The Incidence of Clostridia in the Canine Stomach and Their Relationship to Acute Gastric Dilatation

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The gastric contents of 100 healthy dogs and of nine spontaneous cases of acute gastric dilatation (AGD) were examined post mortem for the presence of clostridia. Clostridium perfringens *was shown to be a normal canine gastric inhabitant. Those isolated from normal dogs were nontoxigenic and were composed of a variety of serologic strains. No other clostridia could be consistently isolated from the stomach contents of normal dogs. A large assortment of viable anaerobes is normally present in the canine stomach.*

Nontoxigenic C. perfringens *organisms also were isolated from six of the nine cases of AGD. No other clostridia could be demonstrated, and mouse-lethal toxin was not found in gastric contents of AGD cases. A gram-positive flora predominated in the majority of the AGD cases. The type and incidence of clostridia in AGD cases did not differ from those found in healthy dogs.*

Introduction

Acute gastric dilatation (AGD) is a sudden and often terminal gastrointestinal disorder which affects many animal species. In dogs it occurs most commonly in the larger breeds. Previous studies suggest a multifactorial pathogenesis in which bacterial fermentation is the source of gastric gas.[18] *Clostridium perfringens*, an anaerobic bacterium noted for its vigorous gas production, has been given particular attention because it was seen in higher numbers and was recovered at necropsy more frequently from AGD cases than from control dogs.[18]

This study was undertaken to further explore the role of gastric clostridia in AGD. Our goals were to (1) identify and determine the incidence of gastric clostridia in 100 healthy dogs, (2) determine the toxigenicity, toxin type, and/or serologic strains of the *C perfringens* organisms that isolated, (3) compare the clostridia from healthy dogs with those found in AGD cases, and (4) seek mouse-lethal *C perfringens* toxins in gastric contents of AGD.

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Materials and Methods

Animals

A total of 100 normal dogs of varying breeds, plus nine cases of spontaneous AGD, were obtained from several sources including pounds, veterinarians, and necropsy accessions. Each of the 100 normal dogs was free of obvious gastrointestinal disorders, was at least two months of age, and had been dead for no longer than 12 hours. Live animals donated to the Department of Pathobiology constituted 32% of those studied. These were killed by intravenous pentobarbital and were sampled immediately.

After opening the abdominal cavity, the surface of the stomach was seared along the greater curvature midway between the cardia and pylorus, and an aseptic longitudinal incision (approximately 15 cm) was made through the seared surface. Stomach contents were sampled by inserting a sterile 100 ml plastic container, scooping the contents, and scraping the mucosa.

Microbiological Examination

The stomach contents were immediately inoculated onto freshly prepared blood agar (5% bovine-supplemented brain heart infusion agar base),[a] clostrisel agar,[b] tryptose-sulfite-neomycin (TSN) agar,[b] and chopped meat medium.[b] Approximately 0.1 ml of contents was used for each inoculum. These media were incubated anaerobically (anaerobic jars and Gaspak[b]) at 37 C and were observed periodically for 72 hours. Samples of the stomach contents also were smeared on glass slides, air dried, stained by Gram's method, and examined microscopically for morphologic types and relative abundance.

Representative colonies from each medium were observed for morphology and stained by Gram's method. Isolates consisting of gram-positive rods were transferred to peptone-yeast (PY) broth.[19,a] Identification was preliminarily based on reactions in litmus milk,[a] gelatin (11%),[a] starch,[a] PY-glucose broth,[a] Ellner's sporulation medium,[5] and egg-yolk agar (modified).[10] Short, nonmotile, sporulating gram-positive rods which hydrolyzed gelatin and starch, acidified glucose, produced lecithinase, and usually gave a stormy fermentation of litmus milk were classified as *C. perfringens*. Hemolytic action on blood agar was variable. Cultures consisting of gram-positive rods which gave varying reactions from those listed above were subjected to further diagnostic tests including the analysis of fermentation endproducts using gas-liquid chromatography.[12,19]

Serology was performed on cells grown anaerobically for 18 hours in chopped meat[b] glucose medium (CMCM). Cells were harvested by centrifugation at 278 g for 15 minutes. The sediment was washed in phosphate-buffered saline solution (PBSS), resuspended in approximately 12 ml of PBSS, and centrifuged at 278 g for 15 minutes. This was repeated twice. Antisera to 13 Hobbs types of *C. perfringens* Type A[c] were used in slide agglutination tests.

Toxicity testing was performed using cell-free supernatant fluid (obtained by centrifugation at 12,350 g for 15 minutes at 4 C) from 6- and 24-hour cultures of *C. perfringens* grown in CMGM or CM-starch medium. Toxicity was determined by injecting 0.5 ml into the peritoneal cavity of each of two white mice (approximately 25 gm each). Several samples which were nonlethal by this method were treated with 1% trypsin,[d] and/or administered in increasing amounts up to 2.0 ml per mouse.

Twenty-eight cultures identified as *C. perfringens*, from both control and AGD cases, which were not lethal to mice and did not agglutinate using any of the Hobbs-type antisera were sent to the Center for Disease Control (CDC), Atlanta, Georgia for further serotyping.

Table 1

Predominant Microorganisms in Gram-Stained Gastric Contents

	Gram-Positive						
	Rods	Cocci	Rods and Cocci	Total	Gram-Negative Rods	Mixed[a]	Spirochetes or Yeasts
Healthy dogs	11	16	12	39	25	31	5
Cases of AGD[b]	3	3	1	7	1	0	0

[a]Mixed gram-positive and gram-negative organisms.
[b]Smears of gastric contents not available for Case 1.

Table 2

Predominant Microorganisms in Primary Culture of Gastric Contents

	Gram-Positive						
	Rods	Cocci	Rods and Cocci	Total	Gram-Negative Rods	Mixed[a]	No Growth on Primary Culture
Healthy dogs	27	14	9	50	11	37	2
Cases of AGD[b]	3	2	1	6	2	0	0

[a]Mixed gram-positive and gram-negative organisms.
[b]Data not available for Case 1.

The testing of stomach contents for lethal toxins was performed on several spontaneous cases of AGD after death, using previously described methods.[14] In some instances, the samples were concentrated to 1/10 of their original volume by pervaporation and treated with 1% trypsin.

Results

Findings in 100 Healthy Dogs

Gram-negative organisms predominated in 25%, while gram-positive organisms predominated in 39% of the samples. In 31% there was an equal distribution of gram-positive and gram-negative forms, while yeasts and spirochetes were dominant in the remaining 5% of the smears [Table 1].

Aerobic cultivation on blood agar revealed a similar heterogeneous assortment of microorganisms. As in the Gram-stained smears, no single group of microorganisms predominated. Gram-negative organisms were most numerous in 11% of the cases, gram-positive types in 50% of the cases, while equal numbers occurred in 37% of the samples. Primary isolation on blood agar was negative in 2% of the cases [Table 2].

Clostridia were isolated from the stomach contents of 72 of the 100 normal dogs [Table 3]. The majority were recovered from primary isolation on the three types of solid media employed. From the 72 clostridial isolates, 70 were identified as *C. perfringens*. *Clostridium bifermentans* was identified in two cases, and two unidentified *Clostridium* spp. were isolated from cases which also yielded *C. perfringens* [Table 4].

Toxins from all cultures of *C. perfringens* were nonlethal to laboratory mice [Table 4]. Seventeen of the *C. perfringens* isolates gave positive agglutination to Hobbs-type antisera. The strain distribution was variable [Table 5]. The isolates processed by CDC all failed to agglutinate with any of the 91 antisera against known strains of *C. perfringens*.

Findings in AGD

Nine cases of AGD were studied. Of these, seven died, and only Case 1 had been treated with antibiotics prior to death. Five of these cases (2, 5, 6, 7, 9) were either found dead or died before any treatment could be attempted. Case 8 had received a simethacone-containing antacid[e] several hours before death occurred. Two additional cases (3 and 4) were submitted during the early stages of bloat, and upon request of the owners were subjected to euthanasia without treatment. A summary of the nine cases of AGD is presented in Table 6.

Table 3

Incidence of *Clostridium* spp. in Gastric Contents

	Healthy Dogs	Cases of AGD
Total number of cases examined	100	9
Total number of cases that yielded clostridia	72	6
Frequency of clostridial isolation	72%	67%

Table 4

Clostridial Species Present in Canine Gastric Contents

	Healthy Dogs	Cases of AGD
Total number of clostridial isolates	74	6
Clostridium perfringens	70	6
Nontoxigenic strains	70	6
Hobbs-types	17	2
Other species	4	0
Clostridium bifermentans	2	
Unidentified *Clostridium* spp.	2	

Table 5

Distribution of Hobbs Types of *Clostridium perfringens*

	Hobbs Types												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Healthy dogs[a]	1	2	1	6	1	2	0	1	0	2	1	0	0
Cases of AGD[a]	0	0	0	0	0	0	0	0	0	2	0	0	0

[a]Total number of positive strains.

Gram-stained smears of gas-producing stomach contents from eight of the nine cases indicated that gram-positive organisms predominated in seven of these samples. As listed in Table 1, there was great variability in the type of gram-positive organisms present. Gram-negative organisms occurred in very low numbers in each case with the exception of Case 9, where they represented the predominant type. In each case of AGD there was

Table 6

Data From the Nine Cases of AGD

Case	Breed	Interval Death to Necropsy (hours)	Predominant Organism(s) in Smears	Clostridial Isolation
1	Mixed	15	Smears not available	*C. perfringens*
2	German shepherd	12	Filamentous gram-positive rods with gram-positive cocci	*C. perfringens*
3	St. Bernard	EB[a]	Filamentous gram-positive rods	*C. perfringens*
4	Irish setter	EB[a]	Gram-positive cocci with short gram-positive rods	*C. perfringens*
5	Great Dane	9	Gram-positive cocci with slender gram-positive rods	*C. perfringens*
6	Great Dane	8	Gram-positive cocci with gram-positive rods	None
7	German shepherd	9	Gram-negative rods	None
8	Great Dane	12	Short gram-positive rods	*C. perfringens*
9	Irish setter	5	Gram-negative rods with gram-positive cocci	None

[a]EB = euthanized while bloating.

good correlation between organisms seen in Gram-stained smears and those which were obtained from primary isolation on anaerobic blood agar plates [Table 2].

Clostridia were isolated from six of the nine cases of AGD. Each was identified as *C. perfringens* [Tables 3 and 4]. Each isolate was nonlethal to laboratory mice, and two isolates agglutinated Hobbs-type antisera. Four isolates gave no positive reactions to any of the 91 serologic strains of *C. perfringens*.

The stomach contents of Cases 4, 5, and 6 were examined for the presence of lethal *C. perfringens* toxins. None could be demonstrated.

Discussion

100 Normal Dogs

After viewing smears and cultures of gastric contents it was obvious that the normal canine stomach is rarely void of microorganisms. This was true even in those stomachs which contained no ingesta and which were sampled immediately after death [Tables 7 and 8]. The presence of microorganisms was not related to the breed and/or weight of the animal [Table 9]. A large number of these gastric microorganisms were viable anaerobes.

As is indicated in Table 1, a variety of organisms including gram-positive rods and cocci, gram-negative rods and cocci, yeasts, and spirochetes were observed in smears of fresh stomach contents. It appears that the distribution of these microorganisms is quite variable and that no single morphological group predominated in the majority of samples. This variability may result from differences in the diet, amount of ingesta, gastric pH, gastric motility, interval between death and sampling, and other factors.[1,4,6,8,11,15]

The presence of clostridia in the stomach contents of 72 of the 100 normal dogs and the identification of 70 of these isolates as *C. perfringens* suggest that this organism is a normal canine gastric inhabitant. Consistent demonstration of this bacterium in the stomach contents of a

Table 7

Frequency of Clostridial Isolation in Relation to the Presence of Ingesta in the Stomach[a]

	Solid Ingesta with or Without Fluids	Fluids Only
Clostridia isolated[b]	35	37
Clostridia not isolated[c]	14	14
Frequency of clostridial isolation	72%	73%

[a]This table applies only to the control sample of 100 dogs.
[b]Total number of positive cases.
[c]Total number of negative cases.

variety of breeds and in stomachs which were void of ingesta and sampled immediately after death substantiates this. Several other investigators also have isolated *C. perfringens* from the stomachs of healthy dogs.[1,3,17,19] The ratio of clostridial organisms to the rest of the flora was highly variable. There were normal dogs in which clostridia represented the predominant microorganism, and there were cases which required repeated isolation attempts to demonstrate them.

The failure to consistently isolate clostridial species other than *C. perfringens* from the normal canine stomach suggests that other species are unable to colonize the canine stomach, that they are present in very low numbers, or else that the sampling techniques were not suitable to demonstrate them. Either of the first two possibilities is favored, since many fastidious clostridia were isolated and identified during preliminary studies with the media employed.

Results from the serology and toxicity testing of the *C. perfringens* isolates from normal dogs suggest that they are composed of an assortment of serologic and nontoxigenic strains. Although it is conceivable that some isolates might have lost their toxigenicity during subculture and storage, it does not seem likely that this would have occurred in each case.

Table 8

The Frequency of Clostridial Isolation in Relation to the Time Interval between Death of the Animal and Necropsy[a]

	Immediate Necropsy	Necropsy Delay (hours)			
		Less than 2	Less than 4	Less than 8	Greater than 8
Clostridia isolated[b]	22	16	11	21	2
Clostridia not isolated[c]	10	6	4	6	2
Frequency of clostridial isolation	69%	73%	73%	78%	50%

[a]This table applies only to the 100 normal dogs.
[b]Total number of positive cases.
[c]Total number of negative cases.

Table 9

The Frequency of Clostridial Isolation in Relation to the Weight of the Animal[a]

	Weight Classes (lb)				
	Less than 15	Less than 25	Less than 35	Less than 50	Greater than 50
Clostridia isolated[b]	9	14	18	25	6
Clostridia not isolated[c]	5	6	7	7	3
Frequency of clostridial isolation	64%	70%	72%	78%	67%

[a]This table applies only to the control sample of 100 dogs.
[b]Total number of positive cases.
[c]Total number of negative cases.

AGD Cases

Viable anaerobic microorganisms were seen and recovered from each of the nine spontaneous cases of AGD which were studied. A gram-positive predominance was noted in smears and cultures of the majority of these cases. As in the control sample there was great variation in the type of gram-positive organism which predominated. The dominance of gram-positive organisms in the stomach contents of AGD cases is consistent with earlier findings.[18] Gram-positive rods resembling clostridia were seen in each case which yielded *C. perfringens*, although they were not present in predominating numbers. Those cases that were subjected to euthanasia during the early stages of bloat and necropsied immediately thereafter yielded low concentrations of short gram-positive rods as compared with filamentous and coccoid forms. The variability observed in these cases may be a reflection of the very large volume of gastric contents present in AGD cases as compared with the normal dogs. It should be emphasized that each sample taken from AGD cases was actively producing gas at the time of culturing. It seems reasonable that if microbial fermentation was the source of this gas, then the causative organism(s) should have been consistently observed in either the smears, inoculated media, or both. Thus the variability encountered is hard to understand.

The incidence and types of clostridia isolated from AGD cases were similar to those obtained from the control sample of 100 dogs. Those clostridia identified as *C. perfringens* from both control and AGD cases were nontoxigenic and were composed of an assortment of serologic strains. There was a higher percentage of gram-positive predominance in AGD cases; however, this study did not reveal a difference in the number of clostridia seen in smears from AGD cases versus controls. Quantitative counts would be helpful in understanding the fermentative potential of clostridia in AGD.

Etiology of AGD

The visible evolution of gas in gastric contents of AGD cases, the flammable nature of this gas, and significant increases in gastric CO_2 and hydrogen in natural and experimental AGD cases suggest that AGD results from bacterial fermentation.[18] Isolation of gas-producing clostridia, experimental production of AGD with heat-treated stomach contents in gastric-ligated dogs, and the demonstration of *C. perfringens* in the majority of bloat cases suggested *C. perfringens* as an etiologic agent.[18] Further inference is obtained by comparing AGD to other gastrointestinal conditions caused by this microorganism.[7,14,16] Features common to AGD and other clostridial gastrointestinal disorders include dietary indiscretion, sudden onset of signs, and the accumulation of large volumes of gas. Factors which stimulate sudden proliferation and/or metabolic activity of *C. perfringens* in the gastrointestinal tract are not well understood, although it has been shown that such increases may result from dietary changes or the feeding of high protein or carbohydrate diets.[2,9,13]

This study demonstrated that clostridia are present in the dog's stomach in 72% of healthy animals and in 67% of AGD cases. Perhaps additional or alternative methods would have found them present in all dogs. As in overeating disease of sheep, an equal incidence of clostridia in healthy animals does not preclude their possible role as pathogens. However, the failure to demonstrate clostridia in three cases of AGD and the observation that smears are not predominated by typical clostridial rods disagree with earlier findings and disturb the hypothesis that *C. perfringens* alone might be responsible for AGD. Lactobacilli, peptostreptococci, sarcinae, and yeasts also are part of the canine gastric flora and have gas-producing capability. Quantitative bacterial counts and gas-liquid chromatographic analyses of contents for bacterial by-products should resolve the questions regarding the source of CO_2 and hydrogen in AGD.

[a] Difco Laboratories, Detroit, MI.
[b] Baltimore Biological Laboratories, Cockeysville, MD.
[c] Center for Disease Control, Atlanta, GA.
[d] Fisher Scientific Co., Pittsburgh, PA.
[e] Mylanta, Stuart Pharmaceuticals, Wilmington, DE.

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