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# *Erysipelothrix rhusiopathiae* isolated from gull-inflicted wounds in southern right whale calves

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**ABSTRACT:** Southern right whales *Eubalaena australis* from Peninsula Valdés, Argentina, show wounds produced by kelp gulls *Larus dominicanus* that feed on the whales' dorsal skin and blubber. During the 2013 whale season, several calves were reported showing kelp gull injuries with a swollen area surrounded by rhomboid-shaped raised edges. Samples from 9 calves were taken in order to establish the etiology of these rhomboid-shaped wounds; 2 calves (one living, one dead) showed gull-inflicted injuries with rhomboid-shaped edges. Samples from the dead calf were histologically characterized by the presence of dermal congestion, suppurative dermatitis and panniculitis, necrotizing vasculitis and vascular thrombosis. *Erysipelothrix rhusiopathiae* was detected by culture and PCR in samples from both calves. In this study we report, for the first time to our knowledge, the isolation of *E. rhusiopathiae* from wounds produced by gull attacks on southern right whale calves, supplying evidence that these wounds may act as an entry route for pathogens. This work provides new information about the consequences of gull-inflicted injuries for whale health.

**KEY WORDS:** Southern right whale · *Eubalaena australis* · *Erysipelothrix rhusiopathiae* · Skin injuries · *Larus dominicanus*

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## INTRODUCTION

*Erysipelothrix* is a genus of Gram-positive, slender, rod-shaped bacteria (Brooke & Riley 1999). The genus consists of 2 main species, *E. rhusiopathiae* and *E. tonsillarum*, and 2 genomospecies represented by *E. rhusiopathiae* serovar 13 (which probably represents the only isolated *E. inapinata*) and *E. rhusiopathiae* serovar 18 (Takahashi et al. 1992, Ludwig et al. 2009). *E. rhusiopathiae*, the only pathogenic species of the genus, is ubiquitous

in nature. It occurs in soil, city sewage and marine locations and can persist for long periods of time in the environment (Reboli & Farrar 1989, Wang et al. 2010). It has been found in a wide variety of domestic and wild animals, including mammals, birds, fish and invertebrates, and is also a commensal organism of some groups of animals (particularly marine fish, mollusks and crustaceans) where the microorganism survives and grows without causing disease (Conklin & Steele 1979, Reboli & Farrar 1989). *E. rhusiopathiae* is an occu-

pational pathogen for humans, prevalent in people whose jobs are closely related to contaminated animals, their products, waste, or soil (Wang et al. 2010). Most cases in humans and other animals probably occur via scratches or cutaneous puncture wounds (Reboli & Farrar 1989).

Swine erysipelas caused by *E. rhusiopathiae* is a disease of great prevalence and economic importance, and domestic swine are the main reservoir of these bacteria (Wood 1992). Three clinical forms have been described in pigs: a severe acute septicemic form of sudden mortality; a milder, subacute urticarial form; and a chronic form with endocarditis or arthritis (Brooke & Riley 1999). The pathognomonic sign of erysipelas in many species is the presence of diamond-shaped skin lesions (Wang et al. 2010). Within marine mammals, cetaceans are most susceptible to the disease. In captivity it is generally believed that they acquire the bacteria from the fish in their diet (Suer & Vedros 1988, Higgins 2000), but other possible sources may be the opportunistic colonization of wounds and vectors between contaminated areas (Wood & Shuman 1981). In cetaceans, dermatologic and acute septicemic forms have been reported in several captive odontocetes, such as *Tursiops truncatus*, *T. aduncus*, *Stenella plagiodon*, *Lagenorhynchus albirostris*, *L. obliquidens*, *Delphinapterus leucas* and *Grampus griseus* (Seibold & Neal 1956, Thurman et al. 1983, Buck & Spotte 1986, Kinsel et al. 1997), and in wild odontocetes such as *Globicephala melas*, *Orcinus orca*, *Phocoena phocoena*, *T. truncatus* and *S. frontalis* (Chastel et al. 1975, Young et al. 1997, Boseret et al. 2002, Melero et al. 2011, Díaz-Delgado et al. 2015). Southern right whales *Eubalaena australis* from Peninsula Valdés, Argentina, show different types of lesions on their skin, including wounds produced by kelp gulls *Larus dominicanus*, which feed on skin and blubber from the dorsum (Bertellotti et al. 2008, Fazio et al. 2012, Fiorito et al. 2015). These ‘gull-peck wounds’ vary in size and severity; in adult whales they are relatively small, but in calves they are often more extensive and severe (Marón et al. 2015). Normal, intact skin provides a physical barrier between the internal milieu and the external world that contains pathogens, but wounds could act as an entry way for microorganisms, generating local or systemic infections (Janeway et al. 2001). During the 2013 whale season, several calves were reported showing kelp gull injuries with raised and rhomboid-shape edges. Due to the particular rhomboid shape of these wounds, *E. rhusiopathiae* colonization was suspected and investigated.

## MATERIALS AND METHODS

### Sample collection

Samples of kelp gull injuries were taken from living (n = 4) and stranded dead (n = 5) southern right whale calves in Peninsula Valdés, Chubut, Argentina, during the 2013 calving season. Samples from living calves were taken from a semi-rigid 5.6 m haul boat powered by a 90 HP outboard engine, using a 5 m long pole tipped with a sterile swab. We approached the mother–calf pairs at a constant speed and at a distance of 3 or 4 m from the pair, waiting for the calf to emerge at the surface and expose its back. When calf surfacing occurred, the sterile swab was rubbed onto the exposed wound to collect the sample. Samples of kelp gull injuries were collected during necropsy of 5 stranded calves, using sterile instruments for later histopathological and bacteriological analysis. Only calves with minimal post mortem skin decomposition were sampled (code 2; Geraci & Lounsbury 2005). Samples from 4 calves were collected by the first author and samples from the fifth calf (BFA02-13) were kindly supplied by personnel of the Southern Right Whale Health Monitoring Program (SRWHMP). Swabs from BFA02-13 were taken from the underside of the lesion, taking all necessary steps to avoid contamination by surface bacteria. Internal organs were unavailable for analysis due to lack of research permits.

For bacteriological analysis, swabs were placed in Stuart’s Transport Medium (Deltalab) and stored at 4°C for 2 d until they were processed in the laboratory. Tissue samples were fixed in 10% neutral buffered formalin, routinely processed for histology, sectioned at 5 µm, and stained with haematoxylin and eosin and Gram stain (Biopur®). Gram stains were also performed from tissue and microbiological samples.

### Isolation and identification of bacteria

At the laboratory, Gram stain was performed for direct observation of bacteria from both samples. Swabs were then plated on brain heart agar with 5% bovine blood, and brain heart broth with 5% bovine blood, and incubated for 48 h at 37°C in microaerophilic conditions. The isolates were identified using classical biochemical assays (oxidase, catalase, sucrose fermentation, motility and sulfide production by triple sugar iron, and sulfide indole motility), as described in Ludwig et al. (2015). The genus of the

isolated strains was confirmed by PCR of 16S rDNA using universal primers designed by Weisburg et al. (1991). The products were sequenced after purification using the Wizard SV Gel and PCR clean-up system kit (Promega) following the manufacturer's instructions. Sequencing was performed on both DNA strands using ABIPrism 3100 BioAnalyzer (Applied Biosystems). The nucleotide sequences were analysed using Blast v.2.0 software ([www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)).

Finally, due to the great variation in serological, biochemical, chemical and genomic properties that exist in species of the genus *Erysipelothrix*, a set of 4 specific 16S rDNA gene primers was used (Takeshi et al. 1999). PCR was performed to distinguish between the 2 species and 2 genomospecies of *Erysipelothrix* (*E. rhusiopathiae*, *E. tonsillarum*, *E. rhusiopathiae* serovar 13 and *E. rhusiopathiae* serovar 18). Samples of observed colonies were taken and suspended in 150 µl of Triton X-100 buffer 1% in TE (1 mM Tris HCl, 0.1 mM EDTA, pH 8) 1× buffer. A DNA extract was prepared by boiling the suspensions for 10 min in a water bath, then a placing in a centrifuge at 10 000 × *g* for 5 min; the supernatant was used as template for PCR. PCR amplification tests were then performed as in Takeshi et al. (1999), with some modifications. Briefly, the PCR mixture (50 µl) was composed of 10 µl of 10× PCR amplification buffer (GoTaq, Promega), 0.2 mM of dNTPs (Promega), 0.1 mM of each primer, 1.5 mM Cl<sub>2</sub>Mg, 1 U of *Taq* DNA polymerase (Promega) and 6 µl of DNA extract. The PCR mixtures were subjected to 2 min at 94°C, 35 cycles of 1 min 30 s at 94°C, 1 min at 58°C, and 1 min at 72°C, and a final 10 min at 72°C using a thermocycler (Eppendorf). The PCR products were electrophoresed in 2% in agarose gels in TBE 0.5X (25 mM Tris borate, 0.5 mM EDTA). Strains HC171 and CLP4 (kindly provided by C. Vay, Buenos Aires, and F. Bessone, Marcos Juarez, Argentina) were used as positive controls and ATCC 12229 as negative control.

## RESULTS

The 9 calves sampled for this study all had wounds caused by kelp gulls. Two of the calves showed injuries with rhomboid-shaped edges: a dead calf (BFA02-13) and a living one (H-BFA02-13). We describe the findings from examination of these 2 calves.

Macroscopically, the injuries from both calves showed severe central loss of epidermis with expo-

sure of dermis. In both cases, the wound was swollen and surrounded by rhomboid-shaped raised edges (Fig. 1). Histologically, skin from BFA02-13 presented a suppurative dermatitis with dermal congestion and severe inflammatory infiltrates that consisted mainly of neutrophils and that extended to deeper layers of the skin into the blubber. The inflammatory infiltrates were within and around the walls of vessels, many of which contained bacterial thrombi and fibrin deposition. Gram-positive, slender filamentous rods, morphologically compatible with *Erysipelothrix* spp., were observed around and within blood vessels (Fig. 2).

Gram-positive, slender filamentous rods were found through direct observation of both samples. Non-motile, non-capsulated and non-endospore-forming, slightly curved rods were isolated in pure culture from both calves. The absence of catalase and oxidase was determined, and acid was not produced from sucrose. We identified the bacterial taxa *Erysipelothrix* spp. after both bacteriological and genetic analysis of 16S rDNA in the isolates from the 2 calves. Sequencing of 16S rDNA confirmed the genus, but could not conclusively identify the species level, showing 88% identity with *E. rhusiopathiae* SY1027. Both isolates were definitively identified by PCR as *E. rhusiopathiae* (Fig. 3) and not as *E. tonsillarum*, *E. rhusiopathiae* serovar 13 or *E. rhusiopathiae* serovar 18 (data not shown), using *Erysipelothrix*-specific primers (Takeshi et al. 1999) designed to differentiate among species.

*Erysipelothrix* spp. was not detected in samples collected from the other 7 calves, but other bacteria such as *Staphylococcus* spp. were isolated (data not shown).

## DISCUSSION

Since the first record of gull attacks on whales in the 1970s, the frequency of attacks and the number of injured whales has increased (Fazio et al. 2012, 2014, Marón et al. 2015). However, little is known about the consequences of these attacks on whale health. In this study we report, for the first time to our knowledge, the isolation of *Erysipelothrix rhusiopathiae* from wounds caused by kelp gull attacks on southern right whales calves, providing evidence that these wounds may act as route of entry for this pathogen. *E. rhusiopathiae* was isolated in purity only from gull injuries surrounded by rhomboid-shaped edges. Even though it is possible that other microorganisms could cause similar skin lesions, we

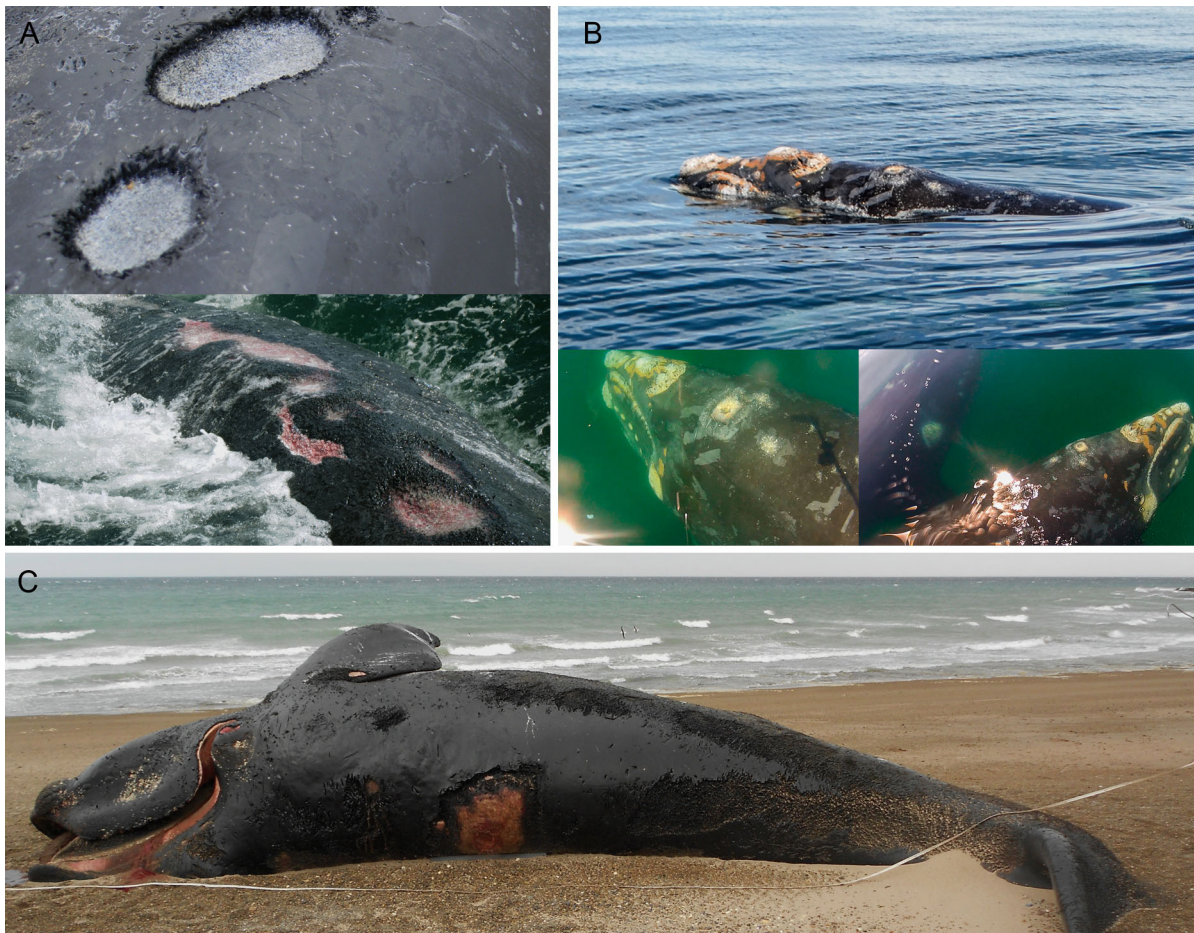


Fig. 1. Macroscopic morphology. Kelp gull *Larus dominicanus* injuries in southern right whale *Eubalaena australis* calves. (A) 'Typical' gull-induced wounds. The epidermis is absent as consequence of gull harassment, and the edges are rounded or irregular. (B) Kelp gull injury on live calf (H-BFA02-13) and (C) stranded dead calf (BFA02-13). Both wounds were swollen and surrounded by rhomboid-shaped edges

believe that in these 2 cases the characteristic appearance of the wounds was the result of colonization by *E. rhusiopathiae*. Because swabs taken from calf H-BFA02-13 were taken from the surface of the lesion, the bacterial isolate could potentially represent surface colonization only; however, we believe that the macroscopic aspect of H-BFA02-13's wound (rhomboid-raised edges) indicates that this is not only a superficial colonization. During the 2013 breeding season, only one stranded calf (BFA02-13) with rhomboid-shaped lesions was reported. Because samples of internal organs were not accessible, we could not test whether BFA02-13 died of erysipelas. Further studies should be conducted to test whether erysipelas could cause septicemia from infected gull wounds. However, the presence of bacteria in blood vessels from skin samples may be indicative of dissemination. Given that cetaceans are

particularly susceptible to the disease, we believe that erysipelas might be dispersed from infected injuries and could potentially be lethal to calves. On the other hand, during 2013 we also saw a calf with a healing wound with rhomboid edges; this may indicate that some calves only develop a local form of disease.

The source of *E. rhusiopathiae* for the whales is still unknown, but there is a particular concern about the role of kelp gulls in the disease pathogenesis, not only as the source of traumatic injury but also because they may act as a source of the bacteria. Although the role of vectors in the transmission of erysipelas is still unclear, some authors have reported that this pathogen could be transmitted by arthropods (Krinsky 1976). Kelp gulls are opportunistic and frequently feed in landfills, where large amounts of waste from the fishing industry and

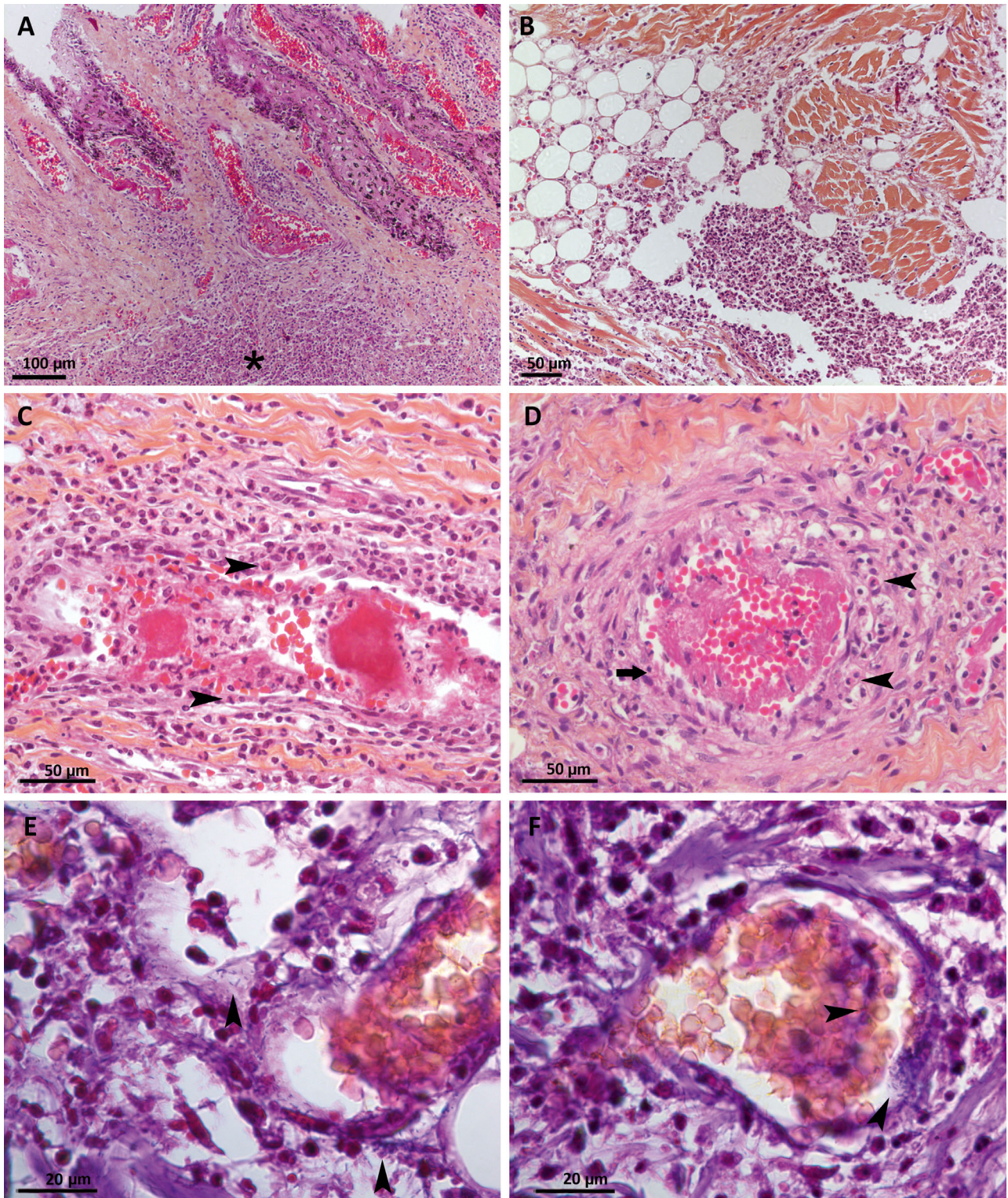


Fig. 2. Microscopic findings from the stranded dead southern right whale *Eubalaena australis* calf BFA02-13. H&E stains showing (A) suppurative dermatitis with loss of epidermis, dermal congestion and neutrophilic infiltration in dermis (asterisk); (B) neutrophilic infiltration of fat tissue (panniculitis); (C,D) vasculitis with moderate neutrophilic infiltrate (arrowheads) and intraluminal thrombi, and moderate fibrin polymerization (arrow) within the wall of a vessel; and (E,F) Gram stain showing slender filamentous rods of Gram-positive bacteria surrounding and within blood vessels (arrowheads)

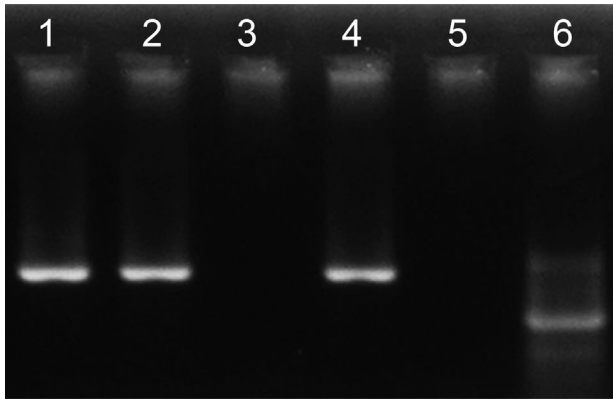


Fig. 3. *Erysipelothrix rhusiopathiae*-specific PCR assay and agarose gel electrophoresis of amplified DNA fragments. Lane 1: BFA02-13; Lane 2: H-BFA02-13; Lane 3: HC171 (positive control for serovar 18); Lane 4: CLP4 (positive control for *E. rhusiopathiae*); Lane 5: ATCC 12229 (negative control); Lane 6: MW 50 bp

slaughterhouse are dumped (Bertellotti & Yorio 2000, Bertellotti et al. 2001). *E. rhusiopathiae* can survive for long periods of time in the external mucous layer of fish (Wood 1975), so these residues may be an important source of bacteria, and gulls may act as vectors. Since 2012, government authorities in the province of Chubut have started to take positive action to reduce gull attacks on whales, including elimination of attacking gulls, reduction of fishery discards and dump remediation. This paper provides new evidence of the effects of kelp gull harassment on whale health, and the results will be useful to strengthen government actions. Further studies should be conducted to establish whether there is a real risk of pathogen transmission from gulls to whales.

Several bacteria and fungi have been reported in association with normal skin in bowhead whales (Shotts et al. 1990), southern right whales (Reeb 2001) and humpback whales (Apprill et al. 2011), so the possibility that the *E. rhusiopathiae* could be a normal microbiota of the whale skin cannot be excluded, and bacteriological samples from healthy skin should be taken in order to further analyze this topic.

Finally, *E. rhusiopathiae* is a potential zoonotic bacterium, so it is necessary to consider the public health implications of this finding. Each year, several calves die of unknown causes at Península Valdes, and infectious diseases are one of the main hypotheses to explain such mortality events (Werner et al. 2011). Stranded whales could be a source of zoonotic bacteria, becoming a risk for people that use the

shore for recreational activities. The results presented here will be useful to promote the implementation of measures to reduce this risk, such as removal of carcasses from beaches frequented by the public.

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