

Cecal and Fecal Bacterial Flora of the Mongolian Gerbil and the Chinchilla

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The Mongolian gerbil is being increasingly used as a laboratory animal and as a pet. Both chinchillas and gerbils are used as animal models for otitis media and other otic research. Previously, only incomplete information was available regarding the indigenous bacterial flora of the lower intestinal tracts of these coprophagic animals. Using the strict anaerobic methodology of the Virginia Polytechnic Institute Anaerobe Laboratory, we studied the predominant bacterial flora of the cecum and fecal pellets of the gerbil and the chinchilla and the bacterial flora of digesta pellets in the proximal colon. We found species of the following anaerobic genera in high dilutions of gerbil fecal pellets: *Bifidobacterium*, *Clostridium*, *Propionibacterium*, *Lactobacillus*, and *Bacteroides*. Only lactobacilli were found in high dilutions of digesta from the upper colon, although the cecum yielded *Peptostreptococcus*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Propionibacterium*, and *Bacteroides* species from high dilutions of cecal contents. The facultatively anaerobic and aerobic flora isolated consisted of species of *Bacillus*, *Streptococcus*, *Staphylococcus*, *Acinetobacter*, *Alcaligenes*, *Escherichia*, *Pasteurella*, and *Pseudomonas* plus several unidentifiable organisms. Species of *Bifidobacterium*, *Bacteroides*, *Eubacterium*, and anaerobic *Lactobacillus* were isolated from chinchillas.

The Mongolian gerbil, *Meriones unguiculatus*, has been used considerably as a laboratory animal (1, 18, 19, 24) and as an animal model of disease (2, 4-8, 14). It is also popular as a pet and is widely available to the public in pet stores. To our knowledge, no complete study of the bacterial fecal flora of this animal has been made. The nasopharyngeal and middle ear bacterial floras are known (24). Knowledge of the intestinal flora of this animal is important both from a public health standpoint and for use of the animal as a model of infectious disease. Carriage of the organisms to be studied as infectious agents could confound the results of experimental disease studies. Since the gerbil is coprophagous, intestinal organisms may appear as transients in the indigenous flora of the oral cavity and nasopharynx. The chinchilla and the gerbil are both used extensively as animal models of otitis media (4, 7-9, 14, 16) and other otic diseases (5); thus, it is important to know the components of the flora that are regular transients in the nasopharynx of animals to be used as models of otic infectious diseases.

The fecal flora of rodents studied to date consists largely of lactobacilli with various numbers of *Bifidobacterium*, *Bacteroides*, *Fusobacterium*, *Eubacterium*, *Propionibacterium*, *Peptostreptococcus*, and *Clostridium* organisms and other reported species (3, 10, 11, 17-20, 22, 23). Majumdar and Carroll (18) and Majumdar and Mosher (19) reported the intestinal flora of conventional gerbils to contain anaerobic lactobacilli as the predominant species with clostridia, enterococci, and flavobacteria present but in numbers of 1 to 3 orders of magnitude lower than those of the lactobacilli. Members of the family *Bacteroidaceae* were not reported by these researchers, although anaerobically cultured coliforms were found in high numbers.

The purpose of this investigation was to isolate and identify the predominant bacterial flora of the gerbil by

commonly employed techniques for enteric organisms (15) and the Virginia Polytechnic Institute (VPI) anaerobic techniques (12) for anaerobic intestinal bacteria. A similar, but less extensive, survey of the flora of the chinchilla was also made.

Species of *Lactobacillus* (166 isolates) were the most often isolated anaerobic organisms in this study, followed by species of *Bacteroides* (124 isolates) and *Bifidobacterium* (31 isolates). A total of 90 anaerobic isolates did not fit any species or were nonviable after isolation.

MATERIALS AND METHODS

Animals. Healthy young adult gerbils were obtained from Tumblebrook Farm and maintained as in our earlier work (4, 7, 8). Adult chinchillas were obtained from chinchilla ranches in western North Carolina. All were acclimatized to laboratory rearing in our Animal Resource Center for 3 or more months. These animals were fed NIH 07 diet and water ad libitum. Chinchilla diets were supplemented with 1 raisin per day. A total of 10 gerbils and 2 chinchillas were used in this study.

Specimen collection and dilution. Fecal pellets were collected in clean cages without bedding. Fecal pellets were removed from the cage as soon as they were passed by the animal. Pellets (0.1 g) were emulsified in a 9.9-ml VPI anaerobic dilution blank (12). From this, 1 to 10 serial dilutions were made in 9-ml VPI anaerobic dilution blanks to a final concentration of 10^{-8} g/ml. To collect cecal contents, animals were anesthetized with an intramuscular injection of 87 mg of ketamine per kg mixed with 13 mg of xylazine per kg. Animals were then sacrificed by cervical dislocation. Abdomens were opened, and the cecum was clamped off, removed, and placed in a sterile petri dish. The cecum was opened with sterile scissors, and cecal contents were removed via a large-orifice pipette. Cecal contents (0.1 g) were placed into 9.9-ml VPI dilution blanks. Serial dilutions (1 to 10 ml) were prepared to a final concentration of 10^{-8} g/ml. Pellets of digesta from the upper part of the large bowel were

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removed, weighed, and diluted as above. Each animal was used for only one specimen (including fecal specimens).

Culture techniques. The methods and media of the VPI Anaerobe Laboratory (12) were used throughout the isolation and identification of anaerobic bacteria. For other organisms, commonly employed techniques were used (15). Enteric bacteria were identified by using the API 20E system (Analytab Products), with additional tests as needed.

Inoculation of culture media. For recovery of anaerobic bacteria, 0.1 ml of the 10^{-6} , 10^{-7} , and 10^{-8} g/ml dilutions were spread on VPI BHIS (12) and/or VPI E (12) roll tubes by spreading 0.1 ml of the dilutions onto the agar surface with sterile glass rods. In addition, two fecal specimens were placed into VPI roll tubes (molten agar at 50°C; tubes were then sealed, mixed, and rolled until agar solidified) (12). For recovery of enteric bacteria and other aerobic bacteria, plates of eosin-methylene blue (EMB), MacConkey, Hektoen enteric, and tryptic soy agars were spread with 0.1 ml of 10^{-4} , 10^{-5} , 10^{-6} , and 10^{-7} dilutions by using sterile glass "hockey stick" spreading rods.

All tubes and plates were incubated at 37°C. Aerobic plates were counted at 24 h, and roll tubes were counted at 48 h. Roll tubes were incubated for an additional 72 h for isolation of slower-growing bacteria.

Counts of organisms. Direct microscopic clump counts were made as described in the VPI Anaerobe Laboratory manual (12). Colony counts for roll tubes were made by counting colonies in tubes having 300 or fewer colonies with a stereomicroscope (12). Plates having 300 or fewer colonies were counted on a Quebec colony counter.

Description of colonies and isolation of pure cultures. All colony types were described, and approximately 10% of each of the colony types were picked for isolation of pure cultures. All colonies were picked from roll tubes with 50 or fewer colonies. Inoculum from each picked colony was transferred to chopped meat (CM) broth medium (12) and to an aerobic BHIS sheep blood agar plate (12) to check for aerobic growth. Smears were made for Gram stain examination. Organisms were streaked on the same medium from which they were isolated to obtain pure cultures. Five colonies were picked, smeared, and Gram stained at each reisolation step until all colonies were in pure culture.

RESULTS

Direct microscopic clump counts of anaerobic bacteria from the gerbils ranged from 1.4×10^9 to 5.3×10^{10} , depending upon the sampling location, while those from the chinchillas were 7.6×10^9 to 2.1×10^{10} organisms per g of digesta. The range of culture counts from the gerbils was 1.5×10^7 to 7.8×10^9 colonies per g of digesta, while those from

the chinchillas were 5×10^7 to 2.6×10^9 . These data are found in Table 1. Direct microscopic clump counts were usually 1 order of magnitude higher than the culture counts.

We isolated and characterized a total of 329 anaerobic organisms from the high dilutions of feces and gut contents of gerbils and chinchillas. Table 2 summarizes the gerbil data in terms of the number of animals from which each species was isolated, the number of animals studied, and the number of isolates of that species of bacterium characterized from those animals from which the species was isolated. A similar, but less extensive, study was made of the organisms found in the feces and cecum of two chinchillas. The chinchilla results are tabulated in Table 3. In gerbils, *Lactobacillus* species (121 isolates) were the most often isolated organisms, followed by *Bacteroides* species (78 isolates) and *Bifidobacterium* species (30 isolates). In chinchillas, the most often isolated organisms were *Bacteroides* species (46 isolates), followed by *Lactobacillus* species (45 isolates). Only one culture of *Bifidobacterium* was recovered from one chinchilla.

Table 4 presents the species of organisms isolated aerobically from 2 dilutions of gerbil feces from four animals. *Escherichia coli* was the only organism found in both dilutions in all four animals. *Acinetobacter calcoaceticus*, a pathogen, was found in only one animal. No other pathogens of consequence were detected in this part of the study.

The predominant flora of each sampling site was determined semiquantitatively, in that representative colonies were isolated in numbers reflecting the number of that colony type in each tube or plate; with some specimens, all colonies from the plate or tube were isolated and characterized. The predominant genus in this study was *Lactobacillus*. Lactobacilli were isolated from all animals in all cultured dilutions of fecal pellets. Culture counts of lactobacilli were greater than 10^9 organisms per g of fecal pellet. Other organisms isolated in counts of greater than 10^9 included species of *Bifidobacterium*, *Eubacterium*, *Propionibacterium*, and *Clostridium*, although these organisms were not found in all specimens. Only two animals contained *Bacteroides* organisms in high dilutions of fecal pellets; however, *Bacteroides* organisms were isolated from all specimens from the cecum in counts greater than 10^9 organisms per g of cecal contents. A total of 90 anaerobic isolates either did not grow in media used for characterization or died out before being characterized. Of these, 50 were gram-positive rods and 19 were gram-negative rods. A total of 21 organisms did not grow on first subculture, and cell morphology could not be confirmed.

Examination of cross sections of the proximal colon of gerbils revealed evidence of a colonic separation mecha-

TABLE 1. Direct microscopic clump counts and culture counts of anaerobic bacteria

Animal ^a	Source	DMCC ^b	Culture counts ^c	
			BHIS medium	E medium
Gerbil	Fecal pellets	0.1×10^{10} – 7.4×10^{10}	0.02×10^9 – 3.7×10^9	0.4×10^9 – 7×10^9
	Upper colon	5.3×10^{10}	7.8×10^9	ND
	Cecum	0.6×10^{10} – 2.5×10^{10}	0.3×10^9 – 1.0×10^9	1×10^9 – 7×10^9
Chinchilla	Fecal pellets	2.1×10^{10}	0.9×10^9 – 2.6×10^9	ND
	Cecum	7.6×10^9	0.5×10^8 – 3.9×10^8	6×10^9

^a Data from 10 gerbils and 2 chinchillas, with one specimen from each animal.

^b DMCC, Direct microscopic clump counts.

^c Includes counts from both inoculation techniques. See Materials and Methods. ND, Not done.

TABLE 2. Summary of anaerobic bacteria isolated from Mongolian gerbils

Organism	No. of animals (no. of isolates) ^a				Upper colon, BHIS medium (n = 1)	Numerical order of species ^b
	Feces		Cecum			
	BHIS medium (n = 3) ^c	E medium (n = 4)	BHIS medium (n = 3)	E medium (n = 3)		
Gram-positive cocci						
<i>Peptostreptococcus</i> species		1 (1)				11
Gram-positive rods						
<i>Bifidobacterium adolescentis</i>	1 (1)	1 (2)				9
<i>B. animalis</i>	1 (2)					10
<i>B. bifidum</i>		1 (1)				11
<i>B. breve</i>	1 (1)					11
<i>B. longum</i>	2 (3)					9
<i>B. magnum</i>	2 (5)	2 (6)				6
<i>B. pseudolongum</i>	2 (4)	2 (2)				8
<i>B. suis</i>	1 (1)					11
<i>Bifidobacterium</i> species		1 (1)	1 (1)			10
<i>Clostridium sporogenes</i>		1 (1)				11
<i>Clostridium</i> species				1 (1)		11
<i>Eubacterium combesii</i>		1 (1)				11
<i>Lactobacillus acidophilus</i>	1 (1)					11
<i>L. brevis</i>	2 (5)	2 (3)			1 (5)	5
<i>L. catenaforme</i>		1 (1)				11
<i>L. crispatus</i>	1 (2)					10
<i>L. delbrueckii</i>		1 (1)			1 (2)	9
<i>L. fermentum</i>	3 (10)	3 (20)			1 (16)	1
<i>L. jensenii</i>	1 (1)					11
<i>L. lactis</i>	2 (3)					9
<i>L. leichmannii</i>	1 (1)	2 (2)				9
<i>L. minutus</i>	1 (1)	1 (1)				10
<i>L. plantarum</i>	3 (33)	2 (8)				2
<i>L. rogosae</i>	2 (2)	1 (1)				9
<i>Lactobacillus</i> species	1 (2)					10
<i>Propionibacterium acnes</i>	1 (1)					11
<i>P. avidum</i>	1 (1)					11
<i>P. granulosum</i>	1 (1)					11
Gram-negative rods						
<i>Bacteroides amylophilus</i>	1 (1)					11
<i>B. capillosus</i>		2 (9)	2 (6)	2 (12)		3
<i>B. coagulans</i>		1 (1)		1 (2)		9
<i>B. furcosus</i>		1 (1)				11
<i>B. intermedius</i>		1 (1)				11
<i>B. microfusius</i>				2 (3)		9
<i>B. nodosus</i>		1 (1)		1 (5)		8
<i>B. oralis</i>			1 (2)	1 (4)		8
<i>B. pneumosintes</i>				1 (3)		9
<i>B. splanchnicus</i>				1 (1)		11
<i>B. succinogenes</i>				1 (2)		10
<i>B. uniformis</i>				1 (1)		11
<i>B. ureolyticus</i>		1 (1)				10
<i>B. vulgatus</i>			1 (6)	2 (8)		4
<i>Bacteroides</i> species		1 (4)	1 (2)	1 (1)		7
Unidentified gram-positive rods	[20] ^d	[10]		[2]	[20]	
Unidentified gram-negative rods			[3]	[12]		
Not viable ^e	[5]	[9]		[4]		

^a The first number represents the number of animals from which that species of bacterium was isolated; the second number (in parentheses) is the number of isolates of that species of bacterium characterized from those animals from which the species was isolated. See Table 1 for culture counts.

^b Ranked by quantity of isolates; number 1 was the species most often isolated, number 2 was the species with the next highest number of isolates, etc.

^c Number of animals.

^d Values in brackets represent the number of unidentified isolates from the entire study.

^e Organisms not viable on first transfer; therefore, morphology was not confirmed.

TABLE 3. Summary of anaerobic bacteria isolated from two chinchillas

Organism	No. of animals (no. of isolates) ^a			Numerical order of species ^b
	Feces		Cecum, BHIS medium (n = 1)	
	BHIS medium (n = 1) ^c	E medium (n = 1)		
Gram-positive rods				
<i>Bifidobacterium</i> species		1 (1)		7
<i>Eubacterium alactolyticum</i>		1 (1)		7
<i>Lactobacillus brevis</i>		1 (1)		7
<i>L. catenaforme</i>		1 (1)		7
<i>L. fermentum</i>		1 (8)		3
<i>L. leichmannii</i>		1 (8)		3
<i>L. minutus</i>		1 (6)		4
<i>L. plantarum</i>	1 (3)	1 (16)		1
<i>Lactobacillus</i> species		1 (2)		6
Gram-negative rods				
<i>Bacteroides capillosus</i>			1 (2)	6
<i>B. coagulans</i>		1 (1)		6
<i>B. distasonis</i>	1 (2)		1 (2)	5
<i>B. eggerthii</i>			1 (1)	7
<i>B. furcosus</i>			1 (1)	7
<i>B. multiaacidus</i>		1 (1)		7
<i>B. oralis</i>		1 (6)		4
<i>B. ovatus</i>	1 (6)	1 (1)	1 (5)	2
<i>B. splanchnicus</i>	1 (2)			6
<i>B. thetaiotaomicron</i>	1 (1)	1 (1)	1 (2)	5
<i>B. uniformis</i>	1 (3)		1 (5)	3
<i>B. vulgatus</i>			1 (1)	7
<i>B. zoogloformans</i>		1 (1)	1 (1)	6
<i>Bacteroides</i> species	1 (1)			7
Unidentified gram-positive rods			[2] ^d	
Not viable ^e		[2]	[1]	

^a The first number represents the number of animals from which that species of bacterium was isolated; the second number (in parentheses) is the number of isolates of that species of bacterium characterized from those animals from which the species was isolated. See Table 1 for culture counts.

^b Ranked by quantity of isolates; number 1 was the species most often isolated, number 2 was the species with the next highest number of isolates, etc.

^c Number of animals.

^d Values in brackets represent the number of unidentified isolates from the entire study.

^e Organisms not viable on first transfer; therefore, morphology was not confirmed.

nism. The cecum and colon of two gerbils were removed, sectioned, and studied without fixing or staining. A furrow between two folds was discernible at the mesenteric side of the wall.

DISCUSSION

The intestinal flora of gerbils and chinchillas does not differ greatly from that of other rodents when the genera of the floras are compared; however, there appear to be differences in the species isolated from the various locations sampled and quantitative differences in the numbers of the species found in the various locations (3, 10, 11, 17, 22, 23, 25). For example, Harris and co-workers (11) found *Bacteroides*, *Fusobacterium*, and *Eubacterium* to be the predominant (10^9 to 10^{11} per g) genera in the large intestine of the mouse followed by *Lactobacillus*, *Propionibacterium*, and *Peptostreptococcus* (10^9 per g). This is similar to earlier

reports in the literature, except that in the earlier work, *Clostridium* species were reported. Harris and co-workers (11) did not identify their isolates to the species level, because the biochemical and other characteristics did not allow matching to the characteristics of the species recognized in the mid 1970s. Macy and co-workers (17) described the intestinal flora of rats during a search for cellulolytic bacteria from ceca and intestines of rats. They found species of anaerobic *Lactobacillus* to be the predominant organism, followed by *Eubacterium*, *Bacteroides*, and *Veillonella* species in that order. In comparison, they found counts of *E. coli* 1 to 2 orders of magnitude lower (10^8) than those of lactobacilli (10^9 to 10^{10} per g). Veilleux and Rowland (25) found the composition of the fecal bacteria of rats to be somewhat different from that found in the intestines in the two previous examples. *Bacteroides* species predominated in feces, followed by lactobacilli. There was a significant difference between the frequency of isolations of each group (anaerobes, lactobacilli, streptococci, etc.) from two different laboratory strains of rats.

In our present work, *Lactobacillus* species predominated in the upper large intestine and feces, while *Bacteroides* species predominated in the cecum of gerbils. In chinchillas, the distribution of genera isolated is approximately the same; however, the species identified differ in the various sampling locations between the two animals. Species of *Bifidobacterium* were the third most frequently found organism in the feces of gerbils, yet only one isolate was found in the chinchillas studied. Thus we found that the anaerobic flora of gerbils and chinchillas, while similar, do have differences, especially in the species found in the various sampling sites. There were also 90 isolates that we could not identify or that did not survive in media used to characterize anaerobic bacteria. These organisms may represent hitherto unidentified species.

Majumdar and colleagues (18, 19) studied the gastrointestinal and fecal flora of the Mongolian gerbil. They did not report many of the details of their method of anaerobiosis and media used. Their method of dilution of specimens in sterile water precluded isolation of extremely oxygen-sensitive anaerobes. It is not surprising that they were unable to isolate gram-negative anaerobes. Despite this, the preponderance of their isolates were anaerobic bacteria (or facultatively anaerobic bacteria which could only be isolated under anaerobic conditions), including lactobacilli, clostridia, enterococci, and flavobacteria. They did not identify the isolates obtained to the species level. Our work supports and extends their results.

Mathieu et al. (20) captured 53 wild specimens of *Chinchilla lanigera*. They collected specimens from the nose aperture and anus of each animal and from the penis of each male. Eighteen of the animals were sacrificed for specimens from the trachea, duodenum, cecum, colon, and rectum. Quantitatively, they found the facultatively anaerobic and aerobic bacterial flora of these chinchillas to be quite similar to the floras of other mammals. Similarly, the anaerobic flora of the sites sampled from the intestinal tract yielded organisms found (*Bacteroides*, *Bifidobacterium*, and *Peptococcus*) in similar locations in other animals. These findings are similar to those of L. G. Miller and S. M. Finegold (Bacteriol. Proc., p. 66, 1967) for chinchillas. The few pathogens found (e.g., *Staphylococcus aureus*) were isolated from normal mucosal surfaces. *Listeria grayi* was the species most often isolated in this study and was proved to be nonpathogenic to laboratory mice by interperitoneal inoculations of 5×10^8 cells per animal. Therefore our study

TABLE 4. Aerobic organisms isolated from gerbil feces

Animal	DMCC ^a	Plate counts	Medium	Species isolated at following dilution:	
				10 ⁻⁵ /g	10 ⁻⁶ /g
1		3.5 × 10 ⁶ 6.8 × 10 ⁷	MacConkey EMB	<i>Escherichia coli</i> <i>Acinetobacter calcoaceticus</i> <i>Alcaligenes</i> sp. <i>Pseudomonas</i> sp.	<i>Escherichia coli</i> <i>Acinetobacter calcoaceticus</i> <i>Alcaligenes</i> sp. <i>Bacillus</i> sp.
2	2.7 × 10 ¹⁰	2.9 × 10 ⁶ 1.1 × 10 ⁶	Tryptic soy agar EMB	<i>Escherichia coli</i> <i>Streptococcus faecalis</i> <i>Staphylococcus xylois</i> <i>Bacillus</i> sp.	<i>Escherichia coli</i> <i>Streptococcus faecalis</i> <i>Staphylococcus xylois</i> Coryneform Unidentifiable gram-positive rod
3	1.9 × 10 ⁹	3.0 × 10 ⁵	MacConkey	<i>Escherichia coli</i> <i>Staphylococcus epidermidis</i> <i>Streptococcus</i> sp.	<i>Escherichia coli</i> <i>Staphylococcus epidermidis</i> <i>Bacillus</i> sp.
4		9.7 × 10 ⁷	MacConkey	<i>Escherichia coli</i> <i>Streptococcus faecalis</i> Gram-positive unidentified <i>Pasteurella</i> sp.	<i>Escherichia coli</i> <i>Streptococcus faecalis</i>

^a DMCC, Direct microscopic clump counts.

expands the information on the anaerobic flora of chinchillas, since Mathieu and co-workers (20) did not identify their isolates to the species level.

The relative infrequency of enteric and similar pathogens in gerbils appears to make these animals safe for use as pets and laboratory animals.

Gerbils are thought to be coprophagous. This would explain the finding of intestinal organisms in the nasopharynx of the gerbil by Thompson et al. (24).

Our study and previous studies found anaerobic organisms of the genera *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Eubacterium*, and others in cecal and fecal contents, while anaerobic lactobacilli predominate in the digesta pellets in the upper colon. Holtenius and Björnhag (13) have found that guinea pigs and chinchillas have a colonic separation mechanism similar in function to that of many other herbivorous animals. In these animals, the proximal colon has a longitudinal furrow which runs along the proximal colonic mucosa between two folds at the mesenteric side of the colon wall. We have preliminary morphologic evidence that the Mongolian gerbil also has such morphologic features in the proximal colon. Material moving down the colon has water and bacteria removed and these, along with mucus, are returned to the cecum via a retrograde fluid transport in the furrow. The result is a concentration of fluid, bacteria, and nitrogenous compounds in the cecum. These materials are periodically delivered into the colon surrounded by a proteinaceous membrane and passed through the large intestine as soft cecal pellets or "cecotrophes." In cecotrophic (coprophagous) animals, these cecotrophes are ingested as soon as they are excreted, thus recycling the bacteria and nitrogenous compounds through the stomach and small intestine for digestion and absorption. These animals, therefore, have a bacterial metabolism similar to that of the rumen taking place in the cecum and by various methods (McBee [21]), such as coprophagy, use the bacterial proteins produced in the cecal fermentation. The methods of separating and recycling bacteria and bacterial products vary with the type of herbivore (13, 21). Thus, a difference would be expected in the bacterial flora of the cecal contents and cecotrophes compared with the dry, hard fecal pellets.

We also found a difference in the species and amounts of bacteria isolated on different media. E medium containing ruminal fluid (12) was able to support the isolation of more species of the genera *Propionibacterium*, *Bacteroides*, *Eubacterium*, and *Clostridium* than BHIS medium (12) (Tables 2 and 3). Although more samples were inoculated into BHIS medium than E medium, overall BHIS supported the isolation of 41 species, while E supported the isolation of 51 species. A total of 24 species were isolated on both media, although not necessarily in experiments in which both media were used in parallel, since the samples were plated on either BHIS or E but not both, except for two fecal samples. On the other hand, BHIS supported the isolation of 48 of the unidentified cultures, while E supported the isolation of 39 of the unidentified cultures.

In the two samples in which both "spreader" roll tubes and the conventional roll tubes (made by inoculating molten agar before rolling the tubes) were inoculated in parallel, we found higher counts in the conventional roll tubes. The counts were 0.5 order of magnitude greater from one sample and 1 order of magnitude greater from the other sample. We have made a similar comparison using *E. coli* (unpublished results); however, in that study the spreader technique yielded higher counts than the conventional roll tube technique. We felt the lower counts were due to the temperature of the molten agar and for that reason used the spreader technique in our present study. Most of our culture counts were about 1 order of magnitude below the direct microscopic clump counts by the spreader technique. Such results are not unusual when using media formulated to isolate a broad range of microorganisms from an ecological niche.

In summary, we found species of the following anaerobic genera in high dilutions of gerbil fecal pellets: *Bifidobacterium*, *Clostridium*, *Propionibacterium*, *Lactobacillus*, and *Bacteroides*. Only lactobacilli were found in high dilutions of fecal pellets from the upper colon, although the cecum yielded *Peptostreptococcus*, *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Propionibacterium*, and *Bacteroides* species from high dilutions of cecal contents. The facultatively anaerobic and aerobic flora isolated consisted of species of *Bacillus*, *Streptococcus*, *Staphylococcus*, *Acinetobacter*,

Alcaligenes, *Escherichia*, *Pasteurella*, and *Pseudomonas* plus several unidentified organisms. Species of *Bifidobacterium*, *Bacteroides*, *Eubacterium*, and anaerobic *Lactobacillus* were isolated from chinchillas.

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