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WILDLIFE

Livestock-associated meticillin-resistant *Staphylococcus aureus* in a young harbour seal (*Phoca vitulina*) with endocarditis

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SUMMARY

A five-month-old male harbour seal was admitted for rehabilitation to the Sealcentre Pieterburen on November 16, 2015. During initial veterinary examination parasitic pneumonia and secondary bacterial pneumonia were suspected. Therefore, the seal received antiparasitic and antimicrobial treatment and appeared to recover but died unexpectedly after several weeks. Postmortem examination revealed a perforation in the aortic wall and histopathological examination of the aorta revealed mural necrosis with haemorrhage and suppurative to mixed inflammation. Bacterial culture resulted in isolation of a meticillin-resistant *Staphylococcus aureus* (MRSA) from the pericardial effusion. Subsequent culture of rectal swabs collected at arrival and during rehabilitation showed that the animal was already colonised with MRSA when admitted to the Sealcentre. MRSA has been isolated from marine mammals before, however, to our knowledge this is the first report of MRSA-associated endocarditis in seals and the first time that livestock-associated MRSA is reported in seals.

BACKGROUND

Staphylococcus aureus is a Gram-positive commensal bacterium in humans and various animal species able to cause disease.[1] The meticillin-resistant variant (meticillin-resistant *S. aureus*, MRSA) is regarded as a serious healthcare threat in hospitals as well as in the community as it is an extremely versatile pathogen also colonising and infecting animals.[2–5] Worldwide, MRSA belonging to clonal complex (CC) 398 and CC9 are associated with livestock, food products and people in contact with livestock. These isolates are referred to as livestock-associated (LA) MRSA.[2] Other types are considered to be primarily human associated. MRSA from wildlife is rare and the result of overflow from livestock or humans. Characterisation of MRSA from wildlife helps to understand the transmission route of resistant bacteria.

To date, several studies have reported the isolation of MRSA from marine mammals, including harbour seals (*Phoca vitulina*), pilot whales (*Globicephala macrorhynchus*) and bottlenose dolphins (*Tursiops truncatus*).[5–10] In the harbour seals, the MRSA was non-LA and related to human isolates. It was detected in a wound of a harbour seal,[6] a harbour seal with brain disease[7] and in the spleen and lymph node of a harbour seal that died at a seal sanctuary in Ireland.[5]

This article documents the isolation of LA-MRSA from a haemorrhagic pericardial effusion of a harbour seal that died during rehabilitation at the Sealcentre Pieterburen, the Netherlands.

CASE PRESENTATION

A five-month-old male harbour seal was admitted for rehabilitation to the Sealcentre Pieterburen on November 16, 2015. During the admission veterinary examination, the seal presented with fever (39.3°C, reference range 36.9°C–38.7°C),[11] dyspnoea, coughing and rhonchi in both lungs at auscultation, superficial wounds on both hind flippers and poor body condition, weighing 17.9 kg. Whole blood samples were processed with a haematology analyser (Medonic CA620/530 vet; Boule Medical). Blood analysis revealed leucocytosis (white blood cell count 34.8 x 10^9/l, reference range 5.9–24.6 x 10^9/l)[12] and normal red blood cell values (haematocrit 0.42 l/l, reference range 0.32–0.58 l/l[12]; red blood cell count 4.46 x 10^{12}/l, reference range 3.6–5.4 x 10^{12}/l).[12] Parasitic pneumonia and secondary bacterial pneumonia were suspected. Therefore, the seal received antiparasitic treatment following the Sealcentre's deworming protocol (ivermectin, Virbamec; Virbac, 0.4 mg/kg, subcutaneous, twice with a 21-day interval, and mebendazol, Mebendoral; AST Farma, 0.2 mg/kg, oral, twice a day for five days) and antimicrobial treatment (tetracycline, Apotheken Groep Groningen, 20 mg/kg, oral, three times a day for 18 days) following the Dutch Formularium for antimicrobial use in dogs and cats.[13] In addition, corticosteroids (Prednoral, AST Farma, 1 mg/kg, oral, once a day for five days), mucolytic (Bronchohexin, AST Farma, 0.4 mg/kg, oral, twice a day for 50 days, and Fluimucil, Zambon Nederland, 12 mg/kg, oral, twice a day for 18 days) and bronchodilator (Ventipulmin, Boehringer Ingelheim Vetmedica, 1 ml/10 kg, oral, twice a day for 48 days) drugs were administered.

The seal showed signs of recovery during the following weeks presenting general condition improvement, body temperature between normal ranges (36.9°C–38.7°C) and a decrease in white blood cell count (14.9 x 10^9/l, reference range 7–14 x 10^9/l). However, four days after finishing the tetracycline treatment, the seal developed fever and

lethargy, and received amoxicillin/clavulanic acid (Clavubactin, AST Farma, 22 mg/kg, oral, twice a day for 12 days, second choice antimicrobial for pneumonia following the Dutch Formularium).[13] However, this change in antimicrobial treatment was not effective, and regular episodes of fever and lethargy were observed. Therefore, it was decided to change to tetracycline (20 mg/kg, oral, twice a day for 12 days). Due to the lack of bacterial culture, the choice of repeating the tetracycline treatment was based on the fact that this antimicrobial treatment was more effective previously on the seal's body temperature and general condition compared with the amoxicillin/clavulanic acid.

The animal appeared to be recovered but died unexpectedly on January 18, 2016. During postmortem examination the pericardial space was found to be filled with blood. The aortic wall showed a perforation between the ascending aorta and the aortic arch. In addition, a perforation was found in the tricuspid valve. Using an ESwab (BD, Sparks) a sample for bacterial culture was collected from the blood in the pericardial space. For histopathological analysis, tissue biopsies were collected and fixed in 10 per cent phosphate-buffered formalin from the perforation areas from the aorta and tricuspid valve, as well as from the liver and the spleen.

INVESTIGATIONS

Liver biopsies revealed multifocal pyogranulomatous hepatitis and focal haemorrhage while spleen biopsies showed no abnormalities. Histopathological examination of the aorta showed mural necrosis with haemorrhage and suppurative to mixed inflammation.

The ESwab was inoculated on blood agar, MacConkey crystal violet agar, and Columbia colistin, aztreonam and peptone selective agar with sheep blood (Oxoid Deutschland). Agar plates were incubated for 48 hours under aerobic conditions with 5 per cent CO_2 at 37°C. An MRSA was isolated from the blood agar as confirmed by antimicrobial susceptibility testing using disk diffusion with a cefoxitin 30 µg disc (Oxoid, UK) using European Committee on Antimicrobial Susceptibility Testing clinical breakpoint tables.[14] The MRSA was resistant to penicillin, flucloxacillin, erythromycin, clindamycin and ciprofloxacin, and susceptible to tetracycline, cotrimoxazole, gentamicin, vancomycin, rifampicin and fusidic acid.

As rectal swabs were collected from the seal at 0 (day of arrival), 8 and 15 days, and stored at −80°C in a 20 per cent glycerol solution, these samples could be analysed retrospectively by culture on MRSA-chrome agar (Oxoid Deutschland) and additionally with the Xpert MRSA assay (Cepheid). All samples tested positive with both assays, indicating that the seal already carried the MRSA at admission. Therefore, the authors concluded the moment of colonisation with MRSA to be prior to the admission of the seal into rehabilitation. Further molecular characterisation using next-generation sequencing revealed that the MRSA had spa-type t1430, multilocus sequence-type ST9 and belonged to CC9. Therefore, this strain could be classified as LA-MRSA.[2] It did not contain the Panton-Valentine leucocidin virulence gene, which is associated with increased virulence of certain strains of *S aureus*.[15]

OUTCOME AND FOLLOW-UP

To investigate if the MRSA was transmitted to other seals in the centre, one rectal and one pharyngeal-nostril ESwab sample were taken from the 120 seals present in the centre at that time. This included samples taken from a grey seal (*Halichoerus grypus*) admitted on January 19, 2016, and placed in the same facility as the harbour seal positive for MRSA one day after the latter's death and after the facility was cleaned with soap and water and disinfected with chlorine. All samples were inoculated on a blood agar plate and a MRSA-chrome agar (Oxoid Deutschland). After 48 hours, a negative result for all the samples was confirmed.

DISCUSSION

In this study, LA-MRSA was isolated from the pericardial effusion of a harbour seal that died during rehabilitation. The most probable cause of death was an aortic perforation due to endocarditis, which led to the filling of the pericardium with blood producing a cardiac tamponade. No other significant findings were found in the postmortem examination that could explain the sudden death of the seal.

S aureus is known as an endocarditis-causing microorganism in humans and in animals.[16 17] In marine mammals, it has been related to endocarditis in a harp seal.[18] In addition, *Escherichia coli* has been reported to be associated with endocarditis in a sea lion.[19] However, to our knowledge this is the first report of (LA-)MRSA-associated endocarditis in seals.

How the seal acquired the LA-MRSA remains unknown. Although CC9 is considered an Asian LA-MRSA, it has been reported in Europe in animals and food of animal origin.[20 21] Furthermore, MRSA belonging to CC9 (ST9; spa-type t1430) has been reported in German pigs as well as human isolates.[22] Therefore, it is possible that this seal that stranded on the Dutch island of Ameland had acquired this bacterium from human or livestock sources that contaminated the environment.

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Learning Points

- Coastal ecosystems can be considered to be important reservoirs for infectious microorganisms and antimicrobial resistance (AMR). Since many marine mammal species share the coastal environment with humans and consume the same food, they also may serve assentinels for the ocean. The isolation of an LA-MRSA in the rectum of this seal strengthens the possible use of seals as bio-indicators for AMR prevalence in marine ecosystems.
- Marine mammals can be a source for zoonotic infections(23,24), including those caused by resistant microorganisms as is shown here. Therefore, handling of wild marine mammals should only be performed by professionals that have the required knowledge and access to accurate protective measurements.
- Prevention of transmission of antimicrobial resistances is of major concern in hospital settings. Here we show that the biosecurity and hygienic measurements taken in the Sealcentre, including separate clothes and boots for each seal enclosure, daily cleaning and disinfection of the enclosures with soap and chlorine, the water filtration and purification system, prevented dissemination of MRSA from the index seal to other seals that were housed at the rehabilitation facilities at the same time.

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