Throat Flora of a Closed Colony of Beagles[1] (34836)

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(Introduced by R. N. Feinstein)

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Bacteremia has long been known to contribute extensively to death in lethally X-irradiated mice (1) and in X-irradiated dogs (2–4). Although Shively, Michaelson, and Howland (5) found that it played a less important role in ^{60}Co irradiated dogs, Norris *et al.* (6) concluded that bacteremia, secondary to granulocytopenia, was the primary cause of death in beagles given a single, 50%-lethal dose of ^{60}Co gamma radiation to the whole body. Subsequently, we concluded that the β-hemolytic streptococci and *Pasteurella* species isolated from blood cultures of beagles exposed to continuous ^{60}Co gamma radiation originated in the throats of these dogs (7). It, therefore, became important for us to study the normal throat flora of dogs in detail. The normal flora of the murine respiratory tract has been associated with bacteremia after X-irratiation of mice (8–12), but except for our own findings, the respiratory flora has not been incriminated in bacteremia of irradiated dogs. In fact, the general literature on the respiratory flora of dogs is scant. The incidence of some select microorganisms in dog throats has been reported (13–17) but only Clapper and Meade (18) reported the complete throat flora of 25 dogs. Accordingly, we began collecting throat swabs from the beagle colony at Argonne National Laboratory. This report covers a 1-year period from May 1968 through April 1969.

Materials and Methods. The dogs in this study were from the purebred beagle colony maintained at Argonne National Laboratory as a closed colony since 1960. The management and the genetic definition of the colony have been described (6, 19). Throat swabs from 467 dogs were collected by swabbing the left tonsillar area of each dog when it received its annual routine physical examination. The swabs were kept moist with sterile saline after collection and the following media were inoculated within 2 hr of collection: blood–agar consisting of 5% sterile sheep blood added to a base of tryptic soy agar and tryptic soy broth for total flora; phenylethyl alcohol agar, for gram-positive bacteria; Chapman Stone agar, for staphylococci; eosin methylene blue agar, for coliforms; and Sabouraud's agar, mycophil agar, and mycosel agar for fungi. PPLO agar for the isolation of *Mycoplasma* species was prepared according to Brennan, Fritz, and Flynn (20) with the addition of fresh yeast extract, 0.002% DNA, and 0.1% DEAE dextran. The media for fungi were incubated aerobically at 25°, all others aerobically at 37°. An additional blood–agar plate was incubated anaerobically at 37° in an atmosphere of nitrogen containing 5% CO_2. Individual colonies of different types were picked from each medium and identified by biochemical serological methods. These included Lancefield grouping of a selected number of beta hemolytic streptococci, and growth-inhibition tests (21) of representative *Mycoplasma* strains with antisera prepared against *M. canis, M. maculosum* and *M. spumans.*

Results. The kinds of bacteria and their incidence are presented in Fig. 1. Alpha streptococci, nonhemolytic streptococci, *Neisseria* species, *Corynebacterium* species, and *Bacillus* species are regarded as normal inhabitants of the throats of dogs and man (18). *Mycoplasma* species were present in all the dogs. Some of the strains we tested were related to *M. canis* but tests with *M. maculosum* and *M. spumans* antisera were negative. Further tests performed on isolates sent

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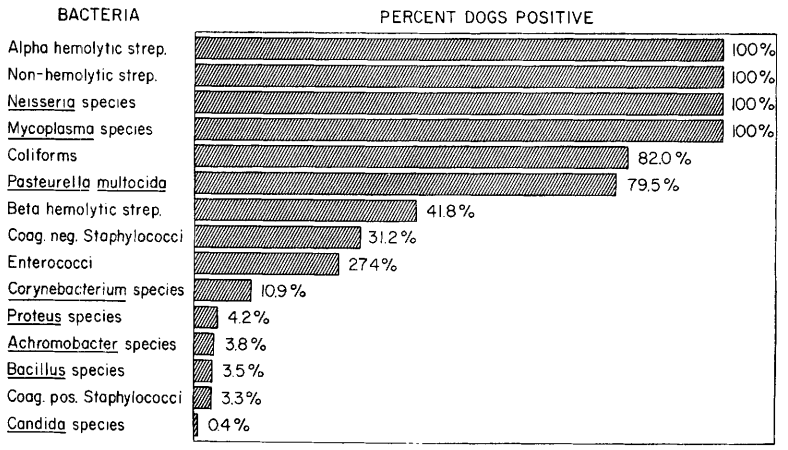

FIG. 1. Kinds of bacteria recovered from throat swabs and their incidence in a closed colony of beagles.

to M. F. Bairle of the National Institutes of Health showed that four were related to *M. edwardii* and two were not identificable (personal communication).

Beta hemolytic streptococci were isolated from 195 dogs, and all that were tested were Lancefield group G. We found that 357 dogs had *Pasteurella multocida* and 382 had coliforms in their throats. *Proteus* species were recovered from 20 dogs, and coagulase positive staphylococci were recovered from only 16 dogs. *Pseudomonas aeruginosa* was recovered from the throat of one dog. Its incidence was, therefore, not included in the figure. Although we cultured for them, *Diplococcus pneumoniae* and *Hemophilus* species were not isolated.

Because of the relatively high incidence of β hemolytic streptococci in the colony in apparently healthy dogs and because of its significance as a cause of bacteremia in irradiated dogs (6, 7), we studied the incidence by age, sex, and pedigree of the dogs and the month in which the culture was obtained. Table I shows the incidence of β hemolytic streptococci in terms of age. Younger dogs (1–4 years old) have a lower incidence of β hemolytic streptococci ($p < .01$) than older dogs.

The incidence of β hemolytic streptococci according to the month of isolation is presented in Table II. The incidence is similar for all months except October and November, when the incidence was about half that found during the rest of the year.

TABLE I. Incidence of β Hemolytic Streptococci in the Throats of Beagles by Age.

Age of dogs in years	Number positive/ number examined	% Positive
1–2	42/113	37.2
2–3	21/65	32.3
3–4	18/53	34.0
4–5	23/42	54.8
5–6	19/50	38.0
6–7	28/54	51.9
>7	44/90	48.9

Table III shows the incidence of hemolytic streptococci by kennel location. Also shown

TABLE II. Seasonal Incidence of β Hemolytic Streptococci in the Throats of Beagles.

Month cultured	Number positive/ number examined	% Positive
January	17/36	47.2
February	16/33	48.5
March	18/38	47.4
April	22/47	46.8
May	32/77	41.6
June	19/37	51.4
July	20/38	52.6
August	16/36	44.4
September	N.D.	N.D.
October	7/35	20.0
November	10/52	19.2
December	18/38	47.4

TABLE III. Incidence of β Hemolytic Streptococci in the Throats of Beagles by Kennel Location.

Kennel	Status	Number positive/number cultured[a]	% Positive
H	Breeding adults	13/41	31.8
M	Experimental	43/78	55.3
N	Experimental	45/94	47.9
O	Colony controls	46/97	47.5
P	Stock	32/90	35.2

[a] The kennel location was recorded on only 400 of the 467 dogs cultured.

is the status of the dogs housed in each kennel. On the basis of status, breeding and stock dogs have a significantly lower incidence than either the experimental or the colony control dogs ($p < .05$).

No sex differences were noted in the incidence of β hemolytic streptococci, nor were differences in incidence observed between the two partially inbred strains of beagles in this colony.

Discussion. The incidence of β hemolytic streptococci is of interest both in terms of its influence in radiation-induced septicemia and in the overall health status of research dogs. Beta hemolytic streptococci have long been recognized as a cause of tonsillitis in dogs (16). Laughton (15) associated group G β hemolytic streptococci with canine clinical disease. Only 5 of 44 healthy dogs she examined harbored group G β hemolytic streptococci, but 54 of 66 strains isolated from clinical disease were group G. They were most often associated with tonsillitis; however, nine strains were isolated from puppies that died soon after birth. We have observed neonatal deaths associated with group G β hemolytic streptococci.[2] Group G β hemolytic streptococci have also been isolated from clinical disease in man (22–25). Clearly, group G β hemolytic streptococci in the throats of the dogs in this colony must be regarded as pathogenic rather than as harmless commensals.

We know of no studies on the epidemiology of β hemolytic streptococci in a closed colony of dogs that would permit direct comparisons with our results. Studies in man (26, 27) indicate that carrier rates are lowest in preschool children and highest in the 5- to 9-year-old group. This is presumed to result from the higher number of contacts with carriers by school children. Presumably some resistance develops with age. One can speculate that higher carrier rates in older dogs may reflect exposure to dogs already harboring the β hemolytic streptococci. The lower incidence in stock dogs compared with control and experimental dogs (Table III) may reflect age differences, because stock dogs are younger than dogs in the other two categories. Alternatively, pups may become infected from the bitch soon after birth and remain infected for life. The similar incidence in young stock dogs and breeding adults lends some support to this hypothesis because many stock dogs are recently weaned additions from the breeding kennel. Currently, we are investigating these possibilities. In man, the seasonal variations in carrier rates show a winter maximum and a summer minimum (14, 26, 28). We did not observe this in our 1-year study. More meaningful comparisons will be possible when we have sampled the same dogs for several years.

Our results indicate that the throat flora of dogs is generally stable, even when different methods are used by different investigators to isolate bacteria and even under the various environmental conditions existing in research kennels. Similar stability was suggested by Clapper and Meade (18) from their experience with 25 dogs. The beagles in our colony carried essentially the same flora as that reported by others (15, 18, 29). Notable differences between our results and those of Clapper and Meade were the absence of Lancefield group A streptococci and the somewhat higher incidence of coliforms in our dogs. They studied an open rather than a closed colony; this may explain the presence of more than one Lancefield group in their dogs. We did not differentiate among the various enteric bacteria which may account for our high incidence of coliforms.

The significance of the high incidence of *Mycoplasma* species in our dogs is not clear.

[2] Unpublished data.

Shoetensack (30–32) first reported the isolation of *Mycoplasma* from the upper respiratory tract of dogs with distemper, and erroneously regarded them as the cause of this disease. Edward and Fitzgerald (33) found a high incidence of *Mycoplasma* species in the throats and vaginas of 39 infertile bitches. The incidence in throats was 74.5%, and the overall incidence from both sites was 90%. A low conception rate has been observed in dogs of one of the partially inbred strains in the Argonne colony (34). While *Mycoplasma* species may contribute to the low conception rate observed in our dogs, this possibility has not been studied.

Norris *et al.* (6) found *Pasteurella pneumotropica* associated with septicemia in dogs given single doses of ^{60}Co radiation. In the present study we included *P. pneumotropica* isolates in the more general category of *P. multocida*. We found these bacteria in the throats of 79.5% of the dogs. Smith (29) also reported a high incidence in the throats of dogs and *P. multocida* has been recovered from dog and cat bites of humans (35, 36). Whether *Pasteurella* species can cause pneumonia in dogs as they do in rodents is not known, but the isolation of *P. pneumotropica* from one case of pneumonia in dogs (37) suggests that possibility.

Summary. Throat cultures from 467 beagles in a closed breeding colony showed a high incidence of *Mycoplasma* species (100%), coliforms (82%), *Pasteurella multocida* (79.5%) and Lancefield group G β hemolytic streptococci (41.8%). The flora was relatively stable over a 1-year period, although younger dogs (1–4 years old) had a consistently lower incidence of β hemolytic streptococci than dogs 4 years old and older. The incidence of other organisms regarded as part of the normal throat flora was similar to that reported in man.

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