

A Case of Generalized Demodicosis and Pyoderma Caused by *Enterobacter cloacae* in a Pug Dog

Pug Irkı Bir Köpekte *Enterobacter cloacae* Kaynaklı Piyoderma ve Generalize Demodikozis Olgusu

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ABSTRACT

This case aimed to evaluate the role of *Enterobacter cloacae* (*E. cloacae*), a rare gram-negative bacterium, in the etiology of pyoderma and generalized demodicosis in a 7-month-old Pug. *Demodex canis* (*D. canis*) and *E. cloacae* were isolated from deep skin scraping and biopsy samples. Miticidal treatment included afoxolaner and milbemycin oxime (2.5–5 mg/kg and 0.5–1 mg/kg, respectively). Antibacterial therapy consisted of cefazolin (20 mg/kg, IV, BID) and benzylpenicillin + dihydrostreptomycin (0.5 mL/5 kg, IM, SID) until culture and sensitivity results were obtained. The dog was returned to its shelter, where treatment was continued by the veterinarian and owner. Unfortunately, it died on the third day post-treatment. This case highlights that rare pathogens such as *E. cloacae* can contribute to severe, refractory demodicosis with secondary pyoderma. Pending antibiogram results, using less commonly employed antibiotics may help address resistance. Regular bacterial cultures and antibiograms are essential for identifying both common and rare pathogens and monitoring their resistance profiles, thus improving treatment success in complex dermatological cases.

Keywords: *Enterobacter cloacae*, generalized demodicosis, pug, pyoderma

ÖZ

Bu olgu sunumunda, 7 aylık Pug ırkı bir köpekte piyodermanın etiolojisinde nadir olarak görülen Gram negatif bir bakteri olan *Enterobacter cloacae* (*E. cloacae*) ve generalize demodikozis değerlendirildi. Parazitolojik ve bakteriyolojik incelemeler sonucunda derin deri kazıntısı ve biyopsi örneklerinden *Demodex canis* (*D. canis*) ve gram negatif bir bakteri (*E. cloacae*) izole edilerek tanıya edildi. Mitisidal tedavi amacıyla afoksolaner + milbemisin oksim (2.5-5 mg/kg afoksolaner + 0.5-1 mg/kg milbemisin oksim, Nexgard Spectra® 3.5-7.5 kg Köpekler için Çiğnenebilir Tablet, Boehringer Ingelheim) kombinasyonu kullanıldı. Antibakteriyel tedavi için bakteriyel kültür ve antibiyogram sonuçları çıkana kadar sefazolin (20 mg/kg dozda, IV, günde iki kez) ve benzilpenisilin + dihidrostreptomisin (0,5 mL/5 kg dozunda, IM, günde bir kez) kombinasyonu kullanıldı. Hayvan sahibi tarafından köpek kendi barınağına götürüldü. Tedavisi buradaki veteriner hekim ve hayvan sahibi tarafından takip edildi. Tedavi sonrası 3. günde köpeğin ex olduğu öğrenildi. Sonuç olarak, klinisyenler tedaviye yanıt vermeyen, inatçı, piyoderma ile komplike generalize demodikozis vakalarının etiolojisinde *E. cloacae* gibi nadir olarak görülebilen bakterilerin var olabileceğini göz önünde bulundurmalıdır. Antibiyogram sonuçları çıkana kadar rezistans sorununu ekarte edebilmek için, klinik rutinde nadir olarak kullanılan antibiyotiklerin tercih edilmesi, zaman zaman bakteri kültürü ve antibiyogram yapılarak yaygın ve nadiren izole edilmiş bakterilerin tanımlanması ve bu bakterilerin direnç profillerinin ortaya çıkarılmasının önemli olduğunu göstermektedir.

Anahtar Kelimeler: *Enterobacter cloacae*, generalize demodikozis, pug, piyoderma

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INTRODUCTION

Dogs are frequently affected by the parasitic skin condition demodicosis, which is characterized by an excessive number of *Demodex* mites on the skin's surface and in hair follicles.¹ Clinical signs of demodicosis include papules, pustules and draining tracts with alopecia, comedones, follicular scaling, hyperpigmentation and secondary bacterial infection.² One of the most common complication of generalized demodicosis is a secondary bacterial infection of skin (superficial or deep pyoderma, folliculitis, furunculosis).¹ Bacterial septicemia is seen especially in severe cases involving frunculosis. *Staphylococcus intermedius* is the most common infecting organism, but secondary infection with *Pseudomonas aeruginosa* or *Proteus mirabilis* may also occur.³

Enterobacter cloacae (*E. cloacae*) are gram-negative bacteria that belong to the family Enterobacteriaceae.⁴ They can be both aerobic and anaerobic. *E. cloacae* is a component of the normal intestinal flora of many mammals.⁵ However, *E. cloacae* can cause wound, respiratory and urinary tract infections.⁶

This case was discussed to bring to the attention of our colleagues about a case that we encounter from time to time in our clinics and sometimes have difficulty in treating. In this case report, generalized demodicosis complicated with pyoderma caused by *E. cloacae*, a gram-negative bacterium that is rarely seen in the etiology of pyoderma in a Pug breed dog, was evaluated.

CASE PRESENTATIONS

The material of the case was an 8-month-old, male, 4.8 kg body weight, Pug breed dog brought to Erciyes University, Faculty of Veterinary Medicine, Veterinary Teaching Hospital, Small Animal Clinic with complaints of loss of appetite, severe itching, hair loss, thickening of the skin and purulent discharge from the skin. It was learned that the dog was owned and kept in their garden of the house. It was determined that the dog was fed with leftover food as a diet and occasionally given dry food. It is known that there are no vaccinations and antiparasitic drugs are not done regularly. It was reported that the disease was noticed 17 days ago and that it first started as a small wound in the head area and then spread to other parts of the body. It was learned that praziquantel + ivermectin, fipronil and *Tarantula cubensis* D6 were used in the treatment. Clinical examination of this dog, which was brought to our hospital after the disease did not improve,

revealed alopecia, itching, hyperpigmentation in the chest, armpit, abdomen and inguinal areas, pustule formation and crusting, hyperkeratosis, foul-smelling and purulent discharge. Additionally, pododemodicosis was present in this case (Figure 1 A-B). When the physical examination findings were examined, it was determined that the dog was depressed and cachectic, its body temperature was 36.0°C, respiratory frequency was 20/min and pulse frequency was 144 beats/min. Mild paleness was detected in the mucosa. Capillary refill time (CRT) was measured at 4 seconds, supporting the clinical estimation of approximately 8% dehydration, consistent with moderate to severe dehydration. No endoparasite eggs were seen in the native examination of the stool.

Complete blood count revealed leukocytosis ($29.4 \times 10^9/L$), lymphocytosis ($6.3 \times 10^9/L$), neutrophilia ($21.8 \times 10^9/L$) and normocytic normochromic anemia [RBC ($3.22 \times 10^{12}/L$), Hgb (6.9 g/dL), Hct (19.9%)]. Interestingly, both the absolute eosinophil count and the eosinophil percentage were not elevated and were reported as zero (Table 1).⁷

Table 1. Complete blood count results

Variables		Ref. Ranges ⁷
WBC ($10^9/L$)	29.4 H	6.00-17.00
Lymph ($10^9/L$)	6.3 H	0.90-5.00
Mono ($10^9/L$)	1.3	0.30-1.50
Neut ($10^9/L$)	21.8 H	3.50-12.00
Eos ($10^9/L$)	0.0	0.10-1.50
Lymp (%)	21.5	9.00-47.00
Mono (%)	4.2	2.00-12.00
Neut (%)	74.3	42.00-84.00
Eos (%)	0.0	1.00-18.00
RBC ($10^{12}/L$)	3.22 L	5.50-8.50
Hgb (g/dL)	6.9 L	12.00-18.00
Hct (%)	19.9 L	37.00-55.00
MCV (fL)	61.8	60.00-72.00
MCH (pg)	21.5	19.50-25.50
MCHC (g/dL)	34.8	32.00-38.50
RDW _a (fL)	46.7	35.00-65.00
RDW (%)	16.3	12.00-17.50
PLT ($10^9/L$)	187	200.00-500.00
MPV (fL)	6.8	5.50-10.50

RBC; Red Blood Cell, Hct; hematocrit, Hgb; hemoglobin concentration, Lymph; lymphocyte, Neut; Neutrophil, Mono; monocyte, Eos; Eosinophil, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin volume, MCHC; mean corpuscular hemoglobin concentration, WBC; White Blood Cell, PLT; platelet, RDW; Red cell distribution, RDW_a; absolute value of the width of the distribution of red blood cells, MPV; mean platelet volume. H: High, L: Low. ⁷Canine and feline hematology reference values.



Figure 1. A Pug dog with generalized demodicosis complicated with pyoderma with (A) purulent discharge on the face and (B) hair loss, crusting, and hyperkeratosis in other parts of the body

Biochemical analysis showed an increase in BUN (66.82 mg/dL), ALP (208 U/L), GGT (12 U/L) enzyme activity and phosphorus (9.2 mg/dL) levels. AST (5.9 U/L) enzyme activity, albumin (1.89 g/dL), and sodium (138.8 mmol/L) levels were found to be lower than normal values (Table 2).⁸

Table 2. Biochemical analysis results		
Variables		Ref. Ranges ⁸
BUN (mg/dL)	66.82 H	19 - 34
Creatinine (mg/dL)	0.77	0.9 - 2.2
Total Protein (g/dL)	6.42	6.0 - 7.9
Albumin (g/dL)	1.89 L	2.8 - 3.9
Glukoz (mg/dL)	84.55	60 - 120
ALP (U/L)	208 H	0 - 45
ALT (U/L)	13.6	0 - 45
AST (U/L)	5.9 L	7 - 38
GGT (U/L)	12 H	0 - 10
CK (U/L)	314	52–368
Total Bilirubin (mg/dL)	0.43	0-0.2
LDH (U/L)	124.8	0–236
Na (mmol/L)	138.8 L	146 - 156
K (mmol/L)	4.18	3.7 - 6.1
Ca (mg/dL)	10.43	8.7 - 11.7
P (mg/dL)	9.2 H	3.0 - 6.1
Mg (mg/dL)	2.36	1.7 - 2.6

ALT; alanine aminotransferase, ALP; alkaline phosphatase, AST; aspartate aminotransferase; CK; creatine kinase, GGT; gamma glutamyltransferase, LDH; lactate dehydrogenase, CRP; C-reactive protein, BUN; blood urea nitrogen, CK; creatine kinase, Na; sodium, K; potassium, Cl; chlorine, Ca; calcium, P; phosphorus, Mg; magnesium.

For parasitological examination, deep skin scraping (until capillary bleeding occurs) samples were taken from 4 different parts of the body (head, right front extremity,

right hind extremity, ventral abdominal wall). These skin scraping samples were treated with 10% KOH and waited for 10 minutes. Then, *D. canis* agents were imaged using 10× and 40× objectives under the light microscope with camera attachment (Olympus BX-50, Japan). To calculate the number of adults, larvae, nymphs, and eggs in the scraping samples taken from each region, 10 microscope fields were examined at 10X magnification in the preparation obtained from each scraping, and the total number of agents obtained was divided by 10, and the average was calculated.⁹ Many *D. canis* agents were seen in these samples (Figure 2). Average mite numbers are as follows; adult (45), larva (15), nymph (102), eggs (48). Since the dog died on the 3rd day of treatment, mite numbers could not be determined after treatment (day 28, day 56) (Table 3).



Figure 2. Demodex canis mites

Adhesive tape technique was used for cytological examination and Giemsa staining was performed. Mallezzia organisms were seen on the slide, although not very densely.

Table 3. <i>D. canis</i> mite numbers					
	Ventral abdominal	Right Forelimb	Right hindlimb	Head region	Mean
Adult	84	44	28	22	45
Larva	12	22	14	12	15
Nymph	48	20	24	10	102
Eggs	26	2	10	10	48

A sample was taken from the abscess in the head area for bacteriological culture and antibiogram. Before taking the sample, the hair in the area was shaved and the area was disinfected with 70% ethyl alcohol and povidone-iodine solution. Samples were placed in separate sterile culture

tubes as appropriate for aerobic or anaerobic culture. The samples were quickly sent to the bacteriology laboratory under appropriate transportation conditions. The transplanted samples were incubated in appropriate media under both aerobic and anaerobic conditions at 37°C for 48-72 hours. The sample sent to the laboratory was inoculated on 5% Sheep Blood Agar, Mannitol Salt Agar and Mac Conkey Agar. After the incubation period, colony morphologies were examined. Suspicious isolates, whose gram characteristics were determined, were uploaded to the automated microbiology testing system (BD Phoenix 100, Biosciences, USA) for bacterial identification and antibiogram (MIC). In addition, an antibiogram was performed using the disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) standards for antibiotics more commonly used in veterinary medicine. The results were evaluated and reported by experienced and expert personnel. As a result of bacteriological cultivation, only *E. cloacae* was isolated and identified. As a result of the antibiogram, it was determined that the agent was resistant to 17 of 33 types of antibiotics, sensitive to 13, moderately sensitive to 2 and slightly sensitive to 1.

Antibiogram results as follows. Sensitive; amikacin, gentamicin, ertapenem, imipenem, meropenem, ceftazidime, ceftriaxone, cefepime, ceftolozane+tazobactam, piperacillin+tazobactam, ciprofloxacin, levofloxacin, trimethoprim+sulfamethoxazole. Less sensitive; streptomycin. Moderate sensitive; marbofloxacin, enrofloxacin, Resistant; cefazolin, ampicillin, amoxicillin+clavulanate, ampicillin+sulbactam, colistin, bacitracin, neomycin, kanamycin, cephalexin, moxifloxacin, peniciline, tigecycline, amoxicillin, lincomycin+spectinomycin, oxytetracycline, doxycycline, cefuroxime.

For miticidal treatment, afoxolaner and milbemycin oxime (orally every 30 days, at the recommended dosage of 2.5-5 mg/kg of afoxolaner and 0.5-1.0 mg/kg of milbemycin oxime was used.⁸ For antibacterial treatment, cefazolin (at a dose of 20 mg/kg IV twice daily for 7 days) and benzylpenicillin+dihydrostreptomycin (at a dose of 0.5 mL /5 kg B.W. /day IM for 7 days) until bacterial culture and antibiogram results are available. They were advised to bathe twice a week with a shampoo containing benzoyl peroxide. Omega-3, Omega-6, Vitamin B₁, Vitamin B₂, Vitamin B₆, Vitamin B₁₂, Biotin and Zinc (oral, once a day, 1 capsule) were prescribed as supportive treatment. The dog was taken to its own shelter by the owner. Additionally, fluid electrolyte therapy was administered. The total amount of fluid (672 mL) to be administered was calculated

by taking into account the dehydration deficit ($4.8 \text{ kg} \times 0.08 \times 1000 = 384 \text{ mL}$) and the daily maintenance fluid requirement ($4.8 \text{ kg} \times 60 = 288 \text{ mL/day}$). Since no vomiting or diarrhea was observed, possible ongoing losses were taken as 0 mL. Intravenous fluids were administered at a rate of 60 mL/kg/hour. In the first six hours, 384 mL (to make up for the lack of water) was given, and the last 288 mL was slowly given over in the next 16 hours. The treatment included: isotonic saline (0.9% NaCl), 5% Dextrose solution and Ringer's lactate in combination. Fluid and electrolyte therapy was planned to be continued for at least 3 days to ensure complete rehydration and stabilisation. His treatment was followed jointly by the veterinarian there and the animal owner. It was learned that the dog died on the 3rd day after the treatment.

DISCUSSION

Juvenile demodicosis is more common in pure bred dogs of particular breeds.² Although the disease is relatively easy to diagnose, it is often difficult to treat. In fact, in some cases, especially in the generalized form complicated by pyoderma, the prognosis can be poor. The generalized form can be one of the most severe canine skin diseases, and can be life threatening if not treated adequately and promptly.^{10,11}

In this case, oral miticidal therapy was started despite the patient being systemically unstable. This decision was made considering the severity of the parasitic load and the need to rapidly control *D. canis* infestation. The treatment protocol included afoxolaner and milbemycin oxime. Both compounds are broadly recognized miticidal agents demonstrated to be safe and efficacious, even in clinically compromised animals.^{2,12} Although caution is generally recommended when using oral medications in systemically unstable patients, in conditions such as generalized demodicosis, early and effective miticidal intervention is critical to preventing secondary infections, systemic inflammation, and clinical deterioration. Therefore, oral miticide use has been considered as part of a supportive care plan, and the patient is carefully monitored throughout treatment.

A study found that owners of American Staffordshire terriers, Staffordshire bull terriers, Chinese shar-pei, and French bulldogs had a more than four-fold increased chance of acquiring generalized demodicosis.¹³ The English bulldog, Pit bull, and Sealyham terrier are breeds that are prone to juvenile onset demodicosis, according to a more extensive study conducted in the United States.¹⁴ However, no information has been found regarding the susceptibility of Pug breed dogs to generalized demodicosis. Only one

report reported that Pug puppies were safely and effectively treated with doramectin at a dose of 0.6 mg/kg/week, with clinical lesions resolving within four weeks and being mite negative by eight weeks.¹⁵ A 12-year-old female pug mix dog was reported to have Demodicosis with *Malassezia* sp.¹⁶ However, according to our clinical experience, cases of generalized demodicosis accompanied by pododemodicosis are common in these breeds.^{1,15,16}

In this case report, generalized demodicosis (juvenile-onset) complicated with pyoderma was described in a 7-month-old Pug puppy. Additionally, pododemodicosis was also present in this case. In humans, two cases of gram-negative bacteria have been reported, one of which was a 17-year-old adolescent male, consistent with pyoderma faciale.¹⁷ *E. cloacae* was found in one patient and *Klebsiella oxytoca* was found in the other.¹⁷ Shumaker et al.¹⁸ reported that deep bacterial infection was present in 29 (94%) of 31 cases of acral lick dermatitis (ALD) in dogs. *E. cloacae* was identified in 2 of these 29 dogs diagnosed with acral lick dermatitis and deep bacterial infection in the skin. It has been reported that these isolated Enterobacter species are highly resistant microorganisms.¹⁸ In the current case report, pyoderma caused by *E. cloacae* accompanying generalized demodicosis was determined. It was observed that the infection was very severe and even two of the signs of sepsis (leukocytosis, hypothermia) were present. On the other hand, in another study, *Escherichia coli* and other Enterobacteria such as *Klebsiella pneumoniae*, *Proteus mirabilis*, *Raoultella ornithinolytica*, *E. cloacae*, *Serratia marcescens* and *Citrobacter youngae* were reported as the predominant Gram-negative bacterial species isolated from skin infections in dogs.¹⁹ Additionally, a multidrug resistance profile was detected in 64% of Enterobacter strains.¹⁹ In the current case report, antibiotics cefazolin and penicillin + streptomycin combination were initially used. Supportive drugs containing Omega-3, Omega-6, Vitamin B₁, Vitamin B₂, Vitamin B₆, Vitamin B₁₂, biotin and zinc are used to correct dry and dull hair, hair loss and dermatological problems that occur on the skin.^{1,11} In this case, supportive treatment was applied in addition to the main treatment. As a result of the antibiogram, it was determined that the identified *E. cloacae* was resistant to cefazolin and penicillin and less sensitive to streptomycin. Additionally, as a result of the antibiogram, it was determined that *E. cloacae* was also resistant to 17 of 33 antibiotics. When the antibiogram result came out 3 days later, it was learned that the dog was dead. Therefore, failure to control the infection due to antibiotic resistance may have caused the case to die.

Nowadays, isoxosaline group antiparasitic drugs such as afoxolaner, fluralaner and sarolaner are used in the treatment of generalized demodicosis.¹ Impressive results from the combination of afoxolaner + milbemycin oxime have been reported by many researchers.^{20,21} In this case, afoxolaner at a dose of 2.5 mg/kg and milbemycin oxime at a dose of 0.5 mg/kg were used orally. However, the effectiveness of the drug could not be determined because the patient died on the 3rd day of treatment.

As a result, improved antibiotic stewardship seems necessary to stop the global spread of antibiotic-resistant Enterobacteriaceae strains. Thanks to the findings obtained from this case, it becomes clear that clinicians should pay attention to antibiotic selection in cases of generalized demodicosis complicated with pyoderma that is refractory to treatment and persistent. Additionally, until the antibiogram results are available, it is important to choose antibiotics that are rarely used in clinical routine (low risk of resistance), to identify common and less commonly isolated bacteria by performing bacterial culture and antibiogram from time to time, and to reveal the resistance profiles of these bacteria.

Ethics Committee Approval: There are no ethical issues with this study.

Informed Consent: Verbal consent was obtained from the owner of the dog whose opinions were consulted in the study for the use of this information in the article.

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