Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.4, No.1, 23-29, 2017

Original Article

Application of a portable gas chromatograph for quantitative measurement of canine oral malodor

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(Received February 3, 2017; Accepted February 13, 2017)

ABSTRACT — Oral malodor is an unpleasant condition experienced by most individuals around the world. Volatile sulfur compounds (VSCs) may be the main source of oral malodor both in humans and dogs. The purpose of this study was to examine the suitability for measurement of oral malodor levels in dogs using OralChroma™, a portable gas chromatograph developed for human use. Oral malodor and periodontal disease in laboratory Beagle dogs (N = 6) were determined by organoleptic assessment and OraStrip® test, respectively. Relatively high concentrations of VSCs in the breath were detected in all six dogs in the order of hydrogen sulfide (H_2S) > methyl mercaptan (CH_3SH) > dimethyl sulfide ($(CH_3)_2S$). There was no significant influence of meals on VSCs levels. Significant correlations ($P < 0.05$) were observed among each VSC. These findings for the first time highlight the potential of OralChroma™, which allows for noninvasive, rapid and quantitative measurement of oral malodor levels in dogs, and warrants further investigation.

Key words: Volatile sulfur compound, Portable gas chromatograph, Malodor, Periodontal disease, Dog

INTRODUCTION

Halitosis is a general term used to define an unpleasant or offensive odor emanating from breath regardless of whether its origin is oral or non-oral (Tonzetich, 1977; Eldarrat, 2011). In approximately 80-90% of all cases, bad breath is caused by oral conditions, defined as oral malodor (Miyazaki *et al.*, 1995; Delanghe *et al.*, 1997). Oral malodor is an unpleasant condition experienced by most individuals around the world. A significant source of oral malodor is from the degradation of organic substances by anaerobic bacteria in the oral cavity. Of several malodorous compounds, volatile sulfur compounds, in particular, H_2S (hydrogen sulfide: HS), CH_3SH (methyl mercaptan: MM) and $(CH_3)_2S$ (dimethyl sulfide: DMS), have been demonstrated to play a significant role in human oral malodor (Tonzetich, 1977). Periodontal disease is considered the most common disease of dogs and its clinical sign frequently reported by owners is oral malodor. However, little is known about the role of VSCs in canine oral malodor.

Currently, an organoleptic assessment is still the gold standard for diagnosis of malodor. The method is easy to perform and requires no extra apparatus. However, the objectivity of this method is rather low because of inter-examiner variation (Rosenberg and McCulloch, 1992). Gas chromatography mass spectrometry (GC-MS) makes it possible to measure the global concentration of sulfur containing gases. However, this approach implies a substantial investment and expense. Recently, portable sulfide monitors have been developed and are used in many human clinical centers (Greenman *et al.*, 2004).

Oral malodor and periodontal disease are very common in humans and dogs, and there is an association between malodor and periodontal disease (Yaegaki and Sanada, 1992). Indeed, some periodontopathic bacteria including *Porphyromonus gingivalis*, *Treponema generate* and *Tannerella forsythia* produce a considerable amount of VSCs (Persson *et al.*, 1990). However, no attempt has been made to develop instruments for quantitative measurement of oral malodor in dogs. Therefore, in this study, we examined the suitability for measurement of VSCs

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levels in dog breath using OralChroma™, a portable gas chromatograph developed for human use.

MATERIALS AND METHODS

Animals

Six laboratory Beagle dogs (2 females and 4 males, 4-11 years old) with no systemic disorders were used in this study. All dogs were individually kept in cages at room temperature (21 ± 2°C) and 55 ± 5% humidity, with a 12-hr light/dark cycle. They were fed (CLEA Dog Diet CD-5M®; CLEA Japan, Inc., Tokyo, Japan) twice daily, once in the morning (10:00) and once in the evening (17:00), and freely provided tap water to drink from a container. All experimental protocols were approved by the Animal Experiment Committee of Azabu University.

Measurement of oral malodor in dogs

To determine the oral malodor and periodontal disease in dogs used in this study, we first performed organoleptic examination and OraStrip® test (DS Pharma Animal Health Co. Ltd, Osaka, Japan), respectively. The organoleptic score was determined by one blinded examiner. A 0-3 score was given, where 0 represented absence of odor, 1 was given for barely noticeable odor, 2 for moderate malodor and 3 for strong malodor. The OraStrip® test was developed for veterinarians to determine periodontal infection by detecting thiol levels due to active periodontal disease in the absence of visual manifestation (Manfra Marretta *et al.*, 2012; http://orastrip.com/). This test paper wipes up gingiva and reacts to the thiol compounds included in the canine mouth, and it is possible to monitor periodontal disease activity, scored 1-5, by the degree of coloring.

Measurement of VSCs levels by OralChroma™

In this study, the OralChroma™ (CHM-2, FIS Inc., Hyogo, Japan) was used to measure the individual concentrations of HS, MM and DMS in ppb (Sutula *et al*., 2013). This portable gas chromatograph was originally developed to quantitatively measure VSCs in human oral air, and thus, in this study, the procedure of measurement of VSCs was slightly modified as follows: Oral air samples were taken from dogs inserting a sterile disposable plastic 2 mL syringe into the dogs' oral cavity between the lips for 30 sec. Then, the plunger was pulled slowly, pushed in again and pulled for a second time before removal from the mouth. The sample of oral air (1.0 mL) was injected into the inlet of the OralChroma™. The measurement was then started automatically, and further operations were handled by the OralChroma™ Data Manager. After 4 min, the process was completed and the concentrations of three VSCs were determined by a calibration curve inside the OralChroma™, and were displayed in either ng/mL or ppb. As spontaneous fluctuation in oral air samples has been demonstrated in several studies (Yaegaki *et al*., 2012; Tangerman and Winkel, 2007), three oral air samples were taken at each time point and these values were averaged.

Statistical analysis

All data are represented as means ± standard deviation (S.D.). Non-paired and paired Student's t-tests were used for statistical analysis using PRISM statistical software (GraphPad software, San Diego, CA, USA). Differences with a P-value of < 0.05 were considered significant.

RESULTS AND DISCUSSION

Background data of Beagle dogs used

We first performed organoleptic and OraStrip® tests on these dogs. The results of the organoleptic examination showed that 2 dogs had moderate (score 2) and the other 4 dogs had strong (score 3) malodor (Table 1). The results of OraStrip® test demonstrated that all dogs had a score of 4.3-5.0, which indicate high thiol levels in their oral cavity reflecting severe periodontal disease.

Table 1. Background data of the beagle dogs (N = 6) used in the present study.

Dog ID	Gender	Age (year)	Body Weight (kg)	Organoleptic score	OraStrip® score
#1	female	4.8	12.0	3	5.0
#2	female	5.3	12.0	2	4.7
#3	male	7.5	14.0	2	4.3
#4	male	7.9	11.0	3	5.0
#5	male	9.1	11.4	3	5.0
#6	male	10.6	12.8	3	5.0

The scores of the organoleptic test and OraStrip® test are the average of 3 determinations.

VSC concentrations measured by an OralChroma™

We next measured VSC levels in these dogs' oral air using an OralCroma™. Measurements were performed on all 6 dogs before and after breakfast for 3 consecutive days. All three VSCs were detected in the dogs' oral air, but levels of VSCs varied among dogs (Fig. 1). There was no significant change in levels of each VSC or total VSCs during the 3 days (Fig. 2).

Influence of meals

Previous studies reported that oral malodor levels vary during day time in humans (Tonzetich, 1977). In addition, fasting is known to cause elevated concentrations of odorous compounds, such as ketones acetone and 2-pentanone, and food intake could have some influence on the detected VSC levels (Statheropoulos *et al*., 2006). Thus, we examined the influence of meals on VSC levels. There was no significant difference between VSC levels before and after meals (Fig. 3).

Comparison of VSCs

Table 2 summarizes the levels of the three VSCs and total VSCs. It is important to take into account that the threshold to smell differs according to the type of gas. The threshold level proposed by the manufacturer of OralChroma™ is 112 ppb for HS, 26 ppb for MM and 8 ppb for DMS, respectively. All six dogs had higher levels above the threshold for MM and DMS, whereas two dogs had lower levels below the threshold for HS. These two dogs (#2 and #3) with lower levels of HS had a low organoleptic score (grade 2) of malodor (Table 1).

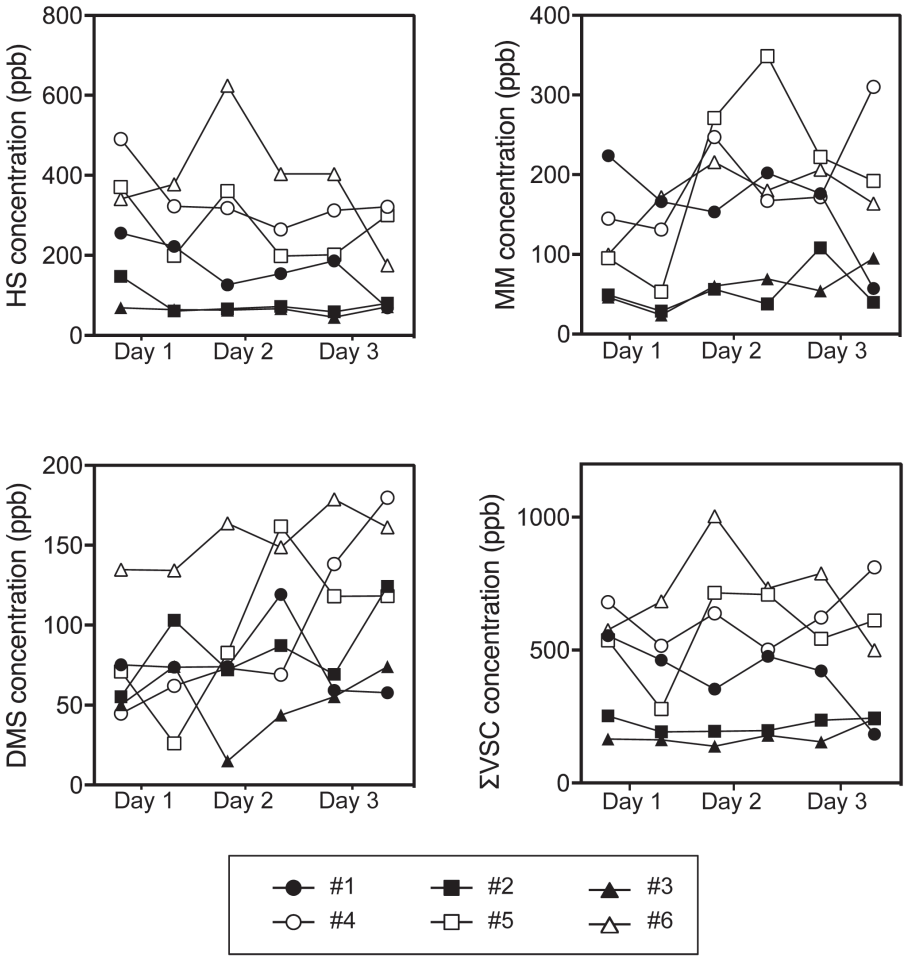

Fig. 1. VSC levels (ppb) in oral air from dogs (N = 6) during 3 consecutive days (day 1-day 3). Data are expressed as means ± S.D. HS: H_2S, MM: CH_3SH, DMS: $(CH_3)_2S$.

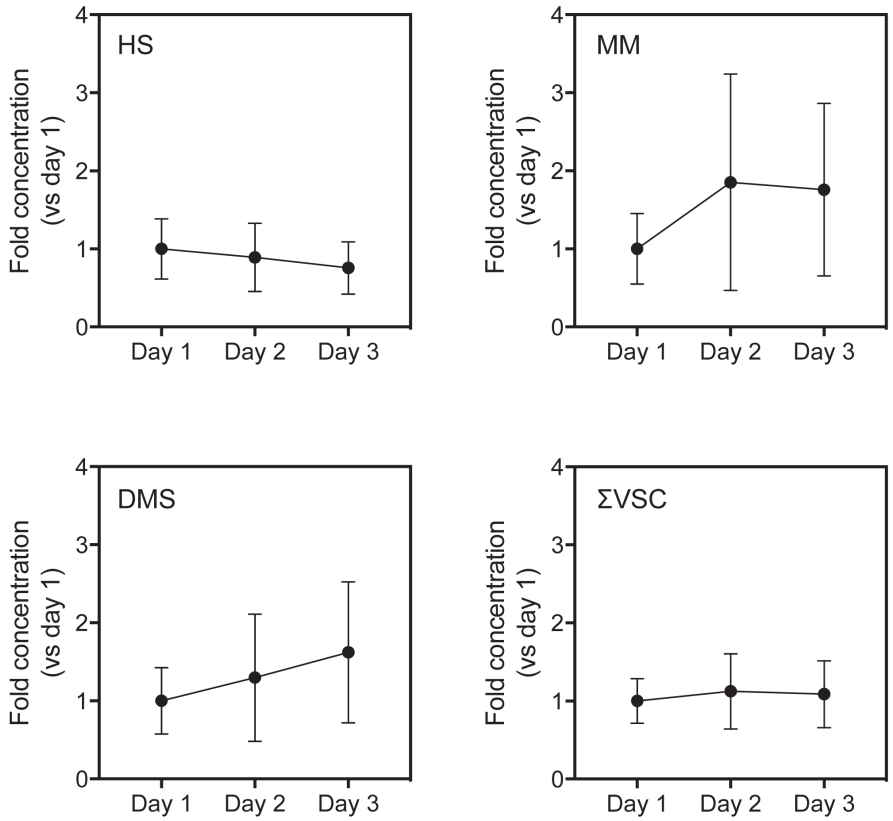

Fig. 2. VSC levels (fold vs. day 1) in oral air from dogs (N = 6) during 3 consecutive days (day 1-day 3). Data are expressed as means ± S.D. The concentration on day 1 was 244 ± 141 ppb for HS, 103 ± 65 ppb for MM, 75 ± 34 ppb for DMS and 422 ± 199 ppb for ΣVSC. HS: H_2S, MM: CH_3SH, DMS: $(CH_3)_2S$, ΣVSC: HS + MM + DMS.

Table 2. The concentrations of hydrogen sulfide (HS) > methyl mercaptan (MM) > DMS and total VSCs (ΣVSC) in oral air from six beagle dogs.

Dog ID	HS (ppb)	MM (ppb)	DMS (ppb)	ΣVSC (ppb)
#1	169 ± 81	163 ± 92	77 ± 28	409 ± 175
#2	81 ± 38	53 ± 31	85 ± 42	220 ± 62
#3	63 ± 24	58 ± 33	52 ± 29	173 ± 59
#4	339 ± 136	195 ± 83	94 ± 51	628 ± 153
#5	272 ± 103	197 ± 139	96 ± 49	565 ± 226
#6	387 ± 187	173 ± 60	154 ± 46	714 ± 242
Average	219 ± 164	140 ± 100	93 ± 51	452 ± 262

Data are expressed as means ± S.D. HS: H_2S, MM: CH_3SH, DMS: $(CH_3)_2S$. ΣVSC = HS + MM + DMS. The threshold concentrations of malodor for HS, MM and DMS are 118 ppb, 26 ppb and 8 ppb, respectively.

The concentrations of three VSCs in the dogs' oral air were in the order of HS > MM > DMS. The comparative ratio (%) of each VSC level to the total VSC level was 45 ± 14% (HS), 31 ± 12% (MM) and 24 ± 13% (DMS), respectively, suggesting that HS and MM play a major role in dogs' bad breath like in humans. One of the advantages of OralChroma™ is that it can measure DMS levels when compared with Halimeter®, the other porta-

ble VSC monitor. Previous findings suggested that DMS is more associated with extra-oral causes of halitosis (Tangerman and Winkel, 2007). Thus, OralChroma™ may be useful for a differential diagnosis in dogs.

Correlations of levels among VSCs

Significant ($P < 0.05$) correlations were found between each VSC level, and the strongest correlation was observed between HS and MM: 0.5824 for HS vs MM, 0.5698 for MM vs DMS and 0.4531 for HS vs DMS (Fig. 4). The implications for strong correlations between VSCs are that certain bacterial groups in the oral cavity may have a common metabolic pathway for production of VSCs. Further work is required to confirm this hypothesis.

Correlations between VSC levels and age:

The influence of periodontal disease on oral malodor development has been well reported (Tonzetich 1977; Rosenberg and McCulloche, 1992; Pham *et al*., 2012). Dogs used in this study had high oral thiol levels suggesting the presence of periodontal disease. Thus, we analyzed the correlation of oral VSC levels and age. The strongest correlation was observed between HS and age (Fig. 5), but this correlation was not significant, probably because of the small number of dogs used in the present study. Our results are in agreement with previous results that periodontal disease is the most widespread oral disease in dogs and incidence increases with advancing age (Kortegaard *et al*., 2008; Hirai *et al*., 2013). Further work is also required to confirm this hypothesis.

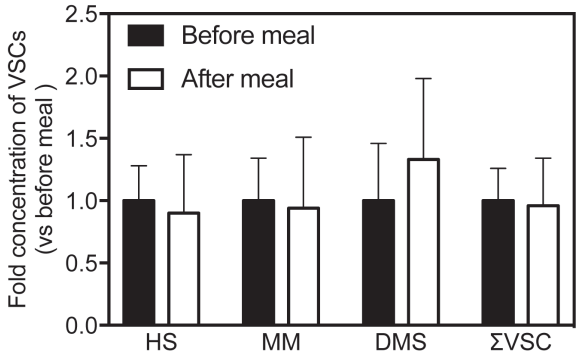

Fig. 3. The influence of meals on VSCs (HS, MM, DMS and ΣVSC) levels in dogs (N = 6). Measurements were done before and after breakfast (10:00) for 3 consecutive days (day 1-day 3). Data are expressed as means ± S.D. HS: H_2S, MM: CH_3SH, DMS: $(CH_3)_2S$, ΣVSC: HS + MM + DMS.

In summary, we examined the suitability for measurement of oral malodor levels in beagle dogs using OralChroma™, a portable gas chromatograph developed for human use, and detected relatively high concentrations of VSCs in the oral air from all dogs tested. These findings for the first time highlight the potential of OralChroma™ for noninvasive, rapid and quantitative measurement of oral malodor levels in dogs, and warrants further investigation.

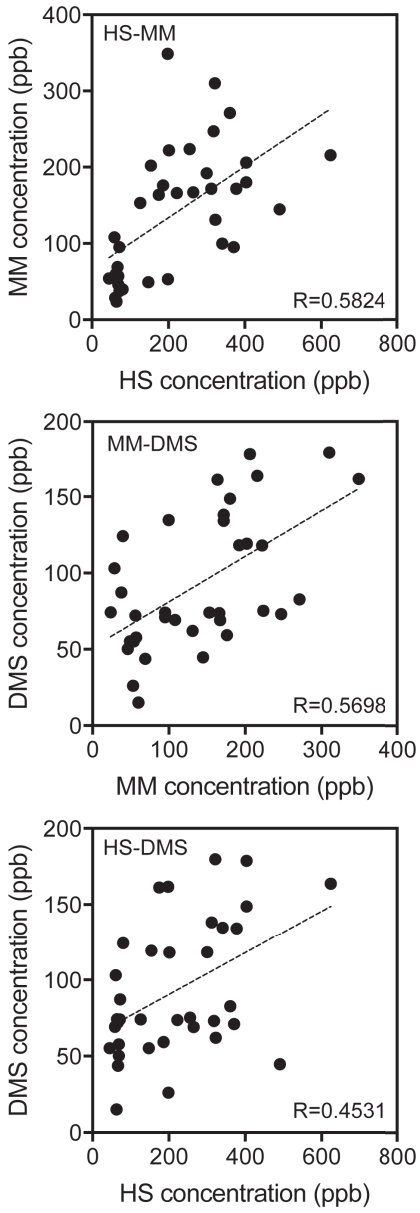

Fig. 4. Correlations between each VSC level. HS: H_2S, MM: CH_3SH, DMS: $(CH_3)_2S$.

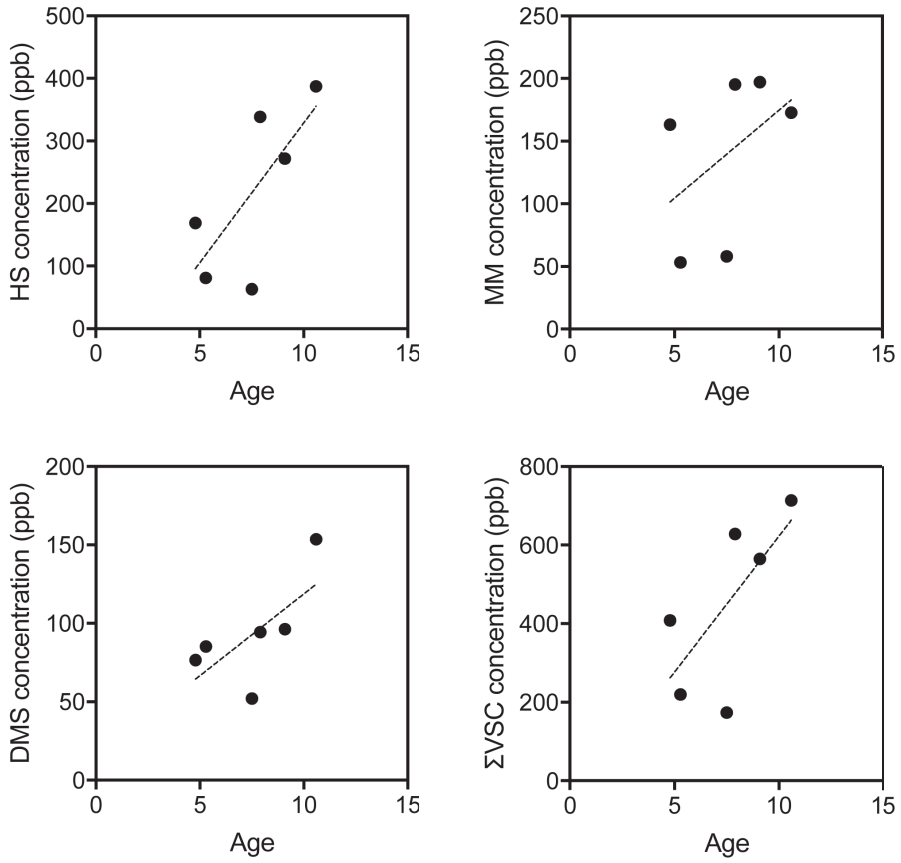

Fig. 5. Correlations with VSC levels and age in dogs. Horizontal axis: dogs' age, vertical axis: levels of VSCs. HS: H_2S, MM: CH_3SH, DMS: $(CH_3)_2S$, ΣVSC: HS + MM + DMS.

ACKNOWLEDGMENTS

This study was supported in part by a research project grant awarded by the Azabu University.

Conflict of interest---- The authors declare that there is no conflict of interest.

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