**Hafnia paralvei** ISOLATED FROM AN EMPHYSEMATOUS PYOMETRA IN A BITCH

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**Abstract:** We report the case of a 9-year-old female Labrador retriever, presented to the Veterinary Teaching Hospital with a history of bloody/purulent and malodourous vulvar discharge, decreased appetite and progressive abdominal enlargement for about 20 days. Clinical examination showed a distended and painful abdomen and tympanic sounds on percussion. The patient also presented systemic inflammatory response syndrome (SIRS) and a leukemoid reaction with leukocyte count of 77.9 x 10⁹/L due to 62.32 x 10⁹/L lymphocytes. The abdominal radiography showed distended tubular structures occupying most of the abdomen; dorsal displacement of the colon in the left-right lateral projection was also found. The ultrasonographic study also revealed tubular structures in the mid-abdomen that contained flowing fluid presenting mixed echogenicity and hyperechoic particles in suspension. The exploratory celiotomy showed that the uterine horns were distended and contained a mixture of gas and liquid, hence an ovariohysterectomy was performed. The uterine fluid was collected with aseptic technique into a syringe and submitted to the clinical pathology laboratory for aerobic and anaerobic culture and antibiotic sensitivity. *Hafnia alvei* (now classified as *Hafnia paralvei*) was identified as the causative bacterial agent. Pyometra caused by gas-producing bacteria is a rare condition and is known as emphysematous pyometra. There are only six reports in the literature of this condition in bitches. A wide range of vaginal bacteria has been found in dogs with pyometra, but to our knowledge this is the first report of emphysematous pyometra caused by *Hafnia paralvei* in dogs.

**Key words:** veterinary surgery; uterus; emphysematous pyometra; *Hafnia paralvei*

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**Introduction**

Pyometra is a common condition that affects intact adult bitches in dioestrus (1). It is a local infection, but is often accompanied by sepsis and/or systemic inflammatory response syndrome (SIRS) (2). Open cervix pyometra is most common, but the closed cervix pyometra have a poorer prognosis (3), and affects about 25% of old nulliparous bitches (4).

Emphysematous pyometra is a rare entity, and has been described in only 6 reports (5-10).

Although the most commonly isolated bacteria in dogs with pyometra is *E. coli*, several other bacteria found in the normal vaginal flora have been reported in canine pyometra (11-13).

*Hafnia paralvei* (formerly *Hafnia alvei*) is a gram-negative, mobile, rod-shaped facultative anaerobe. It is part of the normal microbiota of animals (14). In humans, it behaves as an opportunistic pathogen (15), but it is a rare cause of infection in humans and animals (15-17). However, in veterinary medicine, it has been
reported in equine abortion (18), as a secondary infection in a cat with a nasal tumor (19), and was also isolated from the wound of a dog (20).

In the past, the genus consisted of a single species, *Hafnia alvei*. However, in 2010, organisms previously called *Hafnia alvei* HG 2 were categorized as a novel species, *Hafnia paralvei* (21).

The objective of this study is to present a rare case of an emphysematous pyometra in a bitch, in which was isolated *Hafnia paralvei*.

**Case**

A 9-year-old Labrador retriever nulliparous bitch was submitted to our hospital for evaluation. The animal had a history of vaginal bleeding and a purulent and foul-smelling vulvar discharge. The owner reported that the patient had been hyporexic and that the abdomen had progressively increased in size. The animal weighed 25.36 kg and the last heat had occurred 2 months before.

The clinical evaluation revealed a heart rate of 120 beats per minute, respiratory rate of 40 breaths per minute, and a dehydration rate of 7%, with pale mucous membranes, delayed capillary refill time (>3 seconds) and a temperature of 40°C. Moreover, the patient showed abdominal pain on palpation and tympanic sound on percussion. In addition, the bloody-purulent vulvar discharge was confirmed.

The abdominal left-right lateral and ventro-dorsal projection showed distended tubular structures occupying most of the abdomen (Figure 1). The presence of coprolites was confirmed in both projections.

The abdominal ultrasonography showed tubular structures in the mid-abdomen presenting mixed echogenicity; a flow of hyperechoic particles in suspension and reverberation lines superimposed on the image of mixed echogenicity were also found.

The complete blood count (CBC) findings showed a decrease in hemoglobin values 7.1 g/dL (range 12-18 g/dL), hematocrit 0.204 L/L (range 0.37-0.55 L/L), erythrocytes 3.53 x 10^{12} /L (range 5.5-8.5 x 10^{12} /L) and platelets 160 x 10^9 /L (range 175-500 x 10^9 /L). The mean corpuscular hemoglobin (MCH) 34.9 g/dL (range 31-37 g/dL) and mean corpuscular volume (MCV) 57.9 FL (range 60-72 FL) were within normal ranges. The patient also presented a highly elevated leukocyte count of 77.9 x 10^9 /L (range 5.5-16.9 x 10^9 /L), with 62.32 x 10^9 /L lymphocytes (ranges 1-4.9 x 10^9 /L).

Based on the imaging and laboratory findings we conducted an exploratory celiotomy with a presumptive diagnosis of emphysematous pyometra. The preanaesthetic medication protocol was an intravenous dose of the commercial combination of tiletamine/zolazepam (5 mg/kg) (Zelatol, Fort Dodge, Spain) plus tramadol (3 mg/kg, IV) (Tramadol Jet, Norvet, Mexico) for analgesia. For the induction of anaesthesia an intravenous dose (4 mg/kg) of propofol (Fresofol, Fresenius Kabi, Austria) was used. The inhalant anaesthesia was maintained with 2.5% inspired fraction of isoflurane.

Exploratory celiotomy confirmed the presence of distended uterine horns containing gas and fluid (Figure 2), therefore ovariohysterectomy was performed. Further pre- and postoperative medication included cefazolin (25 mg/kg, IV) (Cefazolin, Apotex Corp, USA), q12 h, gentamicine (6 mg/kg, IV, q 24 h) (Genta Ved, Vedco, USA), metronidazole (15 mg/kg, IV, q 12 h) (Flagyl, Sanofi, Mexico), tramadol (3 mg/kg, IV, q 12 h) and carprofen (2 mg/kg, PO, q 24 h for 7 days) (Novox, Vedco, USA). Uterine and ovarian tissue samples were submitted to the histopathology laboratory. The histopathological report revealed cystic endometrial hyperplasia and suppurative metritis/endometriosis. Fluid from the uterus was collected via aseptic technique into a syringe, air was removed and the syringe was sealed. The sterile sample was sent to the clinical pathology laboratory for aerobic and anaerobic culture and antibiotic sensitivity. *Hafnia paralvei* was recovered in pure culture of the uterine fluid.

Two days after surgery, the patient was discharged with oral cephalixin (22 mg/kg, PO, q 12 h) (Ceporex, Glaxo Smithkline, Mexico) metronidazole (15 mg/kg, IV, q 12 h), and tramadol (5 mg/kg, PO, q 12 h).

The patient recovered completely within a week. Laboratory control tests were taken, being all the biochemical and haematological results within the normal range.

**Discussion**

Canine pyometra is a common disease of intact bitches; with previous reviews indicating that approximately 75% of bitches with pyometra are nulliparous. A delay in onset of treatment of this condition may result in toxaemia, septicaemia
Hafnia paralvei isolated from an emphysematous pyometra in a bitch

and even death of the patient. This condition is more common in older bitches (average age of 7.25 years) (22). Pyometra usually occurs during dioestrus, except when the infection progresses slowly, and dioestrus ends before the diagnosis is confirmed (23). The history and clinical findings in our case matches those commonly described in canine pyometra: age, breed, nulliparity and the resumption of oestrus 2 months prior to the onset of the disease.

The physical examination and laboratory results suggested SIRS in this dog, which has previously been described in 57% of bitches with pyometra, causing the production and release of inflammatory mediators (2). Patients with SIRS can be identified when presenting at least two of the following four criteria: heart rate greater than 160 beats per minute, temperature greater than 39.7 °C or less than 37.7 °C, respiratory rate over 20 breaths per minute or a carbon dioxide pressure lower than 32 mm/Hg, and a WBC count over 12,000/μl or below 4,000/μl or more than 10% of band neutrophils (24). Our patient matched three of these parameters: temperature of 40°C, 40 breaths per minute and a leukocytosis of 77,000/μl. This markedly high leukocyte count is known as leukemoid reaction; this condition has been described in canine pyometra. In the same form that another inflammatory conditions, pyometra stimulating the formation of cytokines

Figure 1: A) Left-right lateral and B) Dorsal-ventral view of the abdomen of a 9-year-old Labrador retriever bitch with emphysematous pyometra. Notice the large tubular structure filled with gas displaced to the ventral side, the presence of coprolites, and complete and incomplete spondylosis deformans from T-10 to L-4

Figure 2: A 9 year old Labrador Retriever bitch with emphysematous pyometra. Notice the uterus filled with gas and liquid on the inside
such granulocyte colony stimulating factor and granulocyte/macrophage colony stimulating factor. This cytokines, stimulating granulopoiesis because promote release of neutrophils in the bone marrow (25). In acute cases, a myeloid leukemoid reaction with neutrophilia and toxic neutrophils has been described (26), although this was not the scenario found in our case. In the present case, the bitch presented an uncommon lymphoid leukemoid reaction.

Although the most common bacterial agent associated with pyometra in bitches is E. coli, several aerobic and anaerobic bacteria have been reported to be involved (10-13). Pyometra caused by gas-producing bacteria is a very rare condition and is also known as emphysematous pyometra. This condition has only been described six times in the literature (5-10). Therefore, this is the first report of an emphysematous pyometra caused by Hafnia paralvei, a gram-negative, mobile, facultative aerobe bacillus capable of producing gas (27). Other studies have isolated different vaginal bacteria in healthy dogs, but in none of them Hafnia paralvei was identified (28, 29). It is the only species of Hafnia in the Enterobacteriaceae family. This bacterium is widely distributed in water, soil and food and is also part of the normal intestinal microbiota of mammals, marsupials, birds, reptiles, fish, invertebrates and insects (15, 30, 31). In humans, it is described as an unusual opportunistic pathogen causing nosocomial (18) or community infections (32, 33). This bacterium is not a common pathogen in animals (15), although it has been isolated in some cases (18-20, 34), but never has been isolated from a pyometra.

In the majority of bitches that develop pyometra, vaginal contamination is the source of infection (35). However, a previous report showed a strong correlation between the normal canine intestinal bacteria and those found in the uterus of dogs with pyometra (36). Although we did not perform faecal culture in this animal, we suspect that the bacteria arrived to the uterus via ascendent faecal contamination, as previously described for other causative agents of pyometra (37).

We conclude that, even though emphysematous pyometra is uncommon in dogs, it should be regarded as a presumptive diagnosis in patients presenting with tubular structures filled with gas in abdominal radiographs, especially in bitches with vulvar discharge presented during dioestrus. Finally, in the present clinical report we describe for the first time a case of canine emphysematous pyometra caused by Hafnia paralvei.

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Povzetek: V članku poročamo o primeru 9-letne samice pasme labradorec, ki je bil sprejet v Bolnišnici za veterinarsko medicino z anamnezo gnojnih in smrdljivih izločkov iz vulve, zmanjšanim apetitom in postopnim povečevanjem trebuha, kar je trajalo približno 20 dni pred obiskom veterinarja. Klinični pregled je pokazal napihnjen in boleč trebuh in timpanične zvoke ob pretrkavanju. Pacientka je imela tudi sindrom sistemskega vnetnega odziva (SIRS) in povečano skupno število levkocitov (77.9 x 10⁹/L) zaradi povišanega števila limfocitov (62.32 x 10⁹/L). Rentgenska slika trebuha je pokazala razširjene cevaste strukture, ki so zasedale večino trebuha; na stranski projekciji (levo-desno) pa so ugotovili tudi dorzalni premik debelega črevesa. Ultrazvočni pregled je prav tako pokazal cevaste strukture v sredini trebuha, ki so vsebovala tekočino mešane ehogenosti s hiperehoičnimi delci v suspoznici. Preiskovalna celiotomija je pokazala, da so bili rogovi maternice raztegnjeni in so vsebovali mešanico plina in tekočine, zato je bila izvedena ovariohisterektomija. Tekočino iz maternice so z aseptično tehniko zbrali v injekcijsko brizgo in jo predali kliničnemu patološkemu laboratoriju za ugotavljanje prisotnosti aerobnih in anaerobnih bakterij ter bakterijske občutljivosti na antibiotike. *Hafnia alvei* (sedaj razvrščeno kot *Hafnia paralvei*) so opredelili kot bakterijskega povzročitelja piometre. Piometra, ki jo povzročajo bakterije, ki proizvajajo plin, je redko stanje in je znana kot emfizematozna piometra. V literaturi je opisanih le šest primerov pri psicah. Pri psicah s piometro so našli širok spekter vaginalnih bakterij, vendar je to prvo poročilo o emfizematozni piometri, ki jo povzroča *Hafnia paralvei* pri psicah.

Ključne besede: veterinary surgery; uterus; emphysematous pyometra; *Hafnia paralvei*