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CASE SET #19

A Microbiology Case:

Reality Bites – And So Do Dogs

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Clinical Laboratory Investigations

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LEARNING OBJECTIVES

Upon completion of reading the case, the learner will be able to:

1. Distinguish *Capnocytophaga canimorsus* from *Pasteurella* spp. using motility, indole, growth requirements, and characteristic staining.

2. Compare the differences between the following conditions: standard aerobic, standard anaerobic, microaerophilic, and capnophilic environments.

3. Discuss the differences in potential for infection between dog and cat bites.

4. Describe the preferred growth conditions of *Capnocytophaga canimorsus,* including temperature, CO_2 level, and media.

5. List the mechanisms that contribute to the pathogenicity of *Capnocytophaga canimorsus.*

6. Discuss which antimicrobial therapies are effective or ineffective against *Capnocytophaga canimorsus.*

7. Describe the symptoms of an animal bite infection.

Reality Bites – And So Do Dogs

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CASE PRESENTATION

Patient History:

A 65 year-old male postal carrier presented in the emergency department with the chief complaint of fever and a hand wound that was not healing. Upon further discussion, the patient revealed that he had been bitten by an aggressive dog while on his mail route 72 hours before. He had treated the wound himself with a topical antibiotic after thoroughly washing the wound, and he kept it covered during the interim. The patient's history included one other dog attack twelve years prior. In that incident, the patient had lost his balance and fallen while trying to elude the animal. He fell off a resident's front porch and was impaled on a decorative fence post. His injuries required that his spleen be removed. The patient returned to work 8 weeks later with no other complications.

Upon inspection of the wound, the physician noted one bite mark with minimal skin tearing which began below the thumb on the right hand and extended to just below the knuckle on the thumb. The skin tear was red and swollen with no exudate evident.

Physical Examination:

The patient's temperature was 101.0° F (37.2°C) during the initial examination. His respirations were normal, but his heart rate was 92 beats per minute (bpm). His weight was 172 pounds (78 kg) which was 24 pounds (10 kg) less than the last time he had been in for his annual visit 9 months earlier. The patient indicated that he had not changed his diet or life style in the past several years. He also reported that he enjoyed having 3-4 beers most nights after work and was looking forward to retirement in 3 months. Blood work was ordered, and the patient was admitted to the hospital. Routine laboratory tests that were ordered included a complete blood count (CBC), basic metabolic panel (BMP), urinalysis (UA), and an erythrocyte sedimentation rate (ESR). In addition to routine tests, the physician ordered 2 sets of blood cultures (aerobic and anaerobic), 30 minutes apart.

Laboratory Findings:

Laboratory test results indicated an elevated white blood cell (WBC) count of $15.6 \times 10^3/mm^3$ (Table I) and an elevated ESR of 84 mm/h (Table II). All other test results including the basic metabolic panel (Table III) and urinalysis (Table IV) were in normal range for this patient.

Table I: Complete Blood Count (CBC)

Test	Results	Flag	Reference Range
White Blood Cell Count (WBC)	15.6	*	5.0-10.0 x $10^3/mm^3$
Red Blood Cell Count (RBC)	5.7		4.6-5.9 x $10^6/mm^3$ (Adult male)
Hemoglobin	14		13-18 g/dL (Adult male)
Hematocrit	47		45-52% (Adult male)
Platelets	350		150-400 x $10^3/mm^3$

Table II: Erythrocyte Sedimentation Rate (ESR)

Test	Results	Flag	Reference Range
ESR	84	*	1-13 mm/h (male)

Table III: Basic Metabolic Panel (BMP)

Test	Results	Reference Range
Sodium	138	137-145 (mmol/L)
Potassium	5.1	3.5-5.1 (mmol/L)
Chloride	105	98-107 (mmol/L)
CO_2, Venous	28	22-30 (mmol/L)
Anion Gap	10	<18 (mmol/L)
Glucose	90	70-99 (mg/dL)

Blood Urea Nitrogen (BUN)	12	7-17 (mg/dL)
Creatinine (Blood)	0.79	0.60-1.00 (mg/dL)
BUN/Creatinine Ratio	15	12-20 (Ratio)

Table IV: Urinalysis (UA)

Test	Results	Reference Range
Color	Straw	Straw - Yellow
Clarity	Clear	Clear - Hazy
pH	5	4.5-8.0
Specific gravity	1.010	1.002-1.035
Protein	Negative	Negative
Glucose	Negative	Negative
Ketones	Negative	Negative
Nitrite	Negative	Negative
Leukocyte esterase	Negative	Negative

In less than 24 hours incubation, the anaerobic blood culture bottle produced a positive result. The technologist Gram stained the broth and observed many Gram negative bacilli. She plated the broth to sheep blood agar (BAP) and chocolate agar plates and incubated them in the CO_2 (5-10%) incubator. She also set up a Brucella blood agar plate supplemented with 5% sheep blood and vitamin K_1 and a Bacteroides bile esculin (BBE) agar plate and placed them in an anaerobic environment for

incubation. After 48 hours neither the plates incubated in CO_2 nor the anaerobic plates showed any growth.

The technologist repeated the Gram stain on the blood culture bottle and confirmed the presence of Gram negative bacilli. She once again plated the broth to BAP and chocolate plates and incubated them in an anaerobic environment and in the CO_2 incubator, but on a hunch the technologist incubated an additional BAP in a microaerophilic (5-6% oxygen) atmosphere. At this point, the physician requested a preliminary report specifically to know if this isolate had been confirmed as a *Pasteurella* species. With the data available at this point, the technologist responded that this isolate was most likely not a *Pasteurella* species but a confirmatory identification would require more testing.

After 48 hours incubation, the plates in both the CO_2 and anaerobic environments again had no growth. However, the BAP incubated in a microaerophilic environment now had a motile, spreading colony growing on it. The organism was a Gram negative bacillus which was found to be catalase positive and oxidase positive. It did not ferment sucrose and was indole negative. Additional testing revealed that the isolate was sensitive to a sodium polyanetholesulfonate (SPS) disk.

At this point, the technologist consulted with a regional reference laboratory. Initially, the microbiologist at the reference lab thought the isolate would be identified as *Pasteurella* species, but upon further discussion of the preliminary results he agreed that the organism was unlikely to be a *Pasteurella*. The reference lab microbiologist asked that the organism be sent to him for further testing. Three days later the

reference laboratory faxed a report identifying the organism as *Capnocytophaga canimorsus*.

Discussion:

There are 83.3 million domestic dogs in the U.S., representing 47% of U.S. households. Comparably, there are 95.6 million domestic cats in the U.S., with 46% of U.S. households owning at least one cat.[1] The potential health benefits of pet ownership have been well-documented. However, there are risks of transmitting potential pathogens as well. According to CDC statistics, 4.5 million people are bitten by dogs each year, and of those bitten about 1 in 5, or 885,000, require medical attention with approximately half of these being children.[2] About 60% of animal bites requiring treatment are attributed to dogs, while 10-20% are related to cats.[3]

The majority of dog bites occur in young children, with the highest risk in boys between 5-9 years of age. Children's lack of knowledge about provoking animals, along with their small stature, increases their bite risk through play, teasing, or abuse.[3,4] Due to a child's height relative to that of a dog, they are more likely to have bites about the face, head, or neck, while adults are more likely to have bites to the hand, thigh, leg, as well as the face and neck.[3,5] In children, head and neck bites can be especially severe and damaging. Large dogs can produce skull fractures, intracranial bleeding, severe scalp lacerations, disfiguring facial damage, and severe wounds to the major blood vessels and nerves of the neck and throat. These types of wounds are associated with high mortality.[3,6,7]

Cat bites tend to be less destructive, but cats' sharp, narrow teeth can inflict deeper puncture wounds and are more likely to create soft tissue abscesses.[3,8] Hands are the most common site to develop rapidly spreading infections, with disabling and permanent damage, due to several distinct features. Bones and joints close to the surface, numerous small compartments, and many small nerves all contribute to morbidity. In particular, cat bites can easily cause deep tissue abscesses and osteomyelitis because of their ability to penetrate deep tissues, especially bones and joints, while leaving small openings for fluid drainage.[3,8]

Bite infections usually contain a mixture of organisms from the patient's skin and the animal's mouth, and can be a mix of aerobes and anaerobes. The most common pathogens isolated from cat and dog bites include: *Pasteurella multocida* (cats), *Pasteurella canis* (dogs), *Streptococcus* spp, *Staphylococcus* spp, *Fusobacterium* spp, *Bacteroides* spp, *Porphyromonas* spp (cats), *Moraxella* spp (cats), and *Capnocytophaga canimorsus* (dogs).[3] The greatest number of non-staphylococcal, non-streptococcal bite-induced septicemia cases are caused by *Pasteurella* spp, and *C. canimorsus*.

Pasteurella spp. are Gram negative, non-sporeforming, non-motile, facultatively anaerobic coccobacilli, which usually appear in pairs or short chains and exhibit bipolar ataining.[3,8,9] Members of this genus have the potential to cause serious infections, such as osteomyelitis, septic arthritis, endocarditis, and necrotizing fasciitis, as well as sepsis, septic shock, and meningitis, especially in immunocompromised patients.[3,8,9] However, there have also been reports of bacteremia in previously healthy individuals.[3] *P. canis* and *P. multocida* are catalase, oxidase, indole, sucrose, and ornithine

decarboxylase positive. They grow well on sheep blood and chocolate agars, where they appear as flat, wet, grey colonies. They can vary in their growth on MacConkey agar, and are easily identified by routine testing protocols.[3,8,9]

Capnocytophaga is a genus within the family *Flavobacteriaceae* and the phylum *Bacteroides*. There are seven species of *Capnocytophaga* found in the normal oral flora of humans, and only two species in that of dogs and cats, *C. canimorsus* and *C. cynodegmi.*[10,11] However, only *C. canimorsus* is known to cause severe disease in humans. *C. canimorsus* is a slow-growing, fastidious, capnophilic ("CO_2-loving"), non-spore- forming, fusiform and filamentous Gram negative bacillus.[8,9,12] The bacilli have tapered or spindle-shaped ends, but coccoid and curved forms can be seen.[9,13] No bipolar staining has been reported. Formerly known as DF-2, or "dysgonic (slow and relatively poor growth) fermenter 2", *C. canimorsus* was first reported in the literature in 1976 by Bobo and Newton as a Gram negative bacillus causing septicemia and meningitis in a patient following a dog bite.[14] The name *Capnocytophaga canimorsus* was proposed in 1989 by Brenner and colleagues. They proposed the genus *Capnocytophaga* because of its requirement for CO_2 and its ability to destroy white blood cells. The derivation of *canimorsus* is from the Latin for "dog bite".[15]

Infections with *C. canimorsus* can range from cellulitis to septicemia, meningitis, endocarditis, pneumonia and gangrene.[3,8,9] Typically, severe infections occur in patients with an underlying immune disorder, including splenectomy, chronic disease, or chronic alcohol use. However, more than 40% of infected patients show no signs of immunosuppression.[16] The incubation period can last from 1-7 days, whereupon an abrupt onset of fever, chills, rash, petechiae, diarrhea, abdominal pain, vomiting,

headache, mental confusion, shortness of breath, myalgia, and malaise may occur.[3,6,13] The infection may rapidly progress to septic shock, and disseminated intravascular coagulation (DIC), leading to multi-organ failure and death.[3,8,9,15] Patients may present with a petechial rash on the trunk and lower extremities which can progress to purpuric lesions and eventually to gangrene. Patients can undergo an extreme inflammatory response, causing microvascular injury of endothelial cells, DIC, acute respiratory distress, and organ damage. Mortality rates for *C. canimorsus* sepsis range from 25-30% up to 60% in cases of septic shock.[3]

Little is known about *Capnocytophaga* spp. pathogenicity. Some studies indicate that *C. canimorsus* may be immunosuppressive by blocking the pro-inflammatory response, and the organism is able to evade the immune system's primary lines of defense by destroying immune cells, including phagocytes.[6,11,16] Evidence from these studies suggests that *C. canimorsus* has a sialidase enzyme exposed on cell surfaces which acts to deglycosylate host cell glycoproteins, enabling the bacteria to feed off of sugars in the glycan chains. Researchers believe such a mechanism probably evolved as commensalism by utilizing carbohydrates in mucosal cells, but also contributes to growth and infectivity of the organism.[11,16] The environment of oral flora is a highly competitive one, and this would enhance the survival of any bacteria competing for nutrition.[16] In addition, researchers have found polysaccharide structures, similar to a lipopolysaccharide, which enable *C. canimorsus* to resist killing by complement. This could also be due to the presence of a capsule. Whatever the structure, this is believed to be responsible for protection not just from complement, but from phagocytosis as well.[11] Any combination of these mechanisms, including resistance to complement,

resistance to phagocytosis, and the ability to feed off of macrophages, all contribute to this organism's pathogenicity.[11,16]

C. canimorsus can be challenging to isolate in the clinical laboratory. It is difficult to grow, often needing 5 or more days of incubation under standard aerobic conditions. Addition of rabbit serum and incubation in a carbon dioxide rich environment can enhance growth.[8,13,17,18] It has been reported to grow best at 35-37°C in aerobic conditions with 5-10% CO_2 or anaerobically on Columbia agar with 5% sheep blood, as well as on chocolate agar.[8,12] Poor growth occurs on tryptic soy agar as compared to brain-heart infusion agar.[17] *C. canimorsus* has been isolated from both aerobic and anaerobic blood culture bottles, but may require 5 days or longer to be detected.[19] Pediatric bottles containing brain-heart infusion media will support growth better than bottles with trypticase soy broth.[20,21]

Colonies of *C. canimorsus* may be flat with an irregular shape, or convex with a narrow flat edge. The organism can exhibit a gliding motility, giving colonies a spreading edge which is not as extensive as the swarming seen in *Proteus* spp.[8,9] Colonies frequently appear light purple in color or produce a yellow-orange pigment.[2,8,9] They are non-hemolytic, and will not grow on MacConkey agar. Positive tests include catalase, oxidase, arginine dihydrolase, o-nitrophenyl-beta-D-galactopyranoside, and alkaline phosphatase.[8,9,12,13,17] *C. canimorsus* can produce acid from D-glucose, lactose, maltose, and, for most strains, dextrin, D-galactose, glycogen, D-mannose, and starch.[8,9,12,17]. Negative tests include indole, urease, nitrate, H_2S production, lysine and ornithine decarboxylases, and gel hydrolysis.[12,13,17,18]

Due to the fastidious nature of *C. canimorsus*, it can be difficult to perform antibiotic susceptibility testing. The organism has been reported to be susceptible to penicillins, amoxicillin-clavulanate, imipenem and other carbapenems, erythromycin, vancomycin, clindamycin, linezolid, tetracycline, ciprofloxacin, third generation cephalosporins, chloramphenicol, rifampin, doxycycline, and quinolones.[3,8,9,12] Resistance to aztreonam, polymyxin, metronidazole, trimethoprim, fosfomycin, and aminoglycosides has been documented. While there have been reports of beta-lactamase producing strains, it has also been reported that these strains respond well to beta-lactamase inhibitor combinations.[3]

Conclusion:

While *Capnocytophaga* spp. are reported to grow well in a CO_2 environment, in this case the only plate on which the organism grew was the BAP incubated in a microaerophilic environment (in a Campy jar). Standard laboratory CO_2 incubators have 5-10% CO_2, which lowers their oxygen from 21% (standard room air) to around 18%. Microaerophilic environments maintain 5-6% oxygen, which increases their CO_2 content accordingly. The increase in CO_2 may have given this isolate the environment it needed to produce growth within 48 hours.

Because of the patient's history of splenectomy, the emergency room physician felt it was necessary to admit the patient. The patient was started on intravenous ampicillin/sulbactam as best practice for empirical antibiotic therapy of the typical organisms isolated from dog-bite wounds. The patient responded well to the antimicrobials. His fever diminished and the redness and swelling of his hand was also

reduced. After three days of intravenous therapy the patient was sent home with a two-week prescription for Augmentin (ampicillin/clavulanic acid), and orders to follow up with his personal physician within five days. The patient recovered from the infection without further complications.

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