

NORMAL FLORA OF THE NOSE, THROAT, AND LOWER INTESTINE OF DOGS

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ABSTRACT

CLAPPER, W. E. (The Lovelace Foundation for Medical Education and Research, Albuquerque, N.M.) AND G. H. MEADE. Normal flora of the nose, throat, and lower intestine of dogs. *J. Bacteriol.* **85**:643-648. 1963.—An attempt was made to isolate and identify the complete normal flora of the rectum, nose, and throat of beagles. For primary isolation, 12 different kinds of media were used. Incubation of blood agar plates and slants anaerobically, and of thioglycolate broth aerobically, allowed the growth of obligate anaerobes. From the rectal specimens, 20 species of bacteria and 10 species of fungi were isolated and identified. The organisms were similar to those found in the human intestine. *Escherichia coli*, *Streptococcus mitis*, enterococci, *S. lactis*, *Bacillus* species, and coliforms other than *E. coli* were most frequently encountered. The frequency of occurrence was approximately the same at both samplings in more commonly cultured bacteria. Pathogenic *E. coli* were isolated from nearly one-third of the first specimens. These were the only human pathogens observed. In the throat cultures, 29 species of bacteria and 2 species of yeasts were identified, and 27 species of bacteria were identified from the nasal cultures. *S. mitis*, *Neisseria*, and coagulase-negative *Staphylococcus* were most often isolated. The flora was similar to that found in human nose and throat cultures, except that more *Haemophilus* and pneumococcus and fewer coliforms are generally found in human throats. Organisms resembling human pathogens were group A streptococci and coagulase-positive staphylococci. These were isolated infrequently. It appears that this kind of examination would reveal any significant changes in normal flora that might be related to the health of the animal.

It is well known that animals exposed to large doses of X irradiation often succumb to an overwhelming septicemia due to organisms commonly

found in the intestinal tract (Benacerraf, 1960), and organs normally sterile in healthy animals are found to be infected with these bacteria when examined at autopsy (Bradner, Bernstein, and McCarthy, 1955). Generalized infection after irradiation has been observed in man (Benacerraf, 1960). An increase in coliform bacilli in the intestines of X-irradiated dogs was reported by Furth, Coulter, and Howland (1952), and an increase in coliforms, accompanied by a decrease in lactobacilli, in the intestines of irradiated rats was observed by Bell, Coneglio, and Hudson (1955).

As an aid in establishing the cause of disease and death in dogs exposed to ionizing radiation, experiments were initiated to identify the bacterial and fungal flora of those areas most likely to contribute causative organisms. Although studies of bacteria found in certain areas of the dog's intestinal tract (Meleney, Berg, and Jobling, 1927; Smith, 1931; Haeren, Dack, and Wilson, 1938; Schweinburg and Sylvester, 1953; Skazaki, Namioka, and Mura, 1956; Bornside and Cohn, 1961) have been made, and large numbers of dogs have been investigated to determine the incidence of specific organisms (Galton, Scatterday, and Hardy, 1952), reports of attempts to isolate all the bacteria and fungi present in the lower intestine, nose, and throat of dogs are either rare or have not been published.

This paper reports the organisms isolated from swabs of the nose, throat, and rectum of 25 healthy dogs, and compares the relative frequency of the various species.

MATERIALS AND METHODS

Specimens: rectal swabs. In collaboration with personnel of the Section of Veterinary Medicine, rectal swabs of 22 beagles were taken during the period from 7 September to 9 October 1961. Of these dogs, 3 were not available for the second study during the period from 9 October to 20 November 1961, but an additional 3 were added to the group, making a total of 25. The swabs

TABLE 1. Media used for the primary isolation of microorganisms from the nose, throat, and intestine of the dog^a

Medium	Use	R ^b	N&T ^c
<i>Aerobic</i>			
Trypticase (BBL) soy blood agar ^d	Total flora	×	×
Phenylethyl alcohol agar.....	Gram-positive cocci	×	×
Tomato-juice agar.....	Lactobacilli, staphylococci, yeasts	×	×
Desoxycholate agar.....	Enteric bacteria	×	×
<i>Salmonella-Shigella</i> agar.....	Enteric pathogens	×	
Selenite broth.....	Enteric pathogens	×	
Chocolate agar.....	Fastidious organisms, <i>Neisseria</i> , <i>Haemophilus</i>		×
Staphylococcus Medium No. 110 (Difco) agar.....	Staphylococci		×
Cystine Trypticase agar.....	Fastidious organisms, <i>Neisseria</i> , <i>Haemophilus</i>		×
<i>Anaerobic</i>			
Trypticase soy blood agar.....	Anaerobes	×	×
Thioglycolate medium.....	Anaerobes	×	×
<i>Fungus</i>			
Sabaraud's dextrose agar.....	Aerobic fungi	×	
Mycosel agar.....	Aerobic fungi, pathogens	×	
Anaerobic blood agar slant.....	<i>Actinomyces</i>	×	×

^a Swabs were placed in Proteose Peptone No. 3 broth, and media were inoculated from this.

^b Rectal swab.

^c Nose and throat swab.

^d Outdated blood-bank blood.

were plastic-enclosed rectal swabs (Falcon) designed to prevent contamination from the outer areas of the anus. These were immediately placed in 2 ml of Proteose Peptone No. 3 (Difco) broth. Gram stains were made from a second specimen on the first eight dogs studied. The media listed in Table 1 were inoculated from the broth. Chocolate and blood agar plates were incubated in 10% CO₂. A blood agar plate and slant incubated anaerobically and thioglycolate broth incubated aerobically were used for the initial isolation of anaerobes. The general scheme employed for primary isolation of all organisms is also presented in Table 1. All colonies on each plate (as many as 25 per specimen) which appeared morphologically different were subcultured to appropriate media to obtain pure cultures. They were identified by Gram stains and by the usual biochemical and serological studies employed in clinical laboratories.

Nose and throat swabs. The tonsillar areas and the anterior nares of 25 dogs were swabbed and the swabs placed in broth. Primary isolation was made in the media listed in Table 1. Individual

colonies were picked from each medium for isolation in pure culture and were then identified by further studies, as indicated above.

RESULTS

The individual species isolated from each area are listed in Table 2. The data are discussed according to the origin of the organisms.

Rectal swabs. Gram stains often revealed a non-culturable spirillum or spirochete, a finding which has been observed by others (Smith, 1931; Craige, 1948).

The percentage of dogs in which the various kinds of bacteria were found is shown in Fig. 1 for the two series of experiments performed. *Escherichia coli* and gram-positive cocci (*Streptococcus mitis*, enterococci, and *S. lactis*) were most prevalent. With the exception of *S. lactis*, there was little difference in the frequency of these organisms in the two samples taken from the same dogs at different times. This is true also for the enteric bacilli: *Paracolobactrum*, *Aerobacter*, *Proteus*, and *Pseudomonas*. Pathogenic *E. coli*, however, were observed in many

more of the dogs during the first period of testing than at the later period. There was a large difference in *Bacillus* species which probably has little significance in relation to the health of the animal and is most likely related to the flora of the environment. *Proteus* organisms were present in approximately one-third of the specimens at both times. *P. mirabilis* was the species most often isolated. Coagulase-negative staphylococci and *Pseudomonas* were seen in only a small percentage and in the same number at both test periods. No coagulase-positive staphylococci were isolated. *Clostridium* and *Lactobacillus* species

were of low prevalence, and both were found less often in the first specimens than in the second. A variety of nonpathogenic fungi were isolated; *Mucor* species were most prevalent, being noted in eight specimens.

Throat swabs. The percentage of dogs in which the various kinds of bacteria were isolated from throat swabs is shown in Fig. 2. *S. mitis* and *Neisseria* species predominated in all of the animals. The next most frequently observed bacteria were the coagulase-negative staphylococci. Coagulase-positive staphylococci were isolated from only two animals, and so were not

TABLE 2. Microorganisms isolated from 25 beagles (listed in order of frequency)

Nose	Throat	Rectum
<i>Staphylococcus</i> , coagulase-negative	<i>Streptococcus mitis</i>	<i>Escherichia coli</i>
<i>Streptococcus mitis</i>	<i>Staphylococcus</i> , coagulase-negative	<i>Streptococcus mitis</i>
<i>Streptococcus lactis</i>	<i>Neisseria flavescens</i>	Enterococci
<i>Neisseria flavescens</i>	<i>Neisseria sicca</i>	<i>Streptococcus lactis</i>
<i>Bacillus</i> sp.	<i>Escherichia coli</i>	<i>Aerobacter aerogenes</i>
<i>Corynebacterium</i> sp.	<i>Streptococcus lactis</i>	<i>Bacillus</i> sp.
<i>Neisseria catarrhalis</i>	<i>Bacillus</i> sp.	<i>Paracolobactrum</i> sp.
<i>Mima polymorpha</i>	<i>Alcaligenes faecalis</i>	Intermediate coliform
Enterococcus	<i>Mima polymorpha</i>	<i>Proteus mirabilis</i>
<i>Pseudomonas aeruginosa</i>	<i>Corynebacterium</i> sp.	<i>Escherichia coli</i> , pathogenic
<i>Aerobacter aerogenes</i>	<i>Neisseria catarrhalis</i>	<i>Escherichia freundii</i>
<i>Neisseria sicca</i>	<i>Pseudomonas aeruginosa</i>	<i>Clostridium perfringens</i>
<i>Lactobacillus</i> sp.	<i>Aerobacter aerogenes</i>	<i>Bacillus subtilis</i>
<i>Clostridium perfringens</i>	<i>Lactobacillus</i> sp.	<i>Staphylococcus</i> , coagulase-negative
<i>Escherichia coli</i>	<i>Streptococcus</i> (β -hemolytic, not group A)	<i>Pseudomonas aeruginosa</i>
<i>Paracolobactrum intermedium</i>	Intermediate coliform	<i>Lactobacillus</i> sp.
<i>Bacillus subtilis</i>	<i>Paracolobactrum intermedium</i>	<i>Proteus vulgaris</i>
<i>Alcaligenes metalcaligenes</i>	<i>Streptococcus</i> , group A	<i>Proteus morgani</i>
<i>Staphylococcus</i> , coagulase-positive	Enterococcus	<i>Pseudomonas</i> sp.
<i>Alcaligenes faecalis</i>	<i>Clostridium perfringens</i>	<i>Staphylococcus</i> , coagulase-positive
Intermediate coliform	<i>Staphylococcus</i> , coagulase-positive	<i>Mucor</i>
<i>Clostridium septicum</i>	<i>Pasteurella multocida</i>	<i>Fusarium</i>
<i>Haemophilus haemolyticus</i>	<i>Achromobacter</i> sp.	<i>Hormodendrum</i>
<i>Paracolobactrum</i> sp.	<i>Paracolobactrum</i> sp.	<i>Diplosporium</i>
	<i>Proteus mirabilis</i>	<i>Geotrichum</i>
	Yeast	<i>Penicillium</i>
	<i>Candida albicans</i>	<i>Cynecephalastrum</i>
		<i>Oospora</i>
		<i>Candida albicans</i>
		Yeast

included in the graph. About 50% of the dogs were noted to have *E. coli* in the throat, with other enteric bacilli seen less frequently; 36% carried β -hemolytic streptococci, nearly one-half of which were group A. *Lactobacillus* and *Corynebacterium* species and enterococci were present, but in small numbers.

This flora is very similar to that which the senior author has observed over a period of 10 years in a clinic diagnostic laboratory where several throat swabs from humans of all ages are cultured daily. The greatest difference was in the lack of *Haemophilus* species and pneumococci in dogs, which are commonly encoun-

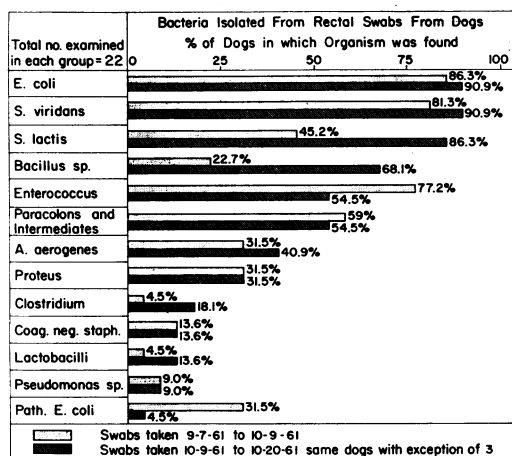


FIG. 1. Bacteria isolated from rectal swabs from dogs.

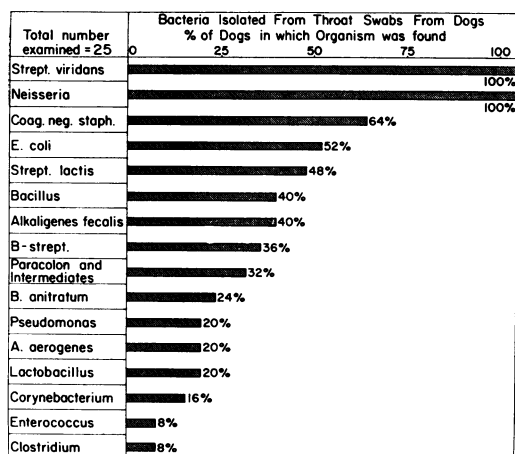


FIG. 2. Bacteria isolated from throat swabs from dogs.

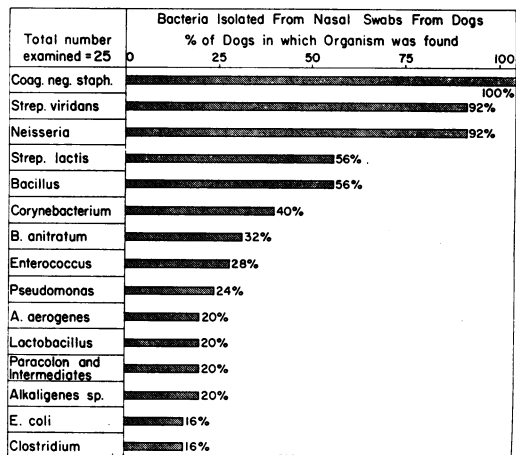


FIG. 3. Bacteria isolated from nasal swabs from dogs.

tered in humans, and the greater number of enteric bacteria found in the dogs. *Proteus* species are not often cultured from throats of humans; they were frequently isolated from the throats of the dogs.

Nasal swabs. Figure 3 shows the results of the study carried out on nasal swabs. The organisms most frequently isolated were the same as those noted in the throat, except that coagulase-negative staphylococci were found in every dog; coagulase-positive staphylococci were not encountered. *Corynebacterium* species were seen frequently. The coliform bacilli were much less in evidence in the nasal swabs than in the throat swabs. β -Hemolytic streptococci and pneumococci were not observed. *B. subtilis* or related organisms were apparent in about one-half of the cultures; this was also true of both throat and rectal swabs.

DISCUSSION

Bacterial counts were not attempted, since it was felt that this would complicate studies involving a large number of animals for an extended period of time to the point of diminishing productivity in results. Furthermore, Smith (1931) abandoned such counts because they varied greatly in the same dog and had no apparent relation to the health of the animal. Since Bornside and Cohn (1961) stated that the most commonly found bacteria were also the most numerous in both control groups and dogs with closed loops in the intestine, it seemed justifiable in the present study to use the percentage occurrence as

a measure of the prevalence of different species or groups. Specimens showing any species in nearly pure culture would be given the same significance as possible causes of disease as they are in a clinical microbiology laboratory. Zubrzycki and Spaulding (1962) recently reported a study in which they found the normal human fecal flora to be remarkably stable. Wide fluctuations occurred only in the number and type of less frequently observed organisms. They believed that a significant variation in the normal flora would affect the health of the individual. A comparison of the results of two specimens taken at different times on the same dogs (Fig. 1) showed little variation in most of the more commonly isolated organisms, with the exception of *S. lactis*. It seems probable that the fecal flora of healthy dogs is also quite stable.

Smith (1931), in her study of the bacterial flora of isolated segments of the small intestine, examined 307 specimens from 40 dogs. No attempt was made to isolate and identify all organisms, as was done in this study, but a comparison of results is of interest. *C. perfringens* was found in 87% of the specimens, and *E. coli* in 85%. Nonhemolytic streptococci (45%) and hemolytic streptococci were next in order. In the present study, *Clostridium* species were noted much less often, perhaps because the sampling was made from the lower intestine.

Haenel and Mueller-Beuthow (1956) examined the fecal flora of several animals including dog and man. Two specimens were cultured from each subject (six times in 4 weeks). The flora of man and dog were observed to be quite similar and to consist of aerobes, anaerobes, coliforms, and enterococci in that quantitative order. Staphylococci were rare. Data reported in the present study confirm the staphylococcal findings, and coliforms, enterococci, and lactobacilli proved to be among the six most frequently cultured organisms.

Mikhlin and Geimberg (1956) reported the fecal flora of dogs to consist chiefly of acidogenic streptococci, coliforms, and lactic acid bacilli, and another group (Skazaki et al., 1956) stated that *E. coli* was the most common organism. In the survey reported in this paper, *E. coli*, *S. mitis*, *S. lactis*, and enterococci were the bacteria isolated most often. Since Zubrzycki and Spaulding (1962) found *Bacteroides* to be the predominating organism in human feces, in spite of coliforms being so considered by others, it might be useful

in future studies to make dilutions as they did, to allow better isolation of these smaller and slower-growing colonies. It would seem doubtful, however, that this would be of importance in evaluating the health of an animal unless they suddenly appeared as the predominating organism of the culture with the usual flora suppressed or absent.

Proteus species, identified in one-third of the specimens examined in the present study, have been isolated by others (Smith, 1931; Bornside and Cohn, 1961; Galton et al., 1952; Gorham, 1949), and *Proteus* is generally considered to be part of the normal flora. Gebert (1953), however, found none in healthy dogs, but did note *P. mirabilis* and *P. morganii* in 60% of those with dysentery.

Although several extensive investigations have shown dogs to be carriers of *Salmonella* (Skazaki et al., 1956; Galton et al., 1952; Wolff, Henderson, and McCallum, 1948), neither *Salmonella* nor *Shigella* were found in the current study. The most interesting finding related to the dog as a carrier of human pathogens (Fig. 1) was the isolation of several pathogenic *E. coli*. Mian (1959) reported that dogs carry pathogenic *E. coli* in their intestines and may be a source of infection to man. Of the 22 dogs first examined here, 7 were carrying these organisms. Five were type O119B14, and two were type O55B5. No further antigen determinations were made. Only one dog was still carrying a pathogenic *E. coli* when examined the second time. This was type O55B5.

Frequent isolation of *Pasteurella multocida* from the tonsils and nose of healthy dogs has been reported (Smith, 1955). It has been occasionally noted in dog bites (Meyer, 1948; Lea and Buan, 1960). β -Hemolytic streptococci have also been cultured from dog tonsils, none of which were human-type strains (Pilot et al., 1936; Laughton, 1948). Mann (1959) reported that 23 of the nasal swabs from 100 dogs yielded coagulase-positive staphylococci. *P. multocida* was found in only two of the throat and one of the nasal swabs in the 25 dogs examined in the present study. Nine throat swabs showed β -hemolytic streptococci, four of which were group A by the bacitracin-disc test (Maxted, 1953). These were not identified serologically. No β -hemolytic streptococci and only two coagulase-positive staphylococci were isolated from the nasal swabs.

It is evident that certain differences will be

found in the intestinal and respiratory flora of dogs, depending upon the methods used for isolation, the manner in which cultures are taken, details relevant to housing, and, perhaps, the contribution of other factors. However, in general, the same organisms have been noted in the various studies reviewed above, and the flora does not seem to differ greatly from that of humans. Therefore, it appears feasible to determine major fluctuations in a variety of experimental situations, including exposure to radiation. To correlate this with the dog's health under various experimental conditions, it will be necessary to conduct similar determinations on a control as well as on the experimental group.

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