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Obligate Anaerobes in Clinical Veterinary Practice

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Clinical specimens obtained from domestic animals were examined to determine the relative prevalence of obligate anaerobic bacteria and the species represented. Of 3,167 samples cultured anaerobically as well as aerobically, 2,234 were bacteriologically positive. Of these positive samples, 583 (26%) contained species of obligate anaerobic bacteria in a total of 641 isolates. Most positive samples contained anaerobes admixed with aerobic species, although 6% of such samples yielded pure cultures of obligate anaerobes. The most common sites from which anaerobes were isolated were abscesses (32% of abscesses cultured contained species of obligate anaerobes), peritoneal exudates (24%), and pleural effusions (20%). *Bacteroides melaninogenicus*, *Bacteroides* spp., *Peptostreptococcus anaerobius*, and *Bacteroides ruminicola* accounted in the aggregate for approximately 50% of all anaerobic isolates. *Bacteroides fragilis* accounted for 1% of all the isolates, and members of the genus *Clostridium* accounted for 8%.

There are numerous reports of the importance of obligate anaerobic species of bacteria in clinical infections in humans (1, 5, 6, 9, 11). There is limited information available concerning anaerobic bacteria and their importance in animals. In 1968, the importance of *Bacteroides melaninogenicus* in clinical veterinary medicine was reported (4). More recent surveys are scarce. In addition to originating from a relatively small sample, the results of these surveys are greatly at odds with those found in human medical practice (2, 3).

For these reasons we report here the results of a survey designed to determine the relative incidence and species of anaerobic bacteria found in clinical specimens obtained from animals.

MATERIALS AND METHODS

Source of specimens. Specimens for culture were obtained from animals brought to the Veterinary Medical Teaching Hospital, University of California, Davis, during a consecutive 35-month period between 1975 and 1978.

Samples generally excluded from anaerobic culture techniques were those obtained from sites possessing a normal flora, i.e., feces, oral cavity, vagina, conjunctivae, and skin and its appendages. Samples of urine were not subjected to anaerobic culture techniques. Samples of the respiratory tract, when obtained from live animals, were collected by transtracheal aspiration.

Cultural techniques. Samples were cultured within 1 h of arrival at the laboratory. Samples were plated on tryptic soy agar containing 5% sheep erythrocytes (BA plates) and on tryptic soy agar containing 5% laked sheep erythrocytes and 0.1 μg of menadione per ml (sBA plates). The latter plates had been stored in an anaerobic environment for at least 24 h before use. The remainder of each sample was placed in a tube of thioglycolate broth.

The BA plates and the thioglycolate broth were incubated at 37°C in an atmosphere of 10% CO_2 and air. The sBA plates were incubated at the same temperature but in an anaerobic jar containing a palladium catalyst. The anaerobic environment was obtained by an evacuation and replacement technique, using an 80% N_2–10% H_2–10% CO_2 gas mixture. The palladium catalysts were never used without first being heated to 160°C for 2 h. Unused heated catalysts were stored in a desiccator containing $CaCO_3$.

All blood plates awaiting inoculation or, if inoculated, waiting to be incubated were stored under flowing O_2-free CO_2.

At 48 h after inoculation, plates incubated anaerobically were examined for bacterial colonies, which, if found, were subcultured to both a BA plate and an sBA plate. The BA plate was incubated in an atmosphere of 10% CO_2 and air at 37°C, and the sBA plate was incubated in an anaerobic environment established as described above.

Obligate anaerobes were inoculated under flowing O_2-free CO_2 into prereduced anaerobically sterilized chopped meat glucose broth (Scott Laboratories, Fiskeville, R.I.) or chopped meat carbohydrate (Carr-Scarborough Microbiologicals, Inc., Atlanta, Ga.). Either broth was incubated at 37°C until maximum growth occurred. Extracts were then prepared and subjected to gas-liquid chromatography. Organisms were Gram stained and then identified to species by established criteria (7). A combination of prereduced anaerobically sterilized media containing various substrates (Scott Laboratories; Carr-Scarborough Microbiologicals, Inc.) or a commercial anaerobe system

(Minitek [BBL Microbiology Systems, Cockeysville, Md.]; API 20A anaerobic system [Analytab Products, Inc., Plainview, N.Y.]) was used for determination of biochemical reactions.

RESULTS

The total number of samples subjected to bacteriological culture techniques during this study was 11,846. Of these, 3,167 were cultured anaerobically as well as aerobically; 2,234 were found to be bacteriologically positive, and anaerobic bacteria were recovered from 583 (26%) of the bacteriologically positive samples.

The findings on all samples cultured anaerobically are shown in Table 1 with respect to clinical diagnosis and species of animal involved. These data are summarized in Table 2 with reference to those conditions having the highest frequency of anaerobic involvement.

Most infectious processes containing an anaerobic bacterium contained an aerobic species as well. The species of aerobic bacterium isolated depended upon the species of animal from which the sample was obtained. For dogs, *Pasteurella multocida* and *Escherichia coli* were the most common; of 181 samples containing a mixture of aerobic and anaerobic species, 39 contained *P. multocida* and 26 contained *E. coli* (22 and 14%, respectively). For cats the most common aerobic species was *P. multocida* (20 of 33 samples, or 61%), for ruminants (bovine, ovine, and caprine) *Corynebacterium pyogenes* was most common (45 of 90, or 50%), and for horses *E. coli* and *Streptococcus zooepidemicus* were most common (32 of 107 and 32 of 107, or 30 and 30%, respectively).

Table 3 lists the genera and species of anaerobic bacteria isolated. Of the 641 isolates, members of the genus *Bacteroides* were isolated in 46% of the cases. The next most frequent isolates belonged to the genus *Peptostreptococcus*. Within the genus *Bacteroides*, *B. melaninogenicus* was the most frequently isolated (of those strains identified to the subspecies level, 91% were *B. melaninogenicus* subsp. *asaccharolyticus*, 7% were *B. melaninogenicus* subsp. *intermedius*, and 2% were *B. melaninogenicus* subsp. *melaninogenicus*), followed by *Bacteroides* spp. and *B. ruminicola*. *Peptostreptococcus anaerobius* was the most frequently isolated species of the genus *Peptostreptococcus*.

DISCUSSION

As in humans, species of obligatory anaerobic bacteria appear to play an important role in

TABLE 1. *Distribution of animal species cultured with respect to disease process*

Disease process	No. of isolates from the following sources:						
	Canine	Equine	Bovine	Feline	Ovine	Porcine	Other[a]
Abscess	394	253	45	46	17	7	55
Respiratory tract	225	352	82	42	69	29	48
Pleural effusions	89	95	11	46	19	1	113
Peritoneal effusions	60	59	7	25	4	1	3
Draining tract	98	38	4	7	1	0	2
Joint	84	75	15	3	2	4	13
Genital	82	22	4	6	2	0	6
Central nervous system	54	15	4	4	6	0	2
Organ (including blood)	195	21	42	16	8	1	8
Osteomyelitis	96	15	4	5	5	1	1

[a] Other refers to the combined frequency of isolation from a variety of animal species, including birds, rabbits, amphibians, reptiles, and goats.

TABLE 2. *Relative frequency of isolation of anaerobic bacteria with respect to disease process*

Rank	Disease process	No. of isolates cultured	No. with anaerobes	No. with aerobes only	% with aerobes only	% with anaerobes
1	Abscess	817	267	439	54	32
2	Peritoneal effusion	159	37	62	39	24
3	Pleural effusion	374	75	138	37	20
4	Draining tract	150	28	92	61	19
5	Organ (including blood)	291	38	116	40	14
6-7	Genital tract	121	16	66	55	13
6-7	Osteomyelitis	127	17	75	62	13
8	Respiratory tract	847	86	575	68	11
9	Central nervous system	85	9	12	14	10
10	Joint	196	10	71	36	6

TABLE 3. *Species of anaerobic bacteria isolated from animals*[a]

	No. of isolates	% of genus	% of total
Bacteroides (total)	293		46
B. amylophilus	5	2	1
B. bivius	3	1	<1
B. capillosus	5	2	1
B. clostridiiformis	7	2	1
B. corrodens	2	1	<1
B. disiens	3	1	<1
B. distasonis	1	<1	<1
B. fragilis	9	3	1
B. furcosus	2	1	<1
B. melaninogenicus[b]	123	42	19
B. oralis	1	<1	<1
B. pneumosintes	3	1	<1
B. putredinis	2	1	<1
B. ruminicola subsp. *brevis*	26	9	4
B. ruminicola subsp. *ruminicola*	12	4	2
Bacteroides spp.	84	29	13
B. succinogenes	3	1	<1
B. thetaiotaomicron	1	<1	<1
B. vulgatus	1	<1	<1
Fusobacterium (total)	36		6
F. aquatile	1	3	<1
F. glutinosum	1	3	<1
F. gonidiaformans	10	28	2
F. naviforme	3	8	<1
F. necrophorum	9	25	1
F. nucleatum	9	25	1
Fusobacterium spp.	3	8	<1
Clostridium (total)	49		8
C. bifermentans	8	16	1
C. difficile	1	2	<1
C. felsineum	1	2	<1
C. hastiforme	2	4	<1
C. perfringens	14	29	2
C. putrefaciens	1	2	<1
C. septicum	1	2	<1
C. sordellii	2	4	<1
C. sporogenes	5	10	1
Clostridium spp.	14	29	2
Eubacterium (total)	19		3
E. lentum	8	42	1
Eubacterium spp.	5	26	1
E. tenue	6	32	1
Propionibacterium (total)	31		5
P. acnes	24	77	4
Propionibacterium spp.	7	23	1
Actinomyces (total)	36		6
A. bovis	1	3	<1
A. odontolyticus	8	22	1
Actinomyces spp.[c]	27	75	4
Peptostreptococcus (total)	97		15
P. anaerobius	79	81	12
P. intermedius	3	3	<1
P. micros	8	8	1
P. parvulus	4	4	1
P. productus	1	1	<1
Peptostreptococcus spp.	2	2	<1
Peptococcus (total)	8		2
P. indolicus	1	13	<1
P. magnus	5	63	1
Peptococcus spp.	2	25	<1
Lactobacillus spp. (total)	7		1
Leptotrichia buccalis	3		<1
Selenomonas ruminantium	1		<1
Selenomonas sputigena	1		<1
Succinivibrio dextrinosolvens	1		<1
Unidentifiable (total)	59		9
Negative rod	26	44	4
Positive rod	10	17	2
Positive coccus	23	39	4

[a] The total number of isolates was 641. See Table 1 for explanation.
[b] Combination of all subspecies.
[c] *Actinomyces viscosus* is not included.

clinical veterinary medicine. However, the incidence we have observed (26% of bacteriologically positive samples) is somewhat lower than that reported previously for animals (2, 3). This may be due in part to the large proportion of samples in our survey that were obtained from the respiratory tract and joints; the respiratory tract was found to rank number 8 in frequency with respect to the isolation of anaerobic bacteria, and with joints the primary diagnosis for the majority of our cases was immune mediated arthritis, with bacteriological work-up as part of the routine handling of such cases.

Our data disagree markedly with those of other recent reports for animals with respect to genera involved. The data presented here are comparable to those reported for humans; i.e., the most frequent genus found was *Bacteroides* rather than *Clostridium* as in the other recent reports dealing with animals (2, 3). Approximately 50% of all isolates were (in decreasing order of frequency) *B. melaninogenicus*, *Bacteroides* spp., *P. anaerobius*, and *B. ruminicola*. *P. anaerobius* was found almost exclusively in canine and feline species. The discrepancies may be due in part to the smaller sample size used

and therefore perhaps more selective nature of the specimens on which previous reports were based and in part to differences in the relative representation of various animal species, organs, and disease processes as contributions to the sample. We do not see sufficient differences in methodology to account for them, nor do we, at this time, believe that geographical factors supply a reasonable explanation.

Of significance is the virtual absence of *Bacteroides fragilis* as a frequently isolated species. This finding is particularly significant in relation to the choice of treatment. *B. fragilis* isolated from humans is almost always resistant to attainable levels of penicillin (5, 10). In animals virtually all anaerobic species are susceptible to attainable levels of this antibiotic (8).

The majority of the infectious processes containing an anaerobic component were found to contain an aerobic one as well. This is an important consideration in selection of an antimicrobic therapy, especially if members of the family *Enterobacteriaceae* are involved.

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