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Acute Gastric Dilatation in Monkeys: A Microbiologic Study of Gastric Contents, Blood and Feed[1]

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Summary | Twenty-one of 24 simian primates with acute gastric dilatation had *Clostridium perfringens* in their gastric contents. Only 2 of 18 normal animals contained this organism in their gastric contents. *Clostridium perfringens* was isolated from monkey biscuits taken from the cages of five affected animals and from five of 11 incoming lots of feed. After these biscuits were fed to normal animals, this organism could be isolated from the gastric contents. There were no other organisms isolated which could account for the voluminous gas production in this condition.

Key Words | Stomach dilatation — *Clostridium perfringens* — *Macaca* — *Papio*

Acute gastric dilatation or bloat in simian primates was reported in 1967 and associated with excessive intake of both monkey biscuits and water (**1**). The isolation of *Clostridium perfringens* and the demonstration of toxin in the colon contents in a case of acute bloat in *Macaca arctoides* was reported in 1971 (**2**). In 1978, 23 cases of bloat were reported in a 10-year review and *C perfringens* was isolated from the stomach of one animal (**3**). A study done in 1974 postulated that acute gastric dilatation was multifactorial in origin in all species and was dependent on the presence of stress, fermentable substrate and fermentable flora (**4**).

Materials and Methods

In the past 3 years, 34 cases of acute gastric dilatation in four species of Old World primates were diagnosed in the authors' facilities. Twenty-six of these were examined bacteriologically in an attempt to document the gastric flora in monkeys suffering from bloat. These cases involved nine *Papio* sp, two *M fasicularis*, 14 *M mulatta* and one *M arctoides*. Fourteen were breeding adults, eight were juveniles, two were adults with diabetes and two were young adults.

The majority of these animals were fed monkey biscuits[3] once daily, supplemented with oranges, apples and a vitamin-mineral sandwich once weekly. Water was available *ad libitum* from an automated system. The two diabetic animals were supplied monkey biscuits and fruit on an *ad libitum* basis. Six of the rhesus monkeys were fed the same commercial diet supplemented weekly with sweet potatoes.

Review of each case indicated that the animals involved had no disruption in their routine schedule, and the prior use of anesthetics or tranquillizers were not associated with the occurrence of bloat.

Necropsy: Necropsies were performed on all animals that died. Samples of selected organs were fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

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[3]Monkey Chow®, Jumbo #5037. Ralston Purina Co, St Louis, MO.

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Microbiological examination: Gastric contents and blood were collected for bacteriological examination from 26 cases of acute gastric dilatation. The samples were taken as soon as possible after death of the animals with the exception of three which were obtained antemortem.

Gastric contents of 18 animals (10 *M mulatta*, six *Papio* sp, one *M fasicularis* and one *M arctoides*) were tested for the presence of *C perfringens* after an overnight fast. The animals were sedated,[4] and the samples collected through a nasogastric tube.[5] In those cases where no gastric contents could be obtained, the stomach was lavaged with sterile saline. Fourteen of the 18 fasted animals (seven *M mulatta*, five *Papio* sp, one *M fasicularis* and one *M arctoides*) were fed monkey diet known to contain high numbers of *C perfringens*, and 4–5 hours after feeding, gastric contents were obtained again.

A Gram-stain was performed on all the samples, (gastric contents, blood and crushed biscuits) in order to determine the relative numbers of clostridial-like organisms present (**5**).

After the initial Gram-stain was performed, the gastric contents were streaked onto two blood agar plates, one EMB agar plate and one egg yolk agar plate. One blood agar and the egg yolk agar plate were incubated anaerobically, the others were incubated aerobically. At the same time, two 10-ml tubes of thioglycollate broth were inoculated with 2 ml each of the gastric contents. One tube was heated at 80°C for 20 minutes to kill the vegetative cells, after which both tubes were incubated at 37°C for 24 hours (**6**). After 24 hours, the tubes were subcultured onto a blood agar and an egg yolk agar plate and incubated anaerobically at 37°C for 24 hours. The agar plates were examined and each type of colony was identified using a commercially available system.[6] Colonies suspicious of *C perfringens* were isolated and confirmed morphologically and biochemically (**7**).[7] The isolates positive for *C perfringens* were tested serologically by the Nagler Reaction using Type A antitoxin (**6**).[8]

Blood was collected from the saphenous vein of two antemortem cases and directly from the heart in the 14 postmortem cases. Five milliliters of blood were inoculated into each of two 50 ml blood culture bottles containing thioglycollate broth.[9] One was kept anaerobic, the other was vented asceptically to make it aerobic. Both bottles were incubated at 37°C for 24 hours, after which the bottles were checked for turbidity and a Gram-stain performed. Bottles positive for growth were subcultured on blood and egg yolk agar and incubated anaerobically. After incubation, colonies suspicious of *C perfringens* were confirmed morphologically and biochemically as described above.

Monkey biscuits taken from the feeders of five animals that died of acute gastric dilatation were examined for contamination with *C perfringens*. Fresh biscuit samples from 11 different lots of monkey diet also were tested for *C perfringens* to determine the presence of that organism in the feed at the time of arrival at our facilities (**7**).

All statistical associations were computed using the Chi Square technique.

Results

Pathology: The only consistent gross abnormality was severe distention of the stomach and small intestines with gas and large volumes of a light-brownish fluid. This fluid had the consistency of water. The wall of the stomach and small intestine were thinner than normal, and in two animals the stomach had ruptured prior to death.

One monkey, which was examined within 1 hour following death, exhibited severe, subcutaneous emphysema. The emphysema was present in muscles as well, but was most prominent in the subcutaneous tissues of the limbs, axillary and inguinal regions. There was no evidence of a ruptured stomach in this case.

The liver, kidney, adrenal, spleen, abdominal lymph nodes, stomach, small intestine, large intestine, lung and myocardium were examined microscopically. There was focal congestion in the liver. The kidneys were congested and showed signs of acute tubular necrosis. There was lymphoid and erythrocyte depletion in the spleen. The adrenal cortex showed extensive congestion and hemorrhages in the zona reticularis and part of the zona fasicularis. The stomach revealed a marked decrease in thickness of the gastric wall which was consistent with extreme dilatation of the stomach. Similar changes were evident in sections of the small intestine.

[4]Ketamine Hydrochloride®, Veterinary Products, Bristol Laboratories, Division of Bristol Meyers Co, Syracuse, NY.
[5]K31 Feeding Tube. Pharmaseal, Toa Alta, PR.
[6]API 20E®. Analytab Products, Plainview, NY.
[7]API 20A®. Analytab Products, Plainview, NY.
[8]Clostridial Diagnostic Serum. Wellcome Reagents Limited, Beckenham, England.
[9]Difco Blood Culture Bottles. Difco Laboratories, Detroit, MI.

Microbiology: Table 1 shows the occurrence of *C perfringens* in the gastric contents, blood and monkey biscuits taken from the cages of affected animals. The two animals that sustained gastric rupture were submitted only for blood culture. Of the 24 gastric samples from monkeys with acute gastric dilatation, 21 (88%) were positive for *C perfringens*. Thirteen were serologically identified as Type A, while the remaining samples could not be classified due to unavailability of the specific typing serum. Fourteen of the samples (58%) revealed *C perfringens* in either large numbers or in pure culture, while seven samples (29%) showed the presence of the organism in small numbers. In three samples (13%), *C perfringens* could not be isolated.

Table 1

Prevalence of *Clostridium perfringens* in gastric contents, blood and monkey biscuits

Estimated numbers of *Clostridium perfringens*	Gastric contents	Blood	Biscuits[a]
In pure culture	1	2	1
In large numbers	13	7	1
In small numbers	7	1	3
None	3	6	0
Total	24	16	5

[a]Biscuits taken from the cages of affected animals

Sixteen blood specimens were available for examination and nine (56%) revealed *C perfringens* in either large numbers or as a pure culture. One sample showed a small number of the organism and six (38%) were negative. Two blood samples were obtained prior to the animals' death and of these, one revealed a pure culture of *C perfringens*, Type A and one was negative.

Biscuits were taken from the cages of five affected animals for bacteriological evaluation, and all were positive for *C perfringens*, Type A. Two contained a pure culture or a large number of the organism, while three revealed a small number of the clostridia.

Table 2 depicts the other organisms isolated in affected animals. In the gastric contents, nonhemolytic streptococcal organisms were found in 19 (79%). Seven (29%) samples showed large numbers of streptococcal organisms. *Escherichia coli* and other Gram-negative bacteria were in 10 (42%) of the gastric samples. Only one (4%) sample had coliforms in large numbers. Other clostridial species were found in four (17%) of the samples with one showing large numbers. Five samples contained staphylococcal organisms and were attributed to improper sampling technique.

Table 2

Organisms isolated from the gastric contents and blood

Organism isolated	Gastric contents[a] Number	%	Blood[b] Number	%
Clostridium perfringens	21	88%	10	63%
Streptococcus sp	19	79%	10	63%
Escherichia coli and other Gram-negative bacilli	10	42%	2	13%
Other *Clostridium* sp	4	17%	1	6%
Staphylococcus sp	5	21%	1	6%
Lactobacillus sp	1	5%	0	0%

[a]Total number of gastric samples examined = 24
[b]Total number of blood samples examined = 16

In the blood samples examined, 10 (63%) contained nonhemolytic streptococcal organisms. Three (19%) of the total samples had large numbers of these organisms. *Escherichia coli* and other Gram-negative organisms were found in two (13%) of the blood samples and in both cases were in small numbers. Staphylococcal organisms were found in one (6%) sample and was correlated with poor sampling technique.

In two (13%) of the blood and four (18%) of the gastric samples, *Streptococcus* sp was the predominant organism. None of the other organisms isolated were ever predominant in any of the blood samples.

A random sampling of incoming feed lots was performed, and of the 11 lot samples tested, five (45%) were positive for *C perfringens*. Four were confirmed as Type A, while one could not be typed due to the unavailability of the antiserum. Of these, four were characterized as showing light growth and one as heavy.

The organisms isolated from the stomach contents from the 18 fasted normal animals are listed in Table 3. In two (11%) of the animals, *C perfringens*, Type A was found in the gastric contents. The predominant organisms isolated were *Streptococcus* sp which were found in 14 (78%) of the animals. *Lactobacillus* sp were found in four (22%) of the samples.

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Table 3

Organisms isolated from fasted animals

Organism isolated	Gastric contents[a] Number	%
Clostridium perfringens, Type A	2	11%
Streptococcus sp	14	78%
Peptococcus sp	1	6%
Bacillus sp	2	11%
Corynebacterium sp	2	11%
Lactobacillus sp	4	22%
Bacteroides sp	1	6%
Other *Clostridium* sp	1	6%

[a]Total number of gastric samples examined = 18

Gastric contents taken from these animals after feeding biscuits containing *C perfringens* revealed the presence of *C perfringens*, Type A in all samples. Ten (71%) samples had heavy growth, and four revealed slight to moderate growth. In all samples with heavy growth, the gas production was so abundant that it forced the feed out of the broth. Three of the fourteen animals in this study developed grossly distended abdomens classified as cases of early bloat. Four other animals became ill and vomited the contaminated food. *Streptococcus* sp were found in 12 (86%) of the animals in this group.

Discussion

In all cases of bloat thus far reported, the outstanding feature has been the presence of voluminous quantities of gas distending the stomach. This phenomenon raises two questions: (1) why is it that these animals seemingly cannot vomit to relieve the pressure; and (2) what is the source of this gas. The presence of large quantities of gas seems to require a fermentable substrate and the presence of flora capable of fermentation (**4**). The substrate is readily available in the form of commercial monkey diets.

The gastric contents of 21 of 24 (88%) animals with acute gastric dilatation contained *C perfringens*, and *Streptococcus* sp was present in 19 (79%) of the animals. In 18 normal, fasted monkeys, low numbers of *C perfringens*, Type A were found in two (11%) of the animals sampled, while *Streptococcus* sp were found in the gastric contents of 14 (78%) of these animals. Thus, *C perfringens* was the only organism isolated whose presence was significantly ($p>0.01$) increased in animals with acute gastric dilatation, and it was also the only gas-producing organism found in significant numbers. This suggests that *C perfringens* is responsible for the gas distention of the stomach in this syndrome.

Although the results of many blood cultures paralleled those of gastric contents, the fact that other typical acute gastric dilatation cases had negative blood cultures tends to exclude a clostridial septicemia as a major factor in this syndrome.

Since the normal empty stomach is devoid of flora with the exception of a few lactobacilli and enterococci, a possible source of these clostridia was sought (**8,9**). Based on findings in man incriminating large numbers of foodborne clostridial organisms with disease outbreaks, the food left in the feeder was examined (**10**). Since all five feeder samples were positive, the incoming shipments of feed were examined and 45% were positive for *C perfringens*. The number of organisms in the feed did not reach the number reported necessary to cause clostridial enterotoxemia in man, but when contaminated biscuits were fed to animals with known stomach flora, *C perfringens*, Type A was isolated from all animals, and three showed clinical signs of early bloat (**10**).

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