



Campylobacter pinnipediorum subsp. *caledonicus* and *C. pinnipediorum* subsp. *pinnipediorum* recovered from abscesses in pinnipeds

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ABSTRACT: *Campylobacter pinnipediorum* was described recently for isolates recovered from pinnipeds. The novel species was further split into 2 subspecies based on host and geography, with *C. pinnipediorum* subsp. *pinnipediorum* recovered from otariid seals in California (USA) and *C. pinnipediorum* subsp. *caledonicus* recovered from phocid seals in Scotland. We report details of the infections of 7 pinnipeds from which *C. pinnipediorum* was isolated: *C. pinnipediorum* subsp. *caledonicus* was isolated from 2 harbour seals *Phoca vitulina* and a single grey seal *Halichoerus grypus*, and *C. pinnipediorum* subsp. *pinnipediorum* was isolated from California sea lions *Zalophus californianus*. Six of the isolates were recovered from samples collected at post-mortem investigation. In 2 of the Scottish seals and in 3 of the California seals, *C. pinnipediorum* was the sole bacterial isolate recovered from abscesses present and suggests they may have resulted from conspecific or intraspecific bite wounds.

KEY WORDS: *Campylobacter pinnipediorum* · Bite wounds

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1. INTRODUCTION

Campylobacter is a bacterial genus that has been associated with asymptomatic carriage and symptomatic infections in humans and other animals (Skirrow

1994). In humans, campylobacteriosis is recognised as the most common cause of bacterial food poisoning in the UK, due mostly to *C. jejuni* and *C. coli* (Strachan & Forbes 2010, Tam et al. 2012). The aforementioned species have also been recognised in

farm and companion animals as well as in wildlife and are established zoonotic pathogens (Sheppard et al. 2009). In addition to the above, further *Campylobacter* spp. of veterinary importance include *C. fetus* subsp. *fetus*, which is associated with abortion in sheep and cattle, and reproductive disease in bovids due to *C. fetus* subsp. *venerealis* (Skirrow 1994).

Increasing microbiological investigations of wildlife have reported the isolation of *Campylobacter* spp., including strains infecting marine mammals. In seals inhabiting Scottish coastal waters, these have included *C. jejuni*, *C. coli*, *C. lari* (Baily et al. 2015) and *C. insulaenigrae* (Foster et al. 2004). First reported from 3 seals and a harbour porpoise *Phocoena phocoena*, the latter has since been reported from seals in other regions of the world (Stoddard et al. 2007, García-Peña et al. 2010, González et al. 2011) but also as a cause of human infections (Chua et al. 2007). Of late, isolates from harbour seals *Phoca vitulina* in the Netherlands were assigned to the novel species *C. blaseri* (Gilbert et al. 2018).

Recently, a polyphasic analysis of *Campylobacter* isolates from pinnipeds resulted in the description of the novel, urease-positive species *C. pinnipediorum* (Gilbert et al. 2017). This novel species could be subdivided further into 2 subspecies based on phylogenetic analysis and catalase reaction. Notably, the 2 subspecies could also be differentiated by the animal hosts infected and their geographic location: *C. pinnipediorum* subsp. *pinnipediorum* was recovered from otariid seals in California (USA), and *C. pinnipediorum* subsp. *caledonicus* was recovered from phocid seals in Scotland. We report here the details of infections with *C. pinnipediorum*: *C. pinnipediorum* subsp. *caledonicus* in 2 harbour seals and a grey seal *Halichoerus grypus* in Scotland and *C. pinnipediorum* subsp. *pinnipediorum* in 4 California sea lions *Zalophus californianus* in California. Six of the isolates were isolated following post-mortem examination.

2. MATERIALS AND METHODS

2.1. Scottish seal cases

Pinnipeds that stranded in Scotland were reported under the Scottish Marine Animals Strandings Scheme (SMASS) funded by Marine Scotland as part of the UK Government's commitment to a number of international agreements and specifically under European Protected Species licence number 120139. Carcasses were transported to Scotland's Rural College (SRUC) Veterinary Services, Inverness, for a post-

mortem examination performed according to a standard protocol (Dierauf 1994). Selected tissues and gross lesions were sampled for microbiological and histopathological diagnoses. Cultures were made on Columbia sheep blood agar (CSBA) (Oxoid) and selectively for *Brucella* on Farrell's medium, incubated at 37°C in air with 5% added CO₂. Selective cultures for *Campylobacter* and anaerobes incubated in microaerophilic and anaerobic environments were also set up. All plates were examined for growth at frequent intervals up to 14 d.

2.2. California sea lion cases

Four California sea lions that live-stranded were rescued and cared for at animal rehabilitation centres in California. Three of the animals were cared for at SeaWorld in California under NMFS Marine Mammal Health and Stranding Response Program Permit 932-1905 and the other at the Pacific Marine Mammal Center in California under NMFS Marine Mammal Health and Stranding Response Program Permit 932-1489. The SeaWorld animals subsequently died and received post-mortem investigations, which included sampling of abscesses. The sea lion at the Pacific Marine Mammal Center in California survived, and abscess exudate was collected for cytology and bacterial culture on 5% sheep blood agar (Hardy Diagnostics) and incubated at 35°C in room air supplemented with 5% CO₂.

3. RESULTS

3.1. Scottish cases

Case S1

A juvenile male harbour seal was found live-stranded at Lossiemouth in the Moray Firth (57°66'13.9" N, 003°61'63.89" W) on 28 October 2000. There was a large poorly circumscribed sub-blubber abscess above the left shoulder, which contained dark red/brown pus. The left pre-scapular lymph node was markedly enlarged. The seal was also suffering from severe pneumonia and was euthanased on welfare grounds. Large numbers of *Campylobacter*-like bacteria were the only organisms observed in a Gram-stained smear from the abscess contents, but they were not seen in tissue smears from lung, liver, spleen, kidney or brain. A pure growth of *C. pinnipediorum* subsp. *caledonicus* (+) was recovered from the abscess on CSBA

after 10 d of microaerophilic incubation at 37°C and after 14 d in a capnophilic atmosphere at the same temperature. Microaerophilic cultures from brain, spleen and lung were negative and cultures using *Campylobacter*-selective media (charcoal cefoperazone deoxycholate agar [CCDA] and Blaser-Wang) were negative for these 3 tissues and the abscess. Additionally, *E. coli* was recovered from lung (+++), spleen (++) mesenteric lymph node (++) kidney (+), liver (few) and in mixed growth from blood and intestine. Selective culture for *Brucella* spp. was negative for all tissues. Histological examination of the lung confirmed the presence of parasitic and suppurative broncho-interstitial pneumonia. Larvae were present in the airways with an associated eosinophilic and lymphocytic inflammatory response, and suppurative infiltrates were associated with small numbers of adult worms in alveoli. The section of liver examined was diffusely congested and contained a focus of necrosis and suppurative inflammation. The thymus was depleted. The spleen was congested with evidence of extramedullary haematopoiesis and the kidney was congested.

Histological examination of the brain showed a severe suppurative meningo-encephalitis, as denoted by the presence of large numbers of neutrophils, and protein-rich oedema around blood vessels in the meninges and in the neuropil along with necrosis in the grey matter of the cerebral cortex and white matter of the internal capsule. These lesions are indicative of a bacterial aetiology, possibly with the production of bacterial toxins.

Case S2

A 3–4 wk old female grey seal pup was found dead at Tynninghame Beach, East Lothian (56° 02' 49.53" N, 002° 59' 84.13" W) on 9 December 2010. A profuse growth of *C. pinnipediorum* subsp. *caledonicus* appeared initially as tiny colonies, which were possibly satellitic around other organisms as a mixed growth on chocolate agar incubated at 37°C in air plus 5% CO₂ from a lung abscess. Other bacteria isolated were *Streptococcus agalactiae*, *Arcanobacterium phocae* and a moderate growth of *Salmonella enterica* serovar Bovismorbificans. In addition, a profuse growth of an unidentified *Fusobacterium* sp. was recovered from anaerobic cultures. Interestingly, culture of non-abscessed lung tissue produced all of the aforementioned organisms except *Campylobacter*. The only other tissues cultured were liver and kidney, which had profuse and moderate growths,

respectively, of *Salmonella* Bovismorbificans, a serovar encountered in grey seals in Scottish waters (Baily et al. 2016). Selective culture for *Brucella* was negative for all tissues.

Case S3

A juvenile female harbour seal was found dead at Findhorn in the Moray Firth (57° 66' 13.9" N, 003° 61' 63.89" W) on 27 November 2011. There was a large abscess within the axillary lymph node under the right scapula, which was confirmed histologically, but no overt evidence of a bite wound. A profuse pure growth of *C. pinnipediorum* subsp. *caledonicus* was recovered from the abscess in anaerobic culture. The only other bacterial isolate was *B. pinnipedialis* recovered as single colonies from lung, spleen and mesenteric lymph node and as a moderate growth alongside a sparse mixed growth of other organisms from the small intestine. No growth was obtained from the liver, kidney or brain. *Campylobacter* cultures of small intestine on Skirrow's agar were negative. Additional histopathology findings included mild drainage reactions in a distant lymph node and the spleen; mildly reactive hepatic sinusoids and mild broncho-interstitial pneumonia associated with adult parasites with gross morphology identical to *Parafilaroides* sp.

3.2. California sea lion cases

Case C1

An emaciated female California sea lion pup, weighing 12 kg, live-stranded for the second time on 9 March 2013 and died at SeaWorld on 11 March 2013. A 9 × 5 × 3 cm abscess was found on the left pelvic limb surrounded by necrotic muscle and chylous peritoneal effusion containing 30 ml of opaque pink/red fluid. *C. pinnipediorum* subsp. *pinnipediorum* was isolated from the effusion along with mixed aerobic and anaerobic bacteria. The primary cause of death was likely sepsis due to the severe focally extensive necrotising myositis, peritonitis and emaciation.

Case C2

An emaciated juvenile female California sea lion, weighing 11.4 kg, died at SeaWorld on 15 February 2013. Open wounds were found on the lower jaw at

the corner of the oral cavity and upper lip just behind the vibrissae. There was also a 6.0 × 3.5 cm abscess in the neck above the right shoulder joint which was full of opaque pink purulent fluid. Histologic examination of the abscess revealed numerous intact and degenerate neutrophils surrounded by abundant fibroplasia extending into the surrounding muscle and adipose tissues with occasional lymphoid aggregates. *C. pinnipediorum* subsp. *pinnipediorum* was isolated from the neck abscess, which was thought to possibly be the result of a bite wound from a conspecific sea lion. The sea lion received injectable antibiotics (ceftiofur), subcutaneous fluids, oral electrolytes and pinniped gruel formula for nutrition. The suspect bite wound abscess was deemed to be the cause of the poor body condition and death.

Case C3

An emaciated male California sea lion pup, weighing 13 kg, was admitted to SeaWorld on 3 March 2013 and died on 4 March 2013. The hypothermic sea lion was housed inside with a thermal heating pad and treated supportively with subcutaneous fluids + 5% dextrose, dilute formula and oral electrolytes. Preliminary cell blood count showed a low white blood cell count (5230 cells μl^{-1} , 31% neutrophils, 18% band neutrophils, 27% lymphocytes, 16% monocytes), haematocrit (46%), and point-of-care I-stat showed hypoglycaemia (27 mg dl^{-1}) and acidosis (pH 7.1). At post-mortem, there was approximately 30 ml of opaque off-white pleural effusion with opaque orange fluid with proteinaceous particles. Both lungs were mottled with dark red regions at the lung periphery. The caudal left lung lobe was enlarged with the edge firm and dark red with clusters of off-white abscesses, from which *C. pinnipediorum* subsp. *pinnipediorum* was isolated in small numbers. Histologic examination demonstrated submassive pneumonia with necrosis of terminal bronchioles and alveoli and tissue replacement by degenerate neutrophils, proteinaceous oedema and fibrin. No microorganisms were identified in any sections of the lung. Cause of death was sub-massive necrosis and pneumonia, pleural effusion and septic necrosis in the live.

Case C4

A California sea lion began treatment at the Pacific Marine Mammal Center on 1 June 2011. Two abscesses were present: 1 on the right dorso-

lateral abdomen and 1 in the right thoracic area. The animal was also moderately underweight, with several raised cutaneous nodules on the thoraco-abdominal dorsum. Two abscesses were opened, suppurative exudate removed, flushed and instilled with Techni-Care® antiseptic. Cytologic examination of the abscess exudate revealed masses of degenerate and/or necrotic leukocytes, approximately 50:50% neutrophils:mononuclear cells with occasional scattered erythrocytes; no bacteria were definitively identified. The right dorsolateral abdominal abscess was aspirated, from which *C. pinnipediorum* subsp. *pinnipediorum* was the sole isolate. Treatment included flushing with salt and/or tap water, amoxicillin-clavulanic acid (17 mg kg^{-1}) orally twice daily for 6 d. Wound care was terminated 12 d after admission, and abscesses were noted to be healed 3 wk after admission. The animal was released on 14 August 2011 weighing 44 kg.

3.3. Characteristics of *C. pinnipediorum* isolates

On CSBA, the isolates of both subspecies of *C. pinnipediorum* presented as α -haemolytic beige, glossy, slightly raised and circular colonies with smooth margins. Growth was observed in microaerophilic and anaerobic environments and at 25°C but not in aerobic environments without added CO₂, nor at 42°C. Growth in air with 5% CO₂ varied with strain. All isolates were sensitive to cephalothin (30 μg) and nalidixic acid (30 μg). Positive reactions were obtained for urea hydrolysis, H₂S production from triple sugar iron agar (TSI), nitrate production and oxidase but negative for hydrolysis of hippurate and indoxyl acetate. The aforementioned characteristics are shared between *C. pinnipediorum* subsp. *caledonicus* and *C. pinnipediorum* subsp. *pinnipediorum* and permitted their distinction from all other validated species of *Campylobacter* as well as urease-positive *C. lari* (UPTC) which is not, as yet, a defined taxon (Miller et al. 2014, Gilbert et al. 2017). Differences in catalase reactions were observed between the Scottish and Californian strains, i.e. catalase-negative for *C. pinnipediorum* subsp. *caledonicus* and catalase-positive for *C. pinnipediorum* subsp. *pinnipediorum*. Extensive molecular testing, including whole genome sequencing, permitted further distinction between the 2 subspecies of *C. pinnipediorum*. A full description of the characterisation methods and results is provided with the species description of *C. pinnipediorum* (Gilbert et al. 2017).

4. DISCUSSION

During the years 2000–2011, *Campylobacter pinnipediorum* subsp. *caledonicus* was isolated from the abscesses of 2 stranded juvenile harbour seals and a grey seal pup that stranded at different locations around the North Sea coast of Scotland. In each of these cases, a single abscess was the only site from which *C. pinnipediorum* subsp. *caledonicus* was recovered: 1 in the shoulder region, 1 in a limb and 1 in the lung. *C. pinnipediorum* subsp. *pinnipediorum* was recovered from abscesses in 4 California sea lions during 2011 and 2013. In 3 of these abscesses *C. pinnipediorum* subsp. *pinnipediorum* was the only organism isolated. It is possible that other cases may have gone undetected, especially if culture under anaerobic or microaerophilic conditions was not performed.

It is interesting to compare these cases with findings from necropsies of 8 harbour porpoises which stranded in Scotland, England, Belgium and the Netherlands, following non-lethal attacks from grey seals (Foster et al. 2019). In all of these cases, *Neisseria animaloris* was recovered from abscesses in skin and lung, similar to the findings for the seals with *C. pinnipediorum* reported here. Grey seals are known to attack harbour seals (van Neer et al. 2015), harbour porpoises (Leopold et al. 2015) and conspecifics (Brownlow et al. 2016). It is possible that the infections in seals follow a similar course to that of *N. animaloris* in porpoises, whereby the seals survive an initial assault only to die of complications later, such as abscesses due to *C. pinnipediorum*, either at the site of puncture or following dissemination and abscessation in the lung. Indeed, a recent microbiome study found that *C. pinnipediorum* is part of the grey and harbour seal oral microbiome, being particularly abundant in some grey seals, and was also detected in very low amounts in porpoise lesions as a result of bites from grey seals (Gilbert et al. 2020). Seal lesions were not part of that study; however, the possibility remains that abscesses in seals become infected with *C. pinnipediorum* following traumatic injury from conspecifics or from other pinnipeds. Furthermore, all strains of *C. pinnipediorum* displayed urease activity, which may be related to a gastric niche and also shared 100% 16S rRNA sequence homology with uncultured bacteria from the gastric microbiota of California sea lions (Bik et al. 2016). *C. pinnipediorum* subsp. *pinnipediorum* was also recovered from the rectal swabs of 4 sea lion pups and a yearling.

We suggest that the most likely pathogenesis for the infections presented in this study are abscessation,

secondary to bite wounds, likely from pinnipeds. Although bite wounds were not observed, their absence was recorded only for the most recent case and it may be that they went unnoticed in the earlier cases or had resolved. The non-reporting of bite lesions may also reflect slow growth of *C. pinnipediorum* or the effects of other stressors on the pathogenicity of this bacterium. It is possible that smaller puncture wounds may partially heal or be difficult to identify but that *C. pinnipediorum* is inoculated into tissues, is able to thrive and replicate in the low-oxygen environment present in damaged or devitalized tissues, and produces the severe cellulitis and debilitation observed in these animals. We therefore suggest that abscesses and bite wounds in pinnipeds should be subjected to appropriate microbiological investigation to determine if either *C. pinnipediorum* subspecies is present to help determine the origin of the wounds/abscesses which may help to determine treatments for live animals and possible ultimate causes of death.

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