

# Effects of Diet on Fecal Occult Blood Testing in Healthy Dogs

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## ABSTRACT

Six dogs were fed each of nine diets to evaluate the effects of diet on fecal occult blood test results. The diets represented a range of different type (i.e. canned, dry or semi-moist), protein and vegetable constituents, and fiber contents. Each diet was fed twice daily for five consecutive days; fecal samples were collected twice daily on days 4 and 5. An o-tolidine test kit and a guaiac paper test kit for fecal occult blood were used.

Two hundred and sixteen fecal samples were analyzed (24 samples/diet). When using the guaiac test the following positive results were obtained from fecal samples from dogs consuming a canned meat- and vegetable-based diet (24/24 samples); a canned meat-based diet (24/24 samples); a dry corn and poultry-based diet (9/24 samples); a dry corn, wheat, and meat meal diet (4/24 samples), a canned poultry-based diet (1/24 sample) and a semi-moist soybean meal-based diet (2/24 samples). A total of 64 samples were positive using the guaiac test. Using the o-tolidine test, no samples were positive. The difference between the number of positive results with each test kit was highly significant ( $p < 0.001$ ). Results indicate that 1) diet affects the specificity of guaiac test fecal occult blood results in the dog and 2) positive o-tolidine test results were not caused by diets fed in the study.

## RÉSUMÉ

Six chiens ont été nourris avec neuf diètes différentes afin d'évaluer l'effet de celles-ci sur la détec-

tion de sang occulte dans les fèces. Les diètes étaient diversifiées quant à leur présentation (en conserve, sèche, semi-humide), et leur contenu en protéines, éléments végétaux et proportion en fibres. Les chiens étaient nourris deux fois par jour pendant cinq jours consécutifs; des échantillons de fèces ont été recueillis deux fois par jour aux jours 4 et 5. La présence de sang occulte a été évaluée par un test utilisant la o-toluidine et par un test utilisant le papier guaiac.

Un total de 216 échantillons ont été analysés (24 échantillons/diète). Avec l'utilisation de papier guaiac, des résultats positifs ont été obtenus des échantillons de fèces provenant des chiens ayant consommé une diète à base de viande en conserve et de végétaux (24/24 échantillons); pour les diètes en conserve à base de viande (24/24 échantillons); pour les diètes à base de maïs séché et de poulet (9/24); pour les diètes à base de maïs séché, de blé et de viande (4/24 échantillons); pour une diète à base de poulet en conserve (1/24 échantillons); et pour une diète semi-humide à base de fèves de soya (2/24 échantillons). Au total, 64 échantillons ont été trouvés positifs en utilisant le test au papier guaiac. Par contre, le test avec la o-toluidine n'a produit aucun résultat positif. La différence entre les deux tests en ce qui concerne le nombre de résultats positifs était fortement significative ( $p < 0,001$ ). Les résultats indiquent que 1 — la diète a une influence sur la spécificité du test au papier guaiac pour la détection de sang occulte chez le chien et, 2 — des résultats positifs avec le test utilisant de la o-toluidine n'ont pas été détectés dans la présente étude suite à l'ingestion des

différentes diètes. (Traduit par Dr Rocky DiFruscia)

## INTRODUCTION

The ability to identify occult fecal blood is important in human medicine for the screening of individuals for gastrointestinal (GI) hemorrhage from neoplasia or other GI disease (1–5). In veterinary medicine, fecal occult blood testing is most commonly used in evaluation of anemic patients, patients with overt GI disease, or patients receiving medication which may initiate the GI bleeding. Although melena is commonly used as a clinical indicator of GI bleeding, several studies have shown that fecal occult blood tests can reliably detect quantities of blood much smaller than those required to cause melena (6–8). However, many of these tests can be influenced by diet (2,9–11).

In the past, it was suggested that canine patients should be fed a meatless/low peroxidase diet (e.g. cottage cheese and rice) for a few days prior to and during fecal occult blood testing to ensure that a positive test was not caused by diet (12). An alternative was to perform an initial test without dietary restrictions, and if the results were positive, then restrict the diet prior to a second test. A diagnosis of GI hemorrhage was confirmed only if the second test result was positive. Recently, Cook *et al* (13) evaluated the effect of several diets on results of an o-tolidine tablet test (Hematest, Miles Diagnostic Division, Elkhart, Indiana) and a modified guaiac paper test (Hemoccult II, Smith-Kline Diagnostics Inc., Sunnyvale, California) for fecal occult blood; they found that only consumption of a canned mutton and rice diet resulted in

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**TABLE 1. Main ingredients and results of o-tolidine tablet test (n = 24) and guaiac paper test (n = 24) in eight healthy dogs fed nine diets**

Diet	Type	Diet characteristics	Fecal occult blood test results	
		Main ingredients	Guaiac	O-tolidine
A	Canned	Beef and meat by-products; chicken and poultry by-products, carrots, potatoes, peas (Pedigree Pal Beef Stew, Effem Foods Ltd, Bolton, Ontario)	24*	0
B	Canned	Beef and meat by-products; chicken by-products, liver, heart (Pedigree Pal Extra Beef, Effem Foods Ltd, Bolton, Ontario)	24*	0
C	Dry	Corn, poultry by-products, oat flour, soybean meal (Cycle Adult Formula, Quaker Oats Company of Canada Ltd, Peterborough, Ontario)	9*	0
D	Dry	Corn, wheat, meat and bone meal, soybean meal (Purina Dog Chow Adult Formula, Ralston Purina Canada Inc, Mississauga, Ontario)	4*	0
E	Dry	Lamb, rice, flour, corn, egg (Iams Lamb and Rice, Iams Company, Dayton, Ohio)	0	0
F	Canned	Poultry by-products, meat by-products, beef by-products, corn, wheat (Pep Chicken Flavored, Quaker Oats Company of Canada Ltd, Peterborough, Ontario)	1	0
G	Semi-moist	Soybean meal, corn syrup, corn, beef (Gaines Burgers, Gaines Pet Foods, Cobourg, Ontario)	2	0
H	Dry	Corn, peanut hulls, poultry meal, soybean mill run, soybean meal (Prescription Diet r/d, Hill's Pet Products, Mississauga, Ontario)	0	0
I	Dry	Meat meal, soybean meal, egg (Science Diet Canine Maintenance Dry, Hill's Pet Products, Mississauga, Ontario)	0	0

\*Chi-square test,  $p < 0.05$

a significant number of false-positive results.

It is not known if other diets, including semi-moist, high-fiber, and vegetable-containing diets, influence fecal occult blood test results. The purpose of the study reported here was to investigate the effect of several different diets on results of two tests for fecal occult blood in the dog.

## MATERIALS AND METHODS

Six laboratory beagles (11–13 kg) were used for the study. Results of a complete blood count, serum biochemical profile, urinalysis and physical examination were within normal limits. To ensure the dogs were free of GI parasites, each dog was treated with fenbendazole (50 mg fenbendazole/kg body weight PO q24h for three days) and metronidazole (25 mg metronidazole/kg body weight PO q12h for five days). Subsequent fecal examinations for parasites were negative. The dogs were housed individually in cement pens and were given water ad libitum. All dogs tested negative for fecal occult blood on three consecutive analyses using both fecal occult blood test kits prior to entry into the study. The dogs were fed a rice and cottage cheese diet during this time. Based on these results it was assumed that the dogs were negative for fecal occult blood.

Nine popular commercial diets were chosen on the basis of differences in diet type (i.e. canned, dry or semi-moist), protein and vegetable

constituents, and fiber content (Table I). Each diet was fed twice daily at the caloric level recommended by the manufacturers. Each diet was fed for five consecutive days and all dog food was consumed. The dogs were fed each diet for 72 hours to allow acclimatization to the new diet and to ensure clearing of feces produced from the previous diet (14) and then fecal samples were collected twice daily on days 4 and 5 of each diet period for a total of four fecal samples per dog per diet. Diets were fed in a randomized sequence with all dogs consuming the same diet at one time. The protocol used was approved by the University of Prince Edward Island Animal Care Committee in accordance with guidelines of the Canadian Council on Animal Care.

An o-tolidine tablet test (Hematest, Miles Canada Inc., Etobicoke, Ontario) and a guaiac paper test (ColoScreen, Helena Laboratories, Beaumont, Texas) were used to test for occult fecal blood. Analyses were performed and evaluated according to the manufacturers' instructions. The o-tolidine test is based on hemoglobin's peroxidase-like ability to catalyze the oxidation of a chromogen, tetramethylbenzidine. The net result is a blue color change. Any blue color, as assessed by visual inspection, which developed on the paper at the periphery of the test tablet within 120 seconds was considered positive. Any color that developed on or directly under the tablet or that developed after 120 seconds was not considered a positive result. This test

generally detects 6 mg of hemoglobin per gram of feces. The guaiac test is also based on hemoglobin's peroxidase-like ability; hemoglobin will oxidize phenolic compounds in guaiac to quinones. Development of a blue color on the test paper following addition of the developer solution was considered a positive result. This test generally detects 10 mg of hemoglobin per gram of feces. All tests were performed by the same investigator (JER).

Chi-square tests were used to identify associations between occult blood tests results and diet (15). McNemar's test was used to compare results from the two test methods (15).

## RESULTS

Two hundred and sixteen fecal samples were analyzed (24 samples/diet). All were grossly normal. The results of the fecal occult blood testing are summarized in Table I. Using the o-tolidine test, no samples had positive results. Using the guaiac test, 64 samples had positive results. The difference between the number of positive results with each test kit was highly significant ( $p < 0.001$ ). Using the guaiac test, fecal samples from dogs consuming diet A (24/24), diet B (24/24), diet C (9/24), diet D (4/24), diet F (1/24) and diet G (2/24) were positive for occult blood. The number of positive samples from diets A ( $p < 0.001$ ), B ( $p < 0.001$ ), C ( $p < 0.05$ ) and D ( $p < 0.05$ ) was significant. No samples from diets E, H or I were pos-

itive. Intra-dog variability in test results was observed.

## DISCUSSION

Previous studies strongly suggest that for the fecal occult blood screening tests to be valid in humans, certain dietary restrictions must be in place (2,9–11). Without these restrictions, a positive test could be due to GI hemorrhage but could also result from consumption of meats or foods containing peroxidases (e.g. fruits and vegetables) (8–9,16). Consumption of a high-fiber diet may decrease (17) or increase (18) false-negative test results. In addition, aspirin (5,19) and iron compounds (20) have been shown to cause false positive results while vitamin C (3) has been shown to cause false negative results.

Much of the confusion about dietary recommendations for fecal occult blood testing in dogs stems from the paucity of clinical studies of the effects of diet on these tests (13). False-positive test results due to meat or peroxidase-containing constituents in the diet can result in added time, expense, and even risk to the patient as a result of diagnostic procedures performed for further investigation of GI hemorrhage. Conversely, false-negative results could result in sub-clinical hemorrhage being missed. However, insistence on extremely rigorous dietary restrictions may also adversely affect client compliance.

In this study, the effects of diet varied based on the test used for detection of fecal occult blood. All the o-tolidine test results were negative regardless of the diet fed. These results would suggest that dietary restrictions are unnecessary when testing dogs for fecal occult blood using this test. However, the lack of false-positive results with the o-tolidine test may be due to a higher threshold for peroxidase detection of occult blood. Inclusion of a positive control would have allowed assessment of possible false-negative results.

The guaiac test results were more variable. The positive guaiac test results seen on testing of feces from dogs consuming several diets suggest that this test is more likely to be positive in the presence of low levels of

peroxidase or peroxidase-like activity than is the o-tolidine test. This is in contrast to the levels of hemoglobin detected by each test as reported by the tests' manufacturers. However, the positive results obtained in our study were also presumed to be false-positive results since the dogs were all healthy and had previously been shown to be negative for fecal occult blood. Because diet was the only variable altered between test periods, the false-positive results are likely due to either peroxidase, hemoglobin, or other peroxidase-like compounds in the foods (8–9, 13,16). In particular, canned diets containing a high proportion of meat products and some dry commercial diets (Diets C and D) were associated with a significant increase in positive fecal occult blood test results. A semi-moist diet (Diet G) and a canned diet containing less meat and more poultry products (Diet F) resulted in fewer positive results.

The only diets that failed to produce any false-positive results with either test in this study were diets E, H and I. Diet H was chosen for its increased fiber content. Dilution of fecal hemoglobin by the increased fecal volume may be responsible for the negative results (17). As with evaluation of the o-tolidine test sensitivity, inclusion of a positive control would have allowed assessment of possible false-negative results occurring with increased dietary fiber. Feces from dogs consuming diet E (lamb meat and rice) also all tested negative for occult blood. This was interesting when compared to a previous study in which consumption of a canned mutton and rice diet resulted in an increased number of fecal samples testing positive for blood (13). In that study, the increased peroxidase activity of mutton was suggested to be responsible for the false-positive results (21). It is possible that the different processing of the dry lamb and rice diet used in our study may have inactivated the peroxidase activity of the lamb. It is also possible that a component of the canned mutton and rice diet other than the mutton was responsible for the positive results in the previous study. The reason for a lack of positive results with feeding of diet I as compared to results with feeding of other dry commercial diets (Diets C and D) is also not known,

although diets C and D have corn as a primary ingredient (and possible source of peroxidase activity) while meat meal is the primary ingredient in diet I. Diet H, which caused no positive results, also contains corn but as a lesser component of the diet and in the presence of increased fiber.

Tests for fecal occult blood do not have 100% sensitivity and specificity (2,4–5,8–10,13,16,20,21). Results of this study and others (2,6,9–11,13, 16,21) suggest that both the diet fed and the test procedure used can influence test results. Restriction of the diet for several days prior to and during testing can lessen the chance of obtaining false-positive results. If using a guaiac test, feeding of a meat-free, low-peroxidase diet such as rice and cottage cheese is recommended during this period of dietary restriction. If patient acceptance of this diet is poor, a period of dietary restriction using another diet [e.g. specific dry cereal based dog foods (Prescription Diet r/d Dry, Hills's Pet Products, Mississauga, Ontario; Science Diet Canine Maintenance Dry, Hill's Pet Products; Purina Dog Chow, Ralston Purina Co., St. Louis, Missouri (13); a dry lamb and rice based diet (Iams Lamb and Rice, The Iams Company, Dayton, Ohio)] shown to have less influence on guaiac fecal occult test results can also be considered. Care must be taken to ensure that the fecal occult blood test kit being utilized has been evaluated with the specific diet, however. The sensitivity of the guaiac test utilized in the study (ColoScreen Helena Laboratories) has not been determined.

Compared to the guaiac test, the o-tolidine test was more specific in this study. If a positive result is obtained from a dog being fed one of the test diets, the dog is likely truly positive for occult fecal blood. The sensitivity of this test has been reported to be 33% in six dogs fed a dry cereal-based dog food (Science Diet Canine Maintenance Dry, Hill's Pet Products) and given 5 mg hemoglobin/kg orally, 67% in dogs given 10 mg hemoglobin/kg orally (the level of detection reported by the manufacturer), and 100% in dogs given 20 mg hemoglobin/kg orally (7). These results and the results of the study reported here would suggest that the o-tolidine test (Hematest, Miles

Canada Inc.) is a specific and moderately sensitive test for fecal occult blood in the dog when dietary restriction is used. Definitive recommendations concerning the optimal fecal occult blood test in the dog must await determination of the desired level of fecal hemoglobin detection and the availability of test sensitivity data for dogs with naturally occurring disease or in dogs consuming other diets.

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