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Effects of Air Travel Stress on the Canine Microbiome: A Pilot Study

Research Article

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Abstract

Behavior assessment is commonly used to identify canines that may have a higher threshold for environmental stress, but no work has established a connection between behavior (as indicated by search performance) and travel stress (as indicated by fecal scores and microbial stability). Six canines (aged 18 months to 8 years), trained according to the standard established by the Federal Emergency Management Agency (FEMA), were utilized to test the effects of airline travel stress on working canines. Our objectives were to test the hypotheses that: 1) working canines can overcome air travel stress with little or no impact on their performance; and 2) fecal score and microbial composition is impacted by airline travel stress. Two groups of dogs, (n=3 per group), were randomly selected from FEMA canine teams rostered in New York City, NY (CONTROL) and in Miami, FL (TRAVEL). TRAVEL dogs were flown in the cabin on a commercial airline to New York. Blood and fecal samples were collected each morning prior to travel (d0) and search work (d1-d3). Fecal bacterial DNA was extracted and 16S rRNA amplicon sequencing was completed using an Illumina MiSeq followed by analysis with QIIME 1.8.0. Fecal scores from TRAVEL were significantly higher ($P = 0.01$) than CONTROL indicating softer stool in the group that travelled. Pre- and post-travel blood samples for the TRAVEL group were compared and demonstrated significant decreases in lactate, bicarbonate, total carbon dioxide, and base excess ($P < 0.05$) following travel. However, these decreases were still within normal range, therefore, may not be biologically significant. In addition, blood samples from TRAVEL and CONTROL were compared on search days and increases were observed in the TRAVEL group for ionized calcium, bicarbonate, total carbon dioxide, base excess, creatinine, and blood urea nitrogen ($P < 0.05$). In contrast, blood glucose was decreased in the TRAVEL group ($P < 0.05$). Search behavior scores were not significant in TRAVEL compared to CONTROL. Principal coordinates analysis (PCoA) of UniFrac distances between samples based on their 97% OTU composition indicated that TRAVEL bacterial communities ($P = 0.01$) and bacterial community abundances ($P = 0.02$) were significantly different from CONTROL. These data demonstrate that airline travel of 2.5h impacts the working canine gut microbiota and some blood metabolites, but has no observed effect on working canine performance.

Keywords: Microbiome; Travel Stress; Working Canine.

Introduction

Stress is defined as a state during which the organism reacts to endogenous and exogenous threats and focuses its energies on coping with the situation of danger [28]. Stress can cause a wide variety of physiological and psychological health issues in canines, including increased activity of the sympathetic nervous system, increased release of catecholamine, and increased blood pressure [28]. The physiological effects of stress on the dog are poorly understood and therefore most likely under-reported. It is important to understand the level of stress working dogs can handle before experiencing physiological consequences in order to prevent negative impacts on job performance.

Working canines are often called upon to travel by air as part of their mission. However, no studies have been conducted identifying the effects of travel on working canines' physiological state and performance ability. Stress can cause a wide variety of physiological and psychological health issues in canines, including increased activity of the sympathetic nervous system,

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catecholamine, blood pressure, and permeability of the intestinal epithelial lining to microorganisms [7, 28]. In addition, a past study demonstrated that gastrointestinal permeability is increased as part of the stress response which may be correlated to acute stress symptoms such as diarrhea [7]. This may result in a change in fecal score. The only data available to assess how working canines handle the stress of air travel is extrapolated from companion animal work. According to a study conducted by Bergeron et al. (2002) [2], both ground and air transportation caused significantly increased levels of stress in dogs. The second most common cause of animal deaths associated with air transportation are secondary illnesses that are thought to be triggered by the environmental stress associated with travel. This travel was followed by acute onset of diseases caused by stress and high altitude, and also may have been due to mishandling of the animals in their carriers [2].

In contrast, search and rescue (SAR) canines have been conditioned to work around and through stressful situations [27]. However, more investigation is warranted regarding the physiological impacts of stress on working canines' performance. Prior research has shown a variety of gastrointestinal issues associated with working canines during deployment [3, 24]. However, no information is available that has connected issues associated with gastrointestinal upset and performance ability. The objectives of the current investigation were to test two hypotheses: 1) fecal scores and fecal microbial composition of working canines are impacted by airline travel stress; 2) working canines can overcome this stress with little or no impact on their performance in the field.

Materials and Methods

Animals and Diets

Southern Illinois University Institutional Animal Care and Use approval was obtained prior to the initiation of this pilot study (#14-047). Standards for animal care were adopted from previously published recommendations [25]. Six canines (two German Shepherds, one Belgian Malinois, two Labrador Retrievers, one mixed breed), aged 5 years ± 3 with a body condition score (BCS) of 4.5 ± 0.5, were randomly selected from two Federal Emergency Management Agency (FEMA) teams and fed commercially available complete and balanced dry kibble diets. Subjects used in this study and for FEMA work were screened according to similar criteria and were trained according to the established federal standard [8]. Dogs were divided into two groups (TRAVEL and CONTROL). Canines in the CONTROL group were housed individually in New York, NY, in temperature- and light-controlled kennels with *ad libitum* access to water. Canines in the TRAVEL group were located in Miami, FL and flew to New York as part of the experimental procedure. Canines in the TRAVEL group were offered water prior to departure and upon landing and were housed individually overnight in temperature- and light-controlled hotel rooms. All canines were fed twice daily at 0600 and 1700h. Canines in the TRAVEL group were screened through standard commercial airline screening procedures (www.tsa.gov/traveler-information) and were flown in the cabin (2.5h flight time). Dogs were transported to the search site and were housed in temperature-controlled kennels (0.962m x 0.635m x 0.685m) at the New York Police Department training site for the duration of the search scenarios. All TRAVEL dogs returned to their hotels each night. All CONTROL dogs returned to their individual kennels each night.

Search Performance

The experiment was conducted in October of 2013, with ambient air temperature of 23.8 ± 2.1°C and relative humidity of 55.4 ± 9.2%, with search behavior scoring taking place on the second and third day of the four day study. The search site was unfamiliar to the TRAVEL dogs, but was in compliance with rubble pile standards established and published by FEMA [8]. The principal investigator and FEMA instructors assigned ranked behavioral scores during the search performance for each subject using an adaptation of a previously published behavior scoring system [17, 18] and by utilizing focal point sampling.

Blood and Fecal Assays

Samples were collected from subjects each day prior to flight (d0) and prior to search (d1 to d3). Parameters collected included blood, rectal temperature, heart rate, and morning fecal samples (Purina Fecal Scoring System, Nestle-Purina, St. Louis, MO). Whole blood was used to monitor blood metabolites, including lactate, pH, partial pressure carbon dioxide, partial pressure oxygen, bicarbonate, sodium, potassium, ionized calcium, hematocrit, base excess, hemoglobin, and oxygen saturation with an Abaxis iSTAT (Union City, CA). Remaining blood samples were collected in EDTA tubes and stored on ice until centrifuged (1,300×g, 15min, 21°C) for serum analysis (Power Spin LX™, Dayton, NJ). Serum samples were analyzed for liver function, alanine aminotransferase (ALT), kidney function, blood urea nitrogen (BUN), total protein, and creatinine, and blood glucose concentrations with an AbaxisVetScan V2 (Union City, California).

Fecal samples were collected in sterile 15mL vials, stored on ice within 15min of defecation, and frozen overnight prior to overnight shipment for laboratory analysis. Upon arriving at the laboratory, samples were stored at -80°C. Fecal bacterial DNA was extracted using MoBio PowerSoil DNA extraction kits (MoBio Laboratories, Carlsbad, CA) and quantified using a NanoDrop (ND-1000 spectrophotometer, (Nanodrop Technologies) and diluted to 10 ng/mL. Electrophoresis using 1% agarose gel, with a 1xTAE buffer at 170V for 60min, evaluated the quality of the extracted DNA (BioRad Cell-Sub, Hercules, CA). A Fluidigm Access Array was used to generate barcoded amplicons of the V4 region of bacterial 16S rRNA from the extracted genomic DNA. Equimolar concentrations of amplicons were then combined and quality was assessed using a 2100 BioAnalyzer (Agilent Tecnologies). High-throughput sequencing was performed at the W.M. Keck Center for Biotechnology at University of Illinois using a MiSeq with Version 3 chemistry (Illumina, San Diego CA). Sequence analysis was completed using QIIME 1.8.0 and data were evaluated using previously published methods [6, 15]. Sequences were clustered into operational taxonomic units (OTUs) using closed reference OTU against the Greengenes 13-8 reference database with a 97% sequence similarity threshold. Following an additional quality filtering [3], samples were rarefied to a depth of 4,088 for subsequent diversity and taxonomic analysis.

Statistical Methods

Fecal and search behavioral scores were analyzed as a chi-square using the PROC FREQ procedure. Blood work was run as an independent *t*-test using PROC TTEST procedure, for pre- and post-travel (TRAVEL dogs only, d0). Additional TTEST procedures were utilized to compare differences in blood parameters of TRAVEL dogs vs CONTROL dogs on search days (d1-d3).

Microbial data were analyzed in three different ways. First, microbial profiles were developed using principal coordinate analysis (PCoA) of UniFrac distances based on a 97% OTU composition. Data were evaluated using both weighted and unweighted models. This technique provides a visual representation of the contrast between both relative abundance and presence or absence of the phylogenetic composition for each treatment group. Second, microbial data were analyzed as a series of *t*-tests comparing day 0 (baseline) for both groups against each successive day. In this way, all dogs served as controls for themselves and potential confounding factors associated with breed, gender, or diet were neutralized. Lastly, microbial taxa sequence percentages were analyzed using the MIXED MODELS procedure with a *Tukey post-hoc* adjustment. Statistical analysis was completed using SAS version 9.4 (SAS Institute, Inc., Cary, NC) with significance established at ($P<0.05$).

Results

Perfect behavior scores (12 of 12 points, Table 1) were assessed at a rate of 44.4% and 33.3% for CONTROL and TRAVEL, respectively. No difference was observed in search behavior between the groups (Table 2). In addition, ranked search behavior scores were not significantly different ($P > 0.05$).

Although all dogs for both groups started with fecal scores between 2-2.5, scores between groups were significantly different ($P=0.0133$) as shown in Figure 1. TRAVEL dogs had higher fecal scores indicating softer stool samples.

Blood work values were different for the TRAVEL group when pre-travel values were compared against post-travel (Table 3). Additionally, TRAVEL and CONTROL blood work demonstrated significant differences between the two groups when measured on days that included search work (Table 4). However, it is important to note that all blood work values were within the normal range and although statistically significant, it is unlikely that there is a biological significance attached.

The phylogenic composition of the treatment groups were compared using weighted UniFrac PCoA [22] of distances between samples based on their 97% OTU composition. Beta-diversity measures revealed that TRAVEL bacterial communities and bacterial community abundances were significantly different ($P=0.02$) from CONTROL (Figure 2A). In addition, when unweighted UniFrac PCoA was applied to test for the presence or absence of the identified microbes, TRAVEL was different ($P=0.01$) from CONTROL (Figure 2B).

Overall, five phyla, 34 families, and 61 genera were identified in these adult canine fecal samples. However, > 70% of all sequences were accounted for by the Firmicutes phyla. Most abundant families for CONTROL and TRAVEL, respectively, included Clostridiaceae (27.06% and 28.32%), Lachnospiraceae

Table 1. Search behavior taxonomy for working Federal Emergency Management Agency (FEMA) canines.

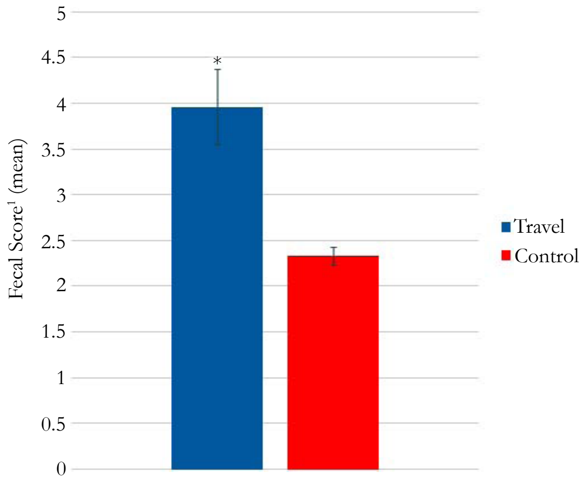
Variable	Behavior Score Definitions		
	Score 0 - Least favorable	**Score 1 - Moderate**	**Score - 2 Most favorable**
Distraction	Fails to search with focus; stops searching to sniff in areas with no human scent	Moves slowly but does not stop searching; is visibly engaged in the search process without stopping	Moves quickly and with visible focus; does not hesitate to navigate difficult obstacles or terrain on the rubble pile
Avoidance	Fails to begin search immediately; hesitates to enter search pile area; requires additional verbal commands from handler	Moves into search upon command but shows some hesitation; no additional verbal commands required	Moves immediately into search area with no hesitation; no additional commands required
Posture	Tail tucked; lowered head; ears tucked; legs or body trembling	Body is stiff; appears reluctant; posture is leaning away from search area or leaning into handler	Canine is visibly focused on search area, ears forward, head is up and alert; intent
Passive	Shows little or no interest in going to search; fails to demonstrate excitement	Shows some interest when the handler gives the command but loses focus soon; excitement wanes quickly	Maintains excited state and moves throughout search area in a purposeful manner without losing intensity
Returns	Returns to handler prior to completion of search; requires more than 1 restart	Returns to handler but moves away again with command	Does not return to handler; completes search and stays with located victim until handler arrives
False alert	Alerts on area where there is no human scent, alerts on food or clothing	Shows interest in hidden food or clothing but moves away without command from handler; may return later to check area again	May check areas with food or clothing but moves away quickly and does not return

Table 2. Effects of air travel on search behavior scores for working Federal Emergency Management Agency (FEMA) canine teams.

Treatment		Search Behavior Scores		
		Search 1	Search 2	Search 3
CONTROL	Canine S	11	12	12
	Canine M	10	9	10
	Canine A	12	10	11
TRAVEL	Canine B	11	12	11
	Canine Y	9	8	10
	Canine Z	12	12	12

Means of TRAVEL behavior scores were not different when compared to CONTROL (*P* > 0.05).

Figure 1. Effects of air travel on post-travel fecal scores[1] in working canines.



*Means of TRAVEL fecal scores were greater (P < 0.05) when compared to CONTROL.

[1.] Fecal scores assigned according to the Nestle Purina Fecal Scoring System

Score 1 – Very hard and dry; requires much effort to expel from body; no residue left on ground when picked up; often expelled as individual pellets.

Score 2 – Firm, but not hard; should be pliable; segmented appearance; little or no residue left on ground when picked up.

Score 3 –Log-like, little or no segmentation visible; moist surface; leaves residue, but holds form when picked up.

Score 4 – very moist (watery), distinct log shape visible; leaves residue and loses form when picked up.

Score 5 – Very moist but distinct shape; present in piles rather than distinct logs; leaves residue and loses form when picked up.

Score 6 – Has texture, but no defined shape; occurs as piles or as spots; leaves residue when picked up.

Score 7 – Watery, no texture, flat; occurs as puddles.

(16.07% and 28.58%), Streptococcaceae (9.71% and 0.14%), and Fusobacteriaceae (9.33% and 11.29%). This is in agreement with work previously published identifying similar composition structure [16] in adult dogs. In addition, the Clostridia class constituted > 50% of the Firmicutes phyla and was significantly higher (*P* < 0.005) for dogs in the TRAVEL group (67.78%) as compared to the CONTROL group (50.99%). Also, the Bacteroidaceae family was greater (*P* < 0.02) in TRAVEL (6.43%) compared to CONTROL (1.09%).

When baseline (d0) sample data was compared against day 1 (24 hours post travel) and each successive search day within each group, there were several changes observed across taxonomic levels for TRAVEL. Relative abundance of Bifidobacteriaceae increased (*P* = 0.03) from d0 to d3, from 0% to 0.23%, respectively, in TRAVEL. At the genus level, *Blautiaincreased* (*P* = 0.05) from 8.99% at d0 to 15.02% at d3. *Bifidobacterium* increased (*P* = 0.001) from 0% at d0 to 0.17% at d3. In addition, *Clostridium* tended to decrease (P = 0.09) when d0 was compared to d1 (0.08% and 0.02%, respectively). No changes were observed in the CONTROL group.

Discussion

The effects of travel on companion animals have not been extensively studied and even fewer data are available on working canines [16, 23, 26, 29, 30]. Past research using laboratory canines revealed that during air transportation, loading and unloading seemed to be the most stressful periods [2]. However, these dogs were transported in kennels and flown in cargo, which provides different traveling conditions than those experienced by canines flown in-cabin. In addition, FEMA canines are vigorously screened prior to acceptance into the training program and are subject to stringent certification requirements [8]. The screening process includes testing for noise sensitivity, surface sensitivity, height sensitivity, as well as a socialization test. Therefore, it seems

Table 3. Effects of air travel on blood work measured pre-and post-travel for TRAVEL canines.

Parameter measured (normal range)	Pre-travel	Post-travel	P value
Lactate (0.60-2.90 µl)	1.21[a]	0.83[b]	0.03
pH (7.35-7.45)	7.37	7.35	0.41
Partial pressure carbon dioxide (40.20-44.10 mmHg)	42.42	37.8	0.47
Partial pressure oxygen (49.30-99.50 mmHg)	32.66	28.66	0.08
Sodium (139-150 mmol/L)	146.20	147.20	0.24
Potassium (3.40-4.90 mmol/L)	4.35	4.13	0.07
Ionized calcium (1.12-1.40 mmol/L)	1.42	1.43	0.85
Glucose (60-115 mg/dl)	89.33	93	0.31
Hematocrit (37-55%)	45.00	44.16	0.57
Bicarbonate (15-23 mmol/L)	24.91[a]	21.93[b]	0.01
Total carbon dioxide (12-27 mmol/L)	26.16[a]	23.16[b]	0.02
Base excess ((-5)-0 mmol/L)	-0.16[a]	-3.33[b]	0.01
Saturated oxygen (80-100 mmHg)	52.83	51.5	0.92
Hemoglobin (12-17 g/dL)	15.31	15.01	0.55
Heart rate (60-140 bpm)	109	103	0.50
Rectal temperature (100.00-102.50°F)	101.8	102.4	0.11
Alanine aminotransferase (10-118 U/L)	42.8	53.66	0.24
Alkaline phosphatase (20-150 U/L)	41	56.5	0.59
Blood urea nitrogen (7-25 mg/dL)	23.00	28.5	0.46
Creatinine (0.30-1.40 mg/dL)	1.50	1.66	0.57
Total protein (5.40-8.20 g/dL)	7.60	9.46	0.30

[a-b] Means within a row with different superscripts differ ($P < 0.05$).

Table 4. Effects of air travel on blood work and vital signs measured prior to search work.

Parameter measured (normal range)	CONTROL	TRAVEL	*P* value
Lactate (0.60-2.90 µl)	1.28	1.25	0.92
PH (7.35-7.45)	7.35	7.36	0.84
Partial pressure carbon dioxide (40.20-44.10 mmHg)	39.47	43.73	0.10
Partial pressure oxygen (49.30-99.50 mmHg)	37.00	35.28	0.82
Sodium (139-150 mmol/L)	146.00	145.7	0.65
Potassium (3.40-4.90 mmol/L)	4.26	4.18	0.50
Ionized calcium (1.12-1.40 mmol/L)	1.37[a]	1.43[b]	0.01
Glucose (60-115 mg/dl)	95.87[a]	86.43[b]	0.00
Hematocrit (37-55%)	48.75	49.14	0.94
Bicarbonate (15-23 mmol/L)	22.21[a]	24.80[b]	0.00
Total carbon dioxide (12-27 mmol/L)	23.57[a]	26.00[b]	0.00
Base excess ((-5)-0 mmol/L)	-3.12[a]	-0.43[b]	0.00
Saturated oxygen (80-100 mmHg)	64.14	57.28	0.61
Hemoglobin (12-17 g/dL)	16.56	15.74	0.58
Heart rate (60-140 bpm)	105.00	98.5	0.38
Rectal temperature (100.00-102.50°F)	101.90	102.00	0.78
Alanine aminotransferase (10-118 U/L)	65.00	43.89	0.12
Alkaline phosphatase (20-150 U/L)	32.67	39.33	0.55
Blood urea nitrogen (7-25 mg/dL)	13.33[a]	18.22[b]	0.01
Creatinine (0.30-1.40 mg/dL)	1.01	1.26	0.06
Total protein (5.40-8.20 g/dL)	7.63	7.59	0.95

[a-b.] Means within a row with different superscripts differ ($P < 0.05$)

Figure 2A. Weighted UniFrac principal coordinates analysis (PCoA) of TRAVEL and CONTROL working canines.

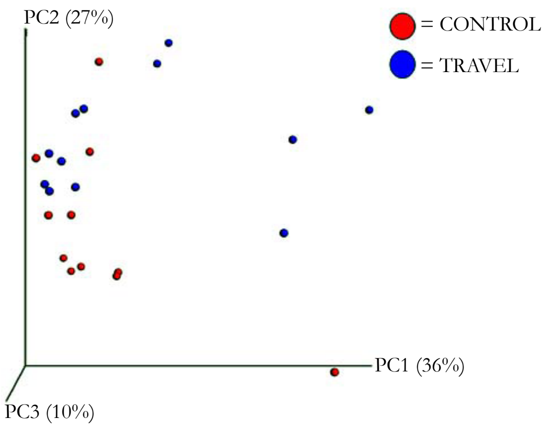


Figure 2B. Unweighted UniFrac principal coordinates analysis (PCoA) of TRAVEL and CONTROL working canines.

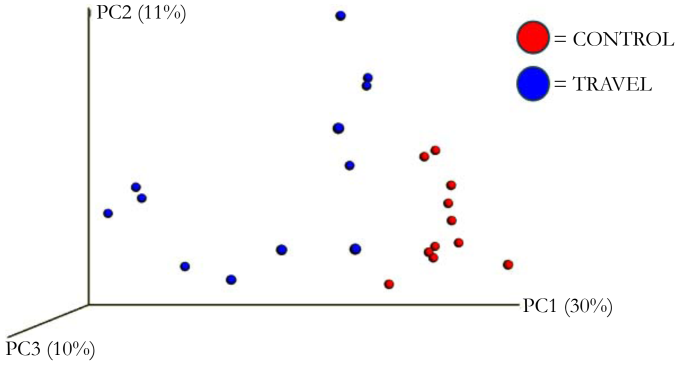
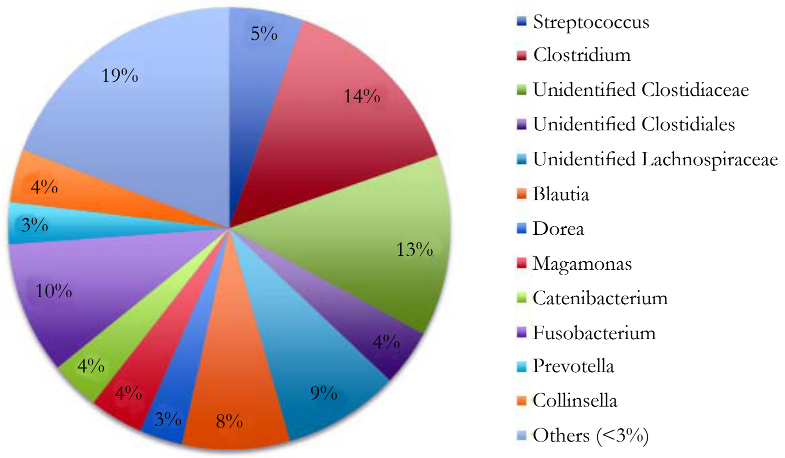


Figure 3. Predominant genera[1] in adult working canines at baseline.



[1] Values expressed are relative abundance for all canines on day 0.

unlikely that a working canine would have a similar response to the airport and environmental stresses when compared to companion animals. Our results highlight the importance of further investigation of travel stress relative to the working canines' gastrointestinal microbial composition.

Although pre-and post-travel blood parameters were affected, it is unlikely that there is any biological significance attached as all values remained within identified normal reference ranges. However, these data indicate that there is an effect on the blood metabolites for canines that travel in cabin. Therefore it is feasible that more stressful travel conditions, e.g. larger flight durations or transport in cargo, could result in biologically significant changes. More work is needed to identify how this change may impact the working canine and what, if any, steps may be taken to prevent these shifts from occurring.

Analysis of data for fecal scores revealed that the TRAVEL group was affected by the travel experience, which resulted in softer stool samples. Softer stool samples may have been due to acute stress during search performance, from the air transportation, or the significant change in the gut microbiome. Fecal scores have been widely used to identify potential fluctuations in the gastrointestinal state of dogs [11, 12]. Changes in fecal consistency are also visible indicators of environmental stress and hydration status [11, 20]. In addition, special consideration should be given to maintaining proper hydration status in working canines. It has been reported that water absorption in the colon is the primary factor associated with fecal quality in the dog, the negative impacts of which may be seen with higher fecal scores [14]. In addition, hydration concerns are often reported during deployment and have been associated with required veterinary intervention in the past [24].

The effects of travel on companion animals have not been well documented. Some research in beagles [2] demonstrated that transportation is stressful, particularly during loading and unloading. However, these dogs were described as "laboratory animals" and were transported in kennels and flown in cargo. Additionally, it seems unlikely that the socialization available for laboratory animals would compare with the socialization experience of a pet or a service animal. Other work has shown a significant (and permanent) effect when animals were exposed to simulated air transport involving hyperthermia [13]. Again, this simulation was typical of dogs flown in cargo, not in cabin. Kennel size was also investigated using greyhounds and showed little effect on the stress response [21].

The stress of travel has been well documented in human travelers. Potential stressors of travel on the ground may include commotion in the airport, baggage transport, long walks through terminals, timely arrival at the gate and potential flight delays. In-flight stressors were identified as turbulence, noise, barometric pressure changes, temperature and humidity fluctuations and general fatigue. Other factors investigated included direction of travel, altitude, and meal and sleep as well as circadian rhythm disruption. Although it is not known how canines perceive ground stressors, it seems logical that the environmental changes identified would likely be experienced similarly in the dog as in the human. This is another area that warrants further investigation.

In addition to work in humans, some work in horses has shown a sensitivity to travel that was linked to a disruption in the circadian rhythm and an interruption in the typical photoperiod. Although the canines utilized in this study traveled during the day with no perceived disruption to their circadian rhythm, it is unclear whether or not the time of travel would have an impact on the working canine. In fact, the working canines used in this study are often unable to plan their travel in advance. Frequently they are called in response to a disaster that has happened elsewhere and travel must occur immediately. More work needs to be done on time of travel in order to more fully understand this variable in the working canine.

The bacterial diversity demonstrated in our novel data set are in agreement with prior work published characterizing a healthy adult canine microbiome [16]. Although the pilot study was limited in its scope, these data reveal an interesting perspective associated with the microbial shift that takes place in the gut of working canines following air travel. Bacterial abundance as well as communities present were different between groups as indicated by the PCoA imaging for this four day study. However, since our data were limited by a four day collection period, it is unknown whether or not there would have been additional differences in the microbial profile for subsequent days. Due to the limitations associated with the sample size in this pilot study, future studies should definitely incorporate methods to test for effects of breed, gender, and diet.

Conclusion

The aim of this pilot study was to examine the effect of travel stress on physical status, search performance, and composition of the gastrointestinal microbiome of the working canine. While search performance scores, and physical exam parameters were not significantly different, the blood parameters, composition of the gastrointestinal microbiome and fecal scores were impacted by travel. Further assessment of working canine fecal microbial composition and subsequent stress response may provide more information to better understand how to effectively manage working dogs in the field after air transportation. In addition, future research needs to be conducted assessing travel stress on a larger scale and utilizing a controlled diet. Lastly, future studies should be directed at identifying factors such as breed, age, dietary changes, gender and others that may result in variability of the canine gastrointestinal microbiome.

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