Opis przypadku *Case report*

Use of the MALDI-TOF technique to identify the cause of pericardial effusion in a cat caused by *Neisseria animaloris* infection – own observations

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Summary

Pericardial effusion is a serious problem in companion animals. *Neisseria* spp. are considered commensal microorganisms of the oral cavity in dogs and cats and are usually isolated from human wounds resulting from bites on these animals. This article presents a clinical case of a cat with hydropericardium, the cause of which was *Neisseria animaloris*. Identification of the pathogen in the pericardial sac fluid was performed using the MALDI-TOF method. This is the first report on a documented case of pericardial effusionin a cat triggered by *Neisseria animaloris* bacterial infection in feline medicine, emphasizing its novelty using the MALDI-TOF method.

Keywords: MALDI-TOF, *Neisseria*, heart, cat

Neisseria are Gram-negative bacteria that are considered commensals in the mucous membranes of the mouth and throat in animals (dogs and cats) and humans (12). The genus *Neisseria* contains 32 species, many of which can infect humans (*N. animalis*, *N. animaloris*, *N. arctica*, *N. bacilliformis*, *N. canis*, *N. chenwenguii*, *N. cinerea*, *N. dentiae*, *N. dumasiana*, *N. elongata*, *N. flava*, *N. flavescens*, *N. gonorrheae*, *N. iguanae*, *N. lactamica*, *N. macacae*, *N. meningitidis*, *N. mucosa*, *N. musculi*, *N. oralis*, *N. polysaccharea*, *N. perflava*, *N. pharyngis*, *N. shayeganii*, *N. sicca*, *N. skkuensis*, *N. subflava*, *N. tadorna*, *N. wadsworthii*, *N. weaveri*, *N. weixii*, *N. zalophi*, *N. zoodegmatis*) (13, 15). Neisseria bacteria are small (0.6 to 1 micrometer) Gram-negative cocci or diplococci (13). Based on the ability to conduct arginine hydrolysis, two biotypes are distinguished: EF-4a and EF-4b (7, 8, 16). Arginine-positive strains (EF-4a) are described as *Neisseria animaloris* (7, 16).

Infection with *Neisseria* can lead to the development of meningitis, meningococcal sepsis (*Neisseria meningitidis*) (4), and gonorrhea (*Neisseria gonorrheae*) (10). Cases of endocarditis after infection with *Neisseria gonorrheae* (6) or *Neisseria elongata* (2) have been reported in humans. Acute right ventricular heart failure can be caused by *Neisseria meningitidis* (15).

Few studies have been conducted on detecting and characterizing *Neisseria spp*. in companion animals. This article aims to describe the use of the MALDI-TOF technique to identify the cause of pericardial effusionin a cat caused by *Neisseria animaloris* infection.

Case description

The observation took place in December 2023. A 3-year-old male European shorthair cat weighing 5 kg was brought to the Clinic of Infectious Diseases of the University of Life Sciences in Lublin due to apathy, weakness, lack of appetite, fainting, and shortness of breath. The cat demonstrated signs of fatigue and panting even after playing briefly. In the past, he had undergone a full cycle of vaccinations against infectious diseases (cat cold, panleukopenia, rabies) and regularly received prophylaxis against intestinal parasites. The animal had never been ill before. The cat had run away from home two weeks before, and after its return, the owners had noticed bite marks on its chest.

On clinical examination, the condition of the cat was described as poor. The mucous membranes of the natural body orifices were moist and light pink, and the capillary

refill time was approximately 4 seconds. The lymph nodes accessible during the examination were enlarged, and the internal body temperature was 41.3°C. The abdominal wall was not swollen or painful under palpation. Her heart rate was 163/min. Auscultation of the chest revealed significant suppression of heart sounds. The breathing rate was 36/min. The heart rate was 111 bpm.

Hematological examination revealed leukocytosis (WBC = $15 \times 10^9/mm^3$). The results of the serum biochemistry tests were normal. The albumin to globulin concentration ratio was 0.9.

Due to severely weakened heart sounds, an echocardiographic examination was performed, which revealed the presence of a significant amount of free fluid in the pericardial sac, containing numerous morphotic elements (Fig. 1). The diastolic collapse of the right atrium lumen and sometimes the right ventricle during expiration was observed (Fig. 2). The heart performs characteristic „swinging" movements in the pericardial sac due to the phase movement of fluid inside it. In addition, enlargement of the hepatic vein and ascites was observed, which suggested right sided heart failure due to pericardial effusion. The anechoic space in the diastolic phase between the wall pericardium and the epicardium was greater than 2.5 cm long, so the patient was classified as demonstrating significant effusion. Echocardiography revealed no signs of neoplasms in the heart or pericardial sac. Differential diagnoses included FIP, purulent pericardium, pericarditis, and a neoplastic process not visible on echocardiography.

Pericardiocentesis was performed to reduce the pressure inside the pericardial sac and remove the accumulated fluid. This procedure resulted in almost immediate relief of symptoms of right ventricular congestive heart failure, a decrease in the number of heartbeats, and as it later turned out, a temporary improvement in the patient's general condition. Approximately 350 ml of opaque, pus-like fluid was removed from the pericardial sac.

The fluid was examined cytologically. The examination revealed numerous neutrophils (mostly without signs of degeneration), macrophages with phagocytic activity, and a small number of lymphocytes and plasmocytes. Bacteria were also observed.

Due to the presence of bacteria, a sample of 100 µl of the fluid was cultivated on a Blood LAB-AGARTM base (Biomaxima Polska) with 5% defibrinated horse blood (Biomaxima Polska). The plates were incubated for 24 hours at 37°C. After that time, the growth of bacteria in the > 10^6 CFU/ml fluid was observed. The bacterial isolates demonstrated the greatest sensitivity to clindamycin.

The bacterial isolates obtained in this study underwent comprehensive analysis and identification using the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique at the Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin, Poland. The analysis was conducted based on the protein profile obtained from a protein extract prepared with a solution of formic acid.

In the experimental procedure, a loop of bacterial culture from the plate was initially diluted in 150 µL of Milli-Q (MQ) water. Subsequently, 450 µL of 99.8% ethanol

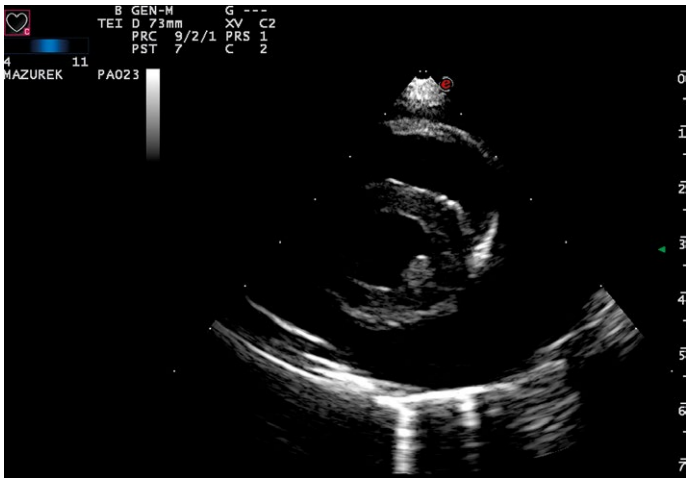

Fig. 1. Right-side parasternal short axis view. The left ventricle of the heart was surrounded by liquid in the pericardial sac

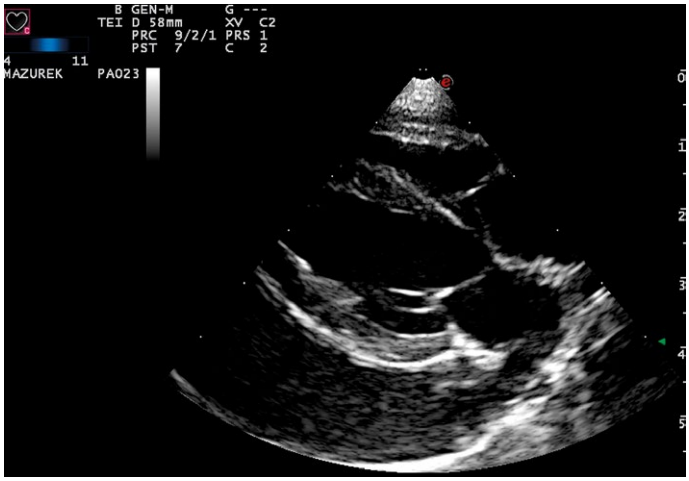

Fig. 2. Right-sided, parasternal four chamber long axis view. A large amount of fluid was visible in the pericardial sac, and a collapse of the right atrium lumen during expiration was present

(POCH, Poland) was added, and the sample was meticulously vortexed. Next, the sample underwent centrifugation for 5 minutes at 13,000 rpm. The supernatant was carefully removed, and the residue was mixed with a solution containing 40 µL of 70% formic acid (FlukaChemie GmbH, Buchs, Switzerland) and 40 µL of acetonitrile (FlukaChemie GmbH, Buchs, Switzerland), followed by vortexing. After a subsequent centrifugation step (13,000 rpm for 2.5 minutes), 1 µL of the resulting supernatant was deposited onto a ground steel plate and air-dried. Finally, 1 µL of a matrix solution containing 10 mg/mL HCCA (α-cyano-4-hydroxycinnamic acid) was applied to the plate and allowed to dry at room temperature. Samples were deposited in three repetitions.

The mass spectra obtained by flex Control Version 3.4 (Build 135) (Bruker Daltonik, Germany) software were subjected to analysis using the MALDI-Biotyper 3.1 database, Build (66) (Bruker Daltonik, Germany), which encompasses 8468 reference spectra. The outcomes are presented as the top 10 identification matches, accompanied by confidence scores ranging from 0.00 to 3.00. Following the manufacturer's criteria, a score below 1.70 is considered insufficient

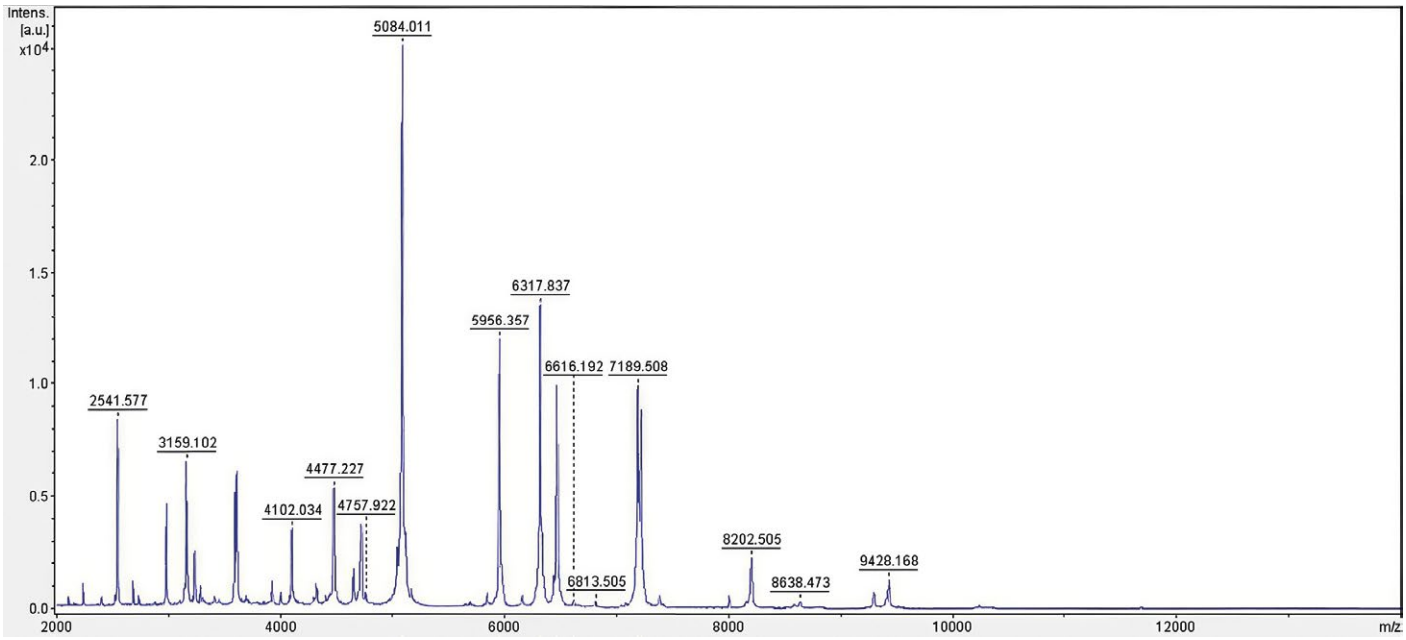

Fig. 3. Mass spectra for *Neisseria animaloris* obtained by the MALDI-TOF technique. The Figure shows a characteristic peptide molecular fingerprint spectrum obtained by the MALDI-TOF technique for the tested sample. The spectrum contains ribosomal proteins distributed in the range of 2,000 Da to 20,000 Da. Comparison of the spectrum with the database allowed it to be matched to the *Neisseria* genus

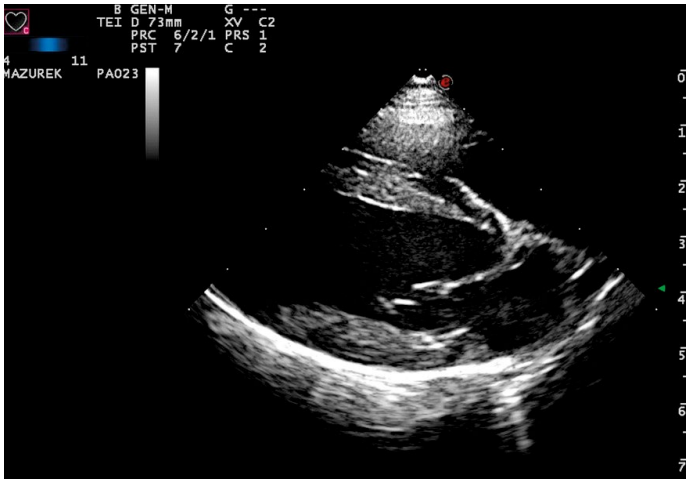

Fig. 4. Follow-up echocardiography

Tab. 1. Results of echocardiography before and after pericardiocentesis

Tested parameter	Value before pericardiocentesis	Value after pericardiocentesis
RVDd (mm)	6.4	3.3
IVSD (mm)	6.4	6.1
LVIDd (mm)	13.9	12.1
PWD (mm)	7.8	7.1
IVSs (mm)	9.4	8.8
LVIDs (mm)	5.8	5.1
EF (%)	91	75
FS (%)	59	38
La/Ao	1.11	1.36
RVOT (m/s)	0.99	0.87
LVOT (m/s)	1.23	1.07

for reliable identification. Scores within the range of 1.70 to 1.99 allowed identification up to the genus level, while scores between 2.00 and 2.29 suggested probable identification at the species level. A score exceeding 2.30 (2.30-3.00) indicates highly probable identification at the species level.

The bacterial colonies were identified by MALDI-TOF MS by comparing highly specific peptide mass fingerprint sample spectra to the BioTyper 3.1 database containing spectra of reference strains. The examined strains were identified as *Neisseria animaloris* in three repetitions. According to Bruker, score values ranging from 1.70-1.99 indicate probable genus identification (Fig. 3).

A sample of the collected fluid was also examined via PCR for FIP (1), but the result was negative. With the cardiological intervention and implemented treatment (clindamycin 5.5 mg/kg body mass every 12 h for 2 weeks), the patient's condition stabilized. A follow-up echocardiographic examination carried out 2 weeks after the cat had been brought to the clinic revealed no accumulation of fluid in the pericardial sac. A follow-up blood test showed no deviations from the norm. The heart rate after pericardiocentesis was 88 bpm. The animal was found to be healthy (Fig. 4).

Discussion and conclusion

This article presents the first clinical case of identification pericardial effusion in a cat as a complication of infection with *Neisseria animaloris* using the Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) method. MALDI-TOF is a state-of-the-art method for identifying bacterial strains from animals that offers a combination of precision and efficiency. This advanced technique relies on protein mass spectrometric profiles to quickly and accurately differentiate between bacterial species. Its

application in animal-derived bacterial identification is pivotal for enhancing our comprehension of microbial diversity in these populations. The high-throughput nature and reliability of MALDI-TOF MS have contributed significantly to revealing intricate microbial ecosystems linked to animals, providing valuable insights into zoonotic diseases.

A cat with symptoms of severe cardiovascular failure was referred to the clinic. Pericardial effusion is more frequently diagnosed in dogs than in cats. In cases of pericardial effusion in cats, the differential diagnosis usually includes FIP and a neoplastic process (especially lymphoma).

A condition that occurs when an excessive amount of fluid accumulates inside the pericardial sac is called pericardial effusion (*hydropericardium*) (10, 14). In cases where fluid accumulates slowly, the pericardium can stretch and accommodate it. With low pressure prevailing in the pericardial sac, this phenomenon is not accompanied by clinical symptoms, and the heart maintains relatively normal function. If fluid accumulates quickly in the pericardial sac or if it is accompanied by an increase in pressure inside the pericardial sac, a situation occurs in which the pressure in the pericardial cavity exceeds the normal diastolic pressure in the right ventricle, and atrium of the heart, which results in tamponade (17).

Determining what type of fluid can accumulate in the pericardial sac based on an echocardiographic examination is difficult. In the case of bacterial infections, fluid contains numerous structures resembling morphotic blood elements or fibrins. Confirmation of the nature of the fluid is possible only based on an additional test.

Infection with *Neisseria* spp. in animals occurs mainly after being bitten by a sick or infected animal (over 90% of cases) and less frequently through indirect contact. Species of the *Neisseriaceae* family belong to the commensal microflora of the upper respiratory tract of animals and humans. They do not usually cause visible or life-threatening conditions. In the described case, bacteria most likely entered the pericardial sac from the bite wound.

In this article, we present the first case of identifying pericardial effusion in a cat resulting from infection with *Neisseria* bacteria using MALDI-TOF method. In the available literature, there are one data on this condition induced by infection with these bacteria (3); however, in humans, bacteria from this genus have been reported to be responsible for the development of endocarditis (2, 10). In cats, *Neisseria* rarely causes infections of the mouth or respiratory tract or sepsis (11, 18). These infections can sometimes be life-threatening for patients.

N. canis and *N. animaloris* are bacteria of zoonotic importance that are present in the oral cavity of felines and can bear the risk of transmission and infection to humans. The fact that they can also be responsible for the development of cardiological disorders indicates that these infections should be taken into account in the investigation of differential diagnosis of heart diseases in cats with fluid accumulation in the pericardial sac, especially in the case of animals that go outside freely and tend to fight.

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