Systemic *Erysipelothrix rhusiopathiae* in seven free-ranging delphinids stranded in England and Wales

RPH: *Erysipelothrix rhusiopathiae* in free-ranging delphinids

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ABSTRACT: Microbiology records for 1,127 cetaceans stranded on English and Welsh beaches and examined at the Institute of Zoology between 1990 and 2019 were reviewed to identify cases of *Erysipelothrix rhusiopathiae*, an uncommon but potentially fatal zoonotic pathogen. Once cases were identified, prevalence was calculated, corresponding postmortem reports were reviewed, common gross and histopathological findings identified, and antibiotic susceptibilities determined. Overall prevalence for *E. rhusiopathiae* was 0.62% (7/1,127; 95% CI: 0.30 - 1.28%). It was isolated from three bottlenose dolphins (*Tursiops truncatus*), three harbor porpoises (*Phocoena phocoena*), and one short-beaked common dolphin (*Delphinus delphis*), with a prevalence of 21.4% (3/14; 95% CI: 7.6 - 47.9%), 0.39% (3/779; 95% CI: 0.13 - 1.13%), and 0.47% (1/212; 95% CI: 0.08 - 2.62%) for each species,

respectively. *E. rhusiopathiae* resulted in septicemia in all cases from which it was isolated. Gross necropsy findings included pulmonary edema (5/7), hemorrhage (5/7) and/or congestion of various organs (4/7), and serosanguineous effusion (3/7: pericardial (3/7), pleural (2/6), abdominal (2/6)). Congestion (5/5), bacterial emboli (4/5), and hemorrhage (4/5) were commonly observed on histopathology and acute renal tubular injury (2/5) and pulmonary edema (2/5) were occasionally observed. Routine bacterial cultures were vital in identifying *E. rhusiopathiae* since gross lesions were often subtle and nonspecific. The liver, kidney, and brain were key organs from which *E. rhusiopathiae* was consistently isolated. Antibiotic resistance was uncommon and was only observed for amikacin and trimethoprim sulfonamide. Penicillins were consistently effective, along with fluoroquinolones, macrolides, clindamycin, cephalexin, and oxytetracycline.

KEY WORDS: *Erysipelothrix rhusiopathiae*, zoonosis, delphinid, septicemia, prevalence, antibiotic susceptibilities

1. INTRODUCTION

The bacterium *Erysipelothrix rhusiopathiae* is a zoonotic Gram-positive rod or non-branching filament found worldwide; it is a catalase- and oxidase-negative facultative anaerobe (Brooke & Riley 1999, Markey et al. 2013). Although *E. rhusiopathiae* is ubiquitous and can persist in the environment for weeks to months, infected animals are thought to be the main sources of infection (Lehane & Rawlin 2000, Wang et al. 2010). It has been isolated from a wide variety of mammals, birds, reptiles, fish, and insects (Reboli & Farrar 1989, Wang et al. 2010). Feces, urine, and respiratory secretions from infected animals

and skin mucus of infected fish are known to harbor *E. rhusiopathiae*; infection is spread via ingestion or through dermal abrasions (Wang et al. 2010, Opriessnig & Coutinho 2019).

Commonly reported manifestations of *E. rhusiopathiae* range from subclinical to rhomboidal cutaneous lesions (pigs), septicemia (birds, pigs), chronic arthritis (sheep, pigs), and/or endocarditis (pigs) (Wang et al. 2010, Bobrek et al. 2013, Opriessnig & Coutinho 2019). Necropsy findings with *E. rhusiopathiae* are well described in pigs and birds with septicemia. Gross findings may be unremarkable, or they may include pulmonary edema, congestion, petechial hemorrhage, and splenomegaly. Rhomboidal dermal plaques resulting from ischemia and lymphadenomegaly may also be observed in pigs. Vasculitis and bacterial emboli are commonly observed on histopathology (Bobrek et al. 2013, Opriessnig & Coutinho 2019).

Cutaneous and septicemic forms of *E. rhusiopathiae* have been described in multiple species of captive cetaceans worldwide (Geraci et al. 1966, Thurman et al. 1983, Kinsel et al. 1997, Venn-Watson et al. 2008). Dunn et al. (2001) reported *E. rhusiopathiae* in 4..2% of the captive population between 1989 and 2000. Cutaneous forms occur most frequently, and usually involve anorexia accompanied by rhomboidal dermal plaques. Early treatment with antibiotics frequently results in resolution (Dunn et al. 2001, Terasawa et al. 2001). The septicemic form often results in death either without premonitory signs or preceded by anorexia, lethargy, and/or rhomboidal dermal plaques (Simpson et al. 1958, Geraci et al. 1966, Kinsel et al. 1997). Necropsy findings with *E. rhusiopathiae* septicemia are varied and may include pulmonary edema, vascular congestion and/or hemorrhage of various organs, dermal infarctions resulting in rhomboidal plaques or ulcerative lesions, enlarged and edematous lymph nodes, and bacterial emboli (Seibold & Neal 1956, Geraci et al. 1966,

Dunn et al. 2001). In contrast to captive animals, current literature on *E. rhusiopathiae* in free-ranging cetaceans is sparse, consisting of only a few case reports (Young et al. 1997, Melero et al. 2011, Díaz-Delgado et al. 2015, Fiorito et al. 2016).

Isolation of pure *E. rhusiopathiae* from at least two internal organs is considered diagnostic of septicemia (Leighton 2008). Recommended sampling sites in cetaceans are gross lesions, particularly the skin, lungs, and lymph nodes (Dunn et al. 2001). Transmission in cetaceans is believed to occur through the ingestion of infected fish, inoculation from the teeth of conspecifics contaminated from infected fish, or opportunistic colonization of wounds (Van Bressem et al. 2008, Fiorito et al. 2016). Although clinical disease in fish has been observed with *Erysipelothrix piscisicarius* (Pomaranski et al. 2020), it has not been reported with *E. rhusiopathiae*.

Zoonotic disease risk from *E. rhusiopathiae* is frequently associated with exposure to infected animals or animal byproducts; fish are often implicated, but cetaceans are also a potential source (Dilborne 1965, Chastel et al. 1975, Reboli & Farrar 1989, Hunt et al. 2008). Many marine mammal rescue, recovery, and disease investigation programs involve staff, students, and volunteers that assist with stranded cetaceans and may potentially be exposed to *E. rhusiopathiae*. Human infection with *E. rhusiopathiae* is uncommon and most often results in a localized cutaneous infection associated with pruritus and painful cellulitis. Rarely, a severe septicemic form can occur which may result in endocarditis and death (Reboli & Farrar 1989, Brooke & Riley 1999). Effective antibiotics are vital for treating severe bacterial infections, including *E. rhusiopathiae* septicemia; however, emerging antibiotic resistance is a global concern in both human and veterinary medicine and is being reported with increasing frequency in marine species (Romney et al. 2001, Wallace et al. 2013, Schaefer et al. 2019).

The Cetacean Strandings Investigation Programme (CSIP) is a multi-partner collaborative research group established in September 1990 to investigate causes of mortality in cetaceans, marine turtles, and basking sharks found stranded on United Kingdom (UK) beaches. Investigations by the CSIP are set through a central government contract at approximately 100 cetaceans per year. Cases are selected out of the 700 – 900 cetaceans which annually strand primarily on the basis of logistical constraints such as carcass accessibility, decomposition code (Deaville et al.(compiler) 2019), and staff availability. Systematic necropsies are performed on all recovered carcasses to determine causes of death, diseases, levels of environmental pollution, reproductive patterns, diet, and population health of cetaceans inhabiting UK waters. The Institute of Zoology (IOZ), one of the CSIP partner organizations, is responsible for the investigation of stranded cetaceans on the English and Welsh coast, excluding Cornwall (the southwestern tip of England).

The aims of this study were to determine the prevalence of *E. rhusiopathiae* in stranded cetaceans with routine microbiology performed at the IOZ between 1990 and 2019, describe the gross and histopathological findings, and determine antibiotic susceptibilities.

2. MATERIALS AND METHODS

Microbiology records for cetacean necropsies performed at the IOZ between 1990 – 2019 were retrospectively reviewed. Bacteriology samples were aseptically collected from the liver, lung, and kidney routinely as well as the brain and grossly abnormal tissues at the discretion of the prosector. Microbiology samples were not collected from tissues where environmental contamination was suspected (for example due to scavenger action) or from

severely decomposed carcasses as determined by the prosector (Deaville et al.(compiler) 2019). Samples were inoculated onto Columbia 5% horse blood agar and chocolate blood agar plates (Oxoid Ltd., Hampshire RG24 8PW, UK), then incubated at 37°C aerobically, anaerobically, and in a CO_2 atmosphere. Plates were examined by a microbiologist at one, two, five, and seven days. Additional culture media was employed for the isolation of other microorganisms as indicated (XLD agar plates for Enterobacteriaceae, Farrell's medium for the selective isolation of *Brucella ceti* and *Brucella pinnipedialis*, and selective enrichment Wilkens-Chalgren agar plates for obligate anaerobic bacteria).

Cases were identified in which *Erysipelothrix rhusiopathiae* was isolated from at least one organ during necropsy and key organs from which *E. rhusiopathiae* was consistently isolated were determined. The identification of *E. rhusiopathiae* was initially determined by Gram's stain and colony morphology and confirmed with the API Coryne system (BioMerieux, Hampshire RG22 6HY, UK) with isolation media that were read after 1, 2, 5, and 7 days. Once identified, isolates were frozen at -80°C on Microbank porous glass beads in a cryopreservative (Pro-Lab Diagnostics, Merseyside CH62 3QL, UK) until accessed for this study. The Microbank storage system is designed for long term storage and retrieval of bacteria without altering antimicrobial susceptibilities (Seidel & Gareis 1995, Veguilla et al. 2008).

Postmortem reports were analyzed for *E. rhusiopathiae* cases. Reports included gross necropsies, which were performed based on a standard protocol (Deaville et al. (compiler) 2019) and where appropriate, included routine histology and parasitology. A standard set of tissue samples along with samples from grossly abnormal tissues were fixed in 10% neutral buffered formalin, routinely processed, stained with hematoxylin and eosin (H&E) with

additional Gram's and Ziehl-Neelsen staining when indicated. Histopathology samples from *E. rhusiopathiae* cases were examined by a single veterinary pathologist using a digital pathology system (Philips IntelliSite Pathology Solution version 3.2).

Relevant details from the postmortem records for the *E. rhusiopathiae* positive cases were summarized. Cetacean age classes were estimated based on dentition and reproductive parameters recorded in the necropsy reports (Raverty et al. 2018). Overall prevalence was determined by dividing the number of *E. rhusiopathiae* positive cases by the total number of cases in which bacterial culture was performed. Confidence intervals (CI) were computed at 95% using Epitools Epidemiological Calculators (Ausvet, Canberra, ACT, AUS). Prevalence for each species from which *E. rhusiopathiae* was isolated was determined in the same manner.

Antibiotic susceptibility was determined for nine antibiotics which were selected for their clinical relevance and variety. Antibiotic disc tests (Mast Diagnostic, Merseyside L20 1EA, UK) were performed using all four agar plates suitable for *E. rhusiopathiae* culture for each isolate (Thermo Fisher Scientific, Hampshire, RG24 8PW): Mueller-Hinton, Mueller-Hinton + 5% horse blood, anaerobe blood with nalidixic acid + tween, and anaerobe blood agar with neomycin as Wilkens-Chalgren anaerobic agar. Multiple plates were employed because a specific antimicrobial susceptibility testing medium for *E. rhusiopathiae* has not been determined (Bridson 2006, Bell et al. 2016). Translucent to semi-transparent alpha-hemolytic colonies are produced by *E. rhusiopathiae* between 18 to 48 hours incubation in CO_2 at 37°C (Markey et al. 2013). An *Enterococcus faecalis* isolate, which also produces translucent alpha-hemolytic colonies, was selected from the lung of a short-beaked common dolphin

(*Delphinus delphis*, SBCD) as a positive control and an uninoculated plate was used as a negative control.

3. RESULTS

3.1 Cases

Necropsies with associated bacterial investigation were performed at the IOZ on 1,127 stranded cetaceans between 1990 and 2019 and included 18 species (Table 1). Pure to predominant isolates of *Erysipelothrix rhusiopathiae* were isolated from multiple organs in seven delphinids: three bottlenose dolphins (*Tursiops truncatus*, BND), three harbor porpoises (*Phocoena phocoena*, HP), and one SBCD. The cause of death was attributed to *E. rhusiopathiae* septicemia in all cases based on gross necropsy, histopathology, and microbiology results.

The *E. rhusiopathiae* positive cetaceans were found in all seasons, stranded along the northeast (n = 2), southeast (n = 2), and northwest (n = 1) coast of Britain; on the banks of the River Thames (n = 1); and on the central west coast of Wales (n = 1) (Fig. 1). Two HP (cases 5 and 6) stranded approximately 55 km apart within the same month. All *E. rhusiopathiae* cases were found dead. Once discovered, carcasses were either necropsied within 24 hours or stored in refrigeration at 4°C until time of necropsy. Necropsies were performed within one day of discovery when possible (n = 4), or within two days (n = 1), three days (n = 1), and nine days (n = 1).

Signalment, month and year of discovery, nutritional state, gross necropsy and histopathology findings attributed to *E. rhusiopathiae*, and comorbidities are summarized in Table 2. The

alimentary tract distal to the esophagus and proximal to the rectum including the pancreas and spleen were missing from the carcass of case 3 and were unavailable for necropsy and bacterial culture; histopathology was not performed on the remaining tissues and bacterial culture was not performed on the liver of this case because of severe autolysis. Tissue samples from case 4 were processed; however, they were too autolyzed for histopathological interpretation and were excluded from this study. The integrity of histopathology samples from cases 1, 2, 5, 6, and 7 varied from no appreciable autolysis to moderate autolysis, except for one pancreas sample which had moderate to marked autolysis (Table 2); all samples were determined to be of sufficient integrity for interpretation by a veterinary pathologist.

3.2 Gross necropsy

Gross findings attributed to *E. rhusiopathiae* septicemia included pulmonary edema (5/7, Fig. 2A), hemorrhage (5/7), congestion (4/7), and effusion (3/7). Petechiae and/or ecchymoses were observed in the stomach (2/6, Fig. 2B-C), heart (1/7, Fig. 2D), pancreas (1/6), blubber (1/7), and acoustic fat (1/7). Vascular congestion was observed in the kidney (2/7), lung (2/7), liver (1/6), adrenal gland (1/7), and thyroid gland (1/7). Effusion was serosanguineous and consisted of pericardial (3/7), pleural (2/6), and abdominal (2/6). Additional finding thought to be associated with *E. rhusiopathiae* septicemia included fibrinous tags on the intestinal serosa (1/6), and cloudy cerebrospinal fluid (1/7). Comorbidities identified grossly included endoparasitism (5/7), lymphadenomegaly (n = 3: pulmonary 3/7, mesenteric 3/6), gastric ulceration (2/6), esophageal ulceration (1/7), gastric mucosal thickening and reddening (1/6), dental abscessation (1/7), and a boney callus along a rib shaft (1/7). The endoparasitism was mild in three cases and severe in two. Nematodes were frequently observed in the respiratory tract (n = 4: *Torynurus convolutus,* 4/7; *Halocercus invaginatus,* 2/7; *Pseudalius inflexus,*

1/7), alimentary tract (n = 4; *Anisakis simplex,* 4/6), and intravascularly (n = 3; *Pseudalius inflexus*, 3/7).

3.3 Histopathology

Histopathology findings associated with *E. rhusiopathiae* septicemia were congestion (5/5), bacterial emboli (4/5), hemorrhage (4/5), acute renal tubular injury (2/5), and pulmonary edema (2/5). Congestion was observed in the spleen (5/5), lungs (3/5), liver (3/5), kidney (2/5), stomach (1/1), thyroid gland (1/2), adrenal gland (1/5), and lymph node (1/5). Gram-positive filamentous/pleomorphic bacterial embolic nephritis (3/5, Fig. 3A-D), adrenalitis (2/5), lymphadenitis (2/5), dermatitis (1/2), cystitis (1/4), pancreatitis (1/4), enteritis (1/4), pneumonia (1/5), splenitis (1/5), and encephalitis (1/5) were observed. Hemorrhage was observed in the lung (2/5), kidney (2/5), stomach (1/1), thyroid gland (1/2), urinary bladder (1/4), heart (1/4), pancreas (1/4), adrenal gland (1/5), and brain (1/5). Necrotizing lymphadenitis, lymphocytolysis, and acute hepatic necrosis (Fig. 3E-F) with intralesional Gram-positive rods and hepatic sinusoidal leukocytosis were observed in one case each and are also believed to be attributed to *E. rhusiopathiae* septicemia. Comorbidities identified microscopically included verminous pneumonia (*P. inflexus*, *T. convolutus, H. invaginatus*), pulmonary arteritis (*P. inflexus)* and lymphadenitis (only chitin present, parasite not identified) in one case and verminous pneumonia (*P. inflexus*, *T. convolutus*), cholangitis (parasite not present), and mild enteritis (*A. simplex*) in one case.

3.4 Microbiology

Erysipelothrix rhusiopathiae was isolated from kidney (7/7), liver (6/6), brain (5/5), lung (3/7), spleen (2/2), heart blood (2/2), peritoneal fluid (1/2), blubber (1/1), adrenal cyst (1/1), tooth abscess (1/1), and rib lesion (1/1). These results and additional bacteria isolated are

recorded in Table 3. The liver, kidney, and brain were identified as key organs for isolating *E. rhusiopathiae*. Zones of inhibition were clearly defined and could be accurately measured in mm on Mueller-Hinton + 5% horse blood (Table 4). All isolates were resistant to amikacin and isolates from cases 1 – 3 were resistant to trimethoprim sulfamethoxazole (TMS); isolates were susceptible to all other antibiotics tested, with penicillins and fluoroquinolones resulting in the largest zones of inhibition.

3.5 Prevalence

Bacterial culture was performed on 1,127 necropsied cetaceans, including 14 BND, 779 HP, and 212 SBCD (Table 1). The overall prevalence for *E. rhusiopathiae* septicemia in stranded cetaceans necropsied at the IOZ between 1990 and 2019 was 0.62% (7/1127; 95% CI: 0.30 - 1.28%). Prevalence for *E. rhusiopathiae* affected species was 21.4% for BND (3/14; 95% CI: 7.6 - 47.9%), 0.39% for HP (3/779; 95% CI: 0.13 - 1.13%), and 0.47% for SBCD (1/212; 95% CI: 0.08 - 2.62%).

4. DISCUSSION

Cases of *Erysipelothrix rhusiopathiae* occurred in all seasons and on various coasts of England and Wales. Two HP stranded less than 55 km apart within the same month; all other occurrences were sporadic. Most cases were observed in juveniles (4/7), followed by geriatric delphinids (2/7). Only one case was observed in an adult, possibly suggesting young and old delphinids are at higher risk for *E. rhusiopathiae* septicemia; however, the sample size is too small for meaningful statistical analysis. The nutritional state was moderate to good in most cases (6/7), consistent with an acute disease process. Prevalence of *E. rhusiopathiae* was significantly higher in BND than in SBCD and HP, suggesting BND are more susceptible to *E. rhusiopathiae* septicemia than other species. Polychlorinated biphenyls (PCBs) are known

to cause immunosuppression (Tryphonas et al. 1991, Schwacke et al. 2011) and have been found in higher levels in BND compared to HP in UK waters (Jepson et al. 2016). The higher prevalence of *E. rhusiopathiae* septicemia in BND in this study may be associated with PCB-mediated immunosuppression; further investigation of a potential toxicological relationship is warranted. It is also possible that BND are inherently more likely than other species to strand with *E. rhusiopathiae* septicemia.

Bacterial septicemia may cause acute systemic inflammation, stimulating a massive release of inflammatory mediators, resulting in endothelial injury/activation followed by edema, effusion, and disseminated intra-vascular coagulation (Bone 1991, Hopper & Bateman 2005, Stearns-Kurosawa et al. 2011). Pulmonary edema, vascular congestion, hemorrhage and cavitary effusion, with bacterial emboli often result, leading to organ failure and death (Semeraro et al. 2012, Iskander et al. 2013). In this study, pulmonary edema and vascular congestion were each observed in six cases; hemorrhage was observed in five; bacterial emboli in four; serosanguineous effusions in three, with suspected effusion (fibrin tags) in a fourth; acute renal tubular injury in two; and necrotic bacterial hepatitis and necrotic bacterial lymphadenitis in one case each. All observed bacterial emboli were Gram-positive and consistent with *E. rhusiopathiae* morphology. These findings along with the pure isolation of *E. rhusiopathiae* from multiple organs are consistent with *E. rhusiopathiae* septicemia (Iskander et al. 2013).

Pulmonary edema, vascular congestion and/or hemorrhage in various organs, and bacterial emboli are commonly reported in cetaceans with *E. rhusiopathiae* septicemia (Young et al. 1997, Terasawa et al. 2001, Díaz-Delgado et al. 2015). Effusions associated with *E. rhusiopathiae* septicemia have been reported previously in three BND as serosanguineous

peritoneal effusion, serous peritoneal effusion, and serous tri-cavitary effusion (Geraci et al. 1966, Díaz-Delgado et al. 2015). Enlarged and edematous lymph nodes are frequently described with cetacean *E. rhusiopathiae* septicemia (Geraci et al. 1966, Kinsel et al. 1997, Díaz-Delgado et al. 2015). Lymphadenomegaly was observed in three cases in the present study, but was attributed to verminous pneumonia, arteritis, lymphadenitis, cholangitis, and/or enteritis in two cases; the underlying cause in the third case was undetermined. Splenomegaly is occasionally reported with *E. rhusiopathiae* septicemia in cetaceans (Simpson et al. 1958, Geraci et al. 1966, Young et al. 1997). Although splenic congestion was observed microscopically in four cases in this study, it was not appreciated grossly, nor was splenomegaly observed.

Raised rhomboidal dermal lesions have been reported in cetaceans with *E. rhusiopathiae*, both with and without septicemia (Simpson et al. 1958, Thurman et al. 1983, Melero et al. 2011, Fiorito et al. 2016); less frequently, ulcerative dermal lesions have been reported (Geraci et al. 1966). Neither rhomboidal nor ulcerative *E. rhusiopathiae*-associated skin lesions were observed in this study. Damage to the epidermis sustained during stranding was common and skin lesions associated with *E. rhusiopathiae* may have gone undetected. Endocarditis is a common sequela in humans with acute to subacute *E. rhusiopathiae* septicemia; it has also been reported in pigs with chronic *E. rhusiopathiae* infections (Reboli & Farrar 1989, Brooke & Riley 1999, Opriessnig & Coutinho 2019). Endocarditis was not observed in this study.

Comorbidities were commonly observed in this study and included mild endoparasitism of the respiratory, gastrointestinal, and cardiovascular systems; verminous pneumonia, arteritis, lymphadenitis, cholangitis, proliferative gastritis, and enteritis; gastric and esophageal

ulceration; dental abscessation; and a healed rib fracture. In addition to *E. rhusiopathiae*, potentially significant bacteria were isolated from case 1 (tooth abscess: *Streptococcus canis*, *Bacteroides* sp.; lung: *Streptococcus agalactiae*) and case 2 (lung: *Vibrio parahaemolyticus*) (Markey et al. 2013). In both cases, pure *E. rhusiopathiae* was isolated from four and three major organs, respectively and considered the primary cause of septicemia and death. Mixed bacterial colonies of *E. rhusiopathiae* and *Aeromonas hydrophila*/*caviae* (Gram-negative rods (Markey et al. 2013)) were isolated from the lung, kidney, spleen, and rib callus in case 7, congruous with a coinfection. However, pure *E. rhusiopathiae* colonies were isolated from the brain and spleen. Additionally, emboli containing Gram-positive filamentous rods (consistent with *E. rhusiopathiae* morphology) were present on histopathology in multiple organs, including the lung and kidney, supporting a diagnosis of *E. rhusiopathiae* septicemia. All other bacteria isolated in these cases were considered normal flora (small intestines: *Clostridium perfringens*, *Escherichia coli*) or attributed to environmental contamination (lungs: *Aeromonas sobria*, *A. hydrophila/caviae*) or postmortem invasion (lungs: *Enterococcus faecalis*, *Shewanella putrefaciens*, *E. coli*; heart blood: *C. perfringens*) (Markey et al. 2013).

Gross changes to affected organs were often subtle or non-specific; performing routine microbiology was valuable in identifying *E. rhusiopathiae* infections. Although some isolation sites were mixed with other bacterial species, most isolates were predominant to pure *E. rhusiopathiae*, supporting *E. rhusiopathiae* as the primary cause of septicemia in all cases. The kidney, liver, and brain were key organs from which *E. rhusiopathiae* was consistently isolated. Although lungs are a recommended *E. rhusiopathiae* sampling site (Dunn et al. 2001), *E. rhusiopathiae* was only isolated from three of seven lung samples and pure *E. rhusiopathiae* colonies were not isolated from the lungs in any case. Aspiration

during live stranding events resulting in perimortem bacterial contamination may account for the frequent isolation of a bacterial species from the lungs of an individual and not from their other organs in this study. Based on the findings in this study, bacterial isolation from the kidney, liver, and brain are recommended for any cetacean in which *E. rhusiopathiae* is suspected, as well as cetaceans without an obvious cause of death on gross postmortem examination.

Antibiotic susceptibility to penicillins and clindamycin; intermediate susceptibility to fluoroquinolones and macrolides; and resistance to aminoglycosides, tetracyclines, TMS, and cefotaxime (third-generation cephalosporine) have been reported previously in *E. rhusiopathiae* isolated from cetaceans (Terasawa et al. 2001, Jones 2004). Similar to previous reports, isolates in this study were susceptible to penicillins and clindamycin, and resistant to aminoglycosides. Isolates from cases 1 – 3 were resistant to TMS, while isolates from later cases had small zones of inhibition, suggesting a decrease in resistance over time. In contrast to previous reports, all isolates were susceptible to fluoroquinolones, macrolides, oxytetracycline, and cephalexin (a first-generation cephalosporine) to varying degrees. Since captive cetaceans are often fed fish sourced from the ocean, it is likely *E. rhusiopathiae* infections in captive cetaceans would have similar antibiotic susceptibility. Based on these results, penicillins continue to be an effective first-line antibiotic for the treatment of *E. rhusiopathiae* in captive cetaceans; aminoglycosides and sulfonamides are not recommended.

Limitations to this study were that sampling for *E. rhusiopathiae* was limited to a proportion of dead cetaceans and cetaceans that have washed ashore, severely decomposed carcasses were not included, and cetaceans stranded on remote beaches may have been excluded because the CSIP were not aware or carcass recovery was not possible. Some cases of *E.*

rhusiopathiae may have been missed if the affected organ was not sampled for microbiology. Additional limitations in this study include variations in postmortem interval, which can result in carcass autolysis or postmortem overgrowth of more proliferative bacteria obscuring the detection of *E. rhusiopathiae*.

This study identified cases of *E. rhusiopathiae* septicemia using microbiology and histopathology. Identification of *E. rhusiopathiae* was confirmed with biochemical tests. Genomic sequencing and genotyping by polymerase chain reaction would have strengthened this study, but financial limitations precluded their use. Despite varying degrees of autolysis, pathological interpretation was possible in five of seven cases. Although *E. rhusiopathiae* was not widely prevalent in this study, when present, it was determined to be the primary cause of death. Previous studies have detected *E. rhusiopathiae* antibodies in free-ranging cetaceans without evidence of clinical *E. rhusiopathiae* infection, suggesting exposure and survival does occur (Suer et al. 1988, Melero et al. 2016).

5. CONCLUSIONS

Overall prevalence of *Erysipelothrix rhusiopathiae* was low in free-ranging cetaceans in England and Wales following necropsy, histopathology, and microbiology performed at the IOZ. Prevalence was significantly higher in BND than in SBCD and HP. Death was attributed to *E. rhusiopathiae* septicemia in the seven cases in which *E. rhusiopathiae* was isolated. Pulmonary edema, petechiae and/or ecchymosis, congestion of various organs, and serosanguineous effusion were commonly observed grossly and congestion, bacterial emboli, and hemorrhage were commonly observed on histopathology. Routine bacterial cultures from the kidney, liver, and brain were an important diagnostic tool since gross lesions were often

subtle or nonspecific; they should be included in cetacean postmortem examinations as standard practice. Antibiotic resistance was uncommon; penicillins continue to be an effective treatment and are recommended for empirical treatment of *E. rhusiopathiae* in captive cetaceans.

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Table 1. Cetacean necropsies that included microbiology conducted by the CSIP[a] at the Institute of Zoology between 1991 and 2019.

Species	No. of necropsies
Harbor porpoise (*Phocoena phocoena*)	779
Short-beaked common dolphin (*Delphinus delphis*)	212
Striped dolphin (*Stenella coeruleoalba*)	37
White-beaked dolphin (*Lagenorhynchus albirostris*)	23
Bottlenose dolphin (*Tursiops truncatus*)	14
Sowerby's beaked whale (*Mesoplodon bidens*)	8
Atlantic white-sided dolphin (*Lagenorhynchus acutus*)	7
Long-finned pilot whale (*Globicephala melas*)	7
Minke whale (*Balaenoptera acutorostrata*)	7
Risso's dolphin (*Grampus griseus*)	7
Fin whale (*Balaenoptera physalus*)	5
Northern bottlenose whale (*Hyperoodon ampullatus*)	4
Sperm whale (*Physeter macrocephalus*)	4
Humpback whale (*Megaptera novaeangliae*)	3
Killer whale (*Orcinus orca*)	3
Pygmy sperm whale (*Kogia breviceps*)	3
Sei whale (*Balaenoptera borealis*)	3
Cuvier's beaked whale (*Ziphius cavirostris*)	1
Total	1127

[a]Cetacean Strandings Investigation Programme

1

Table 2. Signalment, month and year of discovery, nutritional state, and relevant gross necropsy and histopathology findings for seven delphinids with *Erysipelothrix rhusiopathiae* septicemia found stranded on beaches in England and Wales.[a]

Case	**Sex**	**Month**	**Nutritional**	**Findings attributed to septicemia**		**Comorbidities**	
Species	**Age**	**Year**	**state**	**Gross**	**Histopathology[b]**	**Gross**	**Histopathology[b]**
1	F	Nov	Moderate	Tri-cavitary effusion; hemorrhage (ST); congestion (LI, KI)	Pulmonary edema; acute renal tubular injury[2]; hemorrhage (LU, KI[2], ST[1]); bacterial emboli (AG); congestion (LU, LI, KI[2], SP, ST[1], LN); necrotizing lymphadenitis	Gastric ulcer; dental abscess; mild parasitism (RT, AT)	
BND	G	1999					
2	F	Jul	Moderate/ poor	Pulmonary edema; hemorrhage (ST); congestion (AG, TG)	Pulmonary edema; hemorrhage (LU, KI[1], AG, UB, BR[1]); acute renal tubular injury[1]; congestion (LU, LI, KI[1], AG, SP); lymphocytolysis	Mild parasitism (RT, AT, IV)	
HP	J	2001					

3 BND	F J	Dec 2006	Good	Pericardial effusion; hemorrhage (BL, AF)	Samples were not collected because of severe autolysis or they were not available	Esophageal ulcer	
4 BND	F A	Aug 2007	Moderate	Tri-cavitary effusion; pulmonary edema; congestion (LU, KI)	Samples were too autolyzed for interpretation and were excluded from this study	Lymphadenomegaly (PU, ME)	
5 HP	M J	Apr 2012	Good/ moderate	Pulmonary edema; serosal fibrinous tags (IN)	Bacterial emboli (KI, AG, BR); congestion (SP)	Severe parasitism (RT, IV); lymphadenomegaly (PU, ME)	Verminous pneumonia, arteritis & lymphadenitis
6 HP	F J	Apr 2012	Good/ moderate	Pulmonary edema; hemorrhage (HE); cloudy CSF	Hemorrhage (HE); bacterial emboli (KI[1], LN[1], SK); congestion (SP[1])	Gastric ulcer; severe parasitism (RT, AT, IV); lymphadenomegaly (PU, ME)	Verminous pneumonia, cholangitis & enteritis[2]

7 SBCD	M G	Nov 2017	Good/ moderate	Pulmonary edema; hemorrhage (PA); congestion (LU)	Acute hepatic necrosis with intralesional bacteria; hepatic sinusoidal leukocytosis; hemorrhage (PA^{2+}); bacterial emboli (LU, KI^{1}, UB^{1}, SP, PA^{2+}, IN^{2}, LN); congestion (LU, LI, SP)	Gastric mucosal thickening & reddening; mild parasitism (AT); boney callus along rib shaft

[a] **BND** indicates bottlenose dolphin; **HP**, harbor porpoise; **SBCD**, short-beaked common dolphin; **F**, female; **M**, male; **G**, geriatric; **J**, juvenile; **A**, adult; **ST**, stomach; **LI**, liver; **KI**, kidney; **RT**, respiratory tract; **AT**, alimentary tract; **AG**, adrenal gland; **TG**, thyroid gland; **IV**, intravascular; **BL**, blubber; **AF**, acoustic fat; **LU**, lung; **HE**, heart; **CSF**, cerebrospinal fluid; **PA**, pancreas; **LN**, lymph node; **SP**, spleen; **UB**, urinary bladder; **BR**, brain; **SK**, skin; **IN**, intestine; **PU**, pulmonary; and **ME**, mesenteric.

[b] Mildly autolyzed samples are indicated with (^{1}). Moderately autolyzed samples are indicated with (^{2}). Moderate to marked autolysis was observed in one sample and is indicated with ($^{2+}$). All other samples were determined to have no appreciable signs of autolysis by a veterinary pathologist.

1

Table 3. Bacterial culture results for seven delphinids with *Erysipelothrix rhusiopathiae* septicemia found stranded on beaches in England and Wales.[a]

Case Species	*E. rhusiopathiae* positive	*E. rhusiopathiae* negative	Tissue	Additional bacteria isolated
1 BND	Tooth abscess (M) Spleen (P) Liver (P) Kidney (P) Adrenal cyst (P)	Peritoneal fluid Small intestine Lung	Tooth abscess Small intestine Lung	*Streptococcus canis* *Aeromonas sobria* *Bacteroides sp.* *Clostridium perfringes* *Escherichia coli* *Aeromonas hydrophila/caviae* *Streptococcus agalactiae*
2 HP	Liver (P) Kidney (P) Brain (P)	Lung	Lung	*Vibrio parahaemolyticus*
3 BND	Lung (M) Kidney (P) Blubber (P) Brain (P) Heart blood (P)		Lung	*Enterococcus faecalis* *Shewanella putrefaciens*
4 BND	Liver (P) Kidney (P) Heart blood (MP)	Lung	Lung Heart blood	*E. coli* *C. perfringens*
5 HP	Liver (MP) Kidney (P) Brain (NP)	Lung	Lung	*A. hydrophila/caviae*

	Peritoneal fluid (P)		
6 HP	Lung (M) Kidney (P) Liver (P) Brain (P)	Lung	*A. hydrophila/caviae*
7 SBCD	Lung (M) Kidney (M) Liver (M) Spleen (P) Brain (P) Rib callus (M)	Lung Kidney Liver Rib callus	*A. hydrophila/caviae* *Proteus mirabilis* *A. hydrophila/caviae* *A. hydrophila/caviae* *A. hydrophila/caviae*

[a] **BND** indicates bottlenose dolphin; **HP**, harbor porpoise; **SBCD**, short-beaked common dolphin; **M**, mixed bacteria; **MP**, mixed predominantly *E. rhusiopathiae*; **NP**, nearly pure *E. rhusiopathiae*; and **P**, pure *E. rhusiopathiae* isolate.

1

Table 4. Antimicrobial zones of inhibition for *Erysipelothrix rhusiopathiae* isolated from seven free-ranging delphinids found stranded on beaches in England and Wales and plated on Mueller-Hinton + 5% horse blood.[a]

Drug class	Antimicrobial (µg/disk)	Zones of Inhibition in mm						
		Case 1 BND	Case 2 HP	Case 3 BND	Case 4 BND	Case 5 HP	Case 6 HP	Case 7 SBCD
PEN	Amoxycillin 25 µg	23	40	38	30	28	26	30
	Penicillin G 1 µg	20	20	30	18	24	24	22
FLU	Marbofloxacin 5 µg	15	35	25	22	18	20	18
	Pradofloxacin 5 µg	23	34	35	25	28	26	32
LIN	Clindamycin 2 µg	16	32	25	15	21	12	20
TET	Oxytetracycline 30 µg	15	22	20	16	15	12	16
CEP	Cephalexin 30 µg	18	26	25	20	16	19	20
MAC	Erythromycin 5 µg	13	25	28	22	16	14	16
	Gamithromycin 15 µg	22	34	30	18	20	18	26
	Tildipirosin 60 µg	23	24	22	23	18	22	16
SUL	TMS 1.25 /23.75 µg	0	0	0	12	10	12	12
AMI	Amikacin 30 µg	0	0	0	0	0	0	0

[a] **PEN** indicates penicillin; **FLU**, fluoroquinolone; **LIN**, lincomycin; **TET**, tetracycline; **CEP**, first-generation cephalosporine; **MAC**, macrolide; **SUL**, sulfonamide; **AMI**, aminoglycoside; **TMS**, trimethoprim/sulfamethoxazole; **BND**, bottlenose dolphin; **HP**, harbor porpoise; and **SBCD**, short-beaked common dolphin.

1

2

Fig. 1. United Kingdom map with locations of stranded delphinids from which *Erysipelothrix rhusiopathiae* was isolated. Numbers indicate case 1 – 7.

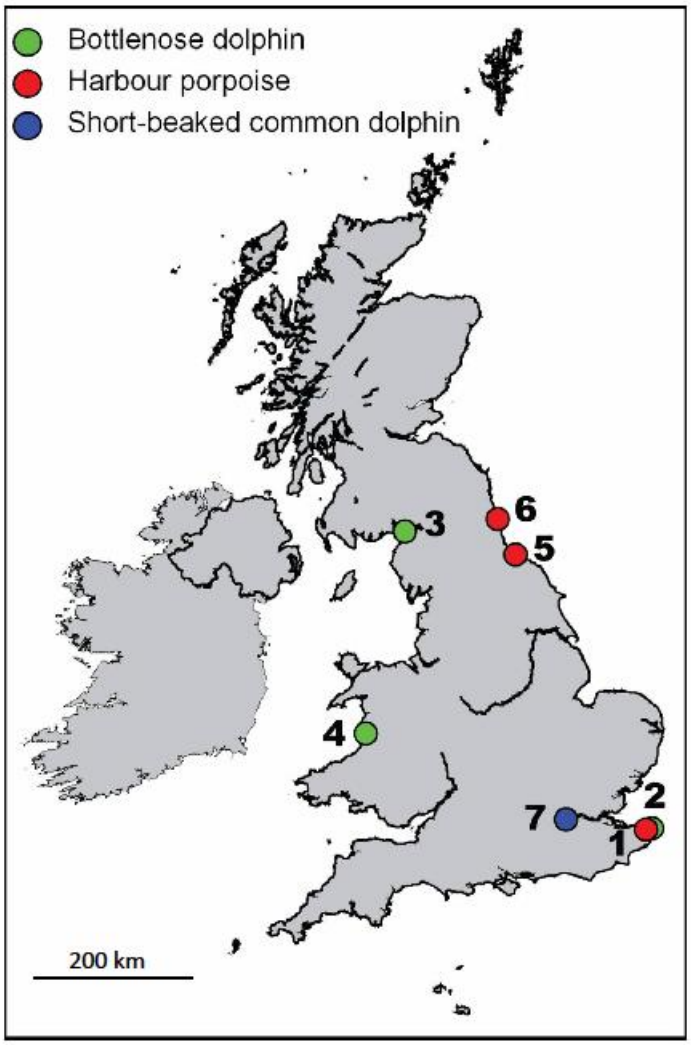


Fig. 2. Gross postmortem images from delphinids diagnosed with *Erysipelothrix rhusiopathiae* septicemia. (A) Pulmonary edema and congestion in a short-beaked common dolphin (*Delphinus delphis*). (B) Gastric ecchymoses in a harbor porpoise (*Phocoena phocoena*). (C) Pyloric petechiae in a harbor porpoise (*Phocoena phocoena*). (D) Endocardial ecchymoses in a harbor porpoise (*Phocoena phocoena*).

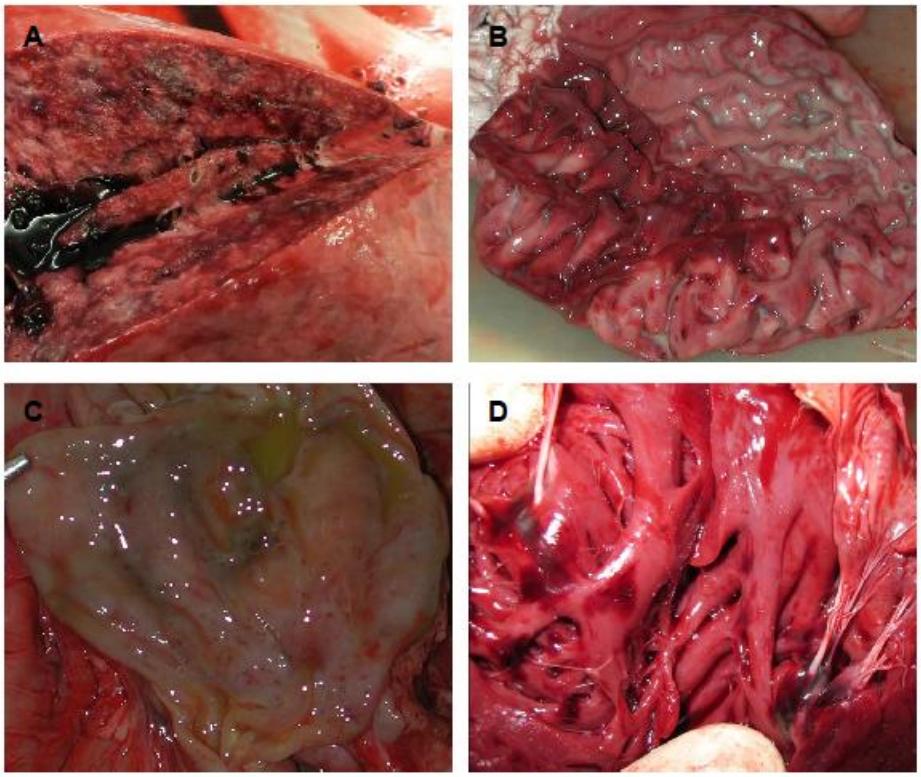
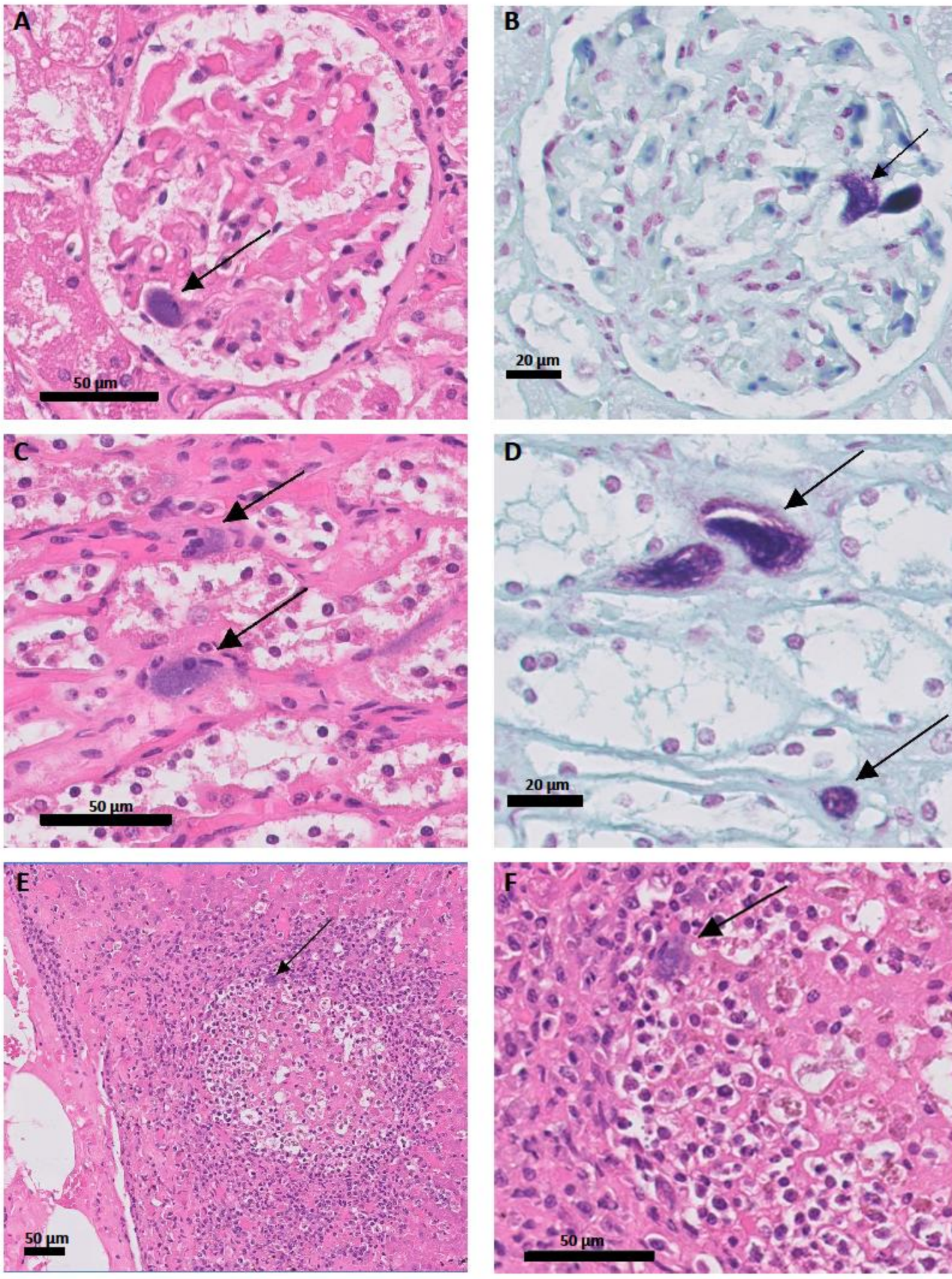


Fig. 3. Histological sections from a short-beaked common dolphin (*Delphinus delphis*) diagnosed with *Erysipelothrix rhusiopathiae* septicemia. (A) Hematoxylin and eosin (HE) stained kidney section with intraglomerular bacterial embolus (arrow). (B) Gram stained kidney section showing an intraglomerular Gram-positive filamentous bacterial embolus (arrow). (C) HE stained kidney section with bacterial emboli in medullary capillaries (arrows). (D) Gram stained kidney section with Gram-positive filamentous bacterial emboli in medullary capillaries (arrows). (E) HE stained liver section showing focal necrotizing hepatitis with an intralesional bacterial cluster (arrow). (F) Higher magnification of focal necrotizing hepatitis with an intralesional bacterial colony (arrow).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