Oral Bacterial Flora of Dogs with and without Rabies : A Preliminary Study in Thailand

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Abstract

The authors studied the bacterial flora of the dog oral cavity and of bite wounds, Aerobic bacteria were isolated from mouth swabs of 16 normal and 5 rabid dogs as well as from infected dog-bite wounds from 18 patients. A total of 20 different microbial species were recovered from mouth swab cultures. The most frequently isolated organisms were *Klebsiella pneumoniae* ssp *pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Citrobacter freundii*, *Enterobacter cloacae*, *Acinetobacter calcoaceticus*, and *Pasteurella* species. There were no differences in the aerobic bacterial flora between rabid and non- rabid dogs. From the cultures of the bite wound swabs, the authors found that almost all of the organisms identified were part of the normal oral flora of the dog. One or more aerobic bacteria were isolated from the infected dog-bite wounds. Two patients had four, 3 had three, 4 had two, and 6 had one of the nine organisms in their wounds. The predominant species of bacteria involved in infection of bite wounds were, as follows: *Staphylococcus aureus*, *Pasteurella multocida*, *E. coli*, *Moraxella* species, *Pasteurella canis*, and *Enterobacter cloacae*. However, three wound cultures had no aerobic bacterial growth. The results of this study show that the infected bite wounds may contain a mixed bacterial flora that colonize human skin and the oral cavity of dogs.

Key word : Aerobic Bacteria, Dog Oral Flora, Bite Wound Infection, Rabies

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The Queen Saovabha Memorial Institute (QSMI) of the Thai Red Cross Society, is a center for rabies diagnosis and includes the primary animal bite clinic for the central part of Thailand. Several hundred thousand Thais are bitten by animals yearly, resulting in approximately 8,000 visits to our clinic alone. Ninety per cent of these bite injuries are from dogs and approximately 13 per cent of transdermal injuries become infected(1). Infection is treated empirically with either amoxicillin or cloxacillin. Neither Gram stains nor cultures are taken at the start of treatment. The authors have no local knowledge of the bacteriology of dog bite infections nor of the indigenous oral bacterial flora of dogs. Such studies have been reported from western countries.

It is known that organisms from the dog oral cavity infect bite wounds. To the best of our knowledge, no such study of local dogs has been reported nor are these studies of the oral bacteriology in proven rabid dogs. This study was done to determine the most prevalent etiological agents of bite-wound infections in Thailand and to study the oral bacterial flora of normal dogs as well as that of living rabid dogs.

MATERIAL AND METHOD

Saliva was collected from dogs admitted to the Rabies Diagnostic Unit at QSMI for quarantine. Sixteen dogs with owners, street dogs as well as 5 rabid dogs were included. Specimens were collected on sterile cotton-tipped applicators and directly streaked on sheep blood and MacConkey's agar plates for aerobic bacteria isolation. Gram stain preparations and biochemical characteristics were made of all subcultured organisms according to the standard procedures(2,3). Whether the dog was rabid or not could be determined, since it might be easily diagnosed by clinical signs of rabies. In some experiments, the reverse transcription-polymerase chain reaction for rabies virus ribonucleic acid (RNA) and the latex agglutination test for rabies virus antigen in saliva were performed in dogs that were ill or showing unusual behavior(4).

All infected (erythema, swelling, exudate) wounds were collected from dog-bite victims presenting to the Animal Bite Clinic at QSMI. Eighteen cases were included in the study. Plain swabs were inserted deeply after the skin surrounding the wound had been cleaned with normal saline. Swab specimens were placed in a transport tube (Swab Transport Pack Amies Medium, Difco, USA) and processed by conventional methods(2,3).

RESULTS AND DISCUSSION

The organisms isolated from oral swabs of 16 normal dogs and 5 pre-terminal proven rabid dogs, and those from dog-bite wound swabs of 18 patients are listed in Table 1. The percentage of specimens in which the various kinds of bacteria were isolated is shown.

Twenty-one offending dogs admitted to the Rabies Diagnostic Unit at QSMI for daily observation of the dog's health were included in the study. Quarantine and observation for a ten-day period is standard practice. Five of them showed signs of rabies during the quarantine periods. All of them were stray dogs. Rabies virus RNA and antigen were also present in the saliva specimens of these 5 dogs (data not shown).

The spectrum of dog oral flora is broad. A total of 20 different bacterial species were recovered from mouth swab cultures. *Klebsiella pneumoniae* was the species most often isolated. Approximately 48 per cent of the dogs examined harbored this organism. The next most frequently observed bacteria were *Escherichia coli, Staphylococcus aureus, Citrobacter freundii, Enterobacter cloacae, Acinetobacter calcoaceticus, Pasteurella multocida*, and *Pasteurella canis*. The other related organisms were of low prevalence. The bacterial flora found in 5 rabid dogs revealed the same major group or genera of bacteria as found in the normal subjects. The frequency and spectrum of the bacterial isolates were similar as well. These indicated that there were no differences in the aerobic bacterial flora between rabid and non-rabid dogs.

In the bite wound cultures, the organisms isolated were similar to those found in the cultures of dog mouth swabs. One or more of the following were recovered from the infected dog-bite wounds: *Staphylococcus aureus, Pasteurella multocida, E. coli, Moraxella* species, *Pasteurella canis, Enterobacter cloacae, Staphylococcus epidermidis*, β-hemolytic streptococcus, and *Proteus mirabilis*. Two patients had four, 3 had three, 4 had two and 6 had one of the nine organisms in their wounds. Three wound cultures exhibited no aerobic bacterial growth (Table 2).

Dog-bite injuries are common in humans. In Thailand, dog bites represent 5.3 per cent of injuries seen in the emergency room. The lower extremities and upper extremities are the most common site for bites. Unprovoked attacks in public places are the most common circumstances. Persons walking along

Table 1. Aerobic bacteria isolated from mouth swabs of 21 dogs, and from dog bite wounds from 18 patients.

Organism	No. of dogs harboring	%	No. of patients harboring	%
Klebsiella pneumoniae ssp *pneumoniae*	10**	48		
Escherichia coli	7*	33	5	28
Staphylococcus				
Staphylococcus aureus	5†	24	7	39
Staphylococcus epidermidis	3‡	14	2	11
Citrobacter				
Citrobacter freundii	5†	24		
Citrobacter amalonaticus	1	5		
Enterobacter cloacae	5‡	24	3	17
Acinetobacter calcoaceticus	4	19		
Pasteurella				
Pasteurella multocida	3	14	6	33
Pasteurella canis	3	14	3	17
Moraxella sp	2‡	10	3	17
β-hemolytic streptococci	2	10	1	6
Corynebacterium jeikeium	2‡	10		
Serratia marcescens	2	10		
Aeromonas sp	2	10		
Proteus mirabilis	1	5	1	6
Alcaligenes faecalis	1	5		
Pseudomonas sp	1	5		
Bacillus sp	1	5		
Simonsiella steedae	1	5		

* Four rabid dogs included
** Three rabid dogs included
† Two rabid dogs included
‡ One rabid dog included

Table 2. Aerobic bacteria isolated from 18 dog-bite wounds

Organism	No. of isolates	%
No bacterial growth	3	17
One bacterial isolate	6	33
Two bacterial isolates	4	22
Three bacterial isolates	3	17
Four bacterial isolates	2	11

streets are at greatest risk(5). A prospective study at QSMI indicated that approximately 13 per cent of animal inflicted deep puncture wounds and lacerations become infected and that infection is usually apparent on days 3-7 post injury(1).

In the present study, all dog-bite victims did not clean their wounds immediately and were not given antibiotics after being bitten. They went to the Animal Bite Clinic at QSMI when the wounds became infected. The bite incident mostly occurred on a street and the majority of offending dogs were not captured. Based on results of bite-wound samples, 83 per cent (15/18) had positive aerobic cultures with 40 per cent (6/15) of those having a single isolate and 60 per cent (9/15) having mixed culture. No bacteria were cultured from 17 per cent (3/18) (Table 3). Devitalized tissues are considered contaminated, both with the dog's oral flora and bacteria from the skin of the victim. Wounds may become infected if treatment is delayed, allowing bacteria to multiply and invade the wounds. The organisms in the wounds were probably present in mixed culture, which are known to have a synergistic relationship(6).

The wound culture results of previous studies are controversial in terms of the frequency of the predominant aerobic organisms(7-10). The spectrum of organisms that cause infection is broad. However, most infections of dog bites are associated with streptococci, *Staphylococcus* species, *Pasteurella* species, and other members of the oral flora. A previous study (11) has shown that more bacteria were cultured per

Table 3. Comparison of aerobic culture results taken from bite wounds in 18 patients and from mouth swabs of 21 dogs.

Sample	Positive cultures		No growth
	Single isolate	Mixed isolate	
Bite wound	6/18	9/18	3/18
Mouth swab	0/21	21/21	0/21

isolate, with a median of 5 bacterial isolates per culture. There were significantly more isolates grown when the samples were sent to a reference microbiology laboratory than when the same samples were sent to local microbiology laboratories. Moreover, the diversity of the wounds' locations, type of wound, degree of severity, and initial wound care also affected the number of total cultivable flora.

The present study revealed that the normal oral flora of the dogs, rather than human skin flora, was the source of most bacteria isolated from bite-wound cultures. Either single or multiple pathogenic bacteria were cultured from bite wounds, ranging from one to four. In patients with multibacterial infection, it is usually difficult to establish which organism(s) are pathogenic. Initial empiric treatment requires combined antimicrobial drugs, in addition to scrupulous wound management is important. The risk of infection can be reduced by proper first aid and wound care. These include prompt washing and irrigation of the bite site. This is of primary importance in reducing not only the high inoculum of the oral bacterial flora from the dog bite but also the risk of rabies virus infection. It cannot be predicted in which dogs rabies infection persists. A brief history of the biting incident can suggest the likelihood of rabies. Early and properly medical treatment can minimize complications.

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แบคทีเรียในปากของสุนัขที่ปกติและที่เป็นโรคพิษสุนัขบ้า : การศึกษาเบื้องต้นในประเทศไทย

ทรงศรี เกษมพิมลพร, ปรด, มณีรัตน์ เบญจวงค์กุลชัย, วทม*, วชิราภรณ์ แสงสีสม, วทบ*, วิศิษฏ์ สิตปรีชา, พบ, ปรด**

ได้ทำการแยกเชื้อแอโรบิคแบคทีเรียในช่องปากสุนัขทั่วไปจำนวน 16 ตัวและสุนัขที่เป็นโรคพิษสุนัขบ้าจำนวน 5 ตัว พบเชื้อประมาณ 20 สปีชีส์ เชื้อที่พบบ่อยคือ *Klebsiella pneumoniae* ssp *pneumoniae, Escherichia coli, Staphylococcus aureus, Citrobacter freundii, Enterobacter cloacae, Acinetobacter calcoaceticus,* และ *Pasteurella species* ไม่พบความแตกต่างของเชื้อในปากสุนัขที่ปกติกับที่เป็นโรคพิษสุนัขบ้า นอกจากนี้ได้ทำการแยกเชื้อจากแผลคนไข้ที่ถูกสุนัขกัดจำนวน 18 คน พบเชื้อแอโรบิคทั้งหมด 9 ชนิด โดยพบเชื้อ 4 ชนิดในแผลคนไข้ 2 คน 3 ชนิด 3 คน 2 ชนิด 4 คน และ 1 ชนิด 6 คน ชนิดที่พบบ่อยได้แก่ *Staphylococcus aureus, Pasteurella multocida, E. coli, Moraxella species, Pasteurella canis,* และ *Enterobacter cloacae* อย่างไรก็ตามมีคนไข้ 3 คนที่ตรวจไม่พบเชื้อแอโรบิค งานวิจัยนี้แสดงให้เห็นว่าเชื้อที่พบที่แผลถูกกัดอาจประกอบด้วยเชื้อหลายชนิดที่ส่วนใหญ่เป็นเชื้อชนิดเดียวกับที่พบตามผิวหนังของคนและในปากของสุนัข

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