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The swab-sampled dry fecal cytology in healthy dogs and in dogs with acute and chronic diarrhea: a pilot study

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Abstract

Dry-mount fecal cytology is a little explored procedure that is potentially useful for dogs with gastrointestinal diseases. This study describes fecal cytology using swab sampling in healthy dogs (HD) and in dogs with acute and chronic diarrhea. Forty HD, 40 dogs with acute diarrhea (AD; diarrhea < 5 days) and 40 dogs with chronic diarrhea (CD; diarrhea > 3 weeks) were also enrolled. Sixteen different cytological features were scored for each fecal sample. Twenty HD were used to establish normal reference range for each cytological feature. Exact tests were used to evaluate associations between the three groups. The presence of red blood cells, cocci bacterial monomorphism and spore-forming bacteria were significantly higher in AD than the HD. Neutrophils, lymphocytes and macrophages were significantly higher in AD and CD groups compared to HD. Plasma cells were significantly higher in CD and the HD and AD groups. Bacterial phagocytosis was significantly higher in AD group than CD and HD. In conclusion, swab-sampled fecal cytology, as a cheap and feasible procedure, can be a useful diagnostic and monitoring technique. Although, our pilot study results showed differences between HD and enteropathic dogs, further investigations are necessary.

Key Words: canine, diarrhea, dry-mount fecal cytology, rectal swab

Introduction

Fecal cytology (FC) is used for fecal examination in dogs with gastrointestinal symptoms [2,9]. There are two types of FC: wet-mount FC and dry-mount fecal cytology (DFC). Wet-mount FC consists of the microscopic observation of a fresh stool smear, which ideally is less than five minutes old. Wet-mount FC is suitable for the detection of *Giardia* spp., trichomonides and amoebae, as well as larvae of nematodes (e.g., *Strongiloides* spp.) and bacteria such as *Campylobacter* spp. [2,4]. With DFC, the smear can be collected by various sampling methods [4] (e.g., rectal scraping, rectal lavage and digital examination) and then is air dried and stained. This method is useful for identifying infectious agents (e.g., *Prototheca* spp., *Cryptococcus* spp.), inflammatory or neoplastic cells, and for evaluating the cytological features of the intestinal microbiota [2,5,9,10].

In the DFC of healthy dogs (HD), an extremely polymorphic population of several different bacilli

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Table 1. Scoring system for fecal cytological feature evaluation.

Cytological features	Power field	Category	
		Normal	Abnormal
Squamous cells	10×	<50	≥50
Columnar cells	10×	<20	≥20
Neutrophils	10×	<10	≥10
Eosinophils	10×	<5	≥5
Lymphocytes	10×	<10	≥10
Plasma cells	10×	<5	≥5
Macrophages	10×	<5	≥5
Immature lymphoid cells	10×	<10	≥10
Red blood cells	40×	Absent	Present
Cyniclomyces spp.	40×	<10	≥10
Yeasts	40×	<30	≥30
Presence of cocci bacterial monomorphism*	100×	Absent	Present
Gull-wing-shaped bacteria	100×	<5	≥5
Spiral-shaped bacteria	100×	<5	≥5
Spore-forming bacteria	100×	<5	≥5
Bacterial phagocytosis	100×	Absent	Present

For cytological features observed with a 10× magnification, all the smear was examined.
For cytological features observed with a 40× and 100× magnification, ten random fields were examined.
* More than 50% of cocci bacteria

is typically observed [4]. There should be fewer than five spore-forming rods/100× field, and cocci should be rare or absent [2,4,11]. Occasionally, a low number of individual or doublet *Cyniclomyces* spp. may be observed, but its real pathogenic power is still unclear [4,6,11]. A monomorphic or oligomorphic bacterial population, an increased number of cocci or an increased amount of yeast (e.g., *Candida*, *Cyniclomyces* spp.) are found in dysbiosis [11]. The presence of neutrophils is abnormal and may be associated with distal colitis or proctitis [2,11]. Fecal cytological patterns in HD have already been reported using several cytological sampling techniques [4]. In Frezoulis' study, FC features collected by various methods, including digital examination (DE), rectal scraping (RS) and rectal lavage (RL) in HD and dogs with diarrhea, are described. In HD, the median number of isolated epithelial cells and lymphocytes was higher when RS was used. This may be because RS is a more aggressive procedure compared to DE and RL, and may collect greater numbers of lymphocytes from the colic lymphoid follicles and columnar epithelial cells from the mucosa [4]. RS may be preferable when the deeper mucosal layers need to be sampled, such as for the diagnosis of colorectal lymphoma [4]. In dogs with diarrhea, the median cluster of epithelial cells and the number of neutrophils in fecal samples have been found to be different among sampling methods. In particular, the presence of clusters of epithelial cells and neutrophils have been shown to be more frequent in RS than in DE and RL [4].

Since there are no available data on swab-sampled DFC on both healthy dogs and dogs with acute and chronic diarrhea, the first aim is to describe swab-sampled DFC features in these populations. In addition, since our second hypothesis is that in dogs with acute and chronic diarrhea may have different various population of inflammatory cells one of each other and different compared to healthy dogs, the second aim is to evaluate potential differences in swab-sampled

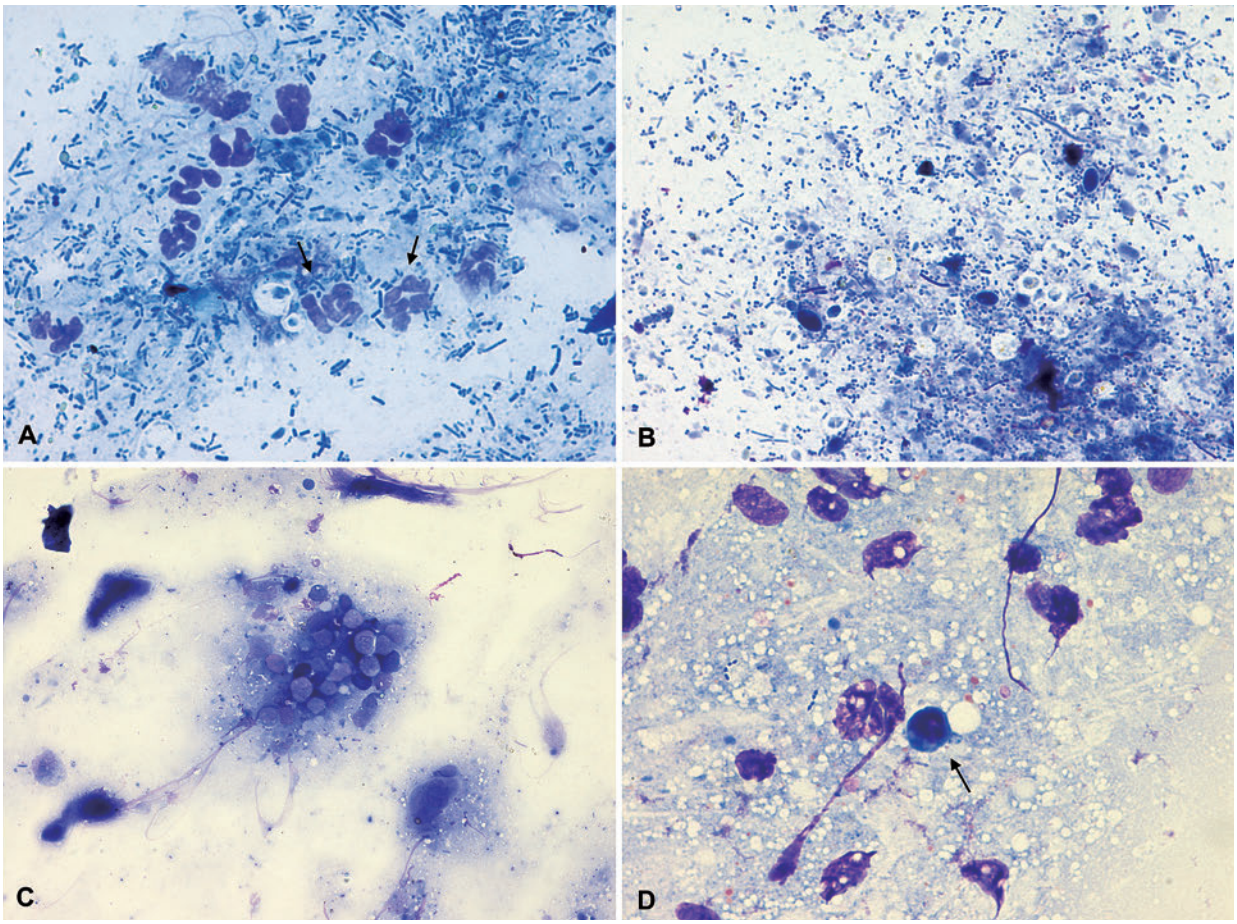

Fig. 1.
Dry-mount fecal cytology findings in canine acute (AD) and chronic diarrhea (CD). Diff Quik® stain. (a) Bacterial phagocytosis. An aggregate of some neutrophils in active phagocytosis (arrows) is present among the microbial flora of an AE dog. 100×, oil. (b) Cocci bacterial monomorphism in AD dog. Abnormal bacterial flora with cocci monomorphism. 100×, oil. (c) Lymphocytes. An aggregate of small lymphocytes from an CD dog. 40×. (d) Plasma cell. An isolated plasm cell in a pale basophilic background with some bacteria from a CD dog. 40×.

DFC between the three study populations.

Materials and Methods

Healthy dogs

Twenty HD were initially used to determine the reference range for each cytological feature (Table 1, column "normal"). A control group was recruited from HD identified during routine physical examination for vaccination. In all dogs, a DFC via rectal swab was performed. A plastic swab was moistened with sterile saline solution, inserted in the rectal ampulla with an inclination of 45° for 1 to 4 cm (according to the size of the patient), rotated on the mucosal surface for 4-5 times, extracted and rolled onto glass slides for cytology. The rectal swab was always performed by the same operator and only one smear for each patient was performed. All smears were stained with a Romanowsky stain (Diff Quik® Bio Optica Milano S.p.A.- Milan, Italy) and examined by a cytopathologist using a light-microscope (Leica DM LB Microscope, Leica Microsystems Srl, Milan, Italy). The highest number for each specific cytological feature detected in the first HD was used as the upper limit of the reference range for that cytological feature. Regarding *Cyniclomyces* spp. [6], gull-wing-shaped

bacteria [2,11], spiral-shaped bacteria [11] and yeasts [11], previously published reference ranges were used. All patients were evaluated using a scoring system established for 16 cytological features (Table 1). The entire smear was evaluated at 10× to estimate the amount of squamous and columnar cells, neutrophils, eosinophils, lymphocytes, plasma cells, macrophages and immature lymphoid cells. The *Cyniclomyces* spp. is a yeast and morphologically identified by its "spectacle case" shape; the length is approximately 1/5th of the diameter of a *Toxocara canis* egg [6]. Presence of red blood cells (RBCs) and number of *Cyniclomyces* spp. and other round-shape yeasts were assessed at 40× magnification in 10 random fields (Table 1). Presence of cocci or rod-shaped bacterial monomorphism and bacterial phagocytosis (Fig. 1-a) and the number of gull-wing-shaped bacteria, spiral-shaped bacteria and spore-forming bacteria were evaluated at 100× magnification in 10 random fields. Cocci bacterial monomorphism was evaluated when cocci exceeded 50% in 10 random fields. (Table 1; Fig. 1-b).

Dogs with acute and chronic diarrhea

Client-owned dogs were enrolled with a diagnosis of acute diarrhea (AD) and chronic diarrhea (CD) that had been referred to our internal medicine service. For each dog, signalment and clinical history were collected and signed informed consent was obtained from their owners. The AD group included dogs with a history of diarrhea for less than five days. The CD group included dogs with a history of diarrhea for at least three weeks. Each CD dog had a hematobiochemical profile, urinalysis, fecal panel and diagnostic imaging performed as a part of routine care. In CD dogs other extra-intestinal diseases, infectious or parasitic diseases and intestinal disease of other etiology (e.g., mechanical obstruction from intussusception, foreign body or intestinal tumors) were ruled out. In all patients, FC was performed at admission or at the time of endoscopy before anesthesia was induced. Dogs with positive fecal flotation or positive in-clinic Giardia test (SNAP® Giardia test, Idexx Laboratories Inc. Europe, Hoofddorp, Netherlands) were excluded. In both groups, administration of symptomatic treatment (spasmolytic, adsorbent, antiemetic or antacid drugs and probiotics) and fleas and tick preventive drugs were permitted. In the AD group, dogs already on antibiotic therapy were excluded. In the CD group, antibiotics, probiotics and immunosuppressive drugs had to have been discontinued at least ten days before the endoscopy. Each dog in CD group had a final diagnosis of chronic lymphoplasmacytic enteritis confirmed at the histopathologic evaluation[3]. Dogs with intestinal lymphoma and with colorectal neoplasia were excluded.

Normal fecal cytological scores were obtained from the HD. The age of the three groups were tested with the D'Agostino Pearson normality test and then compared between the three groups using Kruskal-Wallis test. Fecal cytological features were compared between the three study populations using Chi-square test and then compared between the three pairs of groups (HD vs. AD, HD vs. CD, AD vs. CD) using Fisher's exact test [8]. A P-value < 0.05 was considered significant (GraphPad Prism 7 for Windows, La Jolla, CA).

Results

One hundred and twenty dogs were enrolled and divided into three groups. Forty dogs were included in the HD group, 40 in the AD, and 40 in the CD group. The median age for all dogs was six years (range 1-17 years) and was similar to the three study groups (HD, AD and CD; $P > 0.05$). Fifty-six female dogs (17 spayed) and 64 male dogs (4 neutered) were included in this study and distribution of sex and sexual status was similar between all groups.

Of the 120 dogs included in the study, 51 were mix-breed. The involved breeds were: German Sheperd (13), Labrador Retriever (6), English Setter (5), Cavalier King Charles Spaniel (4), Cocker Spaniel (4), Golden Retriever (3), Jack Russel Terrier (3), West Highland White Terrier

Table 2. Fecal cytological scores for each parameter observed in the three study groups.

Cytological parameters	HD	AD	CD	*P*-value
Presence of RBC	0/40 (0%) [A]	11/40 (27.5%) [B]	4/40 (10%) [A]	0.0008
Squamous cells	0/40 (0%)	0/40 (0%)	0/40 (0%)	NE
Columnar cells	0/40 (0%)	0/40 (0%)	0/40 (0%)	NE
Neutrophils	0/40 (0%) [A]	36/40 (90%) [B]	29/40 (72.5%) [B]	<0.0001
Eosinophils	0/40 (0%)	2/40 (5%)	2/40 (5%)	0.3554
Lymphocytes	0/40 (0%) [A]	7/40 (17.5%) [B]	14/40 (35%) [B]	0.0002
Plasma cells	0/40 (0%) [A]	3/40 (7.5%) [A]	14/40 (35%) [B]	<0.0001
Macrophages	1/40 (2.5%) [A]	9/40 (22.5%) [B]	12/40 (30%) [B]	0.004
Immature lymphoid cells	0/40 (0%)	1/40 (2.5%)	1/40 (2.5%)	0.6014
Cyniclomyces guttulatus	1/40 (2.5%)	1/40 (2.5%)	3/40 (7.5%)	0.4340
Yeasts	14/40 (35%)	12/40 (30%)	15/40 (37.5%)	0.7716
Presence of cocci bacterial monomorphism	0/40 (0%) [A]	12/40 (30%) [B]	4/40 (10%) [A]	0.0003
Gull-wing-shaped bacteria	0/40 (0%) [A]	5/40 (12.5%) [A]	0/40 (0%) [A]	0.0054
Spiral-shaped bacteria	0/40 (0%)	1/40 (2.5%)	1/40 (2.5%)	0.6014
Spore-forming bacteria	2/40 (5%) [A]	9/40 (22.5%) [B]	2/40 (5%) [A]	0.01
Bacterial phagocytosis	0/40 (0%) [A]	25/40 (62.5%) [B]	9/40 (22.5%) [C]	<0.0001

AD = acute diarrhea; CD = chronic diarrhea; HD = healthy dogs; NE = not evaluable; RBC = red blood cell. Fecal cytological scores for each feature were analyzed with Chi-square test between the three groups and if $P < 0.05$ was considered significant. A, B, C, two groups with different letters have a statistically significant difference in the post-hoc Fisher's exact test.

(2), Border Collie (2), Pug (2), Dogo Argentino (2), Shih tzu (2), Rottweiler (2), Poodle (2), Dogue de Bordeaux (2), Yorkshire Terrier (2), Dobermann Pinscher (1), Maremma Sheperd (1), Dalmatian dog (1), Hound (1), Briard (1), Chihuahua (1), Berger Blanc Suisse (1), Maltese (1), Dachshund (1), Beagle (1), Bull Terrier (1), Pinscher (1), Weimaraner (1).

Epithelial cells (squamous and columnar cells), eosinophils, immature lymphoid cells, *Cyniclomyces* spp., other yeasts and spiral-shaped bacteria showed no differences between the three groups, as showed in Table 2.

The presence of RBCs, neutrophils, lymphocytes, plasma cells, macrophages, cocci bacterial monomorphism, as well as gull-wing-shaped bacteria, spore-forming bacteria and bacterial phagocytosis were significantly different between the three groups (Table 2).

In the HD group, no inflammatory cells were observed, except for few macrophages in the sample from one dog. The samples from 35% of dogs in the HD group contained some yeasts, and *Cyniclomyces* spp. was identified in one case. Moreover, samples from two dogs contained increased spore-forming bacteria (Table 2).

The presence of RBCs, cocci bacterial monomorphism and spore-forming bacteria were significantly different between the HD and AD groups ($P = 0.0004$, $P = 0.0002$ and $P = 0.0476$, respectively). HD dogs had significantly different neutrophils, lymphocytes (Fig. 1-c) and macrophages both compared to AD ($P < 0.0001$, $P = 0.0117$, $P = 0.0143$, respectively) and CD groups ($P < 0.0001$, $P < 0.0001$ and $P = 0.0009$, respectively). Plasma cells were significantly different between CD and the HD and AD groups ($P < 0.0001$ and $P = 0.0052$, respectively; Fig. 1-d). Gull-wing-shaped bacteria and bacterial phagocytosis were significantly different between the three groups ($P = 0.0054$ and $P < 0.0001$, respectively). However, gull-wing-shaped bacteria scores were compared between pairs of groups and showed no significant differences. Bacterial phagocytosis scores were significantly different between the three groups (HD

vs AD $P < 0.0001$, HD vs CD $P = 0.0024$, AD vs CD $P = 0.0006$, respectively). Data regarding the scores of each individual cytological feature are shown in Table 2.

Discussion

Dry-mount fecal cytology is not commonly used in clinical practice. To date, few studies have described the technique and interpretation of results [2,4].

In the present study, RBCs and inflammatory cells were absent in HD. In a recent study on thirty-seven healthy dogs, neutrophils and lymphocytes were detected when fecal cytology was collected by DE and RS, however, when RL was used, no RBCs and inflammatory cells were identified in the sample [4]. These findings suggested that more aggressive procedures, such as DE and RS, can lead to falsely increased blood-derived cell levels, making the interpretation of results challenging. In the present study, fecal cytological samples were collected using a moistened plastic swab, which, in the authors' opinion, may be less traumatic than DE and RS, thus reducing the risk of over-diagnosing inflammatory disease or intestinal hemorrhage using FC. In our study, the presence of inflammatory cells in swab-sampled DFC was associated with an intestinal inflammatory condition. According to Mandigers et al., the presence of yeast is not associated to intestinal disease and both round-shape yeast and *Cyniclomyces* spp. can also be present in healthy dogs being its pathogenic role unclear [6].

According to the literature, pleomorphic bacteria (a mixture of cocci and rods) in HD can been seen. As previously reported, our data confirm the microbiota in healthy dogs is variable with a pleomorphic population, with the rod component being the largest [1].

In the present study, macrophages and spore-forming bacteria were detected only in one dog and two dogs of the HD group, respectively. This finding suggests that detection of a low number of macrophages and spore-forming bacteria should be interpreted with caution.

RBCs were significantly more frequently observed in the AD group. During acute enteropathies, RBCs are expected to be detected because hyperemia, inflammatory edema or direct damage of the colonic mucosa are usually present in such conditions [12]. However, in our study population cytologic signs of acute (erythrophagocytosis) or chronic hemorrhage (hemosiderin phagocytosis) were not present.

In our study, neutrophils, lymphocytes and macrophages were significantly increased in dogs with enteropathy compared to HD. Thirty-six out of 40 (90%) dogs in the AE group showed the presence of neutrophils, 25 out of 40 (60%) showed bacterial phagocytosis, and 30% showed cocci bacterial monomorphism. These data suggest the presence of an acute inflammation, besides the involvement of bacteria in the pathological process. With rectal cytology it is not possible to determine whether bacteria are the primary cause or a consequence of the pathological process [1]. In our opinion, it would be interesting to study the associations between bacterial phagocytosis, clinical (e.g., fever) and clinical pathological (e.g., leukocytosis or toxic neutrophils) findings to direct further laboratory investigations (e.g., PCR, specific fecal culture for pathogenic bacteria, immune-enzyme assays for bacterial toxins).

The presence of gull-wing-shaped bacteria was different between groups (Table 2). However, their presence only in the AD group suggests a possible infectious etiology which may be investigated with the laboratory investigation reported above.

In addition, the presence of increased number of spore-forming bacteria in the AD group might warrant further investigations (e.g., *Clostridium perfringens* enterotoxins) into the role of these bacteria in this subset of dogs with acute diarrhea [7].

The presence of small lymphocytes and macrophages was significantly associated with dogs with enteropathy, however the presence of plasma cells (35%) was the only cytological feature associated with CD. Chronic inflammatory

enteropathies are often characterized by the presence of a reactive proliferation of lymphocytes and plasma cells in the gastrointestinal mucosa and submucosa [3]. Regarding inflammatory cells, a previous study described findings in FC in dogs with diarrhea, but no differences were observed between chronic and acute disease [4]. In our case, the findings of plasma cells and bacterial phagocytosis were significantly different between the AD and CD groups.

These results highlight the importance for further studies about the utility of the DFC for the diagnostic work up in enteropathic dogs. This study has some limitations. Primarily, we did not evaluate the absolute number of cytological parameters, however a normal/abnormal basis was estimated from the fecal cytology of twenty healthy dogs. It is therefore not possible to obtain an absolute number in order to establish a ROC curve to determine the actual cut off for each parameter. Furthermore, some evaluations, such as cocci bacterial monomorphism, were a subjective estimation which may differ between cytopathologists. The lack of an etiopathological characterization of the enteropathies and particularly the lack of a clinical severity score, are further limitations of the present study, and therefore the real utility of DFC in clinical practice cannot be determined. On the other hand, since DFC is a cheap, fast and non-invasive procedure that can exclude some life-threating infectious agents (e.g., *Prototheca* spp.), this pilot study is the first step to evaluate the clinical and diagnostic value of DFC.

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