

NOTES

Periodontal Bacteria in Rabbit Mandibular and Maxillary Abscesses

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Despite the high incidence of odontogenic abscesses in pet rabbits, published data on the bacteriology of these infections are lacking, and clinical cultures are often ambiguous, making antibiotic choices difficult. In order to define the bacteriology of these infections, 12 rabbit mandibular and maxillary abscesses were cultured aerobically and anaerobically. All specimens yielded pathogenic bacteria, including *Fusobacterium nucleatum*, *Prevotella heparinolytica*, *Prevotella* spp., *Peptostreptococcus micros*, *Streptococcus milleri* group, *Actinomyces israelii*, and *Arcanobacterium haemolyticum*. These organisms are consistent with the characterized bacteriology of periodontal disease in human and other mammalian studies. The isolates were tested against 10 antimicrobial agents commonly used to treat rabbits; 100% of the strains tested were susceptible to clindamycin, 96% were susceptible to penicillin and ceftriaxone, 54% were susceptible to ciprofloxacin, and only 7% were susceptible to trimethoprim-sulfamethoxazole.

Odontogenic abscesses in rabbits are common and often lead to significant morbidity and mortality (10). Treatment of these abscesses is frequently unsuccessful because of aggressive capsule formation and the development of fistulous tracts. The thick consistency of rabbit pus makes aspiration and drainage of these abscesses very difficult and antibiotic therapy problematic; therefore, surgical excision of the abscesses is often the best treatment option. However, since the abscess tracts may be microscopic and thus visually undetectable, total excision is difficult and recurrence of the infection is likely. Osteomyelitis and retrobulbar involvement are common sequelae.

The lack of published data on the microbiology of odontogenic abscesses in rabbits has made interpretation of culture reports difficult, and the antibiotic treatment options remain unclear. Less-than-optimum specimen collection techniques that do not exclude normal gingival flora can produce ambiguous culture results. Additionally, because rabbits are coprophagic herbivores, cultures of molar pocket pus instead of the abscess capsule may grow organisms not generally considered to be oral pathogens in other mammals; members of the family *Enterobacteriaceae* and *Bacteroides fragilis* group and environmental contaminants such as *Pseudomonas* and *Acinetobacter* spp. are often isolated from such specimens. Therapy

directed against these organisms alone has frequently consisted of an aminoglycoside and has typically been ineffective. In addition, routine culture and identification of oral pathogens have often been unsuccessful (28, 29). And when mixed oral organisms are isolated from these specimens, culture reports of “oral flora isolated” may not preclude such pathogens as the *Streptococcus milleri* group or *Actinomyces israelii*. Finally, susceptibility testing of these organisms is problematic and thus not usually performed

In lieu of adequate clinical data, empirical antibiotic therapy has often been directed against established rabbit pathogens such as *Pasteurella* spp. However, oral delivery of such antibiotics as beta-lactams, macrolides, and clindamycin, which are commonly used in humans and other mammals, can cause fatal enterotoxemia in rabbits. Initial therapies consisting of a fluoroquinolone or trimethoprim-sulfamethoxazole were also largely ineffective. More recently, therapies have been developed based on earlier osteomyelitis studies (23) using antibiotic-impregnated polymethylmethacrylate (AIPMMA) beads (15) or injectable penicillins and have been frequently successful. AIPMMA beads are made by adding antibiotic powder to the polymer and monomer as the resin is mixed; beads are formed by extrusion of the resin through a syringe. Beads implanted in the debrided surgical wound deliver very high concentrations of antibiotic locally for an extended period. This technique is used to treat infections such as abscesses and osteomyelitis when systemic therapy is inadequate.

To determine the bacteriology of these abscesses and improve the selection of proper antimicrobial therapy, 12 rabbits with mandibular and maxillary abscesses were selected by the Veterinary Study Group for aerobic and anaerobic cultures. Ten of the rabbits had received no antimicrobial therapy within seven days of surgery. Two additional rabbits were included because they had failed antimicrobial therapy, one with oral

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trimethoprim-sulfamethoxazole and the other with chloramphenicol-impregnated polymethylmethacrylate bead therapy.

The abscesses were excised percutaneously to avoid contamination by gingival flora. Two specimens were obtained from each rabbit: one was a biopsy specimen of the abscess margin or involved bone, and the second was pus obtained by placing a miniswab into the purulent center of the excised abscess. The specimens were immediately placed in anaerobic transport medium (Anaerobe Systems, Morgan Hill, Calif.) and shipped by overnight courier to R. M. Alden Research Laboratory. Upon receipt, the specimens were processed in an anaerobic chamber (Anaerobe Systems). The biopsy material was mixed with 1 ml of brucella broth and ground with a tissue grinder (Sage Products, Inc., Crystal Lake, Ill.). Purulent swabs were vortexed in 1 ml of brucella broth. One drop of each suspension was used to inoculate various media. Media for aerobic culture included sheep blood agar, chocolate agar, RTF (modified Casman) agar, Columbia agar, and rabbit blood agar (Hardy Diagnostics, Santa Maria, Calif.). These media were incubated for up to 7 days at 35°C in 5 to 10% CO₂. MacConkey agar was incubated for 4 days at 35°C in ambient air. Media for anaerobic culture included supplemented brucella agar, phenylethyl alcohol blood agar, *Fusobacterium* selective agar (Anaerobe Systems), RTF agar, rabbit blood agar, and chopped meat broth (Hardy Diagnostics). These media were incubated for up to 10 days at 35°C in the anaerobic chamber incubator.

Aerobic organisms were identified with biochemical tests, rapid kits, and other standard methods (19). Anaerobic organisms were identified by standard methods, including special potency antibiotic disks, Rapid ANA II panels (Remel, Inc., Lenexa, Kans.), and prerduced, anaerobically sterilized biochemicals (Anaerobe Systems) (16, 19, 25).

While many different types of media have been used to culture periodontal flora, we found RTF agar (Hardy Diagnostics) to be superior to rabbit blood agar and other blood-containing media, including chocolate agar. RTF agar is manufactured as a rabbit blood agar substitute for culture of *Haemophilus* spp. Although we did not isolate *Haemophilus* organisms in this study, most aerobic and anaerobic bacteria produced larger colonies on RTF agar than on the other media.

Susceptibility testing was performed on all isolates against 10 antimicrobials commonly used to treat rabbit infection or equivalents (when the same organism was isolated from both the biopsy and pus specimen, they were considered identical and only one was selected for susceptibility testing). Anaerobes and streptococci were tested by agar dilution and strict aerobes were tested by broth microdilution according to NCCLS guidelines (20, 21). Control strains included *B. fragilis* ATCC 25285 and *Staphylococcus aureus* ATCC 29213. Three of the 34 organisms did not grow with these methods. Standard laboratory powders were obtained from their respective manufacturers: penicillin and chloramphenicol from Sigma (St. Louis, Mo.), ceftriaxone and trimethoprim-sulfamethoxazole from Roche (Nutley, N.J.), cefazolin from Eli Lilly (Indianapolis, Ind.), metronidazole from Searle (Skokie, Ill.), clindamycin from Pharmacia (Kalamazoo, Mich.), ciprofloxacin from Bayer (West Haven, Conn.), azithromycin from Pfizer (New York, N.Y.), and tetracycline from Bristol-Meyers Squibb (New Brunswick, Conn.).

TABLE 1. Aerobic and anaerobic bacteria isolated from rabbit mandibular and maxillary abscesses

Organism	No. of isolations from group (n) ^a			
	Group I (3)	Group II (4)	Group III (4)	Group IV
Anaerobic				
<i>Actinomyces israelii</i>	2			
<i>Arcanobacterium haemolyticum</i>				1
<i>Eubacterium brachy</i>		1		
<i>Peptostreptococcus micros</i>	2	3		
<i>Staphylococcus saccharolyticus</i>				
<i>Fusobacterium nucleatum</i>	2	4		
<i>Prevotella heparinolytica</i>		2		
<i>Prevotella</i> sp., no good fit	1	2		
<i>Desulfomonas pigra</i>				
Aerobic				
<i>Streptococcus intermedius</i>	3		3	
<i>Streptococcus anginosus</i>			1	
Viridans group streptococcus, no good fit	1			
β streptococcus, not groups A-G	1			
<i>Staphylococcus warnerii</i>				1
<i>Bacillus</i> sp.	1			
<i>Achromobacter (Alcaligenes) xylosoxidans</i> subsp. <i>xylosoxidans</i>				1
<i>Neisseria weaverii</i>	1			

^a Group I yielded mixed aerobes and anaerobes; group II yielded anaerobes only; group III yielded aerobes only; group IV yielded only *Arcanobacterium haemolyticum* n, number of rabbits in group.

The cultures from all 12 rabbits yielded potentially pathogenic bacterial species, including *Fusobacterium nucleatum* (6 rabbits), *Streptococcus intermedius* (6 rabbits), *Peptostreptococcus micros* (recently reclassified as *Micromonas micros* [18]) (5 rabbits), *Prevotella heparinolytica* (2 rabbits), *Actinomyces israelii* (2 rabbits), and one each of *Streptococcus anginosus*, *Arcanobacterium haemolyticum*, and a beta-hemolytic *Streptococcus* sp. (not group A, B, C, D, F, or G) (Table 1). *Pasteurella multocida* was not isolated, although it is a common rabbit pathogen and has been presumed to be present in jaw abscesses. *Staphylococcus aureus* and oral viridans group streptococci other than the *Streptococcus milleri* group were not isolated, and although rabbits are coprophagic, members of the family *Enterobacteriaceae* and the *B. fragilis* group were also not isolated. *Staphylococcus warneri*, *Neisseria weaverii*, and *Achromobacter (Alcaligenes) xylosoxidans* subsp. *xylosoxidans* were isolated from two specimens in small quantities. These organisms have not been found to be significant pathogens in other periodontal studies of otherwise healthy individuals (1, 12) and were considered probable skin, oral, or environmental contaminants in this study. While rabbit pus is considered by some to be a poor specimen for culture, in approximately one-half of the abscesses the purulent center yielded a greater variety or quantity of organisms than cultures of the margin in qualitative culture of these specimens.

The types and combinations of organisms recovered formed four distinct patterns. Group I grew a combination of aerobes and anaerobes, group II contained only anaerobes, and group III had *Streptococcus intermedius* or *Streptococcus anginosus* as a single pathogen. Group IV was unusual in that *Arcanobac-*

TABLE 2. In vitro susceptibilities of bacteria isolated from rabbit mandibular and maxillary abscesses

Organism	No. of susceptible organisms/no. tested									
	Penicillin	Ceftriaxone	Cefazolin	Metronidazole	Chloramphenicol	Clindamycin	Azithromycin	Ciprofloxacin	Trimethoprim-sulfamethoxazole	Tetracycline
Anaerobic										
<i>Actinomyces israelii</i>	2/2	2/2	2/2	0/2	2/2	2/2	2/2	0/2	0/2	2/2
<i>Arcanobacterium haemolyticum</i>	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1
<i>Eubacterium brachy</i>	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	1/1
<i>Peptostreptococcus micros</i>	5/5	5/5	5/5	5/5	5/5	5/5	5/5	2/5	0/5	5/5
<i>Staphylococcus saccharolyticus</i>	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1	0/1
<i>Fusobacterium nucleatum</i>	5/5	5/5	5/5	5/5	5/5	5/5	1/5	1/5	1/5	3/5
<i>Prevotella heparinolytica</i>	2/2	2/2	2/2	2/2	2/2	2/2	2/2	0/2	0/2	2/2
<i>Prevotella</i> sp., no good fit	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/1	0/1	0/1
<i>Desulfomonas pigra</i>	0/1	0/1	0/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1
Aerobic										
<i>Streptococcus intermedius</i>	6/6	6/6	6/6	0/6	6/6	6/6	6/6	6/6	1/6	6/6
<i>Streptococcus anginosus</i>	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1
Viridans group streptococcus, no good fit	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1
β streptococcus, not groups A-G	1/1	1/1	1/1	0/1	1/1	1/1	1/1	1/1	0/1	1/1
<i>Staphylococcus warneri</i>	0/1	1/1	1/1	— ^a	1/1	1/1	1/1	1/1	1/1	1/1
<i>Achromobacter (Alcaligenes) xylosoxidans</i> subsp. <i>xylosoxidans</i>	0/1	0/1	0/1	—	0/1	0/1	0/1	0/1	0/1	0/1
<i>Neisseria weaverii</i>	1/1	1/1	1/1	—	1/1	0/1	1/1	1/1	0/1	1/1

^a —, not determined.

terium haemolyticum was the only potential pathogen recovered and the ostensible cause of rapid mortality. Although *Arcanobacterium haemolyticum* is a significant cause of pharyngitis in humans, it can also cause systemic and soft tissue diseases, including septicemia, sinusitis, and orbital cellulitis (2, 24). This organism has not been previously shown to be a rabbit pathogen or part of the normal rabbit flora; thus, the epidemiology of this case is uncertain.

Susceptibility results are found in Table 2. Of all potential pathogens tested, 100% were susceptible to clindamycin and chloramphenicol; 96% were susceptible to penicillin, ceftriaxone, and cefazolin (MIC \leq 2 μ g/ml); 86% were susceptible to azithromycin (MIC \leq 0.5 μ g/ml) and tetracycline; 54% were susceptible to metronidazole and ciprofloxacin (MIC \leq 1 μ g/ml), and only 7% were susceptible to trimethoprim-sulfamethoxazole (MIC \leq 2/38 μ g/ml). All strains of the *Streptococcus milleri* group were susceptible to clindamycin, penicillin, and ceftriaxone, although occasional resistance in humans has been reported (9, 27). Although metronidazole was largely effective against anaerobic gram-negative bacilli and gram-positive cocci in this study, it was ineffective against anaerobic gram-positive bacilli and streptococci. (Because they were considered probable contaminants, *Staphylococcus warneri*, *N. weaverii*, and *Achromobacter (Alcaligenes) xylosoxidans* subsp. *xylosoxidans* were not included in this statistical analysis, although their susceptibilities are included in Table 2.)

Of the 12 rabbits, none had organisms resistant to clindamycin or chloramphenicol, and only 1 had organisms resistant to penicillin, ceftriaxone, or cefazolin. Conversely, 5 had organisms resistant to azithromycin, 9 had organisms resistant to ciprofloxacin, and 11 had organisms resistant to trimethoprim-sulfamethoxazole. Thus, while ciprofloxacin (or enrofloxacin) and trimethoprim-sulfamethoxazole are often prescribed for other rabbit infectious processes, they are ineffective for periodontal infections containing mixed aerobic and anaerobic or-

ganisms. Finally, the rabbit that failed oral trimethoprim-sulfamethoxazole therapy grew mixed aerobic and anaerobic oral pathogens resistant to this agent, and samples from the rabbit that failed AIPMMA chloramphenicol bead therapy grew only a *Streptococcus intermedius* isolate that was susceptible to chloramphenicol.

Our study shows that the etiologic agents of rabbit periodontal infections consist of a mixture of anaerobic gram-negative rods, especially *F. nucleatum*; anaerobic gram-positive non-spore-forming rods, predominantly *Actinomyces* spp.; and aerobic gram-positive cocci, particularly the *Streptococcus milleri* group. These culture results are consistent with the bacteria reported in human (1, 8, 17, 22, 26) and other mammalian (11, 12) periodontal disease studies, and it is therefore important to direct antibiotic therapy for odontogenic abscesses in rabbits against these types of oral pathogens. Specifically, although oral antibiotics such as trimethoprim-sulfamethoxazole and older fluoroquinolones such as enrofloxacin are commonly used in rabbit veterinary practice, our results and previous studies (4, 9) have shown that they are only marginally effective against many oral anaerobes and gram-positive aerobes.

For empirical therapy when an adequate culture cannot be performed, veterinary clinicians should consider the potential susceptibilities of the types of oral microbes isolated in our study, as well as of those reported in human (3-7, 27) and other mammalian (13, 14) studies. New methods of nonoral delivery of clindamycin, ceftiofur, and penicillins are currently under investigation. One clinician has observed that therapy with AIPMMA clindamycin and/or ceftiofur beads resulted in no recurrence in 97 of 104 (93%) rabbits within 90 days of implantation (J. Jenkins, unpublished observation). Although all of the potential pathogens in this study were susceptible to chloramphenicol, its success in AIPMMA beads has been observed to be only marginal by members of the Veterinary Study Group. Another therapy using a combination of injectable

benzathine and procaine penicillins is under study by other clinicians and shows variable yet promising results (E. Hine, personal communication).

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