Published in final edited form as:
Environ Res. 2008 March ; 106(3): 361–364. doi:10.1016/j.envres.2007.09.007.

Environmental tobacco smoke and canine urinary cotinine level

Elizabeth R. Bertone-Johnson[1,2], **Elizabeth Procter-Gray**[1], **Audra L. Gollenberg**[1], **Michele E. Bundga**[1,3], and **Lisa G. Barber**[2]

1*Department of Public Health, School of Public Health and Health Sciences, University of Massachusetts, Amherst, MA*

2*Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA*

3*Division of Gastroenterology, Hepatology and Endoscopy, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA*

Abstract

Epidemiologic studies of companion animals such as dogs have been established as models for the relationship between exposure to environmental tobacco smoke (ETS) and cancer risk in humans. While results from these studies are provocative, pet owner report of a dog's ETS exposure has not yet been validated. We have evaluated the relationship between dog owner's report of household smoking by questionnaire and dog's urinary cotinine level. Between January and October, 2005, dog owners presenting their pet for non-emergency veterinary care at the Foster Hospital for Small Animals at Cummings School of Veterinary Medicine, Tufts University were asked to complete a 10-page questionnaire measuring exposure to household ETS in the previous 24 hours and other factors. A free-catch urine sample was also collected from dogs. Urinary cotinine level was assayed for 63 dogs, including 30 whose owners reported household smoking and 33 unexposed dogs matched on age and month of enrollment. Urinary cotinine level was significantly higher in dogs exposed to household smoking in the 24 hours before urine collection compared to unexposed dogs (14.6 vs 7.4 ng/mL; P = 0.02). After adjustment for other factors, cotinine level increased linearly with number of cigarettes smoked by all household members (P = 0.004). Other canine characteristics including age, body composition and nose length were also associated with cotinine level. Findings from our study suggest that household smoking levels as assessed by questionnaire are significantly associated with canine cotinine levels.

Keywords

tobacco smoke pollution; cotinine; validation studies; dogs

Introduction

Epidemiologic studies of companion animals may help evaluate the relationship between exposure to environmental tobacco smoke (ETS) and cancer risk in humans (Bukowski 1997; Kelsey 1998; Gollenberg 2006; Reif 1992; Reif 1998; Bukowski 1998; Glickman

Address for correspondence: Dr. Elizabeth R. Bertone-Johnson, Arnold House, University of Massachusetts, 715 North Pleasant Street, Amherst, MA 01003-9304, Phone: (413)577-1672; Fax: (413)545-1645, Email: ebertone@schoolph.umass.edu.

1989). Dogs in particular have been well established as appropriate models for a variety of human cancers because of similarities in cancer etiology between species. Canine cancers of the mammary gland, testes, bladder, lung, nasal and sinus cavities, bone and lymphatic system all share clinical, pathological, histologic and prognostic characteristics with their human counterparts, and have been used extensively in therapeutic research (Kelsey 1998; Vail 2000). While results from these studies are provocative, pet owner report of a dog's passive smoking exposure by questionnaire has not been validated.

Cotinine, a metabolite of nicotine, is an established biologic marker of ETS exposure in humans (Benowitz 1996; Emmons 1994) and has been used extensively in validation studies. Previous laboratory research suggests that dogs exposed to tobacco smoke metabolize nicotine to cotinine proportionally to exposure dose (Brazell 1984). We have evaluated the relationship between dog owner's questionnaire report of recent household smoking and canine urinary cotinine level.

Materials and Methods

The protocol for this study was approved by human subjects and institutional animal care and use committees at the University of Massachusetts and Tufts University. Between January and October, 2005, dog owners presenting their pet for non-emergency veterinary care at the Foster Hospital for Small Animals at Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA were asked to complete a 10-page questionnaire on their dog's home environment, health, and care. Owners were asked if anyone in the household smoked cigarettes in the previous 24 hours, and if so, whether smoking took place indoors and the number of cigarettes smoked daily by each smoker (1 – 9, 10 – 19, 20 – 29, 30 – 39, ≥40 cigarettes). We also asked about cigar and pipe smoking. Other questions inquired about factors that could potentially modify a dog's ETS exposure dose, including size, location and type of dwelling; number of hours the dog spent outdoors in the previous 24 hours; household ventilation and vacuuming; and whether the dog had been bathed, groomed, swam or had its teeth cleaned in the past 24 hours. Other characteristics of the dogs were assessed, including age, sex, reproductive status (i.e., reproductively intact or sterilized), year of acquisition, breed, hair length, nose length, body weight, and body composition. We also asked about the use of 40 specific medications in the past week, and the presence of 18 common chronic conditions. To account for nicotine from other sources, we inquired about the use of indoor pesticides, lawn care products and flea control products in the previous 24 hours. Owner characteristics assessed included age, sex, ethnicity, occupation, and highest level of education completed. The questionnaire took 10–15 minutes to complete. Completed questionnaires were obtained from 925 dog owners.

Owners were also asked for permission to collect a urine sample from their pet. Urine samples were collected using the free-catch method from dogs able to urinate. Dogs were taken outdoors and urine samples were collected in sterile containers held in the urine stream. Urine samples were then immediately transferred into one or more labeled cryotubes and stored in a standard freezer at −80° F until laboratory analysis. Urine samples were obtained from 245 dogs whose owners completed questionnaires.

Urine samples from all dogs whose owners reported household smoking were selected for analysis (n = 30), along with a subset of samples from non-smoking households (n = 33). Samples from non-smokers were frequency matched to smoking-exposed samples on dog's age and month of collection. Samples were labeled with subjects' ID number only, and thus blinded to smoking status of the dog's owner. In addition, blinded split samples from dogs from smoking and non-smoking households were interspersed with analytic samples to assess

within-subject variation of the cotinine assay. Internal quality control samples were provided by the analytic laboratory.

Urine samples were assayed for cotinine in January, 2006, using a radioimmunoassay developed and analytically validated by the Foundation for Blood Research (Scarborough, ME) (Knight 1989; Watts 1990). This assay has a lower limit of sensitivity of 3 ng/ml. A cut-off of 10 ng/ml has been used in these studies to reliably distinguish exposed from non-exposed individuals (Chilmonczyk 1990). All samples were analyzed in duplicate with the sample mean used as the ultimate analytic value. Coefficients of variation for pairs of analytic samples ranged from 0.0% to 3.8%.

Total household smoking level was calculated by summing the number of cigarettes smoked per day by each smoker using category midpoints. Data analyses were completed with SPSS (SPSS Inc, Chicago, IL). We first compared mean urinary cotinine level by household smoking (0, 1– 19 and ≥20 cigarettes per day) using one-way ANOVA. We then used multivariable linear regression to assess the relationship between number of cigarettes smoked per day in the household and urinary cotinine levels, after adjusting for covariates found to be related to cotinine level ($P < 0.15$).

Results

Thirty percent of the dogs in the study population were less than 5 years of age, and 29% were aged 10 or older. Approximately half (52%) of subjects were male, and 83% of dogs had been spayed or neutered. Overweight or obese body composition was reported for 38% of subjects.

We observed significant differences in canine urinary cotinine level by level of household cigarette smoking (Table 1). Cotinine level was approximately doubled in dogs exposed to any household cigarette smoking in the previous 24 hours compared to unexposed dogs (7.6 vs. 14.6 ng/mL; $P = 0.02$), and was also higher in the one dog exposed to cigar or pipe smoke but not cigarettes (14.0 ng/mL). Furthermore, mean cotinine level in dogs exposed to a pack or more of cigarettes per day were more than twice that of dogs exposed to less than a pack per day (22.5 vs. 10.4 ng/mL; $P = 0.001$).

The number of cigarettes smoked in the household in the previous 24 hours was significantly related to urinary cotinine level in linear regression analysis. Each one-category increase in the number of cigarettes smoked (0 vs. 1–19 vs. ≥20) was associated with a 6.61 ng/mL increase in cotinine level (beta = 6.61; STE = 1.70; $P < 0.001$). The significant linear relationship between cigarette smoking and cotinine persisted after adjustment for other factors found to be associated with canine cotinine level (beta = 6.04; STE = 1.22; $P < 0.001$; Table 2).

Several other factors were significantly associated with cotinine. In multivariable analyses, cotinine levels were higher in dogs whose owners self-applied lawn care chemicals compared to those reporting no application (beta = 4.18; $P = 0.01$), though use of professionally-applied lawn care chemicals was not associated with cotinine. Cotinine levels were higher in short-nosed dogs as compared to medium and long-nosed dogs (beta = 5.28; $P = 0.03$). Dog's age, body composition and hair length were also modestly associated with cotinine level. We did not find a relationship between urinary cotinine and other factors potentially related to dose of ETS, including dwelling size, hours per day the dog spent outdoors in the previous 24 hours, and household ventilation. Canine characteristics including sex, reproductive status and purebred status were also unrelated to cotinine level.

Discussion

To our knowledge, this is the first study to evaluate the relationship between pet owner report of household tobacco use and canine urinary cotinine levels. We observed measurable urinary cotinine levels in free living pet dogs exposed to environmental tobacco smoke in the home. Cotinine level increased significantly with the number of cigarettes smoked in the household in the 24 hours before urine collection.

Companion animals may serve as useful sentinels for risks associated with environmental exposures they share with humans. Pets living in households are likely exposed to the same environmental contaminants as their human owners, including ETS (Bukowski 1997). Both the routes of exposure and dose of ETS in companion animals may be physiologically relevant to humans. For example, dogs and cats may be exposed to ETS through: 1) inhalation of airborne smoke; 2) trans-dermal absorption of particulates and residues that may be concentrated on fur and skin after contact with carpets, furniture and bedding; and 3) oral ingestion during oral grooming of particulates deposited on their fur. In cats in particular, oral grooming may provide a substantial source of exposure (Bukowski 1997). Because exposure may be through these multiple routes, pets may serve as appropriate models for exposure patterns of young children in a household, who may have closer contact with contaminated carpeting and bedding than adults, and who may mouth contaminated objects (Stanek 1995).

Urinary cotinine levels of dogs exposed to household ETS in our study were similar to levels observed in previous studies of humans with similar exposure (Thompson 1990; Haley 1989). The standard cut-off values for the cotinine assay used are 3–10 ng/ml for no substantial exposure, 11 – 30 ng/ml for passive exposure or light smoking, and 30 – 500+ for active smoking (Knight 1989; Watts 1990). Levels in dogs were also similar to those observed previously in cats exposed to household ETS; mean urinary cotinine levels (ng/ml) in 70 cats exposed to 0, 1 – 19, and 20 or more cigarettes per day were 7.0 (SD 4.1), 12.6 (10.2), and 40.0 (20.8), respectively ($P < 0.001$) (Bertone-Johnson 2003, Bertone 2002).

Several other factors were associated with canine urinary cotinine, including age, body composition and use of owner-applied lawn care chemicals, which have also been associated with risk of cancer in previous studies in dogs (Sonnenschein 1991; Hayes 1991; Hayes 1995). In fact, lawn care chemical use is potentially an important source of nicotine exposure in dogs unexposed to ETS. In our study, among dogs not exposed to ETS, cotinine levels were non-significantly higher in the 12 dogs whose owners reported lawn care chemical use compared to the 14 dogs with no exposure (10.45 ng/ml vs. 6.34; $P = 0.20$). These results indicate the importance of collecting information on a variety of other demographic and environmental factors in studies of ETS and canine cancers to prevent confounding and evaluate effect modification. Lack of control for these factors, as well as small sample sizes, may explain in part non-significant findings in other case-control studies of ETS exposure and cancer risk in canine populations (Bukowski 1998).

Our results also suggested that cotinine levels may be higher in short-nosed (i.e., brachycephalic) dogs such as pugs, boxers and bulldogs, compared to medium (i.e., mesocephalic) and long-nosed (i.e. dolichocephalic) dogs exposed to the same level of ETS. ETS body burden may be greater in short-nosed dogs because less filtration of cigarette smoke occurs in the nasal cavity than in long-nosed breeds, such as collies, greyhounds and Dobermans. This finding is consistent with results from two case-control studies in dogs conducted by Reif and colleagues at Colorado State University. Interestingly, the authors found that ETS was associated with increased risk of lung cancer only in short-nosed breeds (Reif 1992), and was associated with risk of nasal and sinus cancer only in long-nosed breeds (Reif 1998). In long-nosed dogs with extensive nasal filtration, the tissues at greatest risk from ETS

exposure are likely those of the nasal and sinus cavities. In contrast, in short-nosed dogs and in humans, both of whom filter smoke less efficiently in the nasal passages, lung tissue likely has greater exposure to carcinogens in ETS.

In summary, findings from our study suggest that canine cotinine levels are significantly and linearly associated with household smoking level as assessed by pet owner-completed questionnaire. Epidemiologic studies of ETS and risk of cancer in companion animals using these methods may be important tools for further evaluating the health effects of environmental tobacco smoke exposure in humans.

Acknowledgements

Funding source: Supported by grant CA103513 from the National Institutes of Health

The protocol for this study was approved by human subjects and animal care and use committees at the University of Massachusetts and Tufts University.

References

Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol. Rev 1996;18:188–204. [PubMed: 9021312]

Bertone ER, Synder LA, Moore AS. Environmental tobacco smoke and risk of malignant lymphoma in pet cats. Am. J. Epidemiol 2002;156:268–273. [PubMed: 12142262]

Bertone-Johnson ER. The First Author Replies, RE: “Environmental tobacco smoke and risk of malignant lymphoma in pet cats”. Am. J. Epidemiol 2003;158:1227–1230. [PubMed: 14692044]

Brazell RS, Stiff AG, Henderson GM, Henkins RA, Romig PL, Auerbach O. Plasma nicotine and cotinine in tobacco smoke exposed beagle dogs. Toxicol. Applied Pharmacol 1984;73:152–158.

Bukowski JA, Wartenberg D. An alternative approach for investigating the carcinogenicity of indoor air pollution: pets as sentinels of environmental cancer risk. Environ. Health Perspect 1997;105:1312–1319. [PubMed: 9405322]

Bukowski JA, Wartenberg D, Goldschmidt M. Environmental causes for sinonasal cancers in pet dogs, and their usefulness as sentinels of indoor cancer risk. J. Toxicol. Environ. Health 1998;54:579–591.

Chilmonczyk BA, Knight GJ, Palomaki GE, Pulkkinen AJ, Williams J, Haddow JE. Environmental tobacco smoke exposure during infancy. Am. J. Public Health 1990;80:1205–1208. [PubMed: 2400031]

Emmons KM, Abrams DB, Marshall R, Marcus BH, Kane M, Novotny TE, Etzel RA. An evaluation of the relationship between self-reported and biochemical measures of environmental tobacco smoke exposure. Prev. Med 1994;23:35–39. [PubMed: 8016030]

Glickman LT, Schofer FS, McKee LJ, Reif JS, Goldschmidt MH. Epidemiologic study of insecticide exposures, obesity, and risk of bladder cancer in household dogs. J. Toxicol. Environ. Health 1989;28:407–414. [PubMed: 2593174]

Gollenberg, AL.; Bertone-Johnson, ER. Passive smoking and cancer risk in companion animals: sentinels for health effects in humans. In: Jeorgensen, NA., editor. Passive smoking and Health Research. Hauppauge NY: Nova Science Publishers; 2006. p. 185-214.

Haley NJ, Colosimo SG, Axelrad CM, Harris R, Sepkovic DW. Biochemical validation of self-reported exposure to environmental tobacco smoke. Environ. Res 1989;49:127–135. [PubMed: 2721473]

Hayes HM, Tarone RE, Cantor KP, Jessen CR, McCurnin DM, Richardson RC. Case-control study of canine malignant lymphoma: Positive association with dog owner’s use of 2,4-dicholorophenoxyacetic acid herbicides. J. Natl. Cancer. Inst 1991;83:1226–1231. [PubMed: 1870148]

Hayes HM, Tarone RE, Cantor KP. On the association between canine malignant lymphoma and opportunity for exposure to 2,4-dicholorophenoxyacetic acid. Environ. Res 1995;70:119–125. [PubMed: 8674480]

Kelsey JL, Moore AS, Glickman LT. Epidemiologic studies of risk factors for cancer in pet dogs. Epidemiol. Rev 1998;20:204–217. [PubMed: 9919439]

Knight GJ, Palomaki GE, Lea DH, Haddow JE. Exposure to environmental tobacco smoke measured by cotinine 125I-radioimmuoassay. Clin. Chem 1989;35:1036–1039. [PubMed: 2731343]

Reif JS, Dunn K, Ogilvie GK, Harris CK. Passive smoking and canine lung cancer risk. Am. J. Epidemiol 1992;135:234–239. [PubMed: 1546698]

Reif JS, Bruns C, Lower KS. Cancer of the nasal cavity and paranasal sinuses and exposure to environmental tobacco smoke in pet dogs. Am. J. Epidemiol 1998;147:488–492. [PubMed: 9525536]

Sonnenschein EG, Glickman LT, Goldschmidt MH, McKee LJ. Body conformation, diet, and risk of breast cancer in pet dogs: a case-control study. Am. J. Epidemiol 1991;133:694–703. [PubMed: 2018024]

Stanek EJ III, Calabrese EJ. Daily estimates of soil ingestion in children. Environ. Health. Perspect 1995;103:276–285. [PubMed: 7768230]

Thompson SG, Stone R, Nanchahal K, Wald NJ. Relation of urinary cotinine concentrations to cigarette smoking and to other people's smoke. Thorax 1990;45:356–361. [PubMed: 2382242]

Vail DM, MacEwen EG. Spontaneously occurring tumors of companion animals as models for human cancer. Cancer Invest 2000;18:781–792. [PubMed: 11107448]

Watts RR, Lagone JJ, Knight GJ, Lewtas J. Cotinine analytical workshop report: Consideration of analytical methods for determining cotinine in human body fluids as a measure of passive exposure to tobacco smoke. Environ. Health Perspect 1990;84:173–182. [PubMed: 2190812]

Abbreviations

ETS, environmental tobacco smoke.

Table 1

Mean canine urinary cotinine level by household smoking level in the 24 hours before urine collection (n = 63).

Household smoking level	n	Mean (STD) urinary cotinine (ng/mL)	P
Household smoking in last 24 hours			
None	33	7.6 (7.1)	0.02
Cigarettes	29	14.6 (13.9)	
Pipe/Cigar only	1	14.0 (−)	
Number of cigarettes smoked in last 24 hours*			
None	33	7.6 (7.1)	0.001
1 – 19	19	10.4 (6.3)	
≥ 20	10	22.5 (20.4)	

* Excludes 1 dog exposed to pipe/cigar smoke only

Table 2

Relationship between household cigarette smoking in the previous 24 hours and canine urinary cotinine level in multivariable linear regression.

Characteristic*	Beta	STE(Beta)	P-value
Cigarettes smoked in household in last 24 hours (0, 1 – 19, ≥ 20)	6.04	1.22	< 0.001
Owner-applied lawn care chemical use (yes vs. no)	4.18	1.55	0.01
Age (< 5, 5 – 9, 10 – 14, ≥ 15 years)	−2.50	0.90	0.01
Short nose length (vs. medium/long length)	5.28	2.33	0.03
Sex (female vs. male)	−2.70	1.55	0.09
Overweight body composition (vs. thin/normal weight)	−2.70	1.68	0.12
Longer hair length (vs. shorter length)	2.66	1.73	0.14

* Each characteristic adjusted for the effects of all other factors.