Development of a technique for the in vivo assessment of flatulence in dogs

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Objective—To develop a noninvasive method for the in vivo assessment of flatulence in dogs.

Animals—8 adult dogs.

Procedure—Rectal gases were collected via a perforated tube held close to each dog's anus and attached to a monitoring pump fitted with a sensor that recorded hydrogen sulfide concentrations every 20 seconds. Patterns of flatulence were monitored for 14 hours after feeding on 4 days, and within- and between-dog variation was assessed over 4 hours on 4 consecutive days. Rate of hydrogen sulfide production (flatulence index) and frequency and number of emissions were evaluated as potential indicators of flatus characteristics. An odor judge assigned an odor rating to each flatulence episode, and the relationship between that rating and hydrogen sulfide concentration was determined.

Results—Flatulence patterns varied within and between dogs. Variation was most pronounced for flatulence index; mean coefficients of variance within dogs over time and between dogs on each day were 75 and 103%, respectively. Flatus with hydrogen sulfide concentrations > 1 parts per million could be detected by the odor judge, and severity of malodor was highly correlated with hydrogen sulfide concentration. Odor ratings were accurately predicted by use of the equation 1.51 × hydrogen sulfide concentration$^{0.28}$.

Conclusions and Clinical Relevance—The technique described in this report appears to provide sensitive, reliable, and relevant data and will enable further studies of the factors that influence flatulence in dogs. Use of this technique also has the potential to aid in investigations of colonic physiology and pathology. (*Am J Vet Res* 2001;62:1014–1019).

The passage of rectal gas, otherwise known as flatulence, is a frequent everyday occurrence in humans that generally causes no distress or discomfort. Some people, however, do complain of excessive flatulence associated with bloating and variable degrees of pain. Although flatulence in dogs is often a source of humor and occasional scatological interest, little is known of the nature and physiologic origins of odoriferous rectal gases in dogs. Dogs were used in several early studies investigating the role of intestinal bacteria in the production of nonodorous rectal gases.[1,2]

Studies of flatulence in humans have focused on the volume and composition of flatus,[3-5] along with factors that influence the frequency[6] and chemical character of emissions.[7,8] Rectal gases are derived from air that is swallowed, gases that diffuse from the blood, and gas produced as a result of bacterial metabolism and nonbacterial reactions. The major gases in flatus are the atmospheric gases nitrogen and oxygen plus the nonatmospheric gases carbon dioxide, hydrogen, and methane, which are also referred to as fermentation gases. Malodor is generally attributed to sulfur gases, chiefly hydrogen sulfide, which is produced by bacteria that use sulfate during oxidative reactions.[9] The sulfur for these reactions is derived from mucin and dietary sources, which include cruciferous vegetables (eg, cabbage, broccoli), nuts, and sulfates used during the production of beer and bread.

The purpose of the study reported here was to develop a method for the in vivo assessment of flatulence in dogs. The technique incorporated a monitoring pump with a hydrogen sulfide detector to provide real-time measurements of individual episodes of flatulence. Variation in production of rectal gas was assessed, and data processing techniques were used to improve sensitivity by reducing intra- and interdog variability. A predictive model was developed to enable the relative noxiousness of each emission to be determined from the concentration of hydrogen sulfide measured on-line.

Materials and Methods

Animals—8 healthy adult dogs (4 Labrador Retrievers [1 castrated male and 3 spayed females], 1 Golden Retriever [spayed female], 1 German Munsterlander (castrated male), and 2 Mastiffs [1 castrated male and 1 spayed female]) were used for this study. Dogs were housed individually and fed a commercially available complete canned food[a] (experiment 1) or premium complete dry food[b] (experiment 2) once a day at 8:30 AM during and for 3 weeks prior to each experiment. Water was provided ad libitum.

Collection and analysis of rectal gases—Real-time analysis of rectal gases was performed, using a sulfur gas-monitoring pump fitted with a hydrogen sulfide sensor.[c] Gases were sampled from the anal region by means of a 20-cm loop of rigid plastic tubing (bore size, 4.8 mm) with 4 holes pierced at 90° angles to one another around the circumference and spaced at 1-cm intervals along the length. The loop was formed by connecting the 2 ends of the tubing to 2 of the ports on a 3-way tube connector; the third port was connected to the monitoring pump via rigid tubing

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(outer diameter, 10 mm; bore size, 7 mm). The monitoring pump was fitted into a dog jacket that was secured by straps at the cranial and ventral aspects of the thorax, allowing the pump to be carried over the shoulders **(Fig 1)**. The sampling tubing was fitted to each dog by passing the dog's tail through the loop and was held in proximity to the anus by virtue of its connection to the monitoring pump. Paper disposable underpants were placed on each dog to protect the sampling device from external interference and help maintain proximity of the tubing to the anus.

Monitoring pumps were set to measure hydrogen sulfide concentrations in **parts per million (ppm)** at 20-second intervals, and data were downloaded to a personal computer at the end of each sampling period, using the manufacturer's software.[d] This software allowed direct integration of the data into a spreadsheet. We chose to measure concentration of hydrogen sulfide, as it is the most abundant sulfur gas in human flatus[7,8] and the strongest correlate of odor intensity.[8] The hydrogen sulfide sensor we used, however, does detect other sulfur gases with various degrees of cross-reactivity, specifically methyl mercaptan (40% cross-reactivity) and ethyl mercaptan (15%). This cross-reactivity is additive, such that if 100 ppm of hydrogen sulfide and 100 ppm of ethyl mercaptan were present, the pump would register 115 ppm (100 ppm of hydrogen sulfide and 15 ppm of ethyl mercaptan). Although methyl mercaptan (also known as methanethiol) is present in human flatus, we did not believe that cross-reactivity with this gas would have a significant impact on results, because it is generally present in low amounts (one fifth of the concentration of hydrogen sulfide)[7,8] and does not correlate with odor intensity.[8]

Experiment 1—The first experiment was designed to determine flatus production after feeding and intra- and interdog variation in flatulence. Daily flatus production was monitored in 2 Labrador Retrievers (1 castrated male and 1 spayed female) for 14 hours on 4 days over a 10-day period. The sampling devices were fitted immediately after feeding the daily ration and removed at 12:30 and 4:30 PM for brief periods to allow the dogs to urinate and defecate. Within- and between-dog variation was studied in detail, using 1 male and 3 female Labrador Retrievers and 1 female Golden Retriever. Rectal gas production was monitored for 4 hours in these dogs on 4 consecutive days. The sampling devices were placed 3 hours after feeding (ie, at 11:30 AM) and removed at 3:30 PM. This protocol was subsequently repeated in 2 of the Labrador Retrievers approximately 4 weeks later.

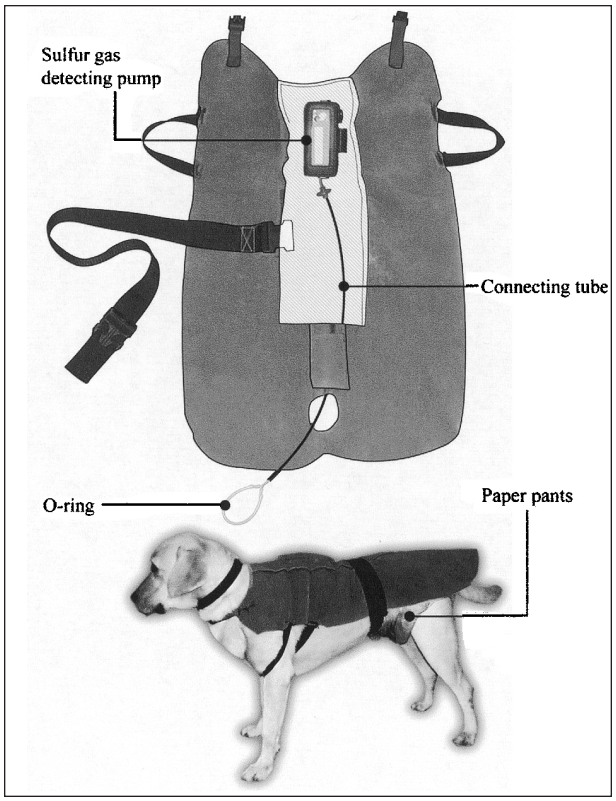

Figure 1—Schematic representation of the device used to measure hydrogen sulfide concentration in rectal gas of dogs.

Three measures of rectal gas production were evaluated: flatulence index, **mean interval free time (MIFT)**, and **number of episodes (NOE)**. Flatulence index was used as a measure of the rate of hydrogen sulfide production and was calculated as the total amount of hydrogen sulfide produced (in ppm) during the sampling period divided by the total sampling time (in minutes). Mean interval free time was used as a measure of the frequency of flatulence and was calculated as the cumulative sum of flatulence-free intervals (in minutes) divided by the number of flatulence-free intervals **(Fig 2)**. Because results of a preliminary study revealed that a human odor judge in the same room was able to detect hydrogen sulfide in canine flatus only when pump readings were > 1 ppm, a flatulence-free interval was taken as any period in which the pump reading was < 1 ppm. A high MIFT indicated prolonged flatulence-free periods. Number of episodes represented the number of flatulence events and was taken as the number of times pump readings, obtained every 20 seconds, were > 1 ppm.

Experiment 2—The second experiment was designed to determine the relationship between hydrogen sulfide concentrations in flatus and organoleptic assessment of flatus odor. Six dogs (1 female and 1 male Labrador Retriever, 1 female Golden Retriever, 1 German Munsterlander, and 1 male and 1 female Mastiff) were used to assess the relationships between hydrogen sulfide concentration in rectal gas and human perception of flatus odor. The rectal gas monitoring equipment was placed on each dog in turn for 4 hours between 11:30 AM and 3:30 PM on 2 days. During the sampling period, dogs were housed in an enclosed room (floor space, 17 m^2) in the presence of an odor judge. The same judge graded flatus from all dogs at all times. Dogs were allowed to roam freely during the sampling period to simu-

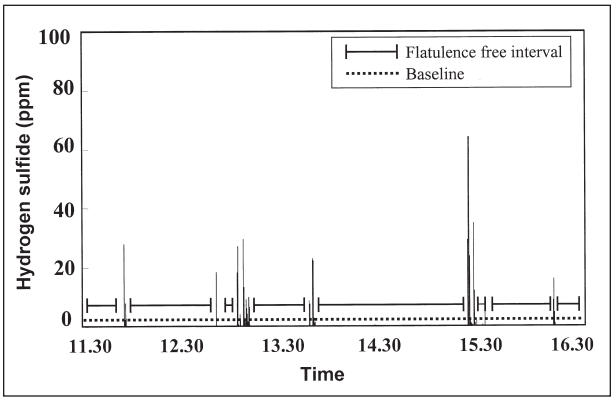

Figure 2—Hydrogen sulfide concentration in rectal gas from an adult dog versus time. The baseline is set at 1 part per million (ppm); readings less than baseline were ignored, and readings greater than baseline were considered a flatulence episode. To calculate mean interval free time (MIFT), total time between flatulence episodes was divided by number of flatulence-free intervals. For the profile pictured here, MIFT is 26.25 minutes (210 min/8).

late an in-home environment. The odor judge noted the time of each flatulence episode and rated the episode on a 1 to 5 scale, where 5 represented an unbearable odor and 1 was noise only with no odor. A rating of 2 represented a slightly noticeable odor, 3 was a mildly unpleasant odor, and 4 was a bad odor. Ratings were subsequently matched with the readings from the sampling pump according to the time of flatulence episode.

Statistical analyses—One- and 2-way ANOVA, with dog as a random factor and day as a fixed factor, were used to test for significant factor effects. Significant differences among group means were further assessed by use of Tukey honest significant difference intervals.[10] Data were transformed prior to analysis, because Box-Cox analysis indicated that natural log transformation of MIFT and NOE was appropriate to ensure data were normally distributed. Measures of spread between groups (ie, **coefficients of variation [CV]**) were tested for significance by use of a paired *t*-test. The relationship between human perception ratings (ie, odor ratings) and concentration of hydrogen sulfide was modeled, using a power law and linear regression techniques. Cross validation was used, whereby a subset of the data was used to identify an appropriate model and estimate its parameters, and the remainder of the data set was independently used to test the fit of the model. The model's parameters were then refined, using the entire data set, and goodness-of-fit was assessed from the percentage of odor ratings predicted by the model that matched those of the odor judge. On the basis of this approach, an equation was derived that allowed odor ratings to be predicted from measured concentrations of hydrogen sulfide. All statistical tests were performed, using a software program.[c] Data were reported as mean ± SD, with the level of significance set at $P < 0.05$.

Results

Experiment 1—No discrete patterns of flatulence were apparent after feeding, and there was substantial variation in hydrogen sulfide production from day to day over the course of the 4 sampling periods **(Fig 3)**. On the basis of flatulence profiles obtained, and for the convenience of dogs and investigators, we obtained all subsequent measurements over a 4-hour period commencing 3 hours after feeding.

Although we did not detect significant differences in flatulence index over the 4 consecutive days of testing, we did detect substantial variation within dogs over time and between dogs on each day; SD were of similar magnitude to mean values **(Table 1)**. Mean CV of the day-to-day measurements of flatulence index was 75% (range, 41 to 144%), whereas mean CV of the dog-to-dog measurements obtained each day was 103% (74 to 145%). In 1 dog, the difference in flatulence index from 1 day to the next was greater than 115-fold (from 17.29 ppm/min on day 2 to 0.15 ppm/min on day 3). There was also substantial difference in flatulence index measured twice at a 4-week interval in 2 dogs; within-dog mean flatulence indices for the two 4-day periods differed by more than 4- and 7-fold.

We detected much less variability in MIFT; no significant differences within or among dogs were detected over the 4 consecutive sampling days **(Table 2)**. Mean CV of the day-to-day measurements of MIFT was 24% (range, 9 to 39%), whereas mean CV of the dog-to-dog measurements obtained each day was 29% (21 to 36%). Individual values were consistent from day to day, and there was no significant difference in MIFT measured twice at a 4-week interval in 2 dogs.

We also did not detect significant differences in NOE within or among dogs over the 4 consecutive days **(Table 3)**. Mean CV of day-to-day NOE values was 26% (range, 5 to 67%), whereas mean CV of dog-to-dog values obtained each day was 34% (21 to 51%). These CV were comparable to those for MIFT. As with MIFT, individual values for NOE were consistent from day to day, and there was no significant difference in NOE measured twice at a 4-week interval in 2 dogs.

We did detect significant differences in CV among flatulence index, MIFT, and NOE for both day-to-day values for individual dogs ($P < 0.01$) and dog-to-dog values obtained each day ($P < 0.005$). Mean CV of flatulence index for day-to-day values for individual dogs was significantly higher, compared with CV for MIFT and NOE; there was no significant difference in CV between MIFT and NOE. Mean CV of MIFT and NOE for dog-to-dog values obtained each day were signifi-

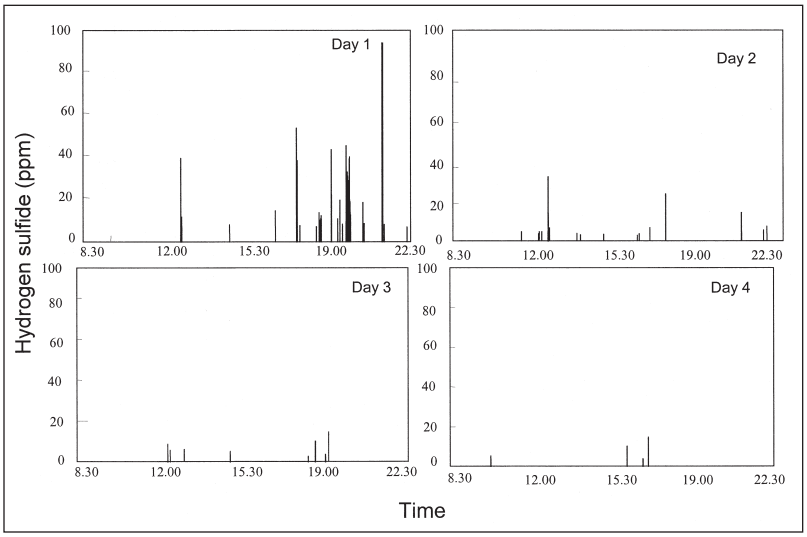

Figure 3—Concentrations of hydrogen sulfide in rectal gas from an adult dog measured for 14 hours after feeding on 4 days.

Table 1—Flatulence index (ppm/min)* in 5 adult dogs determined over a 4-hour period commencing 3 hours after eating on 4 consecutive days

	Day				
Dog†	**1**	**2**	**3**	**4**	**Mean ± SD**
A					
Week 1	1.26	1.34	0.67	1.71	**1.00 ± 0.43**
Week 4	1.48	0.27	1.10	0.40	**0.65 ± 0.57**
B					
Week 1	3.01	0.12	1.43	8.98	**2.71 ± 3.91**
Week 4	17.66	17.29	0.15	1.70	**7.36 ± 9.58**
C	5.24	3.10	3.90	4.40	**3.33 ± 0.90**
D	6.19	6.93	2.67	6.93	**4.54 ± 2.04**
E	0.90	0.90	1.56	1.99	**1.07 ± 0.54**
Mean ± SD	**5.11 ± 5.89**	**4.28 ± 6.20**	**1.64 ± 1.27**	**3.73 ± 2.94**	**NA**

*Flatulence index calculated as the total amount of hydrogen sulfide produced (in ppm) during the sampling period divided by the total sampling time (in minutes). †Measurements were obtained twice for dogs A and B, with 4 weeks between measurements.
ppm = Parts per million.NA = Not applicable.

Table 2—Mean interval free time* in 5 adult dogs determined over a 4-hour period commencing 3 hours after eating on 4 consecutive days

	Day				
Dog†	**1**	**2**	**3**	**4**	**Mean ± SD**
A					
Week 1	2.19	1.79	4.16	2.74	**2.72 ± 1.04**
Week 4	4.20	3.10	1.87	2.03	**2.80 ± 1.08**
B					
Week 1	2.57	3.22	3.42	3.28	**2.08 ± 0.38**
Week 4	2.44	2.29	3.08	2.44	**2.56 ± 0.35**
C	1.38	2.22	2.51	2.76	**2.22 ± 0.60**
D	1.41	0.94	1.36	1.74	**1.36 ± 0.33**
E	2.19	2.66	2.22	2.33	**2.35 ± 0.22**
Mean ± SD	**2.34 ± 0.94**	**2.31 ± 0.79**	**2.66 ± 0.96**	**2.47 ± 0.51**	**NA**

*Mean interval free time calculated as the cumulative sum of flatulence-free intervals (in minutes) divided by the number of flatulence-free intervals. Values were logarithmically transformed. *See* Table 1 for key.

Table 3—Number of episodes of flatulence* in 5 adult dogs determined over a 4-hour period commencing 3 hours after eating on 4 consecutive days

	Day				
Dog†	**1**	**2**	**3**	**4**	**Mean ± SD**
A					
Week 1	3.00	2.77	0.00	2.56	**2.08 ± 1.40**
Week 4	0.69	2.08	3.14	2.83	**2.19 ± 1.09**
B					
Week 1	2.77	2.08	1.95	1.95	**2.19 ± 0.39**
Week 4	2.71	2.94	2.08	3.14	**2.72 ± 0.46**
C	3.74	3.14	2.71	2.48	**3.02 ± 0.55**
D	3.81	4.13	3.78	3.91	**3.91± 0.16**
E	3.14	2.77	3.04	3.00	**2.99 ± 0.16**
Mean ± SD	**2.84 ± 1.04**	**2.84 ± 0.70**	**2.39 ±1.23**	**2.84 ± 0.61**	**NA**

*Number of episodes taken as the number of times hydrogen sulfide concentration, measured every 20 seconds, was > 1 ppm. Values were logarithmically transformed. *See* Table 1 for key.

cantly lower than mean CV of flatulence index. Again, there was no significant difference in CV between MIFT and NOE.

Experiment 2—Odor ratings of flatulence were positively correlated with concentration of hydrogen sulfide in rectal gas ($r = 0.92$, $P < 0.001$; **Fig 4**). Using a training data set consisting of half the data, the relationship between the 2 variables was best fitted by the power function, which formed the basis for our predictive model:

Predicated odor rating = $1.511124 \times$ hydrogen sulfide concentration$^{0.280379}$ where hydrogen sulfide concentrations were measured in ppm.

To assess goodness-of-fit, the predictive model was applied to hydrogen sulfide concentrations from dupli-

Figure 4—Correlation between odor ratings of flatulence and mean (± SD) concentration of hydrogen sulfide in flatus of dogs. An odor rating of 1 represents no odor; 2, a slightly noticeable odor; 3, a mildly unpleasant odor; 4, a bad odor; and 5, an unbearable odor.

cate measures obtained for 6 dogs. The number of predicted ratings that were the same as or different from the actual ratings were calculated. The predicated rating was the same as the actual odor rating for 74% (268/362) of flatulence episodes. The percentage of correct predictions ranged from 81% for an actual rating of 1, 71% for an actual rating of 2, 58% for an actual rating of 3, 61% for an actual rating of 4, and 60% for an actual rating of 5.

Discussion

We have described a technique that provides reliable information on the frequency of rectal gas emissions and their odor characteristics in dogs. The latter is particularly important, as it is the production of odoriferous gases that is of most social relevance to the dog-owning public. This is, to our knowledge, the first description of a technique for the real-time episode-by-episode analysis of rectal gas in any species.

In previous studies, human rectal gases have generally been collected in batches for analysis, rather than collected from discrete flatulence episodes or emissions as our technique allows. Gases are collected from humans, using indwelling rectal tubes,[3-5,7,8] although gastight pantaloons were used in 1 study.[8] Techniques using rectal tubes can result in good quantitative data on volume and gas composition. However, rectal tubes are uncomfortable, tend to plug, and cannot be used for extended periods in free-roaming subjects. The technique described in the present report is noninvasive and was well tolerated by our dogs. This technique could be equally effectively applied to studies of flatulence in humans. However, it would not be suitable for measurement of gas volume and composition, because collection of rectal gases may be incomplete, and atmospheric gases may dilute those gases collected.

The hydrogen sulfide concentrations we measured proved to be reliable markers of flatulence episodes and were predictive of the odoriferous qualities of rectal gases. This is consistent with observations in humans, in which the intensity of odor of flatus is correlated with the concentration of sulfur gases, specifically hydrogen sulfide.[8] Hydrogen sulfide is also the most abundant sulfur gas in human flatus (0.003% vol/vol, compared with methanethiol at 0.0006% and dimethyl sulfur at 0.0019%).[7] Results of our preliminary studies indicated that emissions in which hydrogen sulfide concentrations were < 1 ppm were not detectable by the odor judge. This cutoff value is higher than the odor threshold of hydrogen sulfide in air, which is reported to be 0.005 ppm.[11] The discrepancy between thresholds can be explained by the fact that the pump used in the present study analyzes a more concentrated source of hydrogen sulfide (ie, prior to dilution of hydrogen sulfide by atmospheric gases) than that subsequently analyzed by the odor judge.

The variability in flatulence patterns that we detected, both within and among dogs, is consistent with data from human studies, indicating that there is appreciable intraindividual variation and enormous interindividual variation in the frequency, volume, and gaseous composition of flatus. For instance, daily frequency of flatus varies between individuals by more than 10-fold.[6] Volume of flatus has varied between subjects by as little as 3-fold (476 to 1,491 ml/24 h)[3] to as much as 16-fold (106 to 1,657 ml/4 h).[7] Mean interindividual CV for gas concentrations ranged from 28 to 82% in 1 study, with methane concentrations ranging from 0.006% in 1 person to 17.8% in another, a greater than 10,000-fold difference.[7] This, and variation in volume of each emission, indicates that the expelled gas does not represent the removal of an aliquot from a well-mixed large pool of gas, as occurs with most of the body's excretory products. Rather, the inconsistent gas emissions reflect the variable processes that govern the production, removal, and liberation of rectal gases, which in turn may explain the large variability, both within and between dogs, in the amount of hydrogen sulfide produced as expressed by the flatulence index. Variable capture of rectal gases in the sampling system might also account for some variation in flatulence index. However, the consistently close correlation between measured concentrations of hydrogen sulfide and odor ratings indicated that amount of gas captured likely did not vary significantly among dogs.

Such large typical variability in dog-to-dog and day-to-day results has the effect of diminishing the sensitivity of the technique for discriminating between effect of dogs and effect of treatment factors such as diet. The reduced variability associated with MIFT and NOE, compared with flatulence index, is consistent with the observation in humans that frequency of emissions is less variable than volume and concentration of gases emitted.[6,8]

Any concerns that measurements such as MIFT and NOE may not correlate with the organoleptic qualities of flatus, which are arguably of greater importance to dog owners than either the frequency or volume of emissions, were overcome by the development of the predictive model for odor rating. Thus, the malodor of rectal gas can be accurately predicted from the measure of hydrogen sulfide concentration. This allows for determination of odor ratings for individual flatulence episodes as well as entire sampling periods. Our model thus represents a useful tool for future studies of flatu-

lence in dogs. The relationship between odor and hydrogen sulfide concentration in canine flatus is similar to that in human flatus; odor intensity of human flatus correlated most strongly with hydrogen sulfide concentration ($r = 0.64$, $P < 0.001$).[8]

The present study failed to identify any relationship between time of feeding and pattern of flatulence. This differs from results of human studies, in which production of rectal gas increased significantly following a meal.[3] The increase in rectal gas is believed to reflect gastrocolonic responses that mix colonic bacteria with substrate and thereby increase the rate of fermentation and production of gases. Effects of these gastrocolonic responses is augmented by the dumping of carbohydrate residue from the ileum into the colon. Together, these effects lead to the release of rectal gas composed predominantly of hydrogen and carbon dioxide rather than odoriferous sulfur gases. Thus, because we measured only hydrogen sulfide, it is possible that flatulence profiles are affected by feeding in dogs, but the gases produced after feeding are either not odoriferous or are odoriferous gases other than hydrogen sulfide. Other feeding patterns and diets may affect flatulence profiles, but evaluation of these was beyond the scope of the present study.

The technique described in the present report has been used to evaluate dietary manipulation of the number and frequency of flatulence episodes, as well as the odor characteristics of canine flatus.[12] This technique may enable further studies of flatulence in dogs, with specific attention to those factors that regulate the production of odoriferous gases, and has the potential to provide new insights into colonic physiology and gastrointestinal tract disease.

[a]Pedigree Chum Original, Pedigree Masterfoods, Melton Mowbray, Leicestershire, UK.

[b]Pedigree Formula Advance Adult Supreme, Pedigree Masterfoods, Melton Mowbray, Leicestershire, UK.

[c]MultiRAE plus PGM-50, Rae Systems Inc, Sunnyvale, Calif.

[d]ProRAE 50, Rae Systems Inc, Sunnyvale, Calif.

[e]Statgraphics Plus, version 2.1, Manugistics, Rockville, Md.

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