Early Life Modifiable Exposures and Their Association With Owner Reported Inflammatory Bowel Disease Symptoms in Adult Dogs

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Background: Inflammatory bowel disease (IBD) is an idiopathic multifactorial disease in humans and dogs, usually assigned to the interactions between genes, gut microbiota, diet, environment, and the immune system. We aimed to investigate the modifiable early life exposures associated with IBD in dogs.

Materials and Methods: The study data was extracted from the validated owner-reported DogRisk food frequency questionnaire. This was a cross-sectional questionnaire-based study that tested 21 different early life dietary and environmental, demographic and genetic variables for their association with IBD or not, in adult dogs. A total of 7,015 dogs participated in this study. The study covered early life periods; prenatal, neonatal, early, and late postnatal periods. Two feeding patterns, a non-processed meat-based diet (NPMD) and an ultra-processed carbohydrate-based diet (UPCD) were studied. Data was analyzed using logistic regression analysis with a backward stepwise deletion.

Results: From the final models we found that the NPMD during early and late postnatal periods were significantly associated with lower IBD risk later in life. The UPCD during the same periods was associated with a higher risk of IBD incidence. Also, the maternal diet during the neonatal period showed a non-significant trend of lower IBD risk in the offspring with the NPMD and a higher IBD risk with the UPCD. Additionally, the normal body weight of puppies during the first 6 months of age was associated with a lower risk of IBD in adulthood while, slim puppies associated significantly with IBD in adulthood. From the non-modifiable background variables, we identified the maternal history of IBD as the strongest risk factor for later incidence of IBD. Furthermore, male dogs were twice as likely to develop IBD as female dogs were.

Conclusions: It is reassuring for owners to know that they themselves can have an impact on their dog's health. A high-fat, low-carbohydrate NPMD exposure during early life, and a normal body condition in puppyhood were significantly associated with less IBD in adult dogs. The opposite was true for UPCD exposure and abnormal body condition score in 6 month old puppies.

Keywords: chronic enteropathies, canine, diet, microbiome, gut, immune, prenatal

INTRODUCTION

Canine inflammatory bowel disease (IBD) is a group of chronic idiopathic enteropathies in dogs characterized by persistent and/or recurrent gastrointestinal symptoms (1–3). In this study we will refer to these enteropathies as IBD. The disease usually affects middle-aged dogs with no gender predisposition (4, 5); although intermittent symptoms have been noted even in puppies < 6 months of age (5). Clinical phenotypes of IBD have been identified and used to define specific forms of IBD with varying breed predisposition [(1, 5, 6), **Table 1**]. To date, the etiopathogenesis of idiopathic IBD, both in humans and canines, is not fully understood. Current literature supports the concept that IBD is usually assigned to the interactions between genetics, gut microenvironment (dietary microbiota and diet composition), and the host immune system (1, 2). The genetic component of the disease has been recognized using both genome-wide association and candidate gene approaches in humans (14, 15) and dogs (6, 16, 17).

Mounting evidence from human epidemiological studies suggests that it is wise to focus on exploring the role that early life exposures have on influencing the gut microbiome and immune modulation, as it in turn can modify the disease risk (18–21). Several theories encourage identifying the role of environmental stimulants, including diet, in triggering the inflammatory response. The most prominent of them is the hygiene hypothesis which states that an increased frequency of immune disorders can be attributed to a reduction in enteric microbiota during early life. This has been presumed to be due to exaggerated sanitation, which results in an untrained, and therefore malfunctioning, immune system (22, 23). The newborn immune maturation is mainly driven by the early life exposure to microbes (24). The gut microbiota is the central source of the postnatal microbial exposure (25). The early life diet has a profound effect on the neonate gut microbiota and thereby also on immune regulation (26). Dietary patterns are a fundamental part of a healthy lifestyle and diets can influence gut microbial ecosystems, promoting gut health in dogs and humans. Recently, diet composition has been shown to substantially impact the abundance and modulation of gut microbiome in dogs and humans (27–34). Moreover, the diet processing; whether the diet is offered as a non-processed/raw diet or as an ultra-processed diet, has been observed to impact human and canine health (35, 36).

Research regarding the role of the early diet on IBD incidence in small animals is scarce. One study on cats analyzed the role that early life events and diet had on gastrointestinal symptoms that developed later in life (37). They found that when owners reported diarrhea, vomiting, and/or decreased consumption of commercial diets before their cat was 16 weeks of age, it was also associated with gastrointestinal symptoms occurring at least twice between 6 and 30 months of age (37). Although the disease is frequently presented at animal clinics,

Abbreviations: IBD, inflammatory bowel disease; NPMD, non-processed meat based diet; UPCD, ultra-processed carbohydrate based diet; FFQ, food frequency questionnaire.

TABLE 1 | Inflammatory bowel disease prone breeds.

| IBD phenotypes	IBD prone breeds
Inflammatory bowel disease	Akita[1], Bernese mountain dog[1], Dalmatian[1], English setter[1], German shepherd[1,2,7], Golden retriever[1,2], Irish setter[1], Pointer[1], Rottweiler[1,2], Soft-coated wheaten terrier[1,3,7], Labrador retriever[2], Border colli[2], Boxer[2,7], Staffordshire bull terrier[2], Cocker spaniel[2], West highland white terrier[2], Weimaraner[2], Jack Russell terrier[2], Basenjis[3,7], Mixed breeds[2], French bull dog[3], Doberman pinscher[3], Mastiff[3], Alaskan malamute[3], Shar pei[7]
Intestinal malabsorption	Akita[1], Basenji[1], Chinese Shar pei[1], Chow chow[1], French bull dog[1], Irish setter[1], Old English sheep dog[1], Peruvian inca orchid[1], Rottweiler[1], Shiloh shepherd[1], Soft-coated wheaten terrier[1]
Gluten-sensitive enteropathy	Irish setter[1,2]
Ulcerative colitis	Akbash[1], Boxers[1,4,5,7], German shepherd[1], Skye terrier[1], French bull dog[5,7], Mastiff[5,7], Alaskan malmalute[5,7], Dobermann pinscher[5,7]
Tylosin responsive diarrhea	Irish setter[6], Basenji[6], Lundehund[6], Yorkshire terrier[6], German shepherd[6], Boxer[6], French bull dog[6], Shar-pei[6], Rottweiler[6], Soft-coated wheaten terrier[6]
Antibiotic responsive diarrhea	German shepherds[8]

[1–8]cited from Dodds (7), Kathrani et al. (8), Cerquetella et al. (2), Davies et al. (9), Stokes et al. (10), Westermarck et al. (11), Hall (12), Hall (13) respectively.

there is no data on the true prevalence of IBD in dogs (4). Currently there are no studies that identify early life exposures that might act as risk factors for chronic IBD development in dogs. Furthermore, the possible influence of a non-processed meat based diet and an ultra-processed carbohydrate based diet on the prevalence of IBD in dogs has not been previously assessed. Only one canine study found a significant risk of developing gastrointestinal diseases if a dog had previously suffered from a parvovirus infection (38). Therefore, it is important to investigate modifiable early life risk factors for canine IBD that might have an impact on future immune system stimulation. The main aim of the present study was to investigate possible associations of modifiable early life exposures, dietary and environmental, with owner-reported IBD incidence in later life. In addition, we aimed to test previous known risk factors of the disease, such as maternal history, gender, and breed.

MATERIALS AND METHODS

The Questionnaire and Study Population

The study data was extracted from the DogRisk food frequency questionnaire (FFQ) data. This FFQ is an online validated ongoing questionnaire (39) http://www.ruokintakysely.fi/. It has been available online for dog owners since 2009, when it was launched by us at the University of Helsinki, Finland. The whole questionnaire is in Finnish language. As mentioned elsewhere (36, 39, 40) the FFQ includes 50 questions generating 1,332 data points, reported by the dog owners. It aims to gather information on the owner's dog's diets and lifestyle at different

FIGURE 1 | Flow chart of the study population.
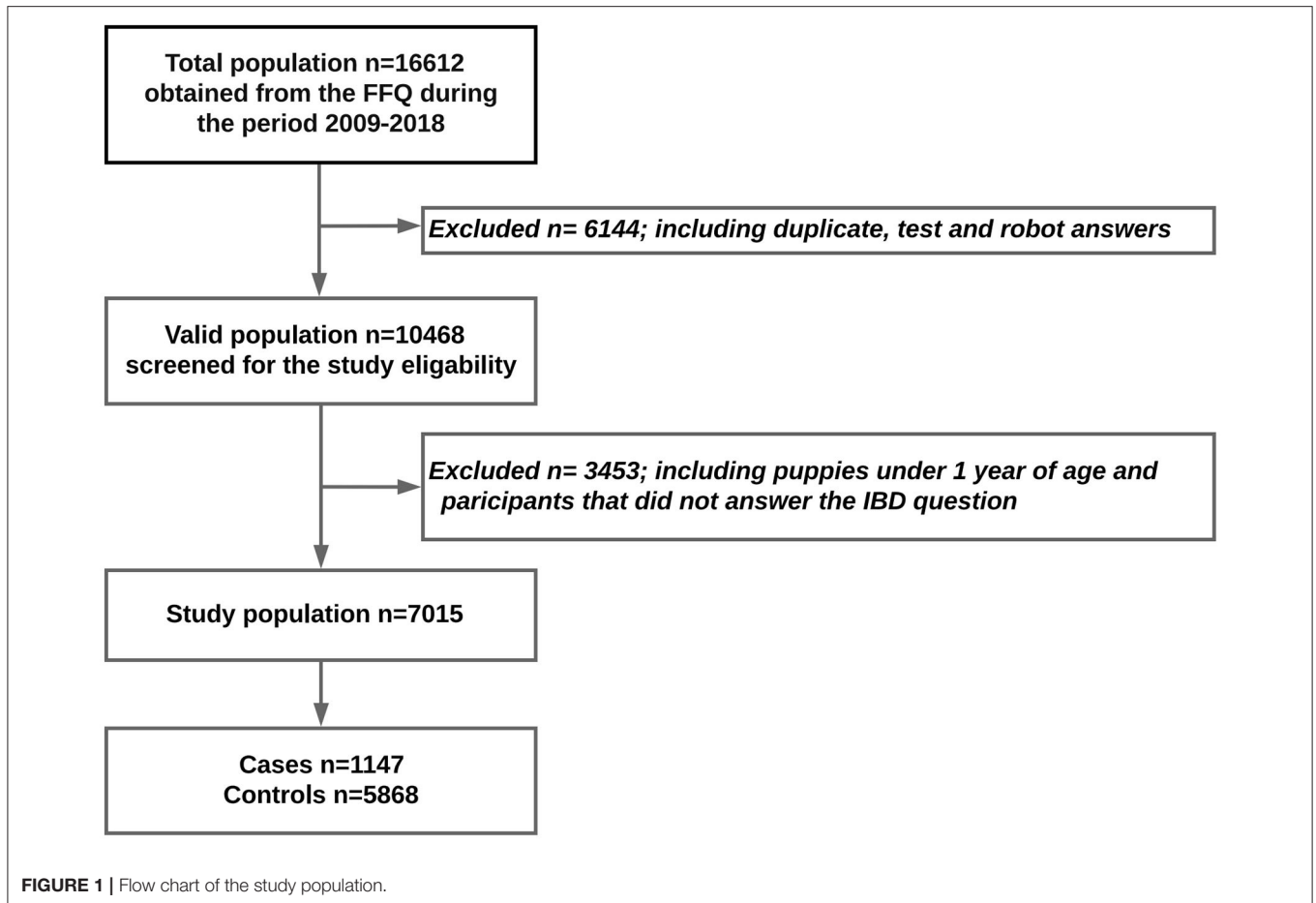
time points throughout its life, as well as data on the dog's health conditions, background, and demographic information. In addition to gathering information on the dog itself, it also contains several questions concerning the dogs' mothers' early maternal diets and their diseases. The FFQ has an ethical approval (29.4.2016) from the University of Helsinki Viikki campus ethical board.

The questionnaire received 16,559 responses between 2009 and 2019. The dogs under 1 year of age were excluded from this study in order to avoid reverse causality. Also, participants who had not answered the question about whether their dogs had been suffering from IBD or not were excluded. Otherwise, all breeds and both sexes were included. After all questionnaire test answers, robot answers, and duplicates had been removed, 7,015 participants were eligible for the study (**Figure 1**).

Study Design and Tested Variables

This is a once-answered, and in that respect cross-sectional, questionnaire-based study asking about multiple exposures at different time points, therefore also making it longitudinal. This study has been carried out to investigate the association between early life modifiable exposures (dietary and environmental) and the incidence of owner-reported IBD later in life. In addition, we tested the associations between non-modifiable genetic and

demographic variables and IBD incidence. The study tested only one dependent categorical dichotomous variable, concerning IBD, for association with 21 different independent categorical and continuous variables (**Table 2**). The dependent variable obtained by responding either "Yes" or "No" to the question: "Has your dog suffered from inflammatory bowel disease (IBD), chronic bowel symptoms, chronic bowel 'allergies'?" The study cases were obtained when the owners responded "Yes," while those who responded "No" were chosen as study controls.

We analyzed four early life periods, prenatal (pregnancy period), neonatal (the 1st 3–4 weeks of life, i.e., the lactation period), early postnatal (from 1 to 2 months of age) and late postnatal periods (from 2 to 6 months of age) as shown in **Figure 2**.

The modifiable early life variables that were tested covered nutritional, environmental and lifestyle factors. We compared two common feeding patterns, the non-processed meat-based diets (NPMD) and the ultra-processed carbohydrate-based diets (UPCD) during the following early life periods: the maternal diet during pregnancy, the maternal diet during lactation, the young puppy first solid diet and the puppy diet in the period between 2 and 6 months of age. The NPMD is a diet rich in fresh animal proteins and fats (red meat, poultry, fish, organs, bones, lard, fish oils etc.) and low in carbohydrates but including raw vegetables,

TABLE 2 | Frequencies of the tested variables in the cases, controls, and total study cohort.
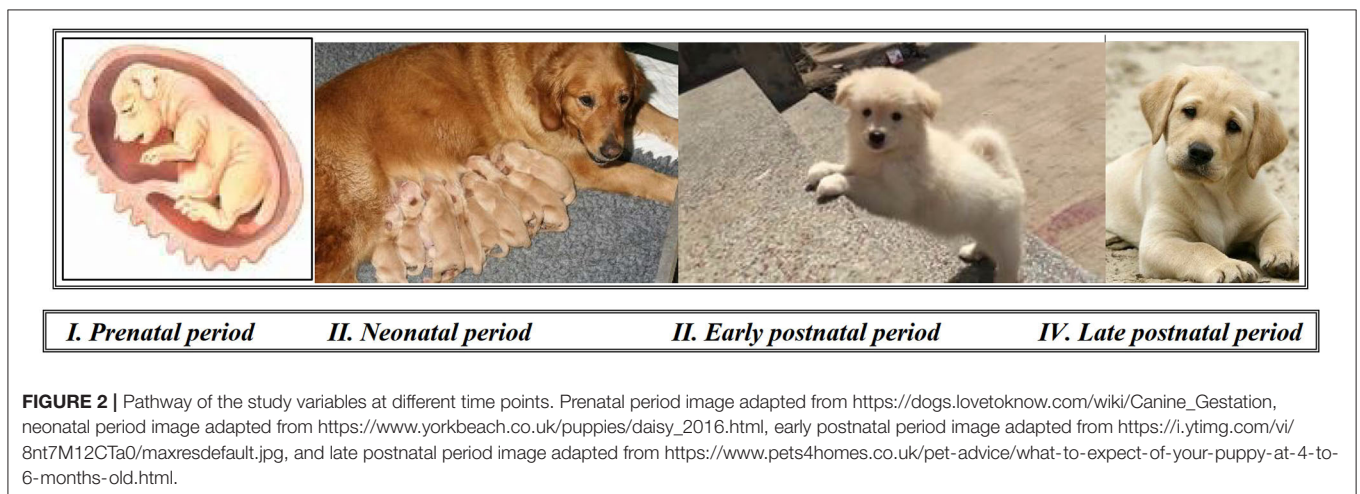
Variables	Categories	Inflammatory bowel disease %(n)		
		Cases, % (n = 1,147)	Controls, % (n = 7,015)	Total, % (n = 7,015)
Non- modifiable; genetic and demographic factors				
Maternal History of IBD	Mothers without IBD	90.0 (260)	98.8 (2,135)	97.7 (2,395)
	Mothers with IBD	10.0 (29)	1.2 (27)	2.3 (56)
Dog breed	IBD prone breeds	55.5 (562)	50.4 (2,583)	51.2 (3,145)
	IBD non-prone breeds	44.5 (450)	49.6 (2,546)	48.8 (2,996)
Dog gender	Males	60.3 (675)	43.4 (2,480)	46.2 (3,155)
	Females	39.7 (444)	56.6 (3,232)	53.8 (3,676)
Dog color	White >50%	21.8 (240)	20.0 (1,107)	20.3 (1,347)
	White <50%	78.2 (862)	80.0 (4,441)	79.7 (5,303)
Dog age, years (mean ± SD)*		5.10 ± 3.06	5.04 ± 3.12	5.05 ± 3.11
Modifiable factors				
Prenatal period (pregnancy)				
Maternal gestation diet	NPMB	6.0 (22)	9.1 (178)	8.6 (200)
	UPCD	94.0 (346)	90.9 (1,788)	91.4 (2,134)
Was the mother dewormed during/just before pregnancy?	Yes	95.9 (446)	96.2 (2,717)	96.2 (3,163)
	No	4.1 (19)	3.8 (107)	3.8 (126)
Was the mother vaccinated during/just before pregnancy?	Yes	59.5 (150)	48.7 (830)	50.1 (980)
	No	40.5 (102)	51.3 (873)	49.9 (975)
Neonatal period (lactation)				
Maternal lactation diet	NPMB	6.2 (21)	9.2 (172)	8.7 (193)
	UPCD	93.8 (319)	90.8 (1,706)	91.3 (2,025)
Early postnatal period (puppy 1–2 months of age)				
Puppy's first solid diet	NPMD	5.9 (22)	10.3 (198)	9.6 (220)
	UPCD	94.1 (353)	89.7 (1,720)	90.4 (2,073)
Frequency of outdoor activity	Many times/day	51.4 (331)	60.0 (2,105)	58.6 (2,436)
	Once/day	15.5 (100)	15.7 (552)	15.7 (652)
	A few times/week	14.4 (93)	11.5 (405)	12.0 (498)
	A few times/month	7.3 (47)	5.0 (176)	5.4 (223)
	Not at all	11.3 (73)	7.8 (273)	8.3 (346)
Rest, hours/day (mean ± SD)*		15.99 ± 3.97	15.98 ± 3.80	15.98 ± 3.82
Type of flooring	Slippery flooring	9.7 (66)	10.9 (399)	10.7 (465)
	Non-slippery flooring	26.3 (179)	27.4 (1,006)	27.2 (1,185)
	Dirt flooring	5.9 (40)	7.5 (275)	7.2 (315)
	Newspaper flooring	30.0 (204)	27.8 (1,023)	28.2 (1,227)
	Carpet flooring	28.1 (191)	26.5 (973)	26.7 (1,164)
Body condition score	Obese puppy	14.6 (109)	14.6 (573)	14.6 (682)
	Normal weight puppy	75.1 (561)	75.9 (2,977)	75.8 (3,538)
	Slim puppy	10.3 (77)	9.5 (371)	9.6 (448)
Late postnatal period (puppy 2–6 months)				
Puppy diet	NPMD	15.1 (45)	22.2 (310)	20.9 (355)
	UPCD	84.9 (254)	77.8 (1,088)	79.1 (1,342)
Outdoor activity, hours/day	< 0.5	1.2 (9)	2.2 (89)	2.1 (98)
	0.5–1.0	28.3 (219)	25.5 (1,019)	26.0 (1,238)
	1.0–2.0	51.8 (401)	51.5 (2,057)	51.6 (2,458)
	> 2.0	18.7 (145)	20.7 (826)	20.4 (971)
Rest, hours/day (mean ± SD)*		14.52 ± 3.21	14.43 ± 3.28	14.45 ± 3.27
Type of flooring	Slippery flooring	23.4 (191)	24.5 (1,040)	24.3 (1,231)

(Continued)

TABLE 2 | Continued

Variables	Categories	Inflammatory bowel disease %(n)		
		Cases, % (n = 1,147)	Controls, % (n = 7,015)	Total, % (n = 7,015)
	Non-slippery flooring	24.9 (203)	25.1 (1,064)	25.0 (1,267)
	Dirt flooring	1.0 (8)	2.0 (85)	1.8 (93)
	Newspaper flooring	0.5 (4)	0.3 (11)	0.3 (15)
	Carpets flooring	12.0 (98)	13.5 (572)	13.2 (670)
Body condition score	Obese puppy	7.3 (59)	6.4 (266)	6.5 (325)
	Normal weight puppy	61.6 (495)	69.0 (2,883)	67.8 (3,378)
	Slim puppy	31.1 (250)	24.7 (1,032)	25.7 (1,282)
Was the puppy vaccinated 2–4 times under 1 year of age?	Yes	98.1 (1,050)	98.7 (5,493)	98.6 (6,543)
	No	1.9 (20)	1.3 (72)	1.4 (92)
Was the puppy dewormed 2–10 times under 1 year of age?	Yes	98.9 (1,018)	98.8 (5,303)	98.8 (6,321)
	No	1.1 (11)	1.2 (64)	1.2 (75)

(n): the number of dogs, IBD, inflammatory bowel disease; NPMD, non-processed meat based diet; UPCD, ultra-processed carbohydrate based diet. *Scale variables presented as (mean ± SD).

I. Prenatal period ***II. Neonatal period*** ***II. Early postnatal period*** ***IV. Late postnatal period***

FIGURE 2 | Pathway of the study variables at different time points. Prenatal period image adapted from https://dogs.lovetoknow.com/wiki/Canine_Gestation, neonatal period image adapted from https://www.yorkbeach.co.uk/puppies/daisy_2016.html, early postnatal period image adapted from https://i.ytimg.com/vi/8nt7M12CTa0/maxresdefault.jpg, and late postnatal period image adapted from https://www.pets4homes.co.uk/pet-advice/what-to-expect-of-your-puppy-at-4-to-6-months-old.html.
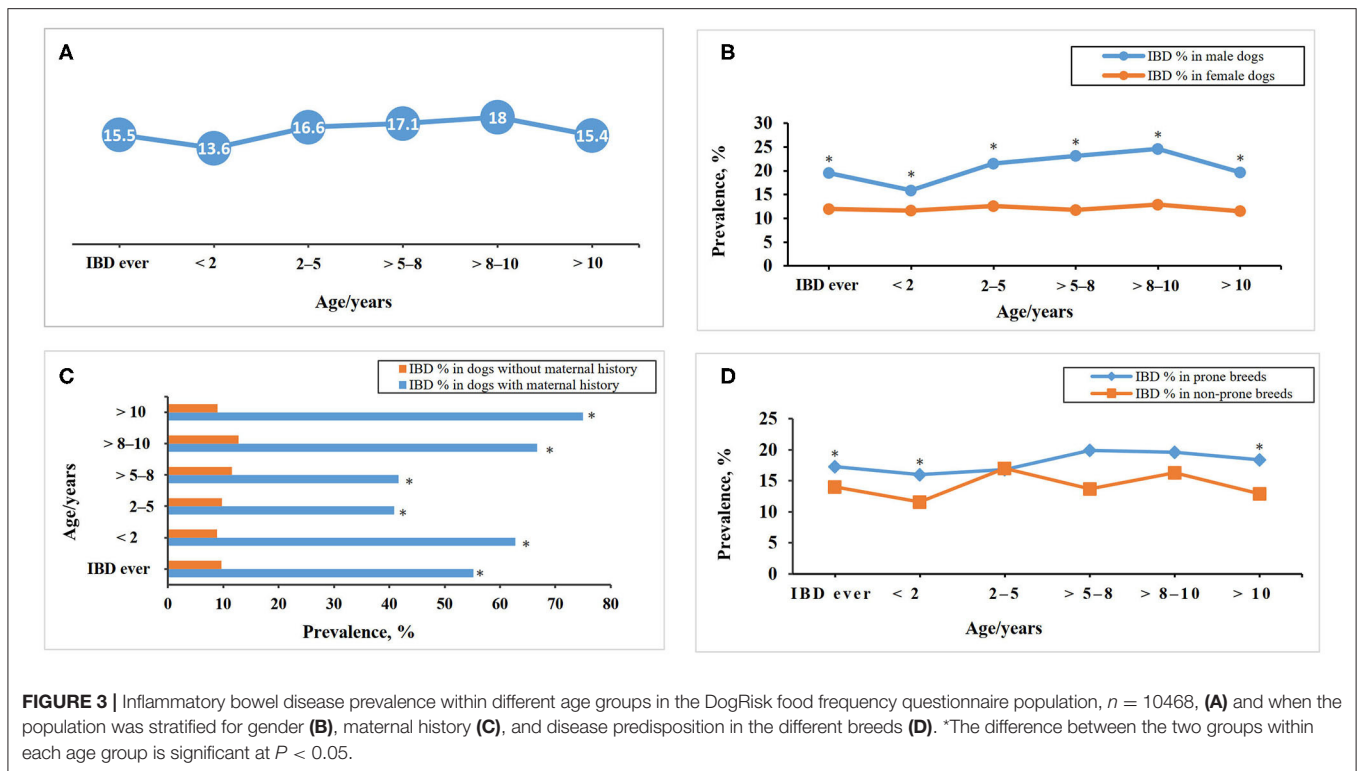
fruits or berries for micronutrients and vegetal fibers. The NPMD is either commercial or prepared at home by chopping, grating, mixing and freezing the ingredients. The UPCD refers to commercially produced extruded kibble. Typically, 40–60% of the contents of the UPCD are processed carbohydrates (mostly grains, rice, potatoes etc. but also some vegetable pulp). The two diets; NPMD and UPCD, exhibit different macronutrient profiles but may also differ in many other ways.

We tested four environmental and lifestyle factors during the early and late postnatal periods including outdoor activity, rest hours, type of flooring, and the body condition score. The body condition score (BCS) was a 5-category scale where the owner could choose between 1 = very slim, 2 = slim, 3 = normal, 4 = round/fat, and 5 = obese.

The questions about vaccination and deworming programs of the pregnant dams and puppies under 1 year of age were also tested for the association with the dependent variable. The question regarding the dog's mother was: "Did you vaccinate/deworm the dam while she was pregnant or just before?" The question regarding the puppy asked whether or not it had received 2–4 vaccinations before the age of 1 year? The answers of these questions were, "yes," "no" or "I do not know/I do not remember." Only the yes and no answers were used in the analyses.

The final five questions regarded the non-modifiable genetic and demographic variables, namely: maternal history of IBD, dog age, dog gender, dog color (specifically wanting to know the amount of white in the coat) and if the dog breed is prone to IBD or not prone to IBD (**Table 1**). The variable of IBD prone breed or not, was gathered from the literature (**Table 1**).

FIGURE 3 | Inflammatory bowel disease prevalence within different age groups in the DogRisk food frequency questionnaire population, $n = 10468$, **(A)** and when the population was stratified for gender **(B)**, maternal history **(C)**, and disease predisposition in the different breeds **(D)**. *The difference between the two groups within each age group is significant at $P < 0.05$.

Statistical Methods

The categorical and discrete variables were presented as frequencies n (%) using cross-tabulation for the cases, controls and the total study population, while the continuous variables were summarized as means and standard deviations (mean $\pm$ SD). The disease prevalence was calculated using cross-tabulation as ratios of the diseased dogs in the total population and within each age group. Moreover, the prevalence was calculated after stratifying the FFQ cohort for gender, maternal history, and disease predisposition in the different breeds.

The association of the tested independent variables with IBD symptoms in adult dogs was calculated using logistic regression analyses. Firstly, the variables were analyzed using univariate logistic regression and the variables with a $p < 0.2$ were used for final modeling. Five final models were created using the backward stepwise regression method. The first model included the demographic non-modifiable variables, the other four models contained the early life modifiable exposures during prenatal life, neonatal life, early postnatal life and late postnatal life, respectively. The five models were adjusted for age and the statistical significance was considered for p-values lower than 0.05 (*), 0.01 (**), and 0.001 (***). To help the veterinarian or nutritionist reader make an informed choice regarding the type of food that they usually prescribes in their practice, we report our regression analysis using two dummy variables that were created for the dichotomous variables instead of one. The missing values were not imputed. To test the fitness of the regression models an Omnibus test p-value should be lower than 0.05, a Hosmer and Lemeshow test p-value should be larger than 0.05 and the

Nagelkerke's R should be as big as possible (41, 42). The statistical analyses were performed using SPSS version 25. The visualization of odds ratios was carried out using the forest plot package (43) in R software version 3.5.1 (44).

RESULTS

Inflammatory Bowel Disease Prevalence (IBD) in the Finnish DogRisk Food Frequency Questionnaire (FFQ) Population

The prevalence of IBD in the FFQ population differs between the age groups and when the population was stratified for gender, history of maternal disease, white color coat ratio, and IBD prone breeds as shown in **Figure 3**.

Variables Characteristics

The distribution of the tested variables within the cases, controls and the total study cohort are presented in **Table 2** as frequencies (%) and numbers (n) for the categorical variables, while the scale variables are presented as means $\pm$ SDs (**Table 2**).

Regression Analysis

The univariate logistic regression analysis showed 10 significant associations ($p < 0.05$). Additionally, six variables ($p < 0.2$) were also included in the final models (**Table 3**).

From the multivariate logistic regression models, four early-life exposures showed a significant association with canine IBD incidence in adulthood and one early-life exposure showed a non-significant "trend" as shown in **Figure 4**. The final models'

TABLE 3 | Associations of early life covariates with inflammatory bowel disease in dogs based on univariate logistic regression analyses.

Covariates	Crude effect estimates		Multivariate models
	cOR (95% CI)	*P*-value	
Non- modifiable; genetic and demographic factors			
Maternal history of IBD			Included in model I
Non-IBD mothers vs. IBD mothers	0.113 (0.066–0.195)	**<0.001***	
IBD vs. non-IBD mothers	8.820 (5.141–15.131)	**<0.001***	
Dog breed			Included in model I
Non-IBD prone vs. IBD prone	0.812 (0.709–0.930)	**0.003****	
IBD prone vs. non-IBD prone	1.231 (1.075–1.410)	**0.003****	
Dog gender			Included in model I
Female vs. male	**0.505 (0.443–0.575)**	**<0.001***	
Male vs. female	1.981 (1.739–2.258)	**<0.001***	
Dog color			Included in model I
> 50% white coat vs. < 50%	1.117 (0.954–1.307)	**0.169**	
< 50% white coat vs. > 50%	0.895 (0.765–1.048)	**0.169**	
Dog age[§]	1.007 (0.987–1.027)	0.505	Included in model I
Modifiable factors			
I. Prenatal period maternal factors			
Mother's diet during pregnancy			Included in model II
NPMD vs. UPCD	0.639 (0.404–1.009)	**0.055**	
UPCD vs. NPMD	1.566 (0.991–2.474)	**0.055**	
Was the mother dewormed during/just before pregnancy?			Not included
Yes vs. no	0.924 (0.562–1.521)	0.757	
No vs. yes	1.082 (0.657–1.780)	0.757	
Was mother vaccinated during/just before pregnancy?			Included in model II
Yes vs. no	1.547 (1.182–2.024)	**0.001****	
No vs. yes	0.647 (0.494–0.846)	**0.001****	
II. Neonatal period (0–1 month of age)			
Mother's diet during lactation			Included in model III
NPMD vs. UPCD	0.653 (0.409–1.043)	**0.075**	
UPCD vs. NPMD	1.532 (0.959–2.447)	**0.075**	
III. Early postnatal period (1–2 months of age)			
Puppy's first solid diet			Included in model IV
NPMD vs. UPCD	0.541 (0.343–0.853)	**0.008****	
UPCD vs. NPMD	1.847 (1.172–2.912)	**0.008****	
Frequency of outdoor activity			Included in model IV
Many times/day vs. not at all	0.588 (0.443–0.780)	**<0.001***	
Once/day vs. not at all	0.677 (0.485–0.947)	**0.023***	
A few times/week vs. not at all	0.859 (0.610–1.210)	0.384	
A few times/month vs. not at all	0.999 (0.661–1.509)	0.995	
Type of flooring			Included in model IV
Slippery vs. dirt flooring	1.137 (0.746–1.734)	0.550	
Non-slippery vs. dirt flooring	1.223 (0.847–1.767)	0.283	
Newspaper vs. dirt flooring	1.371 (0.953–1.973)	**0.089**	
Carpets vs. dirt flooring	1.350 (0.936–1.946)	**0.109**	
Rest, hours/day	1.001 (0.958–1.045)	0.977	Not included
Body condition Score			Not included
Slim vs. normal weight puppies	1.101 (0.848–1.431)	0.469	
Obese vs. normal weight puppies	1.009 (0.807–1.263)	0.934	
IV. Late postnatal period (2–6 months of age)			

(Continued)

TABLE 3 | Continued

Covariates	Crude effect estimates		Multivariate models
	cOR (95% CI)	P-value	
Puppy diet			Included in model V
NPMD vs. UPCD	0.622 (0.442–0.875)	**0.006****	
UPCD vs. NPMD	1.608 (1.143–2.262)	**0.006****	
Outdoor activity, hours/day			Included in model V
0.5–1 vs. < 0.5	2.125 (1.054–4.284)	**0.035***	
1–2 vs. < 0.5	1.928 (0.963–3.858)	**0.064**	
> 2 vs. < 0.5	1.736 (0.855–3.524)	**0.127**	
Rest, hours/day	1.008 (0.983–1.034)	0.528	Not included
Type of flooring			Included in model V
Slippery vs. dirt flooring	1.951 (0.930–4.094)	**0.077**	
Non-slippery vs. dirt flooring	2.027 (0.967–4.250)	**0.061**	
Newspaper vs. dirt flooring	3.864 (0.997–14.973)	**0.051**	
Carpets vs. dirt flooring	1.820 (0.855–3.876)	**0.120**	
More than two types vs. dirt flooring	2.249 (1.078–4.690)	**0.031***	
Body condition score			Included in model V
Slim vs. normal weight puppies	1.411 (1.193–1.669)	**<0.001*****	
Obese vs. normal weight puppies	1.292 (0.959–1.740)	**0.092**	
Was the puppy vaccinated 2–4 times under 1 year of age?			Included in model V
Yes vs. no	0.688 (0.417–1.134)	**0.143**	
No vs. yes	1.453 (0.882–2.395)	**0.143**	
Was the puppy dewormed 2–10 times under 1 year of age?			Not included
Yes vs. no	1.117 (0.587–2.125)	0.736	
No vs. yes	0.895 (0.471–1.703)	0.736	

cOR, Crude odds ratio; CI, Confidence intervals; IBD, Inflammatory bowel disease; NPMD, Non-processed meat based diet; UPCD, Ultra-processed carbohydrate based diet; vs., versus. *p < 0.05, **p < 0.01, ***p < 0.001, Bolded values indicate p < 0.2, §scale variable measured in years.

odds ratios for the associations of the non-modifiable and the early life modifiable factors with IBD in adult dogs are presented in **Figure 4**. A p < 0.05 and an OR > 1 means increased risk for IBD while p < 0.05 and OR < 1 means decreased risk.

DISCUSSION

A growing trove of literature regarding genes and the microbiome confirmed by data from human epidemiological studies advocates the importance of early life exposures in modulating the risk of IBD incidence (18, 45–48). However, the impact that early life environmental and nutritional exposures have on IBD risk in dogs has not been previously examined. This is the first study to investigate the association between modifiable early life exposures and the incidence of IBD in adult dogs. Exploring the most pertinent early life exposures provides clues to the etiopathogenesis of this complex disease and allows for the development of a primary prevention strategy for IBD in dogs. At the same time, as a dog's pregnancy and early life is shorter than a human's, but otherwise similar in both terms of environment and diet, this study might also provide insight regarding the prevention of human IBD. Our results are divided into the non-modifiable

factors that cannot be altered, and the modifiable factors, where the owners' choices can have an impact on their dog's future health.

Non-modifiable Exposures and How They Related to Owner Reported IBD

Our analysis of the non-modifiable background related factors showed that the maternal history of IBD was directly associated with IBD incidence in the offspring in later life. To the best of our knowledge, the family history of IBD in dogs and the risk of the disease morbidity in the offspring later, has not been demonstrated before. The role that the family disease history has on the offspring's subsequent IBD development in adulthood, has been studied in several human studies (18, 49–53). Our findings are similar to a human study, where they found that the greatest risk factor for IBD was having one or more affected first-degree relative (52). In the case of having two parents with IBD, the long-term risk of developing the disease in the offspring was over 30% (53). The increased risk of IBD in dogs with a maternal history of it supports the proposed genetic component of the disease (6). However, other theories exist: Freud et al. (54) concluded that long-term morbidity of pediatric diseases in the offspring up to 18 years of age (such as cardiovascular,
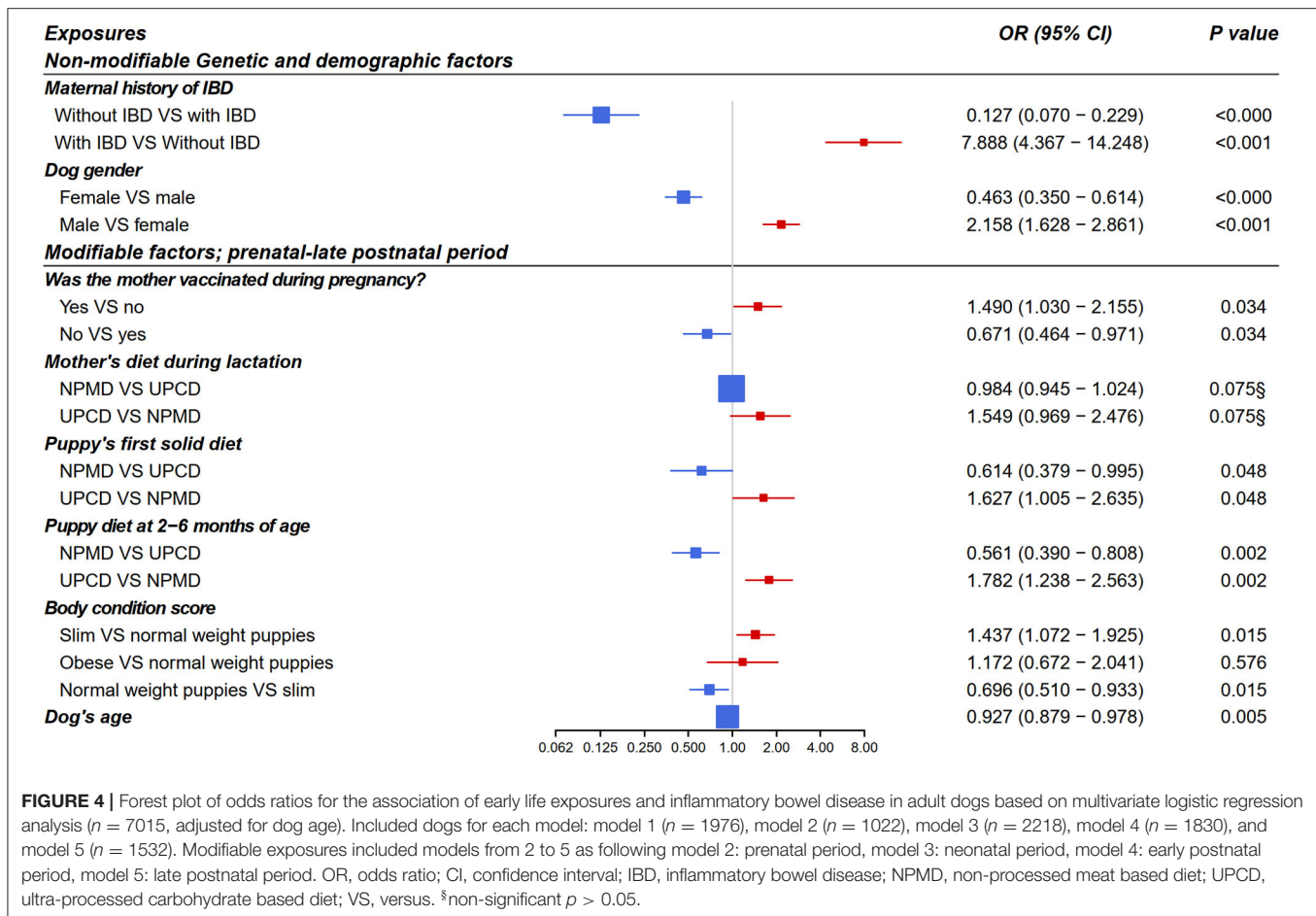
Exposures	OR (95% CI)	P value
Non-modifiable Genetic and demographic factors		
Maternal history of IBD		
Without IBD VS with IBD	0.127 (0.070 − 0.229)	<0.000
With IBD VS Without IBD	7.888 (4.367 − 14.248)	<0.001
Dog gender		
Female VS male	0.463 (0.350 − 0.614)	<0.000
Male VS female	2.158 (1.628 − 2.861)	<0.001
Modifiable factors; prenatal-late postnatal period		
Was the mother vaccinated during pregnancy?		
Yes VS no	1.490 (1.030 − 2.155)	0.034
No VS yes	0.671 (0.464 − 0.971)	0.034
Mother's diet during lactation		
NPMD VS UPCD	0.984 (0.945 − 1.024)	0.075§
UPCD VS NPMD	1.549 (0.969 − 2.476)	0.075§
Puppy's first solid diet		
NPMD VS UPCD	0.614 (0.379 − 0.995)	0.048
UPCD VS NPMD	1.627 (1.005 − 2.635)	0.048
Puppy diet at 2−6 months of age		
NPMD VS UPCD	0.561 (0.390 − 0.808)	0.002
UPCD VS NPMD	1.782 (1.238 − 2.563)	0.002
Body condition score		
Slim VS normal weight puppies	1.437 (1.072 − 1.925)	0.015
Obese VS normal weight puppies	1.172 (0.672 − 2.041)	0.576
Normal weight puppies VS slim	0.696 (0.510 − 0.933)	0.015
Dog's age	0.927 (0.879 − 0.978)	0.005

0.062 0.125 0.250 0.500 1.00 2.00 4.00 8.00

FIGURE 4 | Forest plot of odds ratios for the association of early life exposures and inflammatory bowel disease in adult dogs based on multivariate logistic regression analysis (n = 7015, adjusted for dog age). Included dogs for each model: model 1 (n = 1976), model 2 (n = 1022), model 3 (n = 2218), model 4 (n = 1830), and model 5 (n = 1532). Modifiable exposures included models from 2 to 5 as following model 2: prenatal period, model 3: neonatal period, model 4: early postnatal period, model 5: late postnatal period. OR, odds ratio; CI, confidence interval; IBD, inflammatory bowel disease; NPMD, non-processed meat based diet; UPCD, ultra-processed carbohydrate based diet; VS, versus. §non-significant p > 0.05.

endocrinal, respiratory, hematological, neurological, urinary, and gastrointestinal diseases) were not affected by maternal IBD during pregnancy. This suggests that genetics alone cannot explain the increased prevalence of IBD, instead it synergizes with other potential risk factors, especially diet (1, 2).

In the present study, the male dogs exhibited a higher risk of developing IBD than females did. Similarly, a Japanese study that studied the prevalence of chronic enteropathy in dogs through using an insurance-based population found that there was a slightly higher disease prevalence in males compared to female dogs (55). On the other hand, some studies have reported that there is no gender predisposition of IBD development in dogs (5, 56). As IBD is often used as an umbrella term for different diseases, the different types of IBD and the different breeds studied may be the reason for this discrepancy between different studies.
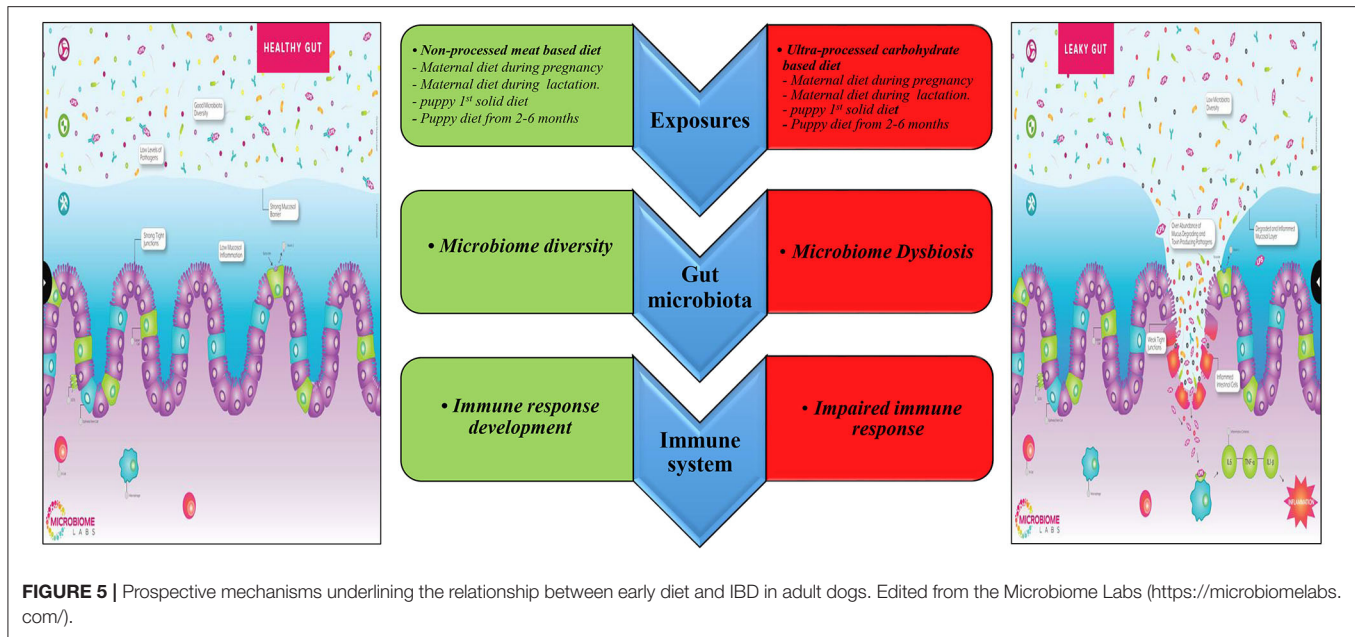
In our study, there was a significant association between the incidence of IBD and the dog's age. We found that the highest IBD prevalence within different age groups was in middle-aged dogs, from 5 to 10 years of age. This finding is in accordance with one study (5), and with anecdotal knowledge that IBD is mainly a disease of middle-aged dogs.

The incidence of IBD showed a significant difference between the IBD-prone and non-prone breed cohorts (**Figure 3**). Breed

predisposition has been recognized for IBD [(7, 8), **Table 1**]. Gluten sensitive enteropathy has been reported with Irish setters (7, 8), while ulcerative colitis has been found to be most common in boxers (7, 9, 10, 12). Chronic enteropathy or protein-losing enteropathy is the most common form of IBD. This form can affect many breeds and mixed-breed dogs (2, 7, 8, 12). German shepherd dogs are more prone to develop antibiotic responsive diarrhea (13), especially tylosin responsive diarrhea (11).

Modifiable Exposures and How They Related to Owner Reported IBD

Next we present the modifiable prenatal exposures that were significantly associated with IBD prevalence in adulthood. Surprisingly, the maternal vaccination during or just before pregnancy was significantly associated with more IBD in the offspring in adulthood, while not vaccinating the dam was associated with less IBD in the offspring at adult age. Literature on the risks of prenatal exposure to vaccines and incidence of IBD later in dogs remains limited. However, our finding is in line with studies of adjuvants such as aluminum salts and mercury-containing compounds such as thimerosal, that have been reported to be involved in the development of inflammatory disorders (57, 58) and stimulation of the immune system in

FIGURE 5 | Prospective mechanisms underlining the relationship between early diet and IBD in adult dogs. Edited from the Microbiome Labs (https://microbiomelabs.com/).

humans (59). A human study testing the effect of childhood vaccination and risk of IBD later found an association between an early life measles vaccination and the risk of IBD later (60), while two other studies found no association between measles vaccines in early life and the risk of IBD (61, 62). Moreover, a meta-analysis showed that the majority of childhood vaccinations against different infectious diseases do not increase the risk for subsequent IBD development (63). We also analyzed the associations between early life puppy vaccinations and IBD and found that these vaccinations had no association with IBD. As there results are controversial and our data only suggestive, we recommend further studies looking at this before any conclusions are drawn.

From the modifiable postnatal exposures, the maternal diet during the lactation/neonatal period appeared to be an important modifiable factor in our study, although it did not reach significance in our final models. Evidence proposes that the maternal gut microbiome may be translocated intracellularly to the mammary glands through the systemic circulation (64). This has been studied in humans, where the authors concluded that the maternal diet in the neonatal period alters the gut microbiome of the offspring, which subsequently modulates the risk of related diseases through breastfeeding (65). The role of the maternal diet during lactation has been confirmed to shape the lifelong health of the newborn human child (66). However, the direct impact of the maternal diet on the milk microbiome during pregnancy and lactation was not established in our study. Future research is needed to test how different feeding patterns in dogs during pregnancy and lactation affects the neonate gut microbiota diversity.

During the early and late postnatal periods, there were significant negative associations between the NPMD and incidence of IBD for the same dogs later in life. These findings are in accordance with several studies, which stated that a raw meat-based diet stimulated the growth of a balanced gut microbiome in healthy dogs which improved their gut function in comparison to dogs fed an extruded dry food (28, 30, 36, 67). Our observations are in accordance with the hygiene hypothesis, which states that the more microbial exposures in the early life, the more developed immune system in adulthood (22, 23). This confirms the role of the NPMD in developing the immune system when given in the early life (14, 68).

Besides the interaction between diet, gut microbiome, and the immune system discussed above (**Figure 5**), there are additional factors that may explain the effect of the early life diet on the offspring's risk of IBD in adulthood. First, the maternal and postnatal diet can permanently modify the epigenetic programming in the newborn during its formative early life. A recent review suggested that the individual becomes resistant or susceptible to diseases by altering inflammatory molecular pathways and immunity via epigenetic modification (69). However, the underlying mechanisms are not clear. Evidence suggests that the maternal gut microbiome can affect the neonate gut microbiome by causing a particular epigenetic signature that can influence the intestinal barrier's properties against inflammatory diseases (69).

Next we discuss the effects that distinct dietary components may have on gut health and physiology. The UPCD contains a high ratio of (carbohydrate and gluten-rich) processed cereal, and hence may increase the dogs' risk of developing gluten sensitive enteropathy (70–72). Furthermore, in mouse studies, the authors found that the consumption of diets rich in refined carbohydrates increased intestinal dysbiosis, permeability, and inflammation (73, 74). As sugars are highly absorbable and carbohydrates are chains of short sugar molecules, a high-carbohydrate diet may cause a high sugar concentration in

the intestinal lumen. This in turn may supply excessive energy for the microbiota and hence lead to intestinal bacterial overgrowth (75). The UPCD also contains a low dietary fiber content. Dietary fibers, also called prebiotics, are non-digestible carbohydrates responsible for maintaining normal intestinal homeostasis through encouraging bacterial diversity, protecting mucosal barriers, and increasing the synthesis of short chain fatty acids (76, 77). Furthermore, the processing methods, which the UPCD or their ingredients, are exposed to, such as rendering, milling, fermentation, heat treatment, and extrusion negatively affects the bioavailability of key minerals and vitamins present in the diet (78, 79). Feed additives, e.g., dietary emulsifiers which are used to improve kibble texture have been found to contribute to the increased incidence of inflammatory diseases both by reducing the gut microbiota diversity and by reducing the thickness of the mucus layer (80). Since dogs have no requirement for carbohydrates (81), this underlines the fact that carbohydrates should not be a main ingredient in a dog's diet (82). As carnivores, dogs have evolved to eat fresh meat-based diets rich in animal proteins, fats, and animal fibers (83, 84). A canine study showed that a raw meat-based diet was both highly palatable and highly digestible when compared to an extruded diet. Moreover, although including lots of fats and proteins, the NPMD decreased blood triglyceride levels, maintained normal serum chemistry and high fecal quality, and altered the fecal microbiota and metabolite concentration (85).

The body condition score during the late puppyhood period revealed that there was a significantly negative association between IBD development and dogs with normal body weight, while puppies with a lower body weight associated positively with developing the disease.

Prior research on IBD shows that there is an association between increase in obesity in the population and IBD prevalence (86). Obesity is a known perpetual factor of systemic low-grade inflammation (87), generating a pro-inflammatory state and immune dysregulation in obese puppies (88). Furthermore, a review also established an association between increased IBD morbidity and malnutrition (89). Additionally, a study found that young IBD patients have been demonstrated to have weight loss prior to diagnosis (90), where 57% of patients with Crohn's disease and 51% of patients with ulcerative colitis exhibited a significantly low body mass index prior to diagnosis. The association of a low body condition score during puppyhood with IBD incidence later in life maybe a reflection of active undiagnosed disease at young age. The loss in weight prior to or at the time of the disease diagnosis is attributed to many factors. As IBD is an inflammatory disease, it stimulates catabolism, alternates metabolic hormones levels, increases the gastrocolic reflux leading to loss of appetite, and causes nutrient malabsorption (90). Although incidences of IBD in puppies < 6 months old have been recorded (5), it remains unclear whether the loss in body weight during puppyhood is a cause or a consequence of IBD. Further research is needed to understand the physiological disturbance underlying the association between the body condition score and active IBD.

Limitations and Strengths of the Study

The present study has some limitations. Firstly, the study design is an owner-reported cross-sectional study which makes it prone to recall bias. In order to overcome this limitation, a thorough questionnaire of the activity and dietary habits of 2,000 different aged Finnish dogs was conducted prior to this FFQ (not published). Based on these results, we constructed this DogRisk internet-based FFQ where all of the foods that were mentioned by owners in the first questionnaire were included as multiple choice questions in the new one. Two empty rows were also given to the owner, if there still would have been things missing. It is considered better to give options to owners that they can choose from when they see them written, that that they should remember all things by themselves. Another limitation is that the FFQ is based on frequencies and not quantity. Also, due to the lack of details regarding the ingredients of the food variables, we were not able to examine the nutrient profiles of the diets. Moreover, the study cases and controls were reported by the owners. We did not ask the owners for a veterinary visit wrap-up, including the diagnosis. To some extent, this has been overcome by validating different types of diseases from the FFQ in a previous validation study (39). Another limitation is that the data has a lot of missing values. This was overcome by retaining a reasonable sample size ($n = 7,015$).

The present study also has strengths. It is the first study to investigate early life environmental and nutritional exposures and their association with IBD incidence in dogs. It is also the first study to investigate the associations between genetic and demographic variables on IBD incidence in dogs. A wide range of covariates were covered and validated data was used (39), both which favor the study's validity, reliability and reproducibility. Furthermore, our data included information about four early life periods, pre- and postnatal, which covered 8 months of the dog's life starting from conception till the age of 6 months. Finally, reverse causality was considered and addressed in this study when choosing the age limits of the cases and controls.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, our study identified many modifiable early life risk and protective factors for canine IBD incidence in adulthood. We conclude that a raw feeding pattern, which typically features a moderate protein, high fat, and low carbohydrate macronutrient profile, during neonatal, early and late postnatal periods, as maternal and puppy diets, was associated with a decreased risk of IBD later in life. Conversely, a dry extruded feeding pattern, which mostly includes a moderate protein, low fat, and high carbohydrate macronutrient profile, during the same periods, was associated with an increased risk of developing IBD later in life. In addition, maternal vaccination during or just prior to pregnancy was significantly associated with a higher risk of IBD incidence in the offspring later. Furthermore, a normal body condition score was associated with a decreased risk of IBD development, while being abnormal in weight (very lean) was associated with an increased risk of IBD at adult age. As foretold,

the identified non-modifiable risk factors from the current study such as dogs with a history of maternal IBD, male dogs, middle-aged dogs, and dogs from breeds prone to IBD development, were all associated with an increased risk of IBD prevalence. Our novel findings regarding the modifiable risk factors provide clues for further research in the disease prevention. The study findings suggest a causal relationship but does not prove it. Future prospective longitudinal dietary intervention studies are needed to confirm our findings, as well as to develop primary strategies for IBD prevention in dogs.

DATA AVAILABILITY STATEMENT

Being funded by commercial sources has not altered our adherence to Frontiers policies on sharing data and materials. The data is still used for theses and will be totally disclosed later. However, for research purposes the data can be obtained upon request from the authors: anna.hielm-bjorkman@helsinki.fi.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethical approval (29.4.2016) from the University of Helsinki Viikki campus ethical board. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

MH and AH-B planned, designed, and drafted the study. AH-B organized the database. MH and AH-B performed the data extraction and did the statistical analysis together with KV who created the figures from statistical software. All authors wrote sections of the manuscript and edited it, contributed to manuscript revision, read, and approved the submitted version.

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