

Cluedo – Source identification in a case of septicemia fatality caused by *Capnocytophaga canimorsus*

L. KNABL¹, M. MANGO¹, B. STÖGERMÜLLER², L. KIRCHMAIR²,
W. POSCH¹, C. LASS-FLÖRL¹, S. FUCHS¹

¹Institute of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

²Department of Anaesthesia and Critical Care Medicine, District Hospital Schwaz, Schwaz, Austria

Ludwig Knabl and Monica Mango contributed equally

Abstract. – The Gram negative pathogen *Capnocytophaga canimorsus* is a frequent commensal in the oral cavity of cats and dogs. Although the bacterium is generally considered harmless, infections in humans can occur displaying a broad spectrum of clinical syndromes. This makes a clinical diagnosis difficult. The patient in the present case was 67 years old and presented to the emergency room (ER) with pain in the upper right abdomen and clinical signs of a feverish infection. The only noticeable record in the patient's medical history was a splenectomy in childhood. The anamnesis revealed that the patient was the owner of two dogs. After a suspected diagnosis of sepsis the patient was transferred to the intensive care unit (ICU), where his medical condition deteriorated rapidly. Despite intensive care measures as well as the fast initialization of a broad-spectrum antibiotic therapy, the patient died 37 h after his presentation in the ER. The search for the causative pathogen turned out to be challenging. Eventually, molecular biological methods assisted in solving the puzzle. It could be demonstrated that the pathogen, found in the patient's blood, was also present in one of his dogs' oral cavity.

Key Words:

Sepsis, PCR, *C. canimorsus*.

Introduction

The pathogen *Capnocytophaga C. canimorsus* has first been isolated in 1976 from blood and cerebrospinal fluid of a man, who had been bitten by a dog¹. It is a common commensal in

dogs' and cats' oral microbiomes². As such, it can be found in 74% and 54% of dogs and cats, respectively³. In rare cases it can lead to infections in humans. A nation-wide survey in the Netherlands revealed an incidence of 0.67 cases per 1,000 000⁴. Regardless of the rare appearance it can lead to life-threatening infections with a relatively high case fatality rate of 31%⁵. A broad variety of clinical symptoms such as fever, abdominal pain, rash, diarrhea and vomiting can occur in association with *C. canimorsus* infection⁶⁻⁸. This makes a precise diagnosis very difficult. Alcoholism and splenectomy are seen as risk factors for infections with this zoonotic pathogen⁹. Its non-specific clinical presentation with diverse symptoms that can also be assigned to many other diseases and its delicate microbiological identification pose major challenges for the physician and the laboratory. Here we describe a case of a 67-year-old man with a lethal *C. canimorsus* infection and the subsequent diagnostic retracing using microbiological and molecular techniques.

Case Presentation

A 67-year-old male presented to the ER with severe pain in the right upper abdomen and clinical signs of a feverish infection. The medical history of the patient was unremarkable. He solely reported a traumatic spleen rupture with subsequent splenectomy in childhood. The further anamnesis revealed that the patient owned two dogs. However, there was no mention of recent scratches or bites from the dogs and no

obvious marks of external injury. After physical examination of the patient a suspected diagnosis of a septic shock was made. Samples for blood culture (BC) were drawn before initialization of an empiric antibiotic therapy with piperacillin/tazobactam. Since his physical condition declined drastically, the patient was transferred to the ICU, where he suffered a cardiac arrest. Despite successful resuscitation the patient's medical condition deteriorated progressively. After consultation with the Institute of Hygiene and Medical Microbiology of the Medical University of Innsbruck a change in the antibiotic therapy to meropenem was decided. The alteration in antibiotic therapy was performed after microscopy of the positive BC had revealed gram negative, pleomorphic rods in the gram stain. Additionally, hemodiafiltration with a specific sepsis filter was started. Due to massive adsorption of toxins and cytokines the filters had to be replaced in hourly intervals. In sum, the patient showed symptoms compatible with acute respiratory distress syndrome (ARDS) and deteriorated rapidly as disseminated intravascular coagulation and liver failure emerged. Due to consequent progression of the multiorgan failure the patient died 37 hours after presentation to the ER.

The automated BC monitor detected microbial growth after 11 hours. Standard Columbia blood agar plates were inoculated with material from the positive BC for subculture and incubated at capnophilic atmosphere. Small grey colonies were visible after 48 h. A MALDI-TOF analysis (Bruker, Billerica, MA, USA) of the isolate and also a broad-spectrum pan-bacterial PCR (Molzym, Bremen, Germany) on the BC identified the pathogen as *C. canimorsus*. However, even after several attempts, an antibiotic susceptibility profile could not be inferred using standard procedures due to the poor growth of the pathogen. In order to trace the source of the causative pathogen mouth swabs of both of the patient's dogs were performed and processed for molecular diagnosis. The swabs were streaked on blood agar and cultivated at 37°C in capnophilic atmosphere. The swabs were also used to inoculate liquid cultures in Müller-Hinton broth (Becton Dickinson, Franklin Lakes, NJ, USA). DNA was extracted from all cultures grown either on solid or in liquid media. Extraction of DNA was performed with the UltraClean Microbial DNA extraction kit (MoBio, Carlsbad, CA, USA) according to the manufacturers' instructions. The extracts contained a mixture of

the microbial flora of the dogs' mouths. Primers developed by Suzuki et al³ were used to specifically detect *Capnocytophaga spp.* (PCR1) and *C. canimorsus* (PCR2). Both PCRs were run in real-time format on a CFX96 thermal cycler (Bio-Rad, Hercules, CA, USA). The PCR results revealed the presence of the pathogen *C. canimorsus* in only one of the patients' dogs, whereas the bacterium was not detectable in the oral swab of the other (Figure 1). Additional controls were included in the PCR runs to assure the specific detection and to confirm the presence of the pathogen in BC.

Discussion

The case presented here is an educational example for the diagnostic intricacies turning up when dealing with uncommon pathogens. First, the major challenge for a practitioner is the correct interpretation and attribution of the clinical symptoms of a potential *C. canimorsus* infection. Secondly, there are certain risk factors (see below) influencing the progression of *C. canimorsus* infections, and thirdly, there are molecular biological tools to assist in a cause analysis of suspected septicemia. Particularly, in the present case the patient was admitted to the ER with fever and pain in the right upper abdomen, which allows a wide range of differential diagnosis. Pers et al⁵ have shown that 26% of all investigated *C. canimorsus* infections presented with abdominal pain. Despite the successful treatment of *C. canimorsus* infections with broad-spectrum antibiotics reported for many patients¹⁰, the prompt administration of piperacillin-tazobactam and the subsequent change to meropenem did not prevent the development of a multiorgan failure in the present case. The medical history of the patient was uneventful apart from a splenectomy in childhood after a traumatic spleen rupture. Splenectomy as well as immunological defects and alcoholism are known risk factors for infections with *C. canimorsus*⁹. Nonetheless, many infections also occur in apparently healthy people¹¹. The infection usually occurs after a dog bite or contact of the dogs' saliva with lesions. However, in the present case neither one nor the other was reported in the anamnesis. Furthermore, there were no signs of external injury or evident lesions. The PCR performed on the cultures grown from the dogs' oral swabs definitely identified *C. cani-*

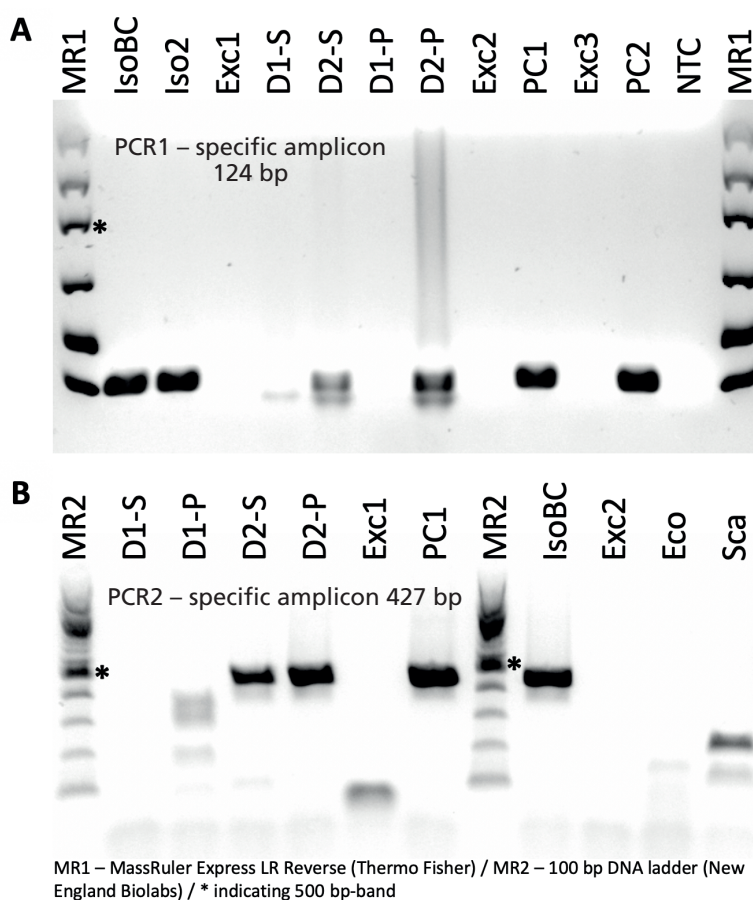


Figure 1. Gelelectrophoresis images of *Capnocytophaga* spp. PCR (A) and *C. canimorsus* PCR (B) with corresponding positive and negative controls. Dog 1 (D1) and Dog 2 (D2) show either no or a specific amplification signal, respectively, using DNA extracted from swab (S) or plate (P) samples. Isolates from the BC sample (IsoBC) or from subculture (Iso2) were run in parallel just like the corresponding extraction controls (Exc). Another independent isolate of *C. canimorsus* served as positive control (PC1/2). Water (NTC) or the extract of other bacterial species (Eco/Sca) served as negative controls.

morsus in the oral microbiome of one dog. We speculate it to be the probable source of the fatal *C. canimorsus* infection in our patient. However, this also demonstrates that animal bite wounds are not mandatory to suspect an infection with this particular pathogen. Janda et al¹² have even shown that 11% of the cases only had close contact to animals without any reported wounds or contact of lesions to the animals' saliva. Interestingly, 22% of the patients did not have any contacts to cats or dogs at all. Perhaps there are other natural reservoirs for *C. canimorsus*. Nevertheless, in the present case the pathogen may have been transmitted from the dog to the patient. How the transfer of the pathogen to the patient occurred cannot be fully elucidated at this point. It could be speculated that the infection happened when the patient had close contact

to his dog or its saliva. Moreover, although *C. canimorsus* is known for its difficult cultivation with slow growth¹³, detection and identification of the causative pathogen was expeditious in the present case by exploiting conventional and molecular methods.

Conclusions

Briefly, this case demonstrates the life-threatening potential of the pathogen *C. canimorsus* and the fatal progression of such an infection despite the instantaneous initiation of an appropriate antibiotic therapy. Additionally, the present case shows the value of molecular biological methods in the diagnostic retracing of intricate infections.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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