisposing it to an opportunistic bacterial infection. To our knowledge, this is the first description of *M. morganii* septicemia with CNS involvement in a wild cetacean.

KEYWORDS

Cuvier's beaked whale, *Morganella morganii*, *Crassicauda*, antimicrobial resistance, septicemia, pollutants(environmental)

Introduction

The Ligurian sea is listed as a high-density area for Cuvier's beaked whales CBW (*Ziphius cavirostris*), the only commonly-observed beaked whale in the Mediterranean Sea (Podestà et al., 2006). CBW's Mediterranean sub-population is listed as "Vulnerable" on the International Union for the Conservation of Nature (IUCN) Red List (Cañadas and Notarbartolo di Sciara, 2018). Being an elusive, deep diver predator, this odontocete is difficult to study in the field and most of the available biological data derive from strandings (Podestà et al., 2006; Heyning and Mead, 2009; Podestà et al., 2016; Carlucci et al., 2020).

Infectious diseases reported in stranded CBW include viral infection by alpha herpesvirus (Arbelo et al., 2010) or morbillivirus (Centelleghé et al., 2017; Felipe-Jiménez et al., 2022), crassicaudiasis (Díaz-Delgado et al., 2016; Febronio et al., 2021), and bacterial diseases (Alstrup et al., 2021; Febronio et al., 2021), including septicemia by *Citrobacter freundii* (Fernández et al., 2011).

Parasitic disease due to *Crassicauda* spp. has been increasingly reported as a significant cause of death in beaked whales (Díaz-Delgado et al., 2016; Febronio et al., 2021; Jerdy et al., 2022) and other cetaceans (Balbuena and Simpkin, 2014) worldwide. For most of the 14 species of the genus, life cycle and transmission are still unclear. Since other marine spirurids usually require intermediate hosts (Anderson, 1988), an indirect cycle involving crustaceans, cephalopods and fishes has been speculated (Lambertsen, 1986; Marcer et al., 2019). However direct transmission cannot be discarded as larvae and/or eggs have been reported in milk (Geraci et al., 2011) and urine (Lambertsen, 1986; Febronio et al., 2021) and transplacental infection has been documented as well (Lambertsen, 1986; Suárez-Santana et al., 2018). These nematodes show different tissue tropisms (urogenital, vascular, integumentary, respiratory) and pathogenic potential, playing a regulatory role in some marine mammal populations (Balbuena and Simpkin, 2014). *Crassicauda boopis* causes severe renal lesions in baleen whales (Lambertsen, 1986; Marcer et al., 2019), *C. grampicola* is associated with moderate to severe sinusitis in Risso's dolphins (*G. griseus*) (Cuvertoret-Sanz et al., 2020) whereas *C. anthonyi* and *C. magna* migration produces verminous arteritis and chronic renal disease in beaked whales (Díaz-Delgado et al., 2016; Febronio et al., 2021; Jerdy et al., 2022). Moreover, several species of crassicaudid

nematodes, including *C. grampicola* (Geraci et al., 2011) and *C. fuelleborni* (Kot et al., 2022) produce parasitic mastitis impacting the reproductive success of endangered cetaceans.

Furthermore, a chronic parasitic infestation may favor infection by opportunistic bacteria, such as members of the family *Enterobacteriaceae* (Paterson and Mathers, 2020), disrupting tissue integrity, translocating pathogens, and/or modulating the immune system of the host (Ashour and Othman, 2020).

Morganella morganii is a gram-negative bacillus, belonging to the *Enterobacteriaceae* family, found in the environment and the digestive tract of humans and animals. It is considered an unusual opportunistic pathogen in animals and nosocomial infections, frequently isolated from the urinary tract or skin wounds (Liu et al., 2016). Its zoonotic potential should not be underestimated, especially in hosts with compromised health status, considering the relatively high mortality rate in human hospitals and the emergence of virulent antimicrobial resistance (AMR) strains.

M. morganii has been identified as a pathogen in ocular lesions in pinnipeds (Thornton et al., 1998), and in two bottlenose dolphins (*Tursiops truncatus*) under human care (Elfadl et al., 2017; Sánchez Contreras and Biancani, 2021). In both cases, the strain exhibited resistance against several antibiotic classes, including new-generation cephalosporins, and in one case was linked to a fatal infection, causing fibrino-hemorrhagic bronchopneumonia and septicemia.

In cetaceans, higher concentrations of *M. morganii* were cultured from diseased or stranded animals compared with the relatively low numbers isolated from their free-ranging counterparts (Martineau et al., 2003; Buck et al., 2006). Moreover, the presence of high numbers of opportunistic pathogens, including *M. morganii* has been linked to immunosuppression in beluga whales (*Delphinapterus leucas*) living in the polluted waters of the Saint Lawrence Estuary (SLE) in Canada (Martineau et al., 2003).

Here we describe the findings of the post-mortem examination and the advanced diagnostic investigations performed on a juvenile male CBW stranded in Italy to determine the cause of death. A case of septicemia caused by *M. morganii* with concurrent renal crassicaudiasis is reported in a threatened cetacean inhabiting the highly polluted waters of the Mediterranean Sea. This study provides valuable information for the conservation of this species and strengthens the role of marine mammals as sentinels for human and ecosystem health (Bossart, 2011).

Materials and methods

Post mortem examination

On October, 3rd 2020, a CBW (IZS number 70726/20) stranded in Sanremo (Italy), along the Ligurian coastline of the Pelagos Sanctuary. A complete field post-mortem examination was performed according to standard protocols (Geraci and Lounsbury, 2005). The animal was a juvenile-subadult male of 498 cm (total length, TL) in good nutritional status, and in moderate decomposition (code 3 – Figure 1A). Blubber thickness was 6 cm. This animal did not display any evidence of interaction with fishing activities, and the stomach content was scarce, consisting of a few, highly-digested cephalopod beaks and lenses. Photo-identification (ID) analysis resulted in a positive match of an individual that was photo-identified in the Ligurian Sea in 2017 and 2018 (CIMA Foundation database, ID: 31705302). Age at first identification was estimated to be 3 or 4yo (weaned, totally brown colored, poorly marked). Photo-ID life history data and natural markings (from marking gain rate; Rosso et al., 2011) suggested an estimated age at death of about seven years.

The necropsy was performed in the field under high environmental temperatures and, due to logistic issues, there was a significant delay between carcass retrieval, visual evaluation of the organs, and sampling. The tissue samples of all the major organs and lesions were collected and split into aliquots for subsequent analyses as previously described (Giorda et al., 2021). Blood serum, aqueous humor, and cerebrospinal fluid (CSF) were collected and kept frozen at -20°C for serological investigations. Parasites were collected in 70%

ethanol and morphologically identified according to taxonomic criteria proposed by Anderson et al., 2009.

Histopathology and immunohistochemistry

Representative tissues (brain, tonsils, lung, prescapular and tracheobronchial lymph nodes, heart, liver, spleen, pancreas, intestine, skeletal muscle, skin, kidney, urinary bladder, adrenal gland, mesenteric artery, and reproductive system) were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at $4 \pm 2 \mu\text{m}$, stained with hematoxylin and eosin (H&E) and examined through a light microscope.

Immunohistochemistry (IHC) for *Morbillivirus* was performed on tissue sections using a monoclonal anti-*Canine Distemper Virus* (CDV) antibody (VMRD) (Di Guardo et al., 2010). *Toxoplasma gondii* IHC was carried out on the brain tissues, using a polyclonal serum of caprine origin (VMRD) (Di Guardo et al., 2010).

Microbiology

Tissue samples including brain, lung, lymph nodes, liver, and spleen were processed for standard aerobic, anaerobic, and microaerobic (5% CO₂) bacterial culture and identification, by biochemical analyses (VITEK[®] MS, bioMérieux SA, Marcy l'Etoile, France) and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen,

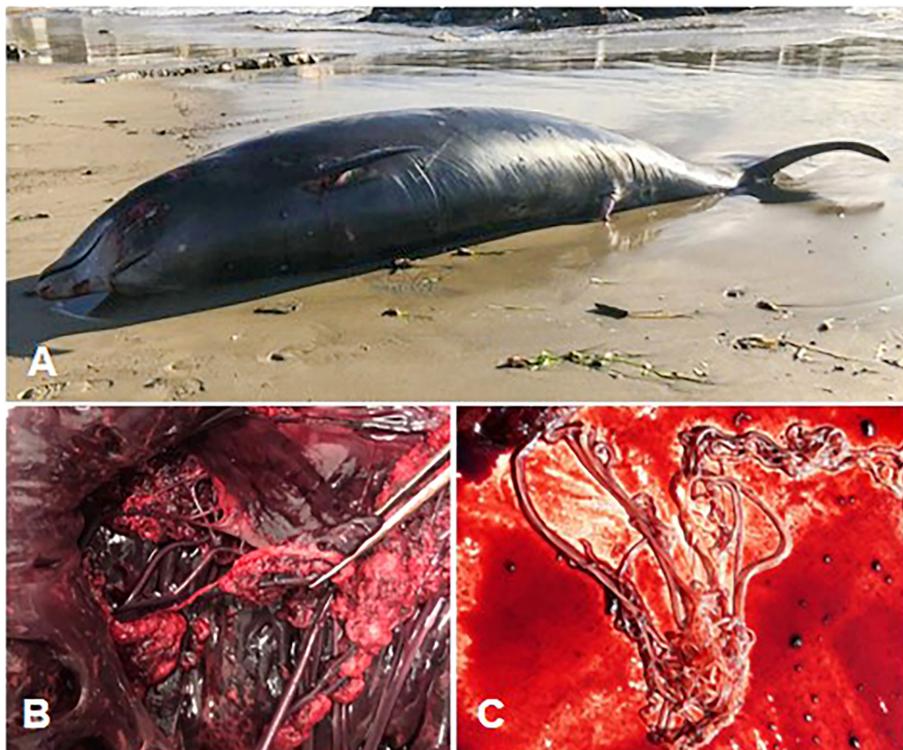


FIGURE 1

(A) Cuvier's beaked whale carcass before retrieval. (B) Right atrioventricular valve - fibrinous valvular endocarditis. (C) Adult *Crassicauda* spp. nematodes retrieved from the renal parenchyma for parasitological analysis.

Germany). Following international recommendations ([World Organisation for Animal Health \(WOAH\), 2018](#)), samples from target tissues underwent specific bacteriological procedures to screen for *Salmonella* spp., *Listeria* spp., and *Brucella* spp.

Urease test on *M. morganii* grown on Blood Agar Base (Liofilchem srl, Roseto degli Abruzzi, Teramo, Italy) was performed according to the manufacturer's instruction (Microbiol srl, UTA, Cagliari, Italy). Intermediate checks of the vials occurred at 30 minutes, 60 minutes, and 4 hours.

To detect and evaluate biofilm formation by *M. morganii*, the Safranin Staining (SS) was performed as previously described ([Ceri et al., 2001](#); [Olson et al., 2002](#)). Briefly, the bacteria were grown into Tryptone Soy Broth (Microbiol srl, UTA, Cagliari, Italy) added with 2% Glucose (Microbiol srl, UTA, Cagliari, Italy) aerobically at 37°C overnight, then resuspended in the same medium at a bacterial concentration of 10⁶ CFU/ml and 2ml were dispensed in a 24-wells plate. The plate was incubated aerobically at 37°C overnight. The wells were washed three times with Phosphate Buffered Saline (PBS, Sigma-Aldrich Merck KGaA, Darmstadt, Germany) and then stained with 2 ml of Safranin 1% (Merck KGaA, Darmstadt, Germany) for 1 minute, due to its affinity to biofilm matrix components (polysaccharides, proteins, lipids, and nucleic acids). After overnight incubation aerobically at 37°C, the wells were washed with Acetic Acid 30% (CH₃COOH, Sigma-Aldrich Merck KGaA, Darmstadt, Germany) to remove biofilm and 200 µL of well content were dispensed in triplicate into 96 wells-microplate and read spectrophotometrically at a wavelength of 492 nm. *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922 were used as positive control (strong biofilm former) and negative control (no biofilm former), respectively.

Antibiotic susceptibility testing

Antibiotic susceptibility of the cryopreserved *M. morganii* strain was tested using the minimum inhibitory concentrations (MICs) method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) ([CLSI, 2018b](#)). Quality controls of the plates used for MIC were performed according to Table 5 of the CLSI VET01S supplement ([CLSI, 2020](#)). MIC breakpoints (expressed in µg/mL) were evaluated and interpretative criteria were retrieved from both human ([CLSI, 2018b](#); [CLSI, 2018a](#)) and veterinary CLSI Standards ([CLSI, 2020](#)) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) ([The European Committee on Antimicrobial Susceptibility Testing, 2022b](#)).

M. morganii strain was tested with cefotaxime for evaluating the synergistic effects when combined with clavulanic acid, which inhibits ESBL β-lactamases, and cloxacillin, which inhibits AmpC β-lactamases. The Combination Disc Tests (Liofilchem srl, Roseto degli Abruzzi, Italy) are carried out using a 10mm paper disk containing cefotaxime alone or in combination with clavulanic acid, cloxacillin, or both of these inhibitors. The inhibition zone around the cefotaxime disc combined with inhibitors is compared with the zone around the disc without the inhibitors according to EUCAST guidelines ([The European Committee on Antimicrobial Susceptibility Testing, 2017](#)).

MIC values were also compared with EUCAST MIC distributions based on collated data from an increasing total of more than 30 000 MIC distributions from worldwide sources ([The European Committee on Antimicrobial Susceptibility Testing, 2022a](#)).

When available on the EUCAST website, Epidemiological Cut-off values (ECOFFs) that distinguish microorganisms without (wild type) and with (non-wild type) phenotypically detectable acquired resistance mechanisms to a specific pharmacological active substance, were reported and compared with the values obtained from *M. morganii* MIC tests.

Toxicology

Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and dichlorodiphenyltrichloroethanes (DDTs) were measured in blubber. Data were expressed in mg/kg dry weight (d.w) or mg/kg lipid weight (l.w) if the Extracted Organic Material percentage (MOE%) was considered. Measurements were made according to the Environmental Protection Agency method 8081/8082, with modifications ([Marsili and Focardi, 1997](#)). To assess the potential toxicological impact of these POP concentrations in the specimen, two different threshold limits proposed in the literature for PCBs were considered. [Kannan et al. \(2000\)](#) and [Jepson et al. \(2005\)](#) proposed the value of 17.0 mg/kg l.w. of ΣPCB in blubber as the tolerance threshold for no deleterious effects in marine mammals; [Helle et al. \(1976\)](#) and [Jepson et al. \(2016\)](#) prefer a much higher value (41 mg/kg l.w. ΣPCB in blubber) as a toxicity threshold for reproductive impairment in Baltic ringed seals (*Pusa hispida*).

Molecular and serological investigations

Molecular detection of *Dolphin Morbillivirus* (DMV) ([Verna et al., 2017](#)), *Herpesvirus* (HV) ([VanDevanter et al., 1996](#)), *T. gondii* ([Vitale et al., 2013](#)), *Brucella* spp. ([Baily et al., 1992](#)) and *Photobacterium damsela* sub. *damsela* ([Osorio et al., 2000](#)) was routinely performed on target tissues.

Serological investigations to screen for the presence of specific antibodies against DMV, *Brucella* spp. and *T. gondii* ([Di Guardo et al., 2010](#)) were performed on serum, CSF, and aqueous humor.

To corroborate bacterial ID obtained by MALDI-TOF MS, the MicroSEQ™ 500 16S rDNA PCR Kit (Thermo Fisher Scientific Inc., Waltham, USA) was used for the amplification of the first 500 base pairs(bp) of the 16S ribosomal RNA gene (rDNA) of the *M. morganii* isolate. DNA extracts were made from pure cultures of *M. morganii* by thermal lyses in PrepMan™ Ultra solution (Thermo Fisher Scientific Inc., Waltham, USA) following protocol for gram-negative bacteria described by the manufacturer. Positive samples were subjected to Sanger sequencing reaction using the MicroSEQ™ 500 16S rDNA Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, USA). Sequencing products were analyzed on the Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzers (Thermo Fisher Scientific Inc., Waltham, USA). Electropherograms were processed with the Bioedit 7.2.5 software (Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.) and the sequences (forward and

reverse) were aligned to obtain a *consensus* sequence. The *consensus* sequence was uploaded to the GenBank[®] server and compared with available sequences retrieved from the National Center for Biotechnology Information (NCBI) database through the Basic Local Alignment Search Tool (BLAST, <http://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 10/08/2022)

Results

Post-mortem findings

The most significant gross findings at necropsy were observed in the vascular system and the kidneys. The vascular wall of the mesenteric arteries and their branches was hard, thickened with endothelial intraluminal pale-yellow plaques of fibrinous-purulent material, partly calcified. Greyish-yellow fibrinous deposits were attached to the right atrioventricular valve leaflets (fibrinous valvular endocarditis - [Figure 1B](#)). Light discoloration of the left myocardium was also observed. Multifocal pyogranulomatous lesions were observed in both kidneys, heavily parasitized by *Crassicauda* sp. nematodes. Multifocal abscess-like lesions of approximately 3 cm in diameter were observed in the perisplenic and perihepatic areas. Red discoloration of the blubber was reported in the cranio-dorsal region and in the melon, alongside multiple, light yellow, abscess lesions of about 0.5 cm in diameter. Upon the opening of the skull, meningeal fibrosis and hemorrhagic CSF were observed.

Renal nematodes were identified as *Crassicauda* sp. according to the morphometrics of the cephalic and terminal portions of adult parasites ([Figure 1C](#)).

Although specific investigations could not be performed, there was no evidence of gas emboli in the tissues examined, as a consequence of gas and fat embolic syndrome, known to affect this species in particular circumstances, such as military exercises ([Fernández et al., 2005](#)).

Histopathology and immunohistochemistry

Histologically, a mixed inflammatory infiltrate associated with foci of necrosis, partly mineralized, was observed mostly in the tunica media of arterial vessels. In the SNC, severe pyogranulomatous encephalitis associated with mild non-suppurative meningitis was diagnosed ([Figure 2D](#)). In all SNC areas, perivascular cuffings and vasculitis were observed ([Figures 2A, B](#)). Microabscesses, granulomas, and a mixed inflammatory infiltrate invaded the cerebral parenchyma ([Figure 2C](#)).

Multiple hemorrhages were scattered in the pulmonary parenchyma. The bronchial submucosa was filled with a mixed inflammatory infiltrate and the associated blood vessels were congested. In the mucosa of the first gastric chamber, foci of pyogranulomatous inflammation (gastritis) were observed multifocally. In the spleen and lymph nodes, lymphoid follicles were depleted and surrounded by multifocal hemorrhages. In all

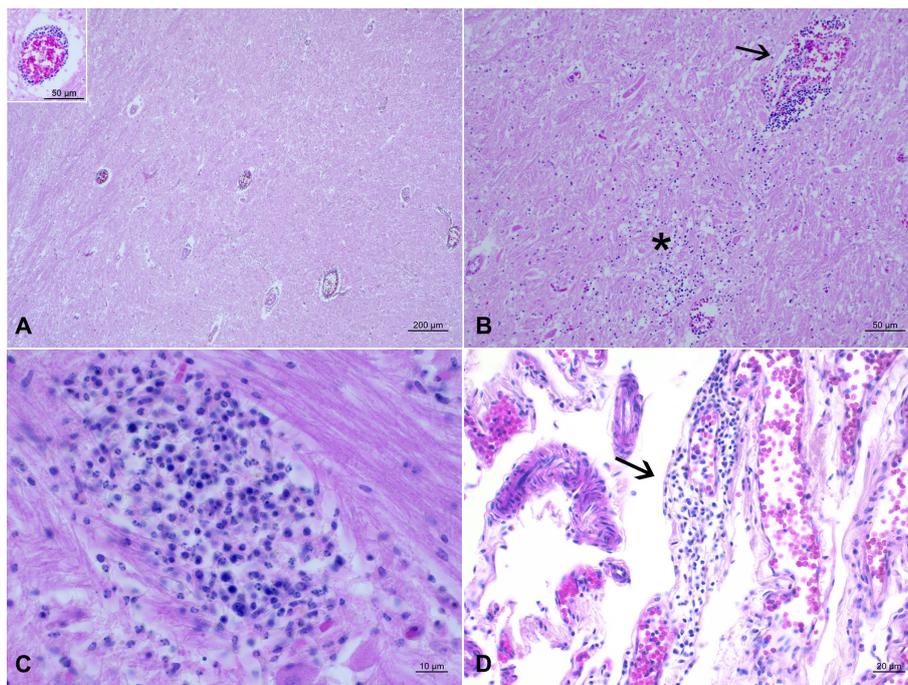


FIGURE 2

Neuropathological lesions observed in the stranded Cuvier's beaked whale. HE. (A) Thalamus. Severe encephalitis with several perivascular mononuclear cuffings. Inset: perivascular cuffing characterized by mononuclear cells infiltration. (B) Pons. Vasculitis with damage to the vessel wall causing leakage of red blood cells with formation of perivascular hemorrhages and presence of mononuclear inflammatory infiltration around it (arrow) and invading the underlying neuroparenchyma (asterisk). (C) Pons. Small granuloma, as an outcome of a microabscess, constituted of macrophages, lymphocytes, and plasma cells. (D) Parietal cortex. Focal and mild non-suppurative meningitis (arrow).

these organs, lesions were described alongside changes compatible with mild to moderate autolysis.

Several other internal organs presented with a severe grade of autolysis due to the delay in sampling and unfavorable environmental conditions. Heart, liver, kidney, and urinary bladder could not be evaluated due to advanced autolysis.

Immunohistochemical investigations against DMV and *T. gondii* were negative in all tissues examined.

Microbiology

M. morganii alpha-hemolytic strain was retrieved in pure culture from liver, lung, prescapular lymph node, spleen, hepatic and renal abscesses, and CNS. *Pasteurella canis* was also isolated from the lungs. There was no evidence of growth for *Salmonella* sp., *Listeria* spp., and *Brucella* spp.

Urease test performed on freshly cultured *M. morganii* strains gave positive results in less than 30 minutes. SS test revealed that the isolated *M. morganii* strain was a weak biofilm former (*M. morganii* OD Mean = 0.082; Negative control OD Mean = 0.057; Positive control OD Mean = 0.273).

Antibiotic susceptibility testing

The results of the MIC test performed on the *M. morganii* isolate, including both CLSI and EUCAST interpretative criteria (clinical breakpoints), are shown in Table SM1. The obtained MIC values were compared to the ECOFFs (and/or TECOFFs) available on the EUCAST site ("European Committee on Antimicrobial Susceptibility Testing. Data from the EUCAST MIC distribution website, last accessed 11/09/2022". <http://www.eucast.org>).

The isolate showed natural resistance to the fixed-dose association Ampicillin-Sulbactam and Amoxicillin-Clavulanic Acid, Oxacillin, Ampicillin, Amoxicillin, most of the first- and second-generation Cephalosporins (Cefazolin, Cefalothin, Cefalexin), Macrolides, Lincosamides, Tetracycline, Nitrofurantoin and Colistin as reported in Morganellaceae. Combination Disc Tests for evaluation of Extended-spectrum β -lactamases (ESBLs) and/or AmpC β -lactamase producers gave negative results.

Based on ECOFF and TECOFF records on the EUCAST website, the strain exhibited MIC values suggestive of a non-wild type *M. morganii* for Trimethoprim-Sulfamethoxazole.

Biomolecular and serological investigations

The biomolecular analysis did not detect any DVM, *Herpesvirus*, *T. gondii*, and PDD DNA in target organs. Serological screening for the presence of DMV, *Brucella* spp, and *T. gondii*-specific antibodies retrieved negative results.

Amplification of the 16S rDNA region yielded an amplicon of approximately 530 bp. Sequence alignment with the BLAST tool allowed the identification of *M. morganii* species with homology greater than 99% (99,25%) with the *M. morganii* strain DG56-16

chromosome (accession number CP032295.1). The new sequence was deposited in GenBank® with the accession number OQ096688.

Toxicology

Blubber concentrations of organochlorine (OC) pollutants are reported in Table 1. MOE% was 92,9%. The blubber levels of organochlorine contaminants, in particularly polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethane and related compounds (DDTs), were extremely high reaching concentrations of 77.831 mg/kg l.w and 68.279 mg/kg l.w respectively,

Discussion

M. morganii septicemia

We diagnosed the stranded cetacean with a septicemic infection by *M. morganii*. Our diagnosis is supported by the systemic isolation of the bacteria in all major organs and by both macroscopic and microscopic lesions suggestive of septicemia such as multifocal hemorrhages, multifocal abscesses and granulomas, severe pyogranulomatous encephalitis, and endocarditis.

In human medicine, bacteremia and sepsis are quite common features (Bandy, 2020) of *M. morganii* infections whereas CNS involvement is rare. When the pathogen succeeds in trespassing the hematoencephalic barrier, meningitis and parenchymal abscesses are the main lesions observed (Abdalla et al., 2006). In animals, to date, this bacterium has been mostly associated with respiratory pathologies (Elfadl et al., 2017) and there are no reports of CNS involvement, unlike in this CBW that suffered from both meningitis and encephalitis.

All of these pathological conditions are reported more frequently in patients with underlying diseases and/or immunosuppression (Bandy, 2020). Urinary tract infections (UTIs) are frequently the original foci of the bacteremia, as biofilm formation and bacterial growth in the urinary tract are enhanced by the urease activity of this organism. Other typical sources of bacteremia in humans are soft tissues and hepatobiliary tract infections (Liu et al., 2016).

In this CBW, dermatological disease or deep wounds were not observed. Gross hepatobiliary abnormalities were not reported either, even though autolysis prevented exhaustive histopathological examination. Therefore, we hypothesize that renal crassicaudiasis and a high burden of organic pollutants have been the most likely predisposing factors for an infection by *M. morganii*.

TABLE 1 OCs levels in the blubber of the stranded CBW.

	mg/kg d.w	mg/kg l.w.
HCB	0.396	0.426
DDTs	63.431	68.279
PCBs	72.305	77.831
Total OCs	136.133	146.538

A parasitized organ represents an optimal pabulum for bacterial growth. For instance, Suárez-Santana et al. (2018) described septic prostatitis and ascending cystitis in two spotted dolphins (*Stenella frontalis*) with heavy prostatic and urethral *Crassicauda* infestation, speculating that nematode parasitism might have favored bacterial colonization. Furthermore, our isolate was phenotypically capable of urease and biofilm production, and alpha-hemolytic strains produce a potent hemolysin with hemolytic and leucocidal properties similar to *E.coli* ones (Eberspacher et al., 1990). However, since autolysis did not allow to confirm or exclude a UTI, it is difficult to determine whether *M. morganii* retrogradely colonized the kidney via the lower urinary tract or if the pathogen could have been translocated during the parasitic migration from the intestine.

Interestingly, another opportunistic pathogen, *P. canis*, was isolated from the lung. This bacterium is also part of the microbiota of the oral and upper respiratory tract mucosa of animals, especially carnivores (biovar 1) and ruminants (biovar 2), and it has been rarely reported as a cause of pneumonia in patients with lung disease or immunodeficiency (Arun et al., 2019). *P. canis* could have overgrown on existing pulmonary lesions observed in this CBW, worsening the respiratory competence of the animal.

The health status of the animal was also compromised by a high burden of organic pollutants with demonstrated detrimental effects on the immune system (Marsili et al., 2004; Marsili et al., 2019; Centelleghé et al., 2019). Blubber concentrations of organochlorine compounds (OCs), particularly DDTs and PCBs, were very high both compared to the values found in other CBWs sampled in the Mediterranean basin (Hatzianestis et al., 1998; Bains et al., 2020) and compared to those measured in BWs from other parts of the world (Knap and Jickells, 1983; Bachman et al., 2014). The blubber PCBs concentrations in the studied specimen of Cuvier's beaked (77.8 mg/kg l.w.) largely exceeded the PCBs toxicity thresholds reported (Kannan et al., 2000; Jepson et al., 2005; Jepson et al., 2016).

A chronic parasitic infestation and the toxic effects of immunosuppressant xenobiotics represented for this CWB a lethal combination that made the animal poorly immunocompetent, as confirmed histologically by the severe lymphoid depletion, and prone to succumb to a non-negligent opportunistic pathogen as *M. morganii* (Liu et al., 2016).

Antimicrobial susceptibility of the *M. morganii* isolate

Since the isolation of antibacterial-resistant (ABR) strains is also increasing in marine animals (Blasi et al., 2020), we performed additional testing to evaluate the antimicrobial susceptibility profile of our strain.

The isolate was confirmed to be sensitive to the antibiotics normally used to treat *M. morganii* infection: aminoglycosides, third- and fourth-generation cephalosporins, carbapenems, quinolones, chloramphenicol, sulfonamides and their association with trimethoprim.

Differently from our case, resistance to third- and fourth-generation cephalosporins has been previously observed in two *M. morganii* strains cultured from captive cetaceans lesions (Park et al., 2020; Sánchez Contreras and Biancani, 2021). However, ESBLs

production was not demonstrated in these cases whereas is increasingly reported in strains related to nosocomial infections. ESBLs and AmpC production has been observed in most *Enterobacteriaceae* species, particularly *E. coli* and *Klebsiella pneumoniae* (Sheng et al., 2013), and can be transferred to other bacterial species by horizontal gene transfer. Furthermore, apart from being plasmid-mediated, AmpC production can derive from the deregulation of natural genetically-encoded β -lactamase enzymes in response to antibiotic exposure (Mizrahi et al., 2020). As expected in a free-ranging wildlife species, our strain did not show any AmpC enzyme production since it is unlikely for a wild CBW to be directly exposed to β -lactam antibiotics.

Nevertheless, the natural wide distribution of *M. morganii* could be the direct consequence of its adaptability to the environment. Several mechanisms are involved in acquiring antibiotic resistance, even if, often, adaptive resistance is a reversible reply of the bacteria to gradual antibiotic increases (Liu et al., 2016).

On the other hand, the MIC value (1mg/L) that was observed for Trimethoprim-Sulfamethoxazole exceeded the (T)ECOFF value proposed by EUCAST of 0,5 mg/L to distinguish between wild and non-wild type microorganisms.

As occurred for *Acinetobacter* species exhibiting the highest abundance of sulphonamide-resistant strains (Xiong et al., 2015) or for bacteria isolated from fluvial sediment samples in India and Spain (Kristiansson et al., 2011; Marti et al., 2013), also for *M. morganii*, the relatively low but constant environmental concentrations of sulpham antibiotics could explain the elevation of the MIC of our strains versus Sulfamethoxazole-Trimethoprim and leading to classify it as a “non-Wild Type” strain.

Wastewaters, frequently insufficiently treated, produced in livestock and poultry breeding, aquaculture, and hospitals (Zhou et al., 2022) are one of the main sources of subinhibitory concentrations of antibiotics, favoring the rise and spreading of ABR bacteria and resistance genes (Lépesová et al., 2019).

Moreover, sulphonamides, apart from being widely used in human and veterinary medicine, including aquatic farmed species, exhibit strong hydrophilicity and easily persist in freshwater (Pruden et al., 2012; Danner et al., 2019) and marine ecosystems where they can exert acute or chronic toxic effects to a wide range of organisms, from algae to fishes (Zhou et al., 2022).

Sulfonamides toxicity has been proven in marine fishes (Zhou et al., 2022) but no ecotoxicological studies have been conducted on marine mammals regarding these antimicrobials. Moreover, the health status of marine species is not impacted just by chronic exposure to environmental levels of antibiotics. Still, it is threatened by other contaminants such as heavy metals, microplastics, OCs, and other chemicals.

In this scenario, the data reported in this study may contribute to the understanding of the combined effects of toxic marine pollutants and pathogens on the health status of an endangered species, such as the CWB, that is challenging to study at sea.

Conclusion

Information regarding bacterial infections in CBW is scattered and mostly incomplete, with no antimicrobial susceptibility testing

reported before (Fernández et al., 2011; Alstrup et al., 2021; Febronio et al., 2021). Moreover, detailed and conclusive necropsies are rare for this species in the Mediterranean region (Podestà et al., 2016). In this report, we present the results of systematic post-mortem investigations stressing their importance to fill knowledge gaps and identify both non-anthropogenic and anthropogenic threats for cetaceans, especially for the elusive ones such as the beaked whales. This study expands information on infectious diseases and chemical contaminants affecting marine mammal health and gives new insights into the interaction between pathogens, host, and environment, whose understanding is still scarce to date (Di Guardo et al., 2018) and contributes to better conservation strategies for marine animals.

To our knowledge, this is the first description of *M. morgani* septicemia with CNS involvement in a wild cetacean. *M. morgani* can represent a threat to marine mammals, especially when they are immunocompromised and inhabit highly polluted environments, such as CBWs in the Mediterranean Sea (Baini et al., 2020). As occurred in terrestrial animals (Franzoni et al., 2022), further research is advised to investigate the cumulative and synergic effect of antibiotics and other contaminants on aquatic species. Although the isolate did not show any antibiotic resistance “*sensu stricto*”, a higher threshold of sensitivity for Trimethoprim-Sulfamethoxazole is reported in this study consisting of the first report of a non-wild *M. morgani* strain in a Mediterranean Cuvier’s beaked whale.

In addition, since adaptive resistance has been well established in *Enterobacteriaceae* (*E. coli*, *S. enterica*), while further investigation for *M. morgani* is suggested (Liu et al., 2016), this report should be the door opener for these studies both in human as well as in veterinary medicine in a One Health approach.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors. The sequence generated in the present study was submitted to the GenBank® database with the accession number OQ096688.

Ethics statement

Ethical review and approval was not required for the animal study because the investigations were performed on a dead stranded cetacean. No live animals were involved.

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Conceptualization, UR-C, CG, FG. Acquisition of data, VM, TA, LM, KV, BI, CM, RZ, RB, AD, FGa, EB, AP, MR, DA. Methodology, UR-C, SZ, TA, FG. Data curation, UR-C, SZ, CG, FG. Software, FG. Supervision, CG, FG. Manuscript drafting, UR-C, SZ. Funding acquisition, CC. Writing review and editing, CC, FG. All authors reviewed and agreed on the current version of the manuscript.

Funding

This research was funded by the Italian Ministry of Health [Ricerca Corrente 2019 IZS PLV 05/19 and Ricerca Corrente 2021 IZS PLV 06/21].

Acknowledgments

The authors are grateful to Sabina Airoidi, Aurelie Moulins and Walter Mignone for their assistance during necropsy procedures.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2022.1058724/full#supplementary-material>

SUPPLEMENTARY TABLE 1

Results of the MIC test performed on the *M. morgani* isolate, including CLSI and EUCAST interpretative criteria (clinical breakpoints). When ECOFF (and TECOFF) were available, they were inserted and compared with the MIC values of the tested strain.

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