APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1977, p. 207-221 Vol. 34, No. 2
 Printed in U.S.A.

Nose, Throat, and Fecal Flora of Beagle Dogs Housed in "Locked" or "Open" Environments

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Received for publication 14 March 1977

The microbial flora of the nose, throat, and feces of male beagle dogs housed in a "locked environment" (i.e. confined to germfree-style isolators and supplied with sterile food, air, and water) or an open environment were assessed between 26 and 30 months into the study. Forty-five genera and 170 different species or types of microorganisms were cultured from the nose, throat, and feces of the beagles. Clostridia, eubacteria, corynebacteria, bacteroides, lactobacilli, and anaerobic, gram-positive cocci accounted for most of the microbial diversity in the flora. Some of the facultative anaerobes, especially streptococci and lactobacilli (in feces), occurred in numbers that were comparable to the most numerous anaerobic species. Confinement to the locked environment resulted in an increased diversity of microorganisms in the flora, but the total microbial counts did not increase to any great extent. Even with the increased diversity of bacteria in the flora of confined dogs, some bacteria seemed to favor certain areas of the gastrointestinal tract over others. The increased diversity of bacteria observed in these confined dogs may pose some infectious disease problems for other mammals (including humans) that may be confined to a locked, ultraclean environment for a prolonged period of time.

The beagle is a valuable research animal and is being used by investigators in a variety of research programs. Its medium size, moderate length of hair, even temperament, ease of handling, excellent disposition, and happy personality are definite assets for research programs (1, 12). There have not been many studies on the microbial flora of the beagle dog (12). Those studies that were carried out did not study the anaerobic bacteria in great detail, and most investigators did not identify as to species the microorganisms they isolated (12).

This study had two purposes: (i) to delineate the aerobic, facultative, and anaerobic bacteria present in the nose, throat, and feces of the beagle dogs; and (ii) to ascertain if confinement to the locked environment for 30 months had any noticeable effect on the qualitative and quantitative composition of the microbial flora of the beagle.

MATERIALS AND METHODS

Animals. Nine purebred male beagles (10 to 12 months old) were obtained from a closed colony at the Argonne National Laboratory, Argonne, Illinois. Seven of the dogs were housed in a "locked environment", in germ-free dog isolators that have been described in detail elsewhere (5, 6). Two of the dogs were housed in regular dog-holding facilities (open cages) at the University of Wisconsin. All dogs were fed a steam-sterilized diet (Purina Dog Meal, Ralston Purina Co., St. Louis, Mo.) and water ad libitum. The dogs were maintained under their designated housing conditions for 30 months. The seven dogs in the locked environment were supplied with sterile air, food, and water throughout their 30-month stay in isolation. All entries into and out of the isolation units were made under sterile conditions. In essence, the locked-environment dogs were treated in a manner that would prevent any exogenous bacteriological contamination. As a control on the integrity of the isolation system, germfree dogs were maintained under similar housing conditions for the 30-month study period (three for 20 months and one for 30 months).

Microbial sampling. During the last 4 months of confinement each dog was sampled twice (control dogs, three times) and an effort was made to identify and quantitate the aerobic, facultative, and anaerobic microorganisms present in the nose, throat, and feces of each isolated and control dog. Dry, sterile cotton swabs in sterile glass tubes that were gassed with CO_2 were used to culture the nose and throat of the isolated and nonisolated dogs. Fresh fecal matter was removed from each dog's rectum with a sterile, stainless steel spatula while the animals were under mild anesthesia with Innovar-Vet (2 ml/30 pounds (about 13.6 kg); Pittman-Moore, Washington Crossing, N.J.). Culture swabs and fecal material were immediately placed into separate tubes of a prereduced transport broth (3). These tubes

were gassed with oxygen-free CO_2 as soon as they came out of the isolators (5 to 15 min). The specimen tubes were sent immediately (after 1 to 3 h) to the anaerobe laboratory and processed in an anaerobic glove box (2, 3) as described below. The transport tubes were put into the anaerobic glove box through a rapid-entry port. Serial 10-fold dilutions were made in prereduced transport broth (3). For swabs, 10^1 to 10^5 dilutions were made, and, for fecal material, 10^1 to 10^9 dilutions were used. Then, 0.1 ml of each dilution was plated onto prereduced A II agar (2, 3) and A II agar with 5% sheep blood. The plates were incubated at 37°C in a Plexiglas incubator especially designed for use in the glove box (4). All plates were held for 5 to 7 days and examined for colonies every 24 h.

The same dilution tubes that were used to inoculate the prereduced A II anaerobic medium were brought out of the glove box, and 0.1 ml of the dilutions was added to a variety of enriched, differential, and selective media that have been described in detail elsewhere (5, 6) to elucidate the qualitative and quantitative aspects of the aerobic and facultative bacteria present in the nose and throat swabs and fecal specimens from the dogs.

Media. A II agar and broth media were prereduced in the glove box for 48 to 72 h before use (2, 3). Transport broth (also used as the dilution medium) was composed of a solution of Trypticase soy broth without glucose but with Na_2CO_3 and cysteine-hydrochloride (2, 3). Tubed transport broth-dilution medium was prepared, sterilized in screw-capped tubes, put into an anaerobic glove box (80% N-10% H_2-10% CO_2) via an airlock and allowed to reduce, with screw caps loose, for at least 48 h before use. After 48 h, the screw caps were replaced with sterile rubber stoppers. For the transport tubes (Kontes; 2.2 by 14 cm), screw caps were used over the rubber stoppers. The enriched, differential, and selective media used to elucidate the aerobic, facultative, and anaerobic bacteria, respectively, detailed elsewhere (5, 6), included Schaedler agar (Difco) and blood agar base (Difco) with 5% defibrinated sheep blood; Mitis-Salivarius agar (Difco); mannitol salt agar (Difco); MacConkey agar (Difco); phenylethyl alcohol agar (Difco) with 5% defibrinated sheep blood; lactobacillus agar (BBL); S.F. agar or enterococcus agar (Difco); kanamycin-vancomycin blood agar; and neomycin blood agar and Mycosel agar (Difco). Aerobic plates were incubated at 37°C for 48 h. Blood and lactobacillus agar plates were incubated in a 10% CO_2 atmosphere at 37°C for 48 h. Anaerobic plates were incubated in GasPak anaerobic jars (BBL) for 72 to 96 h at 37°C. Mycosel agar plates were incubated at 23°C for 48 h.

A quantitative estimate of the aerobic, facultative, and anaerobic bacteria and fungi present in fecal samples was carried out as follows. A weighed amount of fresh feces (usually a 1-g wet weight of the sample) was placed into a tube of transport medium. A 2-ml portion of the 10^1 dilution of each of feces and transport broth (as a control) was dried to estimate the dry weight of the fecal specimen. Bacterial counts in feces are reported as the $\log_{10}$ number of bacteria per gram of fecal material (dry weight). The data on nose and throat swabs are reported as the number ($\log_{10}$) of bacteria per swab.

Identification of microorganisms. (i) Anaerobes. Each morphologically different colony from nose, throat, and fecal specimens found on A II, (or A II agar with 5% blood), Schaedler agar (with 5% sheep blood), kanamycin-vancomycin agar, neomycin agar, phenylethyl alcohol agar (with 5% sheep blood), or blood agar base with 5% sheep blood was Gram stained and subcultured on the same medium used for isolation. Subcultures were incubated in air and under 10% CO_2 to verify the anaerobic nature of the isolate. Facultative bacteria found growing on the anaerobic plates were identified as indicated below. Generally, 50 to 60 different colonial types (anaerobes) were studied from each dog at each sampling.

The strictly anaerobic bacteria were identified by their colonial morphology, Gram reaction, cellular morphology, biochemical tests, and volatile and nonvolatile fatty acid production in PYG broth according to the identification scheme established by the Virginia Polytechnic Institute (VPI) Anaerobe Laboratory (20). Volatile and nonvolatile fatty acids were identified with a gas chromatograph (Dohrman, Anaerobic Bacteriology Analyzer, Mountain View, Calif.). The Minitek procedure (BBL) was also used to assess the biochemical characteristics of the anaerobes (18). The Minitek broth was prereduced in the glove box for 48 h. The broth was inoculated with a loopful of bacteria transferred from an A II agar plate. Other media such as litmus milk, chopped meat-glucose, gelatin, and egg yolk agar were inoculated with bacteria grown on A II agar plates. All biochemical tests were carried out at 37°C in the glove box.

The *Manual of Clinical Microbiology* (23), the eighth edition of *Bergey's Manual of Determinative Bacteriology* (10), and the VPI *Anaerobe Laboratory Manual* (20) were used as guides to identify as to genus and species the anaerobic bacteria. We also used the recent publication of Cato and Johnson (11) in naming the *Bacteroides* species.

(ii) Aerobic and facultative bacteria. Colonies of *Enterobacteriaceae* and other gram-negative bacilli (aerobic and facultative) were recognized by their appearance on selective media. Lactose fermenters and non-lactose fermenters were further characterized by the procedures of Edwards and Ewing (17). *Streptococcus mitis* and *S. salivarius* (referred to in this text as viridans streptococci) and enterococci were identified by their colonial morphology on Mitis-Salivarius and S.F. or enterococcus agar plates and Gram reaction. Beta-hemolytic streptococci were further differentiated by hemolysis on sheep blood agar, Gram reaction, and bacitracin susceptibility. Growth in the presence of high salt concentration and on mannitol salt agar, hemolysis, Gram stain, and coagulase reaction were used to identify *Staphylococcus aureus* and *S. epidermidis*. *Neisseria* spp. were identified by colonial morphology on blood agar, Gram reaction, and the presence of cytochrome oxidase (oxidase differentiation disk; Difco). In general, the procedures and descriptions outlined in the *Manual of Clinical Microbiology* (23) and the

eighth edition of *Bergey's Manual* (10) were used to identify the aerobic and facultative microorganisms that were growing in enriched and selective media. Colonies growing under 10% CO_2 or on anaerobic (GasPak) lactobacillus agar plates that consisted of gram-positive, catalase-negative rods were designated as facultative or anaerobic lactobacilli on the basis of their capacity for growth in the anaerobic glove box. Anaerobic bacteria growing on plates incubated in GasPak jars were identified by the procedures listed above for anaerobic bacteria.

RESULTS

Nose flora. Thirty-seven species of aerobic, facultative, and anaerobic microorganisms were cultured from the nasal swabs of isolated and control dogs. Table 1 shows the aerobic and facultative microorganisms identified. *Staphylococcus epidermidis*, *Streptococcus mitis*, *S. salivarius*, and *Acinetobacter calcoaceticus* subsp. *lwoffi* appeared to be the most prominent (frequency of isolation) aerobic and facultativemicroorganisms in the nares of isolated and control dogs. *Staphylococcus aureus* and *A. calcoaceticus* subsp. *anitratus* were also common to the nares of isolated and control dogs, but they were not as prevalent as *S. epidermidis* or the viridans streptococci and *A. calcoaceticus* subsp. *lwoffi*. It is also obvious from Table 1 that the isolated dogs had a more diverse nasal flora than control dogs. Eighteen species were cultured from the nares of isolated dogs that were not found in the nares of open-environment dogs. *Micrococcus luteus*, *Klebsiella pneumoniae*, six species of corynebacteria, *Nocardia asteroides*, a *Flavobacterium* sp., *Bacillus subtilis*, lactobacilli (three species), and four yeasts were cultured from isolated dogs, but not control dogs, during the last 4 months of the study (Table 1).

The diverse flora of corynebacteria in the nares, primarily of *Corynebacterium renale*, *C. pseudodiphtheriticum*, and *C. aquaticum* (22), is of interest. Diphtheroids are commonly isolated from the nares and throat of dogs (12). The nares of isolated dogs appeared to be a very favorable environment for *C. renale*. Appar-

TABLE 1. *Aerobic and facultatively anaerobic microorganisms cultured from the nares of beagle dogs housed in locked or open environements*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No./swab[b]	No. positive/no. of samples	No./swab[b]
Staphylococcus aureus	7/14	3–5	3/6	3–5
S. epidermidis	13/14	5–6	5/6	4–6
Streptococcus mitis	8/14	5–6	6/6	4–6
S. salivarius	12/14	5–6	6/6	4–6
Acinetobacter species				
A. calcoaceticus subsp. *anitratus*	4/14	4–5	2/6	2–3
A. calcoaceticus subsp. *lwoffi*	12/14	2–5	3/6	2–3
Micrococcus luteus	8/14	4–6	0/6	
Klebsiella pneumoniae	7/14	3–5	0/6	
Corynebacterium species				
C. renale	10/14	4–8	0/6	
C. pseudodiphtheriticum	8/14	6–8	0/6	
C. aquaticum[c]	6/14	5–6	0/6	
C. equi	2/14	4–5	0/6	
C. xerosis	4/14	3–4	0/6	
C. ulcerans	4/14	5–6	0/6	
Nocardia asteroides	4/14	6	0/6	
Flavobacterium sp.	4/14	5–6	0/6	
Bacillus subtilis	4/14	4–5	0/6	
Lactobacillus helveticus	2/14	4–5	0/6	
L. lactis	1/14	5	0/6	
L. crispatus	1/14	4	0/6	
Yeasts				
Rhodotorula sp.	1/14	2	0/6	
Candida krusei	3/14	2–4	0/6	
Geotrichum sp.	1/14	2	0/6	
Trichosporon beigelii	2/14	4	0/6	

[a] Number in parentheses indicates the number of dogs per group.
[b] Log_{10} range of values.
[c] See reference 22.

ently, confinement to a locked environment was conducive for the growth of corynebacteria because they were not isolated from the nares of nonisolated control dogs. *Nocardia asteroides* and yeasts were cultured from the nares of isolated dogs, but not control dogs.

Table 2 demonstrates that anaerobes, although they do not appear to consistently comprise a major part of the nasal flora, did occur in the nares. Eleven different anaerobic species were cultured from isolated dogs, whereas four species were cultured from the open-environment dogs. A *Eubacterium* sp. (unidentified as to species) appeared to be prevalent in the nares of isolated dogs, whereas *Clostridium sporogenes* was isolated three times and *C. perfringens*, *Bifidobacterium infantis*, and *B. adolescentis* were present (isolated twice) in the nares of open-environment dogs. Anaerobes did not appear to be as prominent in nasal flora, at least with respect to the incidence of isolations, as were aerobic and facultative bacteria.

Throat flora. Thirty-six genera and 84 different species (or types) of microorganisms were cultured from throat swabs of isolated and control dogs. The dogs in isolation had more diverse throat flora than the open-environment dogs. The isolated dogs harbored 35 genera (72 species or types of microorganisms), whereas the open-environment dogs had 20 genera (31 species or types) in their throats during the last 4 months of the study. Table 3 shows the 13 genera of aerobic microorganisms that were present in the throats of isolated dogs and the seven genera of aerobic microorganisms that were found in the throats of control dogs. *Micrococcus luteus*, *A. calcoaceticus* subsp. *anitratus* and *A. calcoaceticus* subsp. *lwoffi*, *Pseudomonas aeruginosa*, two *Moraxella* species (*M. lacunata* and *M. nonliquefaciens*), *Branhamella catarrhalis*, and *Candida krusei* were the predominant (>4/14) aerobic species cultured from the throats of isolated dogs. The control dogs had a sparse aerobic throat flora (Table 3) that consisted mainly of *A. calcoaceticus* subsp. *anitratus*, *B. catarrhalis*, and *Neisseria subflava*. *Eikenella corrodens*, *Pseudomonas alcaligenes*, *Haemophilus parainfluenzae*, *B. catarrhalis*, *N. subflava*, *N. flavescens*, and *M. lacunata* were cultured only from the throat swabs. *Neisseria subflava*, present in four of six samples from control dogs, was not isolated from 14 samples of isolated dogs. Conversely, *M. luteus*, *P. aeruginosa*, and *P. alcaligenes* were cultured from the throats of isolated dogs, but not the nonisolated controls.

The eight facultative genera found in the throats of isolated dogs and the six genera cultured from control (not isolated) dogs are shown in Table 4. Citrobacter, enterobacter, and klebsiella were present in the throats of isolated dogs but not in the throats of open-environment dogs. Conversely, *Simonsiella muelleri* was present in the throats of control, but not isolated, dogs (Table 4). The most prevalent facultative anaerobes (present in 50% or more of the samples) in isolated and control dogs were *Staphylococcus epidermidis*, *Corynebacterium*

TABLE 2. *Anaerobic bacteria cultured from the nares of beagle dogs housed in locked or open environments*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No./swab[b]	No. positive/no. of samples	No./swab[b]
Clostridium species				
C. tertium	1/14	4	0/6	
C. putrificum	1/14	5	0/6	
C. oroticum	1/14	4	0/6	
C. sporogenes	0/14		3/6	4–5
C. perfringens	0/14		2/6	3
Bifidobacterium species				
B. infantis	2/14	4–6	2/6	3
B. adolescentis	1/14	4	2/6	2
Eubacterium species				
E. lentum	1/14	4	0/6	
Eubacterium sp. (?)	5/14	5–6	0/6	
Fusobacterium species				
F. nucleatum	2/14	4–5	0/6	
F. symbiosum	1/14	4	0/6	
Peptostreptococcus species				
P. intermedius	1/14	4	0/6	
P. parvulus	1/14	3	0/6	

[a] Number in parentheses indicates the number of dogs per group.
[b] Log_{10} range of values.

TABLE 3. *Aerobic microorganisms cultured from the throats of beagle dogs housed in locked or open environments*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No. swab[b]	No. positive/no. of samples	No./swab[b]
Micrococcus species				
M. luteus	9/14	5-6	0/6	
Acinetobacter species				
A. calcoaceticus subsp. *anitratus*	9/14	4-7	4/6	4-6
A. calcoaceticus subsp. *lwoffi*	7/14	4-5	1/6	2
Branhamella species				
B. catarrhalis	8/14	4-6	4/6	3-4
Pseudomonas species				
P. aeruginosa	5/14	3-5	0/6	
P. alcaligenes	1/14	4	0-6	
Moraxella species				
M. lacunata	4/14	3-5	1/6	5
M. nonliquefaciens	4/14	3-5	1/6	5
Aeromonas species				
A. shigelloides	1/14	6	0/6	
Neisseria species				
N. subflava	0/14		4/6	5
N. flavescens	2/14	4-6	1/6	4
Eikenella species				
E. corrodens	2/14	5-6	2/6	4-5
Haemophilus species				
H. parainfluenzae	2/14	3-4	2/6	2-3
Candida krusei	4/14	2-3	1/6	2
Trichosporon beigelii	2/14	2-3	0/6	
Rhodotorula sp.	1/14	4	0/6	
Torulopsis pintolopesii	1/14	5	0/6	

[a] Number in parentheses indicates the number of dogs per group.
[b] Log_{10} range of values.

pseudodiphtheriticum, *Streptococcus salivarius*, *S. mitis*, *Staphylococcus aureus*, and *Escherichia coli*. The other species of bacteria were, for the most part, isolated less than 50% of the time from the dogs. The latter was particularly noteworthy with the diverse grouping of *Corynebacterium* spp. in the throats of isolated dogs compared with that of the control dogs. *Corynebacterium renale*, prominent in the nares of isolated dogs, was not present in the control dogs. Throats of control dogs did not show the diversity of corynebacteria that was observed in those of the isolated dogs. The diversity of species of streptococci, corynebacteria, lactobacilli, and enterobacteriaceae accounted for the varied flora in the throat of isolated dogs. Particularly noteworthy was the fact that 10 out of 14 throat samples from isolated dogs were positive for *Klebsiella pneumoniae*, whereas, it was not found in any of the six throat samples from the nonisolated dogs. Likewise *Enterobacter agglomerans*, *E. cloacae*, *Citrobacter intermedius*, and *C. freundii* were cultured from isolated, but not from control, dogs.

Anaerobic bacteria were cultured from the throats of both isolated and control dogs (Table 5). Thirteen genera of anaerobes were cultured from isolated dogs, and eight genera were grown from the nonisolated controls (Table 5). Thirty-one anaerobic species (or types) were cultured from the throats of isolated dogs, whereas only 13 anaerobic species (or types) were present in the throats of nonisolated dogs (Table 5).

Bifidobacterium species (*B. infantis* and *B. adolescentis*), *Bacteroides vulgatus*, *B. melaninogenicus* subsp. *asaccharolyticus*, two unidentified clostridia, *Eubacterium parvum*, *Fusobacterium bullosum*, *F. nucleatum*, and *Megasphaera elsdenii* were cultured most frequently from the throats (4 out of 14 samples or better were positive) of isolated dogs. *Clostridium sporogenes*, *Bacteroides distasonis*, *Propionibacterium acnes*, *Fusobacterium russii*, *Peptostreptococcus anaerobius*, and three unidentifiable *Peptococcus* spp. comprised the anaerobes cultured from the throats of nonisolated control dogs. The majority of anaerobic species cultured from the isolated dogs' throats were bacteroides, clostridia, fusobacteria, eubacteria, and bifidobacteria. In contrast to the nonisolated dogs, the isolated dogs had no pro-

TABLE 4. *Facultative anaerobes cultured from throats of dogs housed in locked or open environments*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No./swab[b]	No. positive/no. of samples	No./swab[b]
Streptococcus species				
S. mitis	6/14	4–6	6/6	4–5
S. salivarius	9/14	4–5	6/6	4–5
S. faecium	3/14	5	3/6	3
S. faecalis	2/14	3	0/6	
Beta-hemolytic streptococci (not group A)	2/14	4–6	0/6	
Staphylococcus species				
S. epidermidis	14/14	5–7	6/6	4–6
S. aureus	6/14	5–6	4/6	5–6
Corynebacterium species				
C. aquaticum[c]	2/14	5–6	0/6	
C. pseudodiphtheriticum	14/14	4–5	6/6	4–5
C. xerosis	3/14	5–6	0/6	
C. ulcerans	2/14	4–5	0/6	
C. renale	1/14	5	0/6	
C. equi	2/14	5	0/6	
Corynebacterium sp. (?) no. 1	2/14	4–5	2/6	5–6
Corynebacterium sp. (?) no. 2	2/14	4–5	0/6	
Lactobacillus species				
L. lactis	2/14	4–5	0/6	
L. plantarum	2/14	4–5	0/6	
Lactobacillus sp. (?) no. 3	2/14	4–5	6/6	4–6
Lactobacillus sp. (?) no. 4	2/14	4–5	0/6	
Simonsiella species				
S. muelleri	0/14		2-6	4–6
Klebsiella species				
K. pneumoniae	10/14	4–6	0/6	
Escherichia species				
E. coli	5/14	4–5	4/6	4–5
E. coli (anaerogenic)	2/14	4–5	0/6	
Enterobacter species				
E. cloacae	6/14	3–5	0/6	
E. agglomerans	8/14	5–6	0/6	
Citrobacter species				
C. freundii	4/14	3–4	0/6	
C. intermedius	6/14	3–5	0/6	

[a] Number in parentheses indicates the number of dogs per group.
[b] Log_{10} range of values.
[c] See reference 22.

pionibacteria in their throats. *Lactobacillus catenaforme*, eubacteria, and veillonella, all present in isolated dogs, were not grown from throat swabs of the nonisolated controls. A recently described species of spiral-shaped bacteria, *Anaerobiospirillum succiniciproducens* (14), was also present in the throats of isolated and control dogs.

Fecal samples. Isolated dogs had 34 genera (129 species or types) of microorganisms in their feces, whereas the nonisolated, control dogs had 21 genera (58 species or types) isolated from their stools. Nine aerobic genera, five of bacteria and four of yeasts, were cultured from the feces of isolated dogs. Conversely, the aerobes from feces of nonisolated controls consisted of two genera of bacteria (*Micrococcus* and *Brucella*) and three genera of yeasts (*Candida*, *Trichosporon*, and *Torulopsis*). *M. luteus*, *Candida albicans*, *Trichosporon beigelii*, and *Torulopsis pintolopesii* were the main (four or better positive out of 14 samples) aerobic components of feces from isolated dogs. *Acinetobacter calcoaceticus* (subsp. *anitratus* and subsp. *lwoffi*), *Moraxella nonliquefaciens*, two bacilli (*B. stearothermophilus* and *B. subtilis*), a flavobacterium, *C. krusei*, and a *Rhodotorula* sp., all cultured from isolated dogs, were not cultured from the feces of nonisolated control dogs (Table 6).

The facultative microorganisms present in feces from isolated and nonisolated dogs represented nine genera (37 species or types) and five genera (20 species of types), respectively. The

most prevalent genera in both dog groups were lactobacilli (Table 7). *Klebsiella pneumoniae*, *Enterobacter* species., *Citrobacter freundii*, and *Proteus* species., all present in isolated dogs' feces, were not cultured from the nonisolated control dogs. Both isolated and nonisolated control dogs had streptococci, staphylococci, corynebacteria, lactobacilli, and escherichia. Except for the staphylococci, the isolated dogs had a greater diversity of species within each genus than the nonisolated control dogs. On the basis of frequency of isolation, the isolated dogs' facultative fecal flora consisted primarily of (7 or more positive out of 14 samples) *Streptococcus faecium*, *Staphylococcus epidermidis*, *S. aureus*, *Corynebacterium pseudodiphtheriticum*, *C. xerosis*, *Lactobacillus lactis*, *L. helveticus*, an unidentified *Lactoba-*

TABLE 5. *Anaerobic bacteria cultured from the throat of beagle dogs housed under locked or open environments*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No./swab[b]	No. positive/no. of samples	No./swab[b]
Clostridium species				
C. oroticum	2/14	4–5	0/6	
C. putrificum	2/14	5–6	0/6	
C. sporogenes	0/14		4/6	4–5
Clostridium sp. (?) no. 1 and 2	4/14	5–6	0/6	
Lactobacillus species				
L. catenaforme	2/14	5	0/6	
Bifidobacterium species				
B. infantis	8/14	5–6	0/6	
B. adolescentis	6/14	5–6	0/6	
Propionibacterium species				
P. acnes	0/14		2/6	5–6
Eubacterium species				
E. tenue	2/14	5–6	0/6	
E. lentum	2/14	5–6	0/6	
E. parvum	4/14	4–5	0/6	
Fusobacterium species				
F. bullosum	4/14	4–5	0/6	
F. nucleatum	4/14	4–5	0/6	
F. russii	0/14		2/6	3–4
Fusobacterium sp. (?) no. 1, 2, and 4	2/14	4–5	0/6	
Bacteroides species				
B. corrodens	2/14	4–5	0/6	
B. amylophilus	2/14	4–5	0/6	
B. nodosus	2/14	4–5	0/6	
B. vulgatus	6/14	4–5	0/6	
B. distasonis	4/14	5–6	4/6	5–6
B. ochraceus	2/14	4–5	0/6	
B. melaninogenicus subsp. *asaccharolyticus*	8/14	5–6	6/6	4–5
B. pneumosintes	2/14	4–5	0/6	
Bacteroides sp. (?) no. 2	2/14	4–5	0/6	
B. ruminicola subsp. *ruminicola*	0/14		2/6	4–5
B. ruminicola subsp. *brevis*	0/14		2/6	4–5
Anaerobic gram-positive cocci				
Sarcina ventriculi	2/14	4–5	0/6	
Peptostreptococcus anaerobius	2/14	4–5	2/6	5–6
Ruminococcus flavefaciens	2/14	6	0/6	
Peptococcus species				
Peptococcus sp. (?) no. 4	2/14	4	0/6	
Peptococcus sp. (?) no. 5	0/14		3/6	4–5
Peptococcus sp. (?) no. 7 and 8	0/14		2/6	3–5
Veillonella alcalescens	2/14	5–6	0/6	
Anaerobic gram-negative cocci (not *Veillonella*)	0/14		2/6	4–5
Megasphaera elsdenii	4/14	5–6	0/6	
Anaerobiospirillum succiniciproducens	2/14	5–6	2/6	4–5

[a] Number in parentheses indicates number of dogs per group.
[b] Log_{10} range of values.

TABLE 6. *Aerobic microorganisms cultured from feces of beagle dogs housed in locked or open environments*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No.[b]	No. positive/no. of samples	No.[b]
Micrococcus species				
M. luteus	8/14	6–9	2/6	8–9
Acinetobacter species				
A. calcoaceticus subsp. *anitratus*	2/14	4–6	0/6	
A. calcoaceticus subsp. *lwoffi*	2/14	5	0/6	
Brucella sp.	9/14		2/6	5–7
Bacillus species				
B. stearothermophilus	2/14	7–8	0/6	
B. subtilis	2/14	7–8	0/6	
Flavobacterium species				
Favobacterium sp. (?) no. 1	2/14	5–7	0/6	
Moraxella species				
M. nonliquefaciens	2/14	5–6	0/6	
Fungi				
Candida species				
C. albicans	8/14	3–5	3/6	3–4
C. krusei	2/14	3–4	0/6	
Rhodotorula species				
Rhodotorula sp.	4/14	3–5	0/6	
Trichosporon species				
T. beigelii	8/14	3–5	2/6	3
Torulopsis species				
T. pintolopesii	6/14	3–5	2/6	3

[a] Number in parentheses indicates number of dogs per group.
[b] Log_{10} range of values per gram (dry weight) of feces.

cillus sp. (designated no. 1 in Table 7), *K. pneumoniae*, *E. coli*, and *C. freundii*. Nonisolated control dogs, on the other hand, had a predominant facultative fecal flora (at least three samples out of six positive) of *Streptococcus mitis*, *S. faecium*, *S. faecalis*, *S. epidermidis*, *S. aureus*, unidentified lactobacilli, designated numbers 1 and 2 in Table 7 (number 1 was also present in isolated dogs), and *E. coli*. Minor components of the fecal facultative anaerobes (isolated less than 6 times out of 14 samples) of isolated dogs consisted of *S. faecalis*, *S. salivarius*, *Corynebacterium aquaticum* (22), three other unidentified *Corynebacterium* spp., 13 species of lactobacilli (*L. acidophilus*, *L. cellobiosus*, *L. fermentum*, *L. delbrueckii*, *L. salivarius* subsp. *salivarius*, *L. crispatus*, two varieties of *L. casei*, *L. brevis*, *L. bulgaricus*, and *L. plantarum*), three unidentified lactobacilli, an anaerogenic *E. coli*, *Enterobacter cloacae*, *E. aerogenes*, and two species of *Proteus* (*P. mirabilis* and *P. rettgeri*). Minor components of the nonisolated dogs, less than three isolations from six samples, consisted of *Corynebacterium pseudodiphtheriticum*, *C. xerosis*, and an unidentified *Corynebacterium* sp. (designated no. 2 in Table 7 and similar to *Corynebacterium* sp., no. 2 of the isolated dogs), *Lactobacillus acidophilus*, *L. fermentum*, *L. helveticus*, *L. crispatus*, *L. casei* subsp. *rhamnosus*, *L. bulgaricus*, *L. plantarum*, *L. leichmannii*, and an unidentified *Lactobacillus* sp. (no. 2, Table 7). *Enterobacteriaceae*, except for *E. coli*, even though they were minor components (except for *K. pneumoniae* and *C. freundii*) of the fecal flora, were often present in the feces of isolated dogs but noticeably absent from the feces of control (nonisolated) dogs.

Strict anaerobes were found in greater diversity in the feces of isolated dogs (13 genera or groups, 78 species or types) than in nonisolated controls (10 genera or groups, 31 species or types). Most of the anaerobes were clostridia, bacteroides, eubacteria, peptococci, peptostreptococci, bifidobacteria, and fusobacteria. Veillonellae, present in controls, were not cultured from the feces of the isolated dogs.

Eleven species and 12 different types of clostridia unidentified as to species were cultured from the feces of isolated dogs (Table 8). Conversely, *C. sporogenes* and four types of clostridia unidentified as to species (similar to those found in isolated dogs) were cultured from the feces of nonisolated dogs. The diversity of clostridia and the large number of clostridia that we could not identify as to species indicate that the confined environment enhances the growth of members of this genus in the dogs'

intestinal tracts. The most prevalent (7 or more isolations out of 14 samplings) clostridia in the feces of isolated dogs were *C. putrificum*, *C. perfringens*, and three unidentified clostridia (designated numbers 1, 2, and 6 in Table 8). Minor clostridial components (i.e., fewer than 7 isolations out of 14 samplings) were *C. cochlearium*, *C. tyrobutyricum*, *C. oroticum*, *C. ramosum*, *C. limosum*, *C. sporogenes*, *C. acetobutyricum*, *C. chauvoei*, *C. putrefaciens*, and nine other clostridia that we could not identify as to species by our methodology.

Two species of anaerobic lactobacilli (*L. minutus* and *L. catenaforme*) were cultured from isolated dogs. However, the frequency of isolation was low; two positive cultures for the latter

TABLE 7. *Facultative microorganisms present in feces of beagle dogs housed in locked and open environments*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No.[b]	No. positive/no. of samples	No.[b]
Streptococcus species				
S. mitis	6/14	9–10	6/6	9–10
S. salivarius	6/14	9–10	0/6	
S. faecium	12/14	9–10	4/6	8–10
S. faecalis	6/14	8–10	3/6	8–9
Staphylococcus species				
S. epidermidis	14/14	4–10	6/6	8–9
S. aureus	10/14	4–6	6/6	4–6
Corynebacterium species				
C. aquaticum[c]	6/14	8–10	0/6	
C. pseudodiphtheriticum	8/14	9–10	2/6	8–10
C. xerosis	8/14	8–10	2/6	8–10
Corynebacterium sp. (?) no. 1	4/14	8–9	0/6	
Corynebacterium sp. (?) no. 2	2/14	9–10	2/6	9–10
Corynebacterium sp. (?) no. 3	2/14	8–9	0/6	
Lactobacillus species				
L. acidophilus	2/14	9	2/6	10/11
L. lactis	8/14	9–10	0/6	
L. fermentum	6/14	9–10	2/6	10
L. cellobiosus	2/14	8–9	0/6	
L. casei subsp. *rhamnosus*	4/14	9–10	2/6	10
L. delbrueckii	4/14	9–10	2/6	9–10
L. crispatus	2/14	10–11	2/6	8–9
L. salivarius subsp. *salivarius*	6/14	8–9	0/6	
L. helveticus	10/14	8–10	2/6	7–9
L. brevis	6/14	8–10	0/6	
L. bulgaricus	2/14	10	2/6	9–10
L. plantarum	2/14	5–7	2/6	8–10
L. leichmannii	0/14		2/6	8–10
Lactobacillus sp. (?) no.1	12/14	8–10	3/6	8–10
Lactobacillus sp. (?) no. 2	4/14	8–10	2/6	8–10
Lactobacillus sp. (?) no. 3	3/14	7–9	0/6	
Lactobacillus sp. (?) no. 4	6/14	8–9	0/6	
Klebseilla species				
K. pneumoniae	8/14	4–6	0/6	
Escherichia species				
E. coli	10/14	5–7	4/6	
E. coli (anaerogenic)	4/14	4–6	0/6	
Enterobacter species				
E. cloacae	6/14	5–7	0/6	
E. aerogenes	4/14	4–6	0/6	
Citrobacter species				
C. freundii	14/14	4–6	0/6	
Proteus species				
P. mirabilis	6/14	4–6	0/6	
P. rettgeri	2/14	4–6	0/6	

[a] Number in parentheses indicates number of dogs per group.
[b] Log_{10} range of values per gram (dry weight) of feces.
[c] See reference 22.

TABLE 8. *Anaerobic microorganisms present in feces of beagle dogs housed in a locked or open environment*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No.[b]	No. positive/no. of samples	No.[b]
Clostridium species				
C. cochlearium	2/14	9	0/6	
C. tyrobutyricum	6/14	9–10	0/6	
C. oroticum	4/14	7–8	0/6	
C. ramosum	2/14	8–9	0/6	
C. limosum	2/14	6–8	0/6	
C. putrificum	8/14	8–9	0/6	
C. sporogenes	0/14		4/6	10–11
C. perfringens	7/14	7–9	0/6	
C. acetobutylicum	2/14	7–9	0/6	
C. chauvoei	2/14	8–9	0/6	
C. putrefaciens	2/14	8–9	0/6	
Clostridium sp. (?) no. 1	9/14	8–10	4/6	9–11
Clostridium sp. (?) no. 2	9/14	9–10	0/6	
Clostridium sp. (?) no. 3, 7, and 13	4/14	8–9	0/6	
Clostridium sp. (?) no. 4	5/14	9–10	2/6	8–9
Clostridium sp. (?) no. 5	2/14	8–9	0/6	
Clostridium sp. (?) no. 6	7/14	8–9	0/6	
Clostridium sp. (?) no. 8 and 9	2/14	8–9	0/6	
Clostridium sp. (?) no. 10 and 14	2/14	8–9	2/6	9–10
Lactobacillus species				
L. catenaforme	2/14	10–11	0/6	
L. minutus	2/14	8–9	2/6	8–9
Bifidobacterium species				
B. infantis	7/14	9–10	2/6	10
B. adolescentis	12/14	9–11	2/6	10–11
Eubacterium species				
E. tenue	2/14	7–8	0/6	
E. ruminantium	6/14	9–10	0/6	
E. aerofaciens	2/14	9–10	2/6	9
E. alactolyticum	3/14	9–10	0/6	
E. parvum	4/14	10–11	2/6	10
E. ventriosum	4/14	7–9	0/6	
E. contortum	4/14	8–9	0/6	
E. tortuosum	0/14		2/6	8
Eubacterium sp. (?) no. 1 (lysis)	4/14	8–9	0/6	
Eubacterium sp. (?) no. 2	6/14	9–10	3/6	9
Eubacterium sp. (?) no. 3	8/14	9–11	0/6	
Eubacterium sp. (?) no. 4	6/14	9–10	2/6	9
Eubacterium sp. (?) no. 5	10/14	9–10	3/6	9
Eubacterium sp. (?) no. 6, 7, 8, 12, and 13	2/14	7–9	0/6	
Eubacterium sp. (?) no. 9 and 10	0/14		2/6	7–9
Bacteroides species				
B. ruminicola subsp. *ruminicola*	2/14	10	2/6	9–10
B. ruminocola subsp. *brevis*	2/14	9	0/6	
B. clostridiiformis susbp. *clostridiiformis*	0/14		1/6	9
B. corrodens	8/14	9–10	2/6	8–10
B. amylophilus	6/14	9–10	0/6	
B. nodosus	4/14	9–10	0/6	
B. capillosus	2/14	10	0/6	
B. vulgatus	6/14	10	0/6	
B. distasonis	4/14	10	0/6	
B. fragilis	2/14	10	0/6	
B. ovatus	2/14	10	0/6	
B. ochraceus	2/14	10	0/6	
Bacteroides sp. (?) no. 1 and 4	2/14	10	0/6	
Bacteroides sp. (?) no. 6	0/14		2/6	10

TABLE 8—*Continued*

Microorganism	Locked (7)[a]		Open (2)[a]	
	No. positive/no. of samples	No.[b]	No. positive/no. of samples	No.[b]
Propionibacterium species				
P. jensenii	6/14	8–10	0/6	
P. acidi-propionici	2/14	6–7	0/6	
P. acnes	4/14	9–10	0/6	
Propionibacterium sp. (?) no. 1	2/14	8–9	2/6	8–9
Fusobacterium species				
F. russii	4/14	9	0/6	
F. prausnitzii	2/14	8–9	0/6	
F. glutinosum	0/14		2/6	7–8
Butyrivibrio species				
B. fibrisolvens	2/14	8–9	0/6	
Anaerobic gram-positive cocci				
Peptostreptococcus intermedius	6/14	8–9	4/6	9–10
P. productus	6/14	8–10	4/6	9–10
P. micros	4/14	9–10	0/6	
P. magnus	4/14	8–10	2/6	
Peptococcus prevotii	4/14	9–10	2/6	8–9
P. variabilis	2/14	8–9	0/6	
P. constellatus	0/14		2/6	9–10
P. saccharolyticus	2/14	8–9	0/6	
P. asaccharolyticus	0/14		2/6	9–10
Peptococcus sp. (?) no. 1, 2, 9, and 10	2/14	8–9	0/6	
Peptococcus sp. (?) no. 3	2/14	8–10	2/6	9–10
Ruminococcus albus	2/14	8–9	0/6	
R. flavefaciens	2/14	7–8	0/6	
Anaerobic gram-negative cocci				
Veillonella parvula	0/14		2/6	9–10
Not *Veillonella* sp. (?) no. 1	0/14		1/6	9
Megasphaera elsdenii	6/14	9–10	0/6	

[a] Number in parentheses indicates the number of dogs per group.
[b] Log_{10} range of values per gram (dry weight) of feces.

two lactobacilli (out of 14 samplings) from isolated dogs and only two of six samples from nonisolated controls were positive for *L. minutus*.

Bifidobacteria (*B. infantis* and *B. adolescentis*) were also cultured from the isolated and nonisolated dogs' feces; however, the frequency of their isolation was better with feces from the isolated dogs (Table 8).

Eubacterium species were recovered frequently and in very large numbers from the feces of isolated dogs. Seven species of eubacteria and eleven others that could not be identified as to species were cultured from the feces of isolated dogs (Table 8). Three species of eubacteria (*E. aerofaciens*, *E. parvum*, and *E. tortuosum*) and five nonclassifiable types of eubacteria were present in the feces of the nonisolated dogs. The most prevalent eubacteria present in the feces of isolated dogs could not be identified as to species (designated numbers 3 and 5 in Table 8). *Eubacterium ruminantium* and unidentified *Eubacterium* spp. numbers 2 and 4 were present in 6 out of 14 samplings of feces from isolated dogs. *Eubacterium tenue*, *E. aerofaciens*, *E. alactolyticum*, *E. parvum*, *E. ventriosum*, *E. contortum*, and seven other unidentifiable eubacteria were cultured from two to four out of 14 fecal samples from isolated dogs. The two most prevalent eubacteria cultured from the feces of nonisolated dogs (*Eubacterium* spp. numbers 2 and 6, Table 7) also could not be identified as to species by our methodology. Both of the latter eubacteria were also found in the isolated dogs and, along with *E. aerofaciens*, *E. parvum*, *E. tortuosum*, and three other unidentified types, comprised all of the eubacteria cultured from the control dogs.

Bacteroides spp. were also well represented in the feces of dogs. The isolated dogs had eleven bacteroides species and two other types that could not be identified as to species by our methodology. The nonisolated control dogs had three recognized species and one other *Bacte-*

roides sp. (number 6, Table 7) that were not present in any of the samples of isolated dog feces.

The most prevalent bacteroides in dog feces, which was present in 8 out of 14 fecal samples taken from isolated dogs, was *B. corrodens*. The latter species plus *B. ruminicola* subsp. *ruminicola*, *B. clostridiiformis* subsp. *clostridiiformis* (*Clostridium clostridiiformis*) (21), and an unidentified *Bacteroides* sp. (number 6, Table 8) were the only bacteroides species cultured from nonisolated dogs. *Bacteroides amylophilus*, *B. vulgatus*, *B. nodosus*, and *B. distasonis* were cultured from the feces of isolated dogs four to six times out of 14 samples. The remaining *Bacteroides* species, i.e., *B. ruminicola* subsp. *ruminicola*, *B. ruminicola* subsp. *brevis*, *B. capillosus*, *B. fragilis*, *B. ovatus*, *B. ochraceus*, and the two unclassifiable bacteroides (numbers 1 and 4, Table 8), comprised minor components (2 out of 14 samples were positive) of the isolated dogs' feces.

Three propionibacteria (*P. jensenii*, *P. acidipropionici*, *P. acnes*) and one unclassifiable propionibacterium were cultured from the feces of isolated dogs, whereas only the latter unidentified *Propionibacterium* sp. was cultured from the nonisolated dogs' feces.

Fusobacteria were not very prevalent in the feces of isolated or nonisolated dogs. Two fusobacteria (*F. russii* and *F. prausnitzii*) were present in less than 50% of the samples from isolated dogs, and only *F. glutinosum* was cultured from the feces (two out six samples were positive) of nonisolated dogs.

Anaerobic, gram-positive cocci comprised another major group of anaerobic bacteria present in the feces of isolated and nonisolated dogs.

Four peptostreptococci, *Ruminococcus albus*, and *R. flavefaciens* along with five unidentifiable peptococci comprised the anaerobic, gram-positive cocci in the feces of isolated dogs. Three peptostreptococci (*P. intermedius*, *P. magnus*, and *P. productus*) along with three peptococci (*P. prevotii*, *P. constellatus*, and *P. asaccharolyticus*), and one unclassifiable *Peptococcus* sp. (number 3, Table 8), which was also found in the isolated dogs, comprised the gram-positive cocci present in the feces of the nonisolated control dogs.

Peptostreptococcus micros, *Peptococcus variabilis*, *Peptococcus saccharolyticus*, *Ruminococcus albus*, and *R. flavefaciens* along with unclassifiable *Peptococcus* spp. numbers 1, 2, 9, and 10 were cultured from isolated dogs, but not from the nonisolated controls. Conversely, *Peptococcus constellatus* and *P. asaccharolyticus* were cultured from nonisolated controls, but not from the isolated dogs.

Veillonella parvula and another unidentified gram-negative coccus were cultured from two out of six and one out of six fecal specimens from nonisolated dogs, respectively. Contrary to the nonisolated dogs, veillonella were not isolated from 14 fecal specimens from isolated dogs (Table 8). *Megasphaera elsdenii* was found in feces of 6 of the 14 isolated dogs and not the nonisolated dog feces.

DISCUSSION

This study on the microbial flora of beagles was carried out to identify (and identify as to species where possible) the various aerobic, facultative, and anaerobic bacteria present in their noses, throats and feces and to ascertain if confinement to a locked environment (i.e., no exogenous microbial contamination) for a prolonged time period had any effect on their microbial flora.

Previous studies on the microbial flora (nose, throat, feces) of beagles revealed a diverse grouping of aerobic and facultative bacteria (12, 13). Studies that have attempted to quantitate and identify as to species the microorganisms in the flora of dogs are few in number (5–9, 12, 13, 25, 27, 28) and those studies that have attempted to elucidate the anaerobic bacteria present in the flora of dogs are even fewer (6, 7, 12, 13, 25). The early studies indicated that *E. coli*, viridans streptococci, *C. perfringens*, and bacteroides were the predominant microorganisms in the feces of dogs (12, 13, 27, 28).

This report and other data on the microbial flora of beagles (5, 6, 9, 12, 13) demonstrate that their microbial flora is diverse and is made up of a variety of aerobic, facultative, and anaerobic bacteria, and several genera of yeasts. Our studies (5, 6, 14, 15) also demonstrate that the flora of dogs becomes more complex when they are confined to a locked environment and supplied with sterile food, air, and water. The increased complexity of the flora during confinement is a good indication that there are many microorganisms, in low numbers, in the flora of conventional (nonisolated) dogs. These microorganisms are apparently repressed by microorganisms in the "normal" flora or by some other physiological, biochemical, environmental, or immunological mechanism(s). The increased diversity of the flora under locked-environment conditions also indicates that prolonged confinement may interfere with a very important host defense mechanism, namely, the capacity of a normal microbial flora to inhibit or suppress the growth of potentially pathogenic microorganisms. The reduction of the microbial-inhibiting capacity of the flora could take place through changes in the normal flora itself, or

by an alteration of innate, (i.e., gastric pH, intestinal enzyme secretions, redox potential, nutritional competition, etc.) or acquired immunity (i.e., thymus-dependent T cells or antibody production, especially secretory immunoglobulin A). The alterations in the environments of body cavities that would enhance the growth of microorganisms in the host that is housed in a closed environment are not known. We have, however, demonstrated that confined dogs, compared with nonisolated controls, have lower hemoglobin, packed cell volume (PCV), eosinophils, lymphocytes, and alkaline phosphatase enzyme activity (30). The confined dogs also manifest increased values for bilirubin, serum glutamic oxalacetic transaminase and lactic dehydrogenase enzyme activity, serum protein (total), alpha 2 and beta globulins, and immunoglobulin (30). How these blood cell, serum protein, and serum enzyme changes are related to the alterations in the flora, if at all, are purely speculative at this time.

The increased diversity of the flora in the isolated dogs was manifested by the culture procedures used in this study and by tissue Gram stains and scanning electron microscopy of gastrointestinal tract sections of these same dogs when they were killed and autopsied at the conclusion of this study (15). The latter data indicated that the confined dogs had a gram-positive layer of bacteria in their intestinal tract. Normally a layer of gram-negative bacteria is present over the epithelial cells in the ilea, cecum, and colon of dogs (15) and rodents (26). Davis et al. (15) also observed a diminished population of bacteria in the crypts of Lieberkuhn of isolated dogs, and their culture work on the ilea, cecum, and colon (tissue and contents) again verified that the dogs in the locked environment had a more diverse microbial flora than did the nonisolated control dogs.

"Microbial shock" has been alluded to as a potential hazard for astronauts who would venture into space and be confined to a locked environment for a prolonged period of time (24). It was speculated that under locked-environment conditions, the microbial flora of the astronauts would simplify to one or two microbial species because of the lack of exogenous microbial contamination (24). The microbial shock for the astronauts would supposedly occur if the surviving species in the flora are pathogens or if pathogens colonize the astronaut when he returns to earth or some other contaminated environment. Based on our study of isolated dogs, the danger of microbial shock would come about not because the flora had simplified but because it had increased in diversity. Many of the latter microorganisms showing an increase in numbers under isolated conditions are pathogens or opportunists. One added bit of speculation on space flights could be brought out by the increased incidence of yeasts (especially *C. albicans*) in the locked environment. An increased incidence of *C. albicans* has occurred on space flights and other confinement studies with male astronauts (29, 31). If *C. albicans* increases in numbers in the vaginas of future female astronauts, they could possibly be troubled with candida vaginitis on their long-term space flights.

The nose and throat flora of the dogs in this study contained aerobic, facultative, and anaerobic bacteria. A number of microorganisms found in the noses and throats of these dogs were those that are commonly associated with the fecal flora (i.e., *Enterobacteriaceae*). The presence of *Enterobacteriaceae* in the noses and throats of beagle dogs has been reported by other investigators (9, 12). The major differences in the flora appeared to be associated with confinement to the locked environment and not with coprophagy.

Studies on the human fecal flora have demonstrated that there is a difference in the predominating bacteria from individual to individual (19). A similar situation also was evident in the dogs in this study. Although most groups of bacteria were common to the flora of all dogs, each dog had different microorganisms predominating in his flora, and no two dogs were exactly alike when the most predominant microorganisms in their flora were compared.

Even though there was an increased diversity of bacteria in the flora of isolated dogs, there were still areas and regions of the gastrointestinal tract that were more suited to certain bacteria than were others. Many bacteria found in the feces were not cultured from the nose and throat, and bacteria that were common in the nose and throat were not cultured from the feces. Although we stressed the increased diversity of bacteria that was present in isolated dogs, but not in the nonisolated controls, we also observed several bacteria that were cultured from the nonisolated control dogs, but not from the isolated dogs. An example of the latter was *C. sporogenes*, which was cultured from noses, throats, and feces of nonisolated dogs, but not from the isolated dogs. Apparently, conditions in the isolated dogs had changed and were no longer conducive to large populations of *C. sporogenes*, but they were conducive to a wide variety of other clostridia, as is evident in Table 8.

During this study, we observed (by microscopic examination) many spiral-shaped bacteria in the dilutions of throat swabs and fecal

material. We isolated one of these spiral bacteria, *Anaerobiospirillum succiniciproducens*, and described it as a new genus of bacteria (14). Other spiral-shaped bacteria were observed, but we were not able to culture them. It is obvious that the dogs' flora is even more complex than the data in this report indicate since there are many other microorganisms in the flora that we are still not able to culture and identify.

We used two methods to study the microbial flora, the anaerobic glove box and primarily A II media for isolation (2, 3); the same nose, throat, and fecal samples used in the glove box were also diluted and plated on a variety of enriched, selective, and differential media and incubated in GasPak jars (5, 6). Although we did not make a detailed study of the efficiency of these methods, it was obvious to us that more species of anaerobes were isolated and identified with the glove-box methodology than in the plating of enriched, selective, and differential media and incubating the plates in GasPak jars.

It is also worth pointing out that many studies on rodent cecal contents and human feces indicate that strictly anaerobic bacteria outnumber aerobic and facultative bacteria by 1,000:1 or more. Although the latter situation was true of the aerobic bacteria found in the flora of dogs, it did not hold for many of the facultative bacteria, especially streptococci (*S. mitis* and *S. salivarius*), lactobacilli, and staphylococci. The latter bacteria were very often present at 10^{10} per gram (dry weight) of fecal material. Although differences in experimental design have to be taken into consideration, these data on dog flora indicate that facultative bacteria, in dogs, can and do achieve numbers that we would ordinarily associate with the number of viable anaerobic bacteria in the feces of other mammals and man (19).

ACKNOWLEDGMENTS

This research was carried out with the support of grant NGR50-002-91 from the National Aeronautics and Space Administration.

We thank D. W. P. Norris, Tom Fritz, and C. Poole of the Argonne National Laboratory, Argonne, Ill., for their assistance and advice on this study.

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