Utrecht University Faculty of Veterinary Medicine

Department Clinical Sciences Companion Animals - Clinical Nutrition

Master Thesis

Guardian reported effect of raw meat based diets in young animals on adult development of food adverse reactions

Lynn van der Ende (6375340)

Universiteit Utrecht

Date: June 2024

Under the supervision of Ronald Jan Corbee, DVM, PhD, Dipl. ECVCN, a Dutch expert in Veterinary Animal Nutrition and a European specialist in Veterinary and Comparative Nutrition.

Assisted by biostatistician Hans Vernooij, MSc.

Preface

The following pages contain the definitive version of my master thesis: "Guardian reported effect of raw meat based diets in young animals on adult development of adverse food reactions". Written to meet the requirements of a master's degree in Veterinary Science from the faculty of veterinary science at Utrecht University and was completed in 15 weeks.

The research question was inspired by my junior external internship, where raw meat was not endorsed by veterinarians but nevertheless adopted by them as a preventive measure against food adverse reactions in some breeds they deemed high risk for development of such diseases. Triggered by these claims of preventive qualities associated with raw meat and knowing that students are taught to critically evaluate such statements, I found no scientific evidence to support this relationship, yet.

No current research on this topic was ongoing and so a new study had to be created. I was given the opportunity to do so and am incredibly grateful for this. I would like to extend my special thanks to my supervisor Ronald Jan Corbee for giving me the opportunity and faith to set up my own research. Thank you also, for creating time in your schedule to guide me, allowing me to discover on my own whilst steering me to the absolute best version of this thesis. I have certainly learned very much of this process. A significant amount of gratitude is also owed to Hans Vernooij, without whose patience I would have had a hard time presenting my findings in a statistically correct manner. Thirdly I am grateful for all participants of my survey; without their contributions to the data, this thesis would not have been possible. Lastly, I would like to thank my brother for his help in fine tuning the design of my thesis and its figures and tables.

Hopefully, this thesis will be appreciated as much as I am proud of it.

Lynn van der Ende

Abstract

Raw feeding practices and their increasing popularity, despite concerns being raised on this diet, among pet owners are an interesting subject for observing preventative qualities of nutrition. Using the hygiene hypothesis as foundation, this study examined the relationship between puppyhood diet and adult health status, focusing on food adverse reactions in dogs in the Netherlands. Data from 327 survey entries was analysed, leading to 266 respondents eligible for study entry. No significant benefit on risk of food adverse reaction development of raw feeding practices in puppyhood were found in the final analysis, however: tendencies towards lower odds were seen for the raw fed dogs. This study, due to its owner-based data, is very susceptible for bias. Further research into the preventative qualities of raw meat based diets are therefore necessary before recommendations on feeding practices can be made.

Contents

1 Introduction

Many diets in human nutrition claim positive health effects (Łuszczki et al., 2023). In the petfood industry, this is no different (Knight, Huang, Rai & Brown, 2022; Empert-Gallegos, Hill & Yam, 2020). Though there is an enormous spectrum of pet diets, one diet in particular stands out: the Bones and Raw Feed (BARF) diet. It distincts itself in its approach to canine nutrition by emphasizing the importance of raw and unprocessed ingredients, mirroring the ancestral canine diet (Freeman, Chandler, Hamper & Weeth, 2013). Ingredients typically used are raw meat, bones, fruits and vegetables thereby responding to the growing trend and need of pet owners seeking an alternative, more natural diet than processed and cooked kibble (Michel et al., 2008).

The relevance of the BARF diet, however, extends beyond its popularity. Its impact on the health of companion animals opens the conversation on the impact of the overall used kibble as pet nutrition on companion animal well-being. For years veterinarian professionals have been supportive of commercially sold kibble as this presented a streamlined approach to dog nutrition, free of the risk for nutritional deficiencies. Supporters of BARF however, argue their choice of diet improves their dog's dental health, gut health, skin condition and overall energy and liveliness when compared to these more processed diets (Empert-Gallegos et al., 2020; Freeman et al., 2013). Concurrently, concerns have been expressed by professionals on potential risks associated with feeding BARF diets including, but not limited to, nutritional imbalances and contamination and infection of guardians with antibiotic resistance microbes (Freeman et al., 2013; van Bree et al., 2018).

To broaden the scope of this thesis, not just the original BARF diet was taken into consideration but the influence of any BARF derivative diet. The derivative diets allowed in this thesis must be similar to BARF in their emphasis on raw and unprocessed ingredients but may differ slightly from the original BARF principles as they may include modifications. Examples of modifications are the inclusion of supplements or different ingredient ratios. For example, other raw meat based diets can contain a certain amount of grain fillers, thereby not complying with the BARF principle of the exclusion of grains, fillers and artificial additives (Michel et al., 2008). By including these raw meat based diets in the study as well, a more nuanced understanding of the impact of raw meat based feeding on canine health can be provided. This nuance is needed for the BARF diet may not be the exclusive benchmark for enhancing health, as it is now often presented by its supporters.

Examining the raw meat based diets' possible implications, using its popularity as an advantage, is necessary. Not only is knowledge needed to properly advice and guide raw feeding practices. If a dietary basis of raw meat indeed does improve organ health, for example gut health, its preventive working would help keep our population healthy and reduce the necessity of medical interventions. This paper aims to investigate the use of the raw meat based diet in the puppyhood of a dog, in prevention of developing food adverse reactions (FAR) at a later stage in life. In other words, the aim is to gain an answer to the question: "Does feeding a raw meat based diet, when compared to feeding a heat-processed diet, to dogs of <6 months reduce chances of developing FAR in the first 2 years of their life?". As there is no exact prevalence and food allergies have been diagnosed in dogs of all ages, an estimate had to be made as to what age limit would be used. The age limit was produced after initial descriptive analysis of the retrieved dataset.

Sub-questions relating to the main objective that also to be answered in this thesis are:

- What cut-off value for age limits in the case and control groups presents the best balance between remaining with a large study group and having a large number of cases?
- If a protective quality of raw meat based diets against FAR development exists, does quantity of the raw meat proportion matter?

To include the multifactorial nature of FAR, a second aim was created. This paper explores and compares the relative risk of developing food allergies for a variety of management, disease and character related variables. Hereby gaining comprehensive and useful insights in correlations between these variables and FAR development in adulthood.

To fully understand the possible implications and challenges of the raw meat based diets, firstly its influence on socio-economic and environmental aspects must be discussed. Secondly, it is important to understand the underlying etiology and pathophysiology of FAR such as food allergy and to address existing struggles of managing it. This information will be provided in the background section (section 2). Lastly, of course, data is needed. The data used in this thesis will be retrieved from guardians residing in the Netherlands, gathering information on their dogs' puppyhood and their adulthood health status, using an online questionnaire. For more details on the materials and methods used, see the 'Materials and Methods' section (section 3).

In conclusion, raw meat based diets, such as BARF, are emerging and notable in the broad spectrum of available pet diets. There are several BARF derived diets on the marked that are similar in their emphasis on raw and unprocessed ingredients. As the potential implications for the use of raw meat based diets as preventive medicine and improving overall canine health will be discovered, its challenges will also be discussed. Furthermore, other aspects of puppyhood will be examined to better understand the interplay between the choices and events in puppyhood and food allergy development in adulthood. The goal being to offer valuable, comprehensible and straightforward advice to pet owners and their respected veterinarians.

2 Background and literature

In the introduction, only a brief exploration of the BARF diet and its attributes on canine health and guardian popularity was outlined. This background section will be delving deeper into the attributes, implications and the potential role of the BARF diet and its derivative diets, henceforth referred to as raw meat based diets, in prevention of FAR development.

Before this can be discussed however, it is essential to consider the multiple facets on which (any) diet choice has an influence. This background will provide its readers with a comprehensive understanding of/ a brief overview on important considerations when choosing a diet, taking the BARF diet as an example. This thesis will explore the health, environmental, social and economic impact of the BARF diet and further dive into raised concerns on it. A more detailed description of food allergies and their struggles will also be discussed, including its relationship to *Giardia spp.* infections. After this, the attention was transferred to its possibly preventive qualities and alleged beneficial effect on canine health by reviewing the results of the questionnaire. Through this approach, both endorsements of BARF and raised concerns are incorporated in the context of FAR prevention in a clear and well-structured manner. With this

foundation, readers are provided with the means necessary to develop their own perspective on recommendations presented in the conclusion of this thesis.

2.1 General diet considerations

What we consume does not only affect ourselves. It has an (enormous) environmental, social and economic impact as well. For example, eating fair trade chocolate like Tony's Chocolonely provides sustainable use of resources. It also ensures a fairer distribution of profits and overall improvement of the economy of the country providing us with the raw materials (Beacom, 2024). This concept also applies to the petfood industry (Swanson, Carter, Yount, Aretz & Buff, 2013). Examining the environmental, social and economic consequences in the context of the BARF diet, a complex interplay is revealed between dietary choices and their benefits and broader sustainability concerns. For instance the excessive consummation of animal-based proteins in pet food raises ethical questions regarding resource allocation as well as environmental questions on use of resources, such as land and water and unnecessary high emissions of greenhouse gasses (Knight, 2023). From these perspectives, the Bones And Raw Feed diet with its raw meat based ingredients is not a desirable pet food diet. The intended purpose, however, can justify the means used. If no other diet can achieve healthiness in a companion animal or prevent disease, the BARF diet might be the only choice left. It is the responsibility of the owner to choose the right balance between health and sustainability and the responsibility of the veterinarian to provide the information to make such decisions.

2.2 The Hygiene hypothesis

Consequently, veterinarians need evidence to support the positive effect of a certain diet on companion animal health. A hypothesis that lies at the start of research into allergy development, is the hygiene hypothesis. It was firstly developed in the context of hay fever and states that an increase in microbial exposure stimulates the immune system thereby protecting the host from developing inflammatory or allergic diseases (Strachan, 1989). Later, a similar relationship between parasitological exposure and immune system development was described (Yazdanbakhsh & Matricardi, 2004). Building on the hygiene hypothesis, it was also stated that a lack of microbial exposure in childhood creates a lack of microbiome development, thereby influencing both the innate and the adaptive immune system leaving the unexposed individual prone to an increased prevalence of (allergic) diseases (Stiemsma & Michels, 2018). This is a possible explanation for rising counts of allergies in developed countries as described by Sicherer and Sampson (2018). Research conducted on dogs showed that microbial communities and metabolome vary significantly between BARF and commercially fed dogs (Schmidt et al., 2018; Butowski, Moon, Thomas, Young & Bermingham, 2022). Whether the BARF diet fed in puppyhood minimizes the risk of food allergy development currently remains unknown.

A guardian reported study in Finland already disclosed a relationship between the puppyhood diet and the adulthood incidence of atopic dermatitis in dogs. Their paper suggested both positive and negative influences of diet choice on the incidence of atopic dermatitis (allergies for surrounding allergens) exist. This could mean that the puppyhood diet is both the key and the culprit to FAR related challenges presented to guardians of dogs suffering from this unfortunate fate.

2.3 Food adverse reactions

Because suffering is what having a FAR entails. To determine the importance of preventing FAR a brief review of the underlying etiology, pathophysiology, clinical presentation and challenges will be presented, using food allergies as an example.

Firstly, the difference between a food allergy and FAR must be made clear. The most important difference is that while food allergies always involve a type of immune-mediated response, this is not the case for FAR or intolerances (Craig, 2019). These reactions may involve toxic, pharmacologic, or idiopathic mechanisms, which do not necessarily involve the immune system. An example would be food intolerances as they result from the inability to properly digest or metabolize certain components of food. Clinical presentation of food intolerance usually involves the gastrointestinal tract, while the food allergies can present themselves in multiple organs including the skin and gastrointestinal tract (Sicherer & Sampson, 2018; Jackson, 2023). The focus of this study is primarily based on preventing food allergies, food intolerance and idiopathic FAR with a chronic nature.

2.3.1 Food allergies

As discussed, FAR are divided into true immunological food allergies and food intolerance resulting from toxic, pharmacological, metabolic or idiopathic reactions to food (Jackson, 2023; Bhagat, Sheikh, Wazir, Mishra & Maibam, 2017). Food allergy development and its origin is very complex. It is supposedly a multi-factor process involving genetics, epigenetics and the environment that leads to an intolerance to dietary components (Jackson, 2023). The true etiology and pathophysiology of food allergies are therefore not yet fully understood(Bhagat et al., 2017). In addition, there is currently little information available about the prevalence of food allergies in dogs, only estimates were made (Jackson, 2023). The risk of a puppy in a certain environment developing a food allergy can therefore not accurately be estimated. What is known is that clinical signs related to food allergies are the result of immune disorders in the production of immunoglobulin E (IgE) or non-IgE mediated immune disorders. When any food is eaten it is presented to dendritic cells in the gastrointestinal tract. Being transported to the local lymph node by this cell, normally a regulatory T-cell response is initiated. Individuals with a food allergy however, develop an excessive effector T-cell response as well. Subsequently, these individuals' target cells (B-cells) are triggered to initiate an allergen specific IgE response causing the clinical symptoms of an allergy. This process can occur with or without the non-IgE mediated (cell mediated) reaction (Yu, Freeland & Nadeau, 2016).

To address a gap of information considering challenges presented to affected companion animals and their guardians three arguments will hereafter be presented. First of all, the first step to a diagnosis is excluding other causes for presented clinical signs. As the clinical signs of a food allergy can vary from ear infections and skin lesions to diarrhea (Olivry & Mueller, 2020; Bhagat et al., 2017) this can become a costly endeavour both in terms of time and money.

In addition to this, an elimination diet trial (EDT) is the only way to make a reliable diagnosis (Jackson, 2023; Sicherer & Sampson, 2018; Mueller & Olivry, 2017; Bhagat et al., 2017; Olivry & Mueller, 2020). Any deviation from this diet, however small, means that no clinical improvement can be a false negative and the diet has to be started from day 0. The EDT appears to be an intensive diagnostic tool for guardians. Painter, Tapp and Painter (2019) concluded that most prescribed EDTs were not followed strictly by guardians. This study also showed that owner

compliance and education were essential in the performance of an EDT, marking the substantial commitment asked of guardians. Unfortunately, in the best case scenario this major commitment is not temporary: even if success is achieved, a food allergy is a chronic illness (Baker & Nowak-Wegrzyn, 2020; Jackson, 2023; Sicherer & Sampson, 2018). There is a possibility that the earlier described diet and management adjustments and thereby the dedication they demand, extend for the remainder of the lifespan of their companion. In the worst-case scenario the aforementioned management changes can even fall short (Baker & Nowak-Wegrzyn, 2020; Jackson, 2023; Sicherer & Sampson, 2018).

2.4 *Giardia spp.*

As explained earlier, the usual role of IgE is normally most prominent in the defence against parasites. If no serum IgE against parasites is present, IgE is being directed to innocuous antigens instead (Fitzsimmons, Falcone & Dunne, 2014). Consequently, a reduction in helminth infections is associated with a rise in the prevalence of allergic diseases (Rook, 2009). The basis for this phenomenon lies in the earlier described hygiene hypothesis (Yazdanbakhsh & Matricardi, 2004; Rook, 2009). *Giardia spp.* infections are thus not a probable predisposition factor for food allergy development according to the previously mentioned studies, but there is no concrete evidence to support this statement yet.

However, there remains a belief among veterinarians that *Giardia spp.* infections might contribute to FAR (Skubic-Vengust, 2003). A possible explanation for this discrepancy lies in the treatment of *Giardia spp.* detections. Despite *Giardia spp.* sometimes being a commensal in dog populations (Covacin, Aucoin, Elliot & Thompson, 2011), the protozoa is often treated when detected (Biancardi, Papini, Giuliani & Cardini, 2004). It could be that FAR development in dogs is an effect of antimicrobial use, suggesting that the use of antimicrobial agents, rather than the *Giardia spp.* infection itself, might be a risk factors for FAR development. A relationship that has already been studied and observed in humans (Aguilera, Dagher & Kloepfer, 2020). Influence of antimicrobials on FAR development is beyond the scope of this thesis but this piece of background information justifies including *Giardia spp.* infections in the questionnaire.

2.5 Conclusion of background

In conclusion, raw meat based diets are complex and contain advantages and drawbacks. Careful consideration should be given to the excessive consumption of animal-based proteins in dogs. The notable variations in microbial communities and metabolome between BARF and commercially fed dogs could, following the hygiene hypothesis, contribute to lower prevalence of food allergies. Using the same train of thought and contrary to popular belief in the field, *Giardia spp.* infections could have a protective property against FAR development. Given that food allergies and to some extend FAR, require substantial dietary, management and possible therapeutic interventions, the relevance of its prevention is marked and crucial.

3 Materials and methods

3.1 Study design

The 'Puppyhood diet and food allergy' questionnaire is an epidemiological, owner reported cross-sectional, retrospective questionnaire. It was created at the end of 2023 using Google Forms, ensuring ease of accessibility and user-friendly interaction. The questionnaire was distributed

online via social media and by QR codes in the waiting rooms of a variety of veterinarian clinics in The Hague. All dogs for whom the puppyhood diet was known were eligible. To ensure this criterium was met, a question in the first section of the questionnaire asked owners if they knew the puppyhood diet of their dogs. If the answer was 'No', owners were send to the last section asking for permission of use in this study and could not fill in the rest of the questionnaire. No specific call for cases and controls were performed; the questionnaire differentiated between case and control dogs with a separate set of questions, as described in the 'Data curation' section (section 3.2). As part of this thesis, the researcher underwent theoretical training in literature review, study design and ethical considerations.

Participants were informed about the study's purpose and confidentiality measures. Consent was obtained from all participants after they finished the questionnaire. No laboratory animals were used.

3.2 Data curation

3.2.1 Survey respondents

A total of 328 respondents completed the survey, one respondent did not consent to the response being used in the study, leaving 327 consenting respondents. The first round of data cleaning procedures involved those who did not raise their dog from puppyhood and respondents lacking detailed information on puppy diet, 15 exclusions were made. Furthermore, one dog had not had any animal derived protein in their puppyhood diet. For this dog alone could not fill a category and its diets character was not similar to any of the other categories, this dog was excluded from the dataset.

3.2.2 Preliminary case and control groups

A binary question inquiring about veterinarian-diagnosed FAR in the dog determined a preliminary case and preliminary control group assignment. Dogs that had not received their diagnosis through a veterinarian ($n = 4$), of which diagnostic procedure was unknown ($n = 2$) or dogs that were still in the diagnostic process ($n = 19$) were excluded due to ambiguity in classification. The same was done for the 3 dogs that were not diagnosed with an EDT or available blood tests for food intolerances or allergies. Dogs that were diagnosed with a blood test but are not on any hypoallergenic diet or did not improve clinically on this diet were also to be excluded, however this was not the case for any of the dogs in this study. Additionally, exclusions were made for all dogs under six months old as they had not yet finished their puppyhood diet of 6 months. The aim of this was to mitigate reverse causality concerns. After exclusions were made, the study group consisted of 266 dogs. This group was used for descriptive and survival analysis purposes.

3.2.3 Statistical case and control groups

Dogs of all ages can develop FAR and allergies (Sicherer & Sampson, 2018; Carlotti, Remy & Prost, 1990). Subsequently, one can imagine a risk for bias in the preliminary case and control groups as devised above. To address this bias hazard it was considered to further thin the selection by creating a cutoff value and excluding dogs younger than the cutoff value for they may not have yet developed a food allergy. Without this cutoff value, there is a risk that younger dogs, which have not yet had the opportunity to develop food allergies, would be misclassified

as controls.

Choosing this cutoff age proved a challenge: because dogs can be diagnosed with food allergies at any age and no specific age cutoff has been identified in existing literature. The cutoff value was thus determined using the collected data from the survey entries. After the first descriptive statistics were performed, the results of which are discussed in detail in the results section (section 4), a cut-off value of 2 years was chosen for this. It presented the best balance between remaining with a large study group and having a large amount of cases. Though the misclassification bias is still present, this approach minimized its influence on the results.

By introducing the cutoff value, the effects of the diet on the chance of being diagnosed with a food allergy at the age of 2 are examined in this study. All animals diagnosed after 24 months of age did not belong to the control or case group and were therefore eliminated from the statistical study group. The same applies to animals younger than 24 months that have not yet been diagnosed with a food allergy: they are not a case and they have not yet completed the full period of 24 months and can therefore not be included in the control group either. This resulted in 79 exclusions from control and case group combined.

3.2.4 Summary of classification for statistical case and control group

- **Case group:** The statistical case group consisted of 30 dogs of both sexes, diagnosed with a FAR by a veterinarian using an EDT or a blood test. The EDT could be performed with and without provoking the animal after the 8 week period when improvement of clinical signs of the FAR was seen. The criteria for all case dogs was that their clinical symptoms had to have improved after a diet change to a hypoallergenic type of diet, either commercial or home cooked. Furthermore of these dogs the puppyhood diet was known, the owner had detailed information on puppyhood diet and health status and the reporting guardian was over 18 years of age. The minimum age to be in the case group was 6 months. There was no maximum age limit but the diagnosis had to be made at an age of 24 months or less. There was no exclusions based on type of puppyhood diet. Dogs of all breeds, sexes and both neutered and intact animals were included.
- **Control group:** The statistical control group consisted of 157 dogs who had not been diagnosed with a food allergy by a veterinarian. Furthermore of these dogs the puppyhood diet was known, the owner had detailed information on puppyhood diet and health status and the reporting guardian was over 18 years of age. The minimum age to be in the control group was 24 months. There were no exclusions based on type of puppyhood diet. Dogs of all breeds, sexes and both neutered and intact animals were included.

3.2.5 Conclusion of data curation

In conclusion the total study group consists of 266 dogs. They comprised the dataset used for descriptive analysis and survival analysis. The possible misclassification bias in the descriptive analysis are tolerated because the goal of conducting a descriptive analysis is to summarize and understand the data, not to draw conclusions or advices from it. This study group could also be used for the survival analysis, for the result of this analysis is minimally affected by censored dogs[1]. For the censored dogs, the survival function calculates the odds of developing a FAR over

[1]Censored: When measuring time-to-event data, censored observations are present. These are incomplete observations where the event has not happened but could still take place after data retrieval periods. An example:

time, factoring in the number of events and the available information on the remaining dogs involved.

For statistical analyses of which the results are not as minimally affected by censored dogs, the earlier mentioned cut-off value was used to determine case and control group. After 79 exclusions, 187 dogs remained for use in the chi-square tests and logistic regressions.

A flowchart of the exclusions can be found in Figure 1.

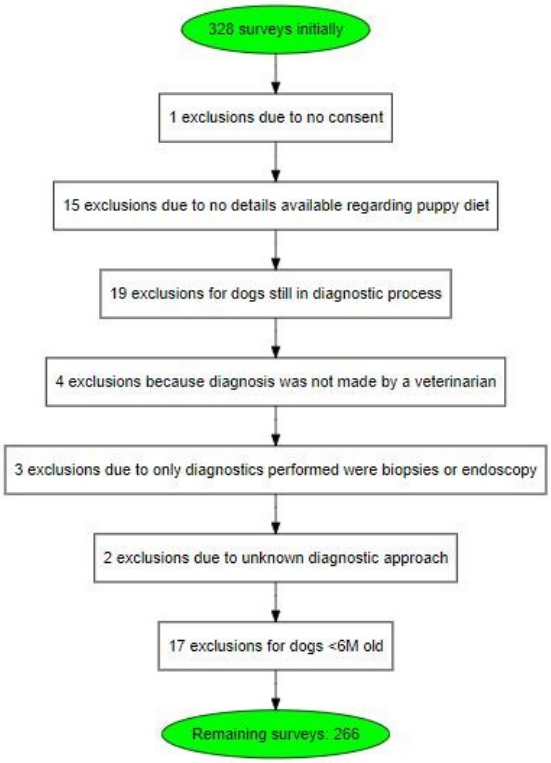

Figure 1: *Data curation process of survey responses: The initial dataset consisted of 328 responses. One respondent did not give consent for their survey response to be used in the study. Furthermore 15 exclusions were made due to no details available regarding puppy diet. Subsequently, 19 exclusions were made for dogs still in the diagnostic process. There were 4 exclusions because the diagnosis was not made by a veterinarian. Additionally, 3 exclusions were due to only diagnostics performed were biopsies or endoscopy, and 2 exclusions were made due to unknown diagnostic approach. Finally, 17 exclusions were made for dogs less than 6 months old, resulting in the remaining surveys being 266.*

3.3 Study variables and data analysis

3.3.1 Structure

The questionnaire comprised of ten sections, of which eight were of an informative nature and two were made only for data curation purposes. Of the eight informative sections, four were obligatory and four were non obligatory follow up sections. The original dataset is available upon request. Upon completion of the data collection phase, the acquired responses were exported from Google Forms and subjected to data cleaning procedures in Excel (version 2405 Build

dogs in our study can develop FAR their whole life, the endpoint of the timeline on which the observations can happen being death. If a dog has not yet received the diagnosis, all we know now is that this dogs' time-to-event is longer than the age at which the observation was made.

16.0.17628.20006) as described earlier in the 'Data curation' section (Section 3.2).

3.3.2 Questionnaire content

The questionnaire gathered information on four primary subjects: signalment, puppyhood diet, current health status and adulthood diet. A map of obtained variables from the questionnaire is projected in Figure 2. Not all variables were used in the data analysis.

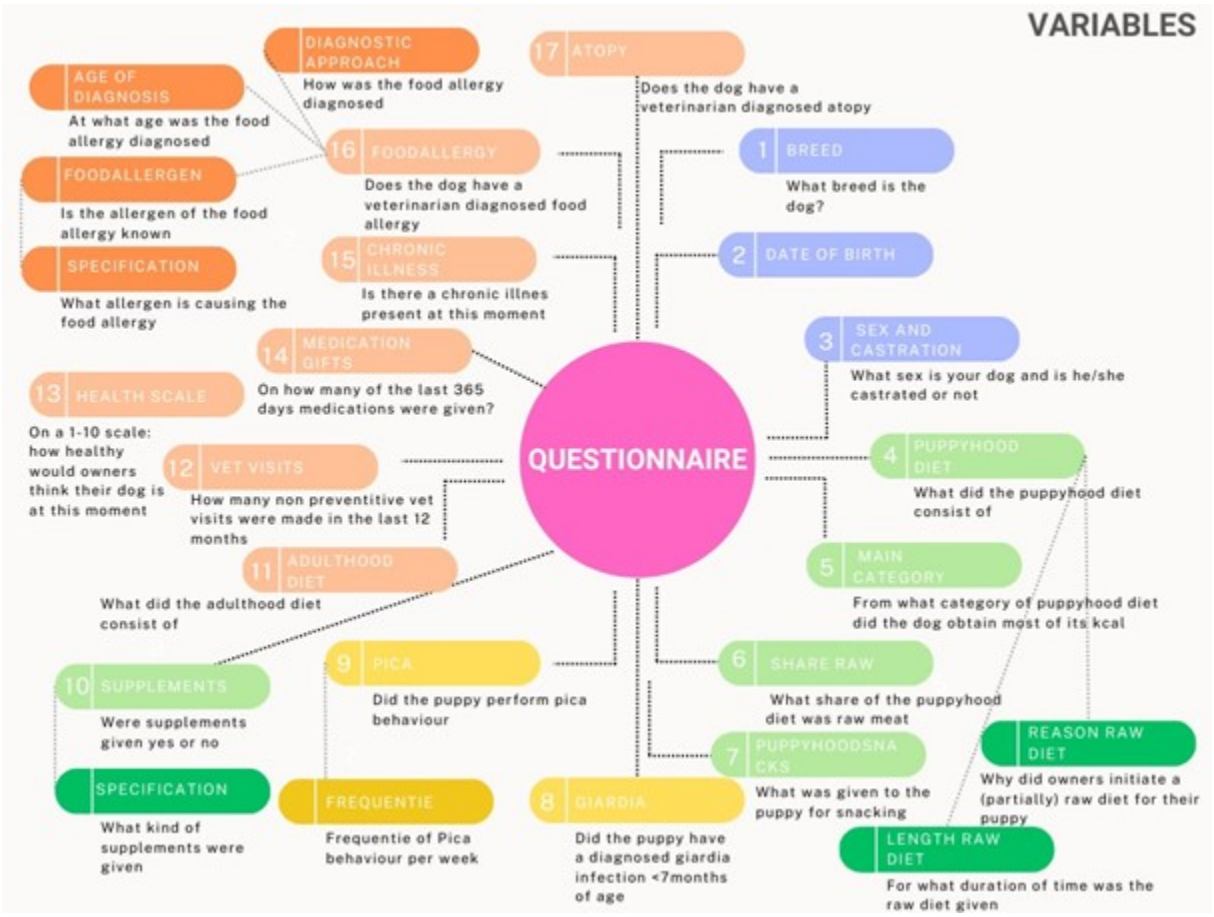

Figure 2: *Map of data obtained from the questionnaire. Questions are color-coded for clarity: blue questions are related to signalment, green questions pertaining to puppyhood diet, yellow to information from puppyhood not categorized under 'puppyhood diet' or 'signalment' and orange regards information on adulthood diet and health status. The numbers indicate the order in which questions were asked. Non-mandatory follow-up questions are not numbered.*

3.3.3 Categorization of non-quantitative questions

The qualitative questions used in all sections included both open-ended, multiple choice and selection menu questions. Open-ended questions were manually categorized in categories by clustering similar answers. This was amongst others performed for the answers on the question about main category of puppyhood diet in which respondents could tick a box from a pre-made list or write their own answer. The identical food items with similar nutrient profiles and processing methods were put together in 2 categories: heat processed meat and unprocessed/raw meat. For a total overview of all variables available for use in data analysis, see Table 9 in Appendix B.

3.3.4 Analysis

The data analysis has been performed using RStudio software, version 4.3.3, and was primarily aimed to explore potential associations between the puppyhood diet, with a specific emphasis on the raw meat based diets, and the development of FAR in adult dogs. However, during the initial examination of the dataset, additional variables that appeared to be potentially relevant were identified. Consequently, the focus of the analysis shifted towards a more comprehensive, multicentred approach, taking into account a broader range of factors beyond just puppyhood diet. Further discussion on these factors can be found in the 'Discussion and conclusion' section (section 5).

3.3.5 Categorization into binary categories

Additionally, each respondent's diet was assessed to determine if any part contained raw meat, resulting in a binary categorization. The question on sex and castration status offered four possible answers: intact female, intact male, neutered female, and neutered male. The responses were split into two binary variables: sex and castration status. This was done to be able to more specifically take into account the effect of these individual variables on food allergy development. Similarly, breed information provided under the question "What breed is your dog?" was used to derive a binary categorization: crossbreed or purebred dog. Breeds that were filled in but of which no recognized pedigree exists were labelled as crossbreed. Examples are the Labradoodle, Maltipoo and Pomchi.

3.3.6 Classification: case or control

Ultimately, after the data retrieving period had ended dogs were classified as control, case or neither using the criteria as described under the 'Data curation' section (section 3.2).

3.4 Statistical Analysis

Initially, descriptive statistics were used to summarize the demographic information of the participants and their dogs. Although not statistically significant, this information provided insight in and familiarity with the dataset. This proved useful later, when statistical approach had to be determined. Among other insights, the descriptive analysis provided the basis for the selection of a two-year cutoff age for dogs participating in either control or case group, more details on this can be found in the 'Results' section (section 4).

After gaining familiarity with the data, comparative analysis and statistical testing were started. The main objective of this study was to assess the contents of the puppyhood diet as a risk increasing or reducing factor for developing FAR. The secondary objective was to find other risk factors in FAR development. The investigated risk factors were qualitative and nominal variables, as was the outcome. Thus, for comparative analyses, chi-square tests and logistic regressions were performed. Because for a large part of the case group the age at diagnosis was also known, a survival analysis was performed as well.

3.4.1 Chi-square tests

Chi-square tests were initially performed to assess univariable association between binary or categorical variables and food adverse development. Validation of chi-square tests is dependent on the following conditions:

- A minimal sample size of 5 for each cell.
- Independence of observations.
- Both variables tested must be categorical.

For all variables, these conditions were met thus ensuring validation of the results.

3.4.2 Univariable logistic regressions

To further assess the hazard ratio for all variables, univariable logistic regressions were performed. The regressions were used to assess the relationship between various factors, including the puppyhood diet, and the incidence of FAR in adulthood. Model assumptions for the univariable logistic regression are as follows:

- Independence of observations.
- Dependent variable is binary of nature.
- The relationship between predictor variable and logarithmic odds of the outcome variable is linear.

Again, all conditions were met ensuring validation of the results. When compared to the chi-squared test, a second advantage of using logistic regressions is that they have a predictive value. The coefficients of the logistic regression model indicate a relationship between the predictor variables and the log odds of the outcome if there is one.

3.4.3 Multivariable logistic regressions

Though useful in providing indications of relevant variables, statistical significance was achieved in few. The multifactorial nature of FAR calls for a multifactorial approach. For this purpose a multivariable logistic regression was also performed. This model takes into account that there is more than one variable of influence on the outcome. The same model assumptions apply as in the univariable logistic regression.

The regression was then finetuned by excluding irrelevant variables. Exclusions were made based on Akaike Information Criterion (AIC) value. The AIC value is used to compare regression models: the model with the lowest AIC value has the most power. Following this logic, variables that contributed to a higher AIC value were excluded. This resulted in a regression where only relevant variables were left. However, to anticipate the results section, significancy of the regression was not frequent.

3.4.4 Survival analysis

As discussed before, dogs of all ages can develop FAR. The previously mentioned analysis were performed to assess the risk that a dog developed a FAR at the age of 2 years (24 months). To visualize the survival probabilities over time for different groups from different variables, a Kaplan-Meier survival curve was generated. To determine the significance of survival differences between groups, cox proportional hazards regression models were used. This was done with the coxph() function in R. A comparison between the hazard ratios for both groups was made and a p-value for significance was provided by this function as well.

The assumptions for the survival model are as follows:

- Independence of censoring on survival times.
- Non-informative censoring.
- Time-to-event data with a well-defined time frame

The assumptions for the cox proportional hazard regression model are as follows:

- Proportional hazards.
- Linearity.
- Independence of observations.
- Correct specifications of covariates.
- Non-informative censoring.

4 Results

4.1 Descriptive analysis

4.1.1 Signalment

As described in the material and methods section (section 3), 266 dogs were enrolled in the study after eliminations were made from 328 survey entries. For more details on this process, see Figure 1.

Dogs enrolled in this study were 55% female. Overall, 55% of the dogs were neutered. Females in this study were more often neutered: 60% of the females was no longer intact, compared to 50% in the male proportion. For 1 out of 266 dogs, no reliable value or estimate of the age was available. Age of the remainder of the study population ranged from 182 to 5536 days (Median = 1278 days, mean = 1621 days), minding that the dogs of <182 days were already excluded from participation. No exclusions were based on breed or pure bred status. 56% of the study subjects were crossbreed dogs. The remaining pure bred dogs ranged from toy-breeds (Toy-Poodle, Mini dachshund) to large breed (Staffordshire Bull Terrier, Rottweiler) dogs. Notable in the crossbreed proportion of the dogs was that Labradoodles comprised a large proportion, making up at least 33% of the crossbreed dogs. Interestingly also, Labradoodles were often categorized as a distinct breed by their owners, rather than recognized as a crossbreed. These observations underscore the popularity of the Labradoodle and the interests that owners of Labradoodles take in the subject of BARF feeding and FAR in the Netherlands.

4.1.2 Diet choice

Dogs that had not completed their puppyhood diet because they were less than 6 months of age were already excluded. 38% of the study population was fed a predominantly raw meat based diet and 62% predominantly a heat processed diet. 88% of the heat processed diets for puppies consisted of puppy kibble of various brands, the remaining 12% consisted of puppy wet food (4%), cooked meat (4%), adult kibble (3%) and therapeutic diets (1%).

4.1.3 General health status

40 of 284 dogs in the study population were affected by a *Giardia spp.* infection before 7 months of age. Of these 40 dogs, 80% had not developed any type of FAR at the time of survey entry which subjectively suggests there is no relationship between *Giardia spp.* infections and FAR development. Approximately 95% of the 266 dogs received a health rating of 6 or higher on a 10-point scale indicating generally good health, for more details see in Table 10 in Appendix B. 61% was even given a rating of 8 or higher. Appropriately, only 41 of 266 dogs in this study population were diagnosed with a chronic illness, 56% of which were allergies. The remainder of the chronic illnesses present in the study group was made up of various diseases ranging from Cushing's disease to valvular dysplasia. Osteoarthrosis was not recognised as a chronic illness but as a third category: degenerative joint disease. 7 of the dogs in this population suffered from osteoarthrosis according to their owners.

4.1.4 Atopic dermatitis

In all dogs enrolled in the study ($n = 266$), 22 dogs suffered from atopic dermatitis, making up 8% of the total population, and 4 were suspected but not, yet, diagnosed. Comorbidity of atopic dermatitis in dogs with a FAR was present in 18 dogs (7%). Notable is that 81% of 22 atopic dogs was also describe to suffer from FAR according to their guardians report. These numbers are likely to support earlier described relationships between atopic dermatitis and food allergies or FAR in human infants (Baker & Nowak-Wegrzyn, 2020) and the fact that they are not unlikely to be comorbidity factors. Though not completely within the spectrum of this thesis, this finding highlights the value of considering dietary factors alongside environmental allergens when diagnosing and managing atopic dermatitis in dogs.

4.1.5 Food adverse reactions

In the group fed a predominantly raw puppyhood diet (≥50%) the proportion of dogs with FAR was 0.13 compared to 0.19 in the group that mainly ate a heat processed diet. When considering only whether a dog had any raw meat in its puppyhood diet, regardless of quantity, the same slight increase in the proportion of adverse food reactions is observed in dogs that did not have any raw meat, as shown in Table 1 and Table 2. Subjectively, this leads to think that adding any raw meat components into a puppies' diet reduces the risk of developing FAR. However, statistical testing is necessary and was performed, the results will follow in the 'Statistical analysis' section (section 4.2). As can be seen, the proportion of dogs with an adverse food reaction is slightly higher in the population where the quantity of raw meat components was ignored. This raises the question if quantity of raw meat in the puppyhood diet is of influence on FAR development. For 214 dogs the proportion of raw in their puppyhood diet was estimated by their owners. Dogs were categorized in 5 categories and proportions of dogs suffering from FAR were calculated for each category. As shown in Table 3, the highest proportion of dogs with FAR was found in the group that ate less than 5% raw meat in their puppyhood diet. The proportions of dogs with FAR were similar in the 30-60% and >90% raw meat groups, suggesting no significant influence on the risk of developing FAR of the quantity of raw meat in puppyhood diets: it only seems to be relevant if >5% raw meat was present at all.

Table 1: *Proportion of dogs diagnosed with a food allergy for dogs with and without presence of raw meat components in their puppyhood diet. Note that a dog will be put in the 'yes' section also if less than 50% of their diet consisted of raw meat.* $n = 266$.

	Raw in puppyhood diet (No)	Raw in puppyhood diet (Yes)
FAR (No)	0.815	0.185
FAR (Yes)	0.873	0.127

Table 2: *Proportion of dogs diagnosed with any type of FAR for dogs that had a 50% or higher share of heat processed diet or a 50% or larger share of raw meat diet.*

	FAR (No)	FAR (Yes)
Heat-Processed	0.817	0.183
Raw	0.892	0.108

Table 3: *Proportion of dogs diagnosed with any type of FAR in various categories based on quantity of raw meat in puppyhood diet (in %). Note that not alle dog owners filled in this question in the survey and the number of NA's is therefore high.*

Diagnosis	Quantity of raw meat in puppyhood diet (in %)					
	0-5 ($n = 124$)	5-30 ($n = 10$)	30-60 ($n = 14$)	60-90 ($n = 30$)	90-100 ($n = 37$)	NA ($n = 52$)
FAR (No)	0.453	0.040	0.044	0.124	0.151	0.187
FAR (Yes)	0.537	0.024	0.073	0.049	0.073	0.244

Further exploring the descriptive analysis of FAR development, it can be seen that most diagnoses are made in dogs aged 6 to 12 months, see Table 4.

Table 4: *Distribution of dogs diagnosed with food allergies by range of age on diagnosis.*

Interval (months)	Frequency (n)
(0,6)	7
(6,12)	18
(12,18)	3
(18,24)	2
(24,97)	9

4.2 Statistical analysis

All logistic regression analysis were performed using the age corrected group of 187 dogs were cases had to be diagnosed before the age of 24 months and control cases must be 24 months of age minimum. Logically, the following conclusions on odds for FAR development apply to the odds of having developed such a reaction at the age of two years. For odds later in life, survival analysis were performed using the complete study group of 266 dogs.

4.2.1 Univariable logistic regressions

To look further into what variables might be relevant in themselves, univariable regressions were made for each individual variable. This approach determines not just if a correlation exists but also what the coefficient would be. As can be seen, there is a borderline significant increase in risk of developing food allergies for males compared to females ($p = 0.062$) leading to an

increase in odds of FAR development of 2.11 (Confidence Interval (CI): 0.96–4.74) If gender is ignored, there is no significant difference between neutered and intact animals. Combining these variables it can be seen that being an intact females comes with a non-significant exponentiated coefficient ($\exp\beta$) or odds ratio of approximately 0.169 ($p = 0.09$). For a predictor variable, for example 'sex including castration status', this number represents the change in the odds of developing a food allergy, in this case: intact females have about 0.169 times the odds of developing a FAR compared to neutered females. This trend was also observed in the survival analysis, see Appendix A, Figure 7.

Dietary factors

As can be seen in Table 5, no significance was achieved in the logistic regressions for dietary related variables. However, presence of raw in the puppyhood diet did show a tendency for lower odds ratio for FAR development that was borderline significant ($p = 0.082$).

Table 5: *Outcome of the univariable logistic regressions on dietary related variables. Presented are the exponentiated coefficients ($\exp\beta$), their 95% confidence intervals, and p-values for each predictor. $n = 187$.*

Predictor variable	$\exp\beta$	95% CI Lower	95% CI Upper	p-value
Model 1: presence of raw in puppyhood diet				
Presence of raw in puppyhood diet (No)	0.264	0.155	0.428	
Presence of raw in puppyhood diet (Yes)	0.490	0.213	1.083	0.083
Model 2: Supplements				
Supplements (No)	0.17	0.107	0.260	
Supplements (Yes)	1.868	0.676	4.713	0.202
Model 3: predominantly fed puppyhood diet				
Heat processed	0.237	0.145	0.369	
Raw based	0.528	0.210	1.218	0.15
Model 4: share of raw in puppyhood diet				
<30%	0.240	0.139	0.392	
30-60%	0.926	0.134	3.995	0.926
60-90%	0.208	0.011	1.108	0.138
>90%	0.174	0.009	0.912	0.097

Atopy

Notable results came from the univariable regression between atopy and FAR. As can be seen in Table 6, almost no dogs without FAR suffered from atopies. In the group with FAR, almost 50% had an atopy as well. In the univariable logistic regression, this leaded to an almost 48 times increase in odds for food allergy ($p < 0.001$). Please note that it was not known if dogs were diagnosed with an atopy before or after diagnosis with a food allergy: the predictive quality of an atopy is therefore disputable. The variable is also highly susceptible to bias: the difference between a true FAR and an atopy might be hard to understand for owners as the therapy is often similar. This can lead to confusion and the believe that the dog is suffering from both diseases when in truth, this is not the case. No survival analysis was made for this variable for these two reasons. Atopy was also removed from the multivariable model.

Table 6: *Cross-table by atopy and FAR diagnosis* ($n = 266$).

	Atopy		
FAR:	No	Yes	NA
No	221	4	0
Yes	19	18	4

No other variables showed to be any significant risk for FAR development.

4.2.2 Multivariable logistic regressions

All variables from the univariable logistic regressions were put in a multivariable model as well, except atopy for reasons discussed previously. Also, because independence of variables is needed certain variables had to be removed. Three dietary related variables were dependent on each other (main puppyhood diet, raw meat and share of raw meat in puppyhood diet). It was chosen to perform the initial analysis with the variable presenting information on the presence of raw meat in any quantity in the puppyhood diet. A second analysis was done for the presence of a minimum of 50% raw meat in the puppyhood diet. Multivariable logistic regression considered all variables left at first. Based on AIC score further selections were made in the model to make it more accurate. Diet related variables however, were kept in the model at all times for they are part of the study's objectives. Variables were excluded in a one by one manner starting with the variable which exclusion provided the biggest reduction AIC score. Also, when <2 points of the AIC score were gained by adding a variable, insufficient contribution was made to the accuracy of the model and the variable was removed.

The first model contained variables providing information on sex, neutering status, crossbreed status, pica behaviour, history of *Giardia spp.* infections, presence of any quantity of raw meat in the puppyhood diet and supplements in puppyhood diet. To summarize its contents, key findings are listed below including odds ratios and their confidence interval (CI).

- Presence of raw in puppyhood diet: Feeding (partially) raw significantly decreases the odds of developing FAR at the 5% significance level ($p = 0.048$). The odds ratio is 0.41 indicating raw fed animals have a 0.41 (CI: 0.17-0.97) chance at FAR when compared to animals that were fed heat processed food only.

- Sex: Male dogs seem to have an tendency for higher odds of FAR development, though non-significant at the 5% significance level ($p = 0.086$). The odds ratio for being male is 2.07 (CI: 0.91 – 4.85).

- Neutering status: No significant difference was seen in odds of food adverse development between neutered and intact animals ($p = 0.867$)

- History of *Giardia spp.* infection: Dogs that had gone through a *Giardia spp.* infection before the age of 7 months did not have a higher risk of FAR development ($p = 0.401$). the odds ratio for dogs with an infection was 0.58 (CI: 0.14-1.90) when compared to those that had not been diagnosed.

- Pica behaviour: presence of pica did not indicate any significant differences in odds of FAR development ($p = 0.476$).

- Crossbreed status: crossbreed dogs seem to have a tendency for higher odds of FAR development, though non-significant at the 5% significance level ($p = 0.085$). The odds ratio for being crossbreed is 2.28 (CI: 0.92 – 6.10).
- Supplements: dogs receiving supplements seem to have an tendency for higher odds of FAR development, though non-significant at the 5% significance level ($p = 0.062$). The odds ratio for being crossbreed is 2.76 (CI: 0.92 – 7.99).

To summarize: in this model, raw meat in the puppyhood diet significantly reduces the odds of FAR. Being fed supplements, being crossbreed and being male seem to increase the odds of FAR though insignificantly. These findings suggest potential areas for further research. No level of significance was seen in pica behaviour, history of *Giardia spp.* infections or neutering status.

Based on AIC scores as showed in Table 7, the accuracy of this model will be attempted to improve. The base AIC value of this model is 167.23 removals will be made individually in a one by one matter. The dietary related variables will not be removed as they are part of this thesis' objectives.

Table 7: *AIC scores of the multivariable regression model and their p-value.*

Factor	AIC score	*p*-value
Presence of raw in puppyhood diet	167.23	0.043
Sex	169.33	0.084
Neutering status	168.21	0.867
Giardia spp.	165.26	0.384
Pica	165.99	0.470
Crossbreed status	168.37	0.076
Supplements	168.50	0.070

The first removal was the neuter status. In the improved model, Pica was removed based on AIC score, after which *Giardia spp.* was removed. For the fourth removal, based on AIC score, the raw meat factor had to be removed. For reasons already given, this was not done so the next removal that was considered was the supplements variable. However: no exclusion would lead to a decrease in AIC score and no variable contributed to an AIC improvement of >2 points. The best version of this model was thus reached and included presence of raw meat, supplements, crossbreed status, and sex. It is presented in Table 8.

Table 8: *summary of the multivariable logistic regressions after exlusions based on AIC score*

Variable	Estimate	Odds Ratio (expβ)	95% CI (Lower)	95% CI (Upper)	*p*-value
(Intercept)	-2.210	0.110	0.042	0.255	<0.001
Presence of raw meat - yes	-0.808	0.446	0.183	1.030	0.065
Sex - male	0.677	1.969	0.879	4.497	0.101
Crossbreed - yes	0.706	2.025	0.878	4.997	0.108
Supplements – yes	0.894	2.445	0.834	6.762	0.090

To summarize the table contents, it can be said that at the significance level of 5%, no significance was achieved. However, presence of raw meat and supplementation of puppyhood diet seemed to have a tendency for altered odds of FAR development. Raw meat would decrease the odds whilst supplementation increased the odds.

4.2.3 Survival analysis

As discussed earlier, survival curves were created using the study group of 266 dogs, not corrected for age or age at diagnosis. For some variables, additional observations were deleted due to missingness in the data, the number of observations for each survival curve is described in their plot subtitles. For each variable from the univariable logistic regressions, a survival curve was made. The survival analysis does not provide odds ratios for FAR development but calculates the estimated proportion of the population that remains FAR free over a certain amount of time for each group. Each line in the plot represents a certain group. The proportions are displayed at the y-axis under the name 'survival proportion'. To accurately represent the survivaltime, for control cases age at the moment of survey entry was used and for cases age at diagnosis was used.

All survival curves can be found in Appendix A. Overall, gender showed a significant association with the risk of FAR, with males having a higher risk compared to females ($n = 263$, p=0.0465). Other factors such as castration status for both sexes and history of *Giardia spp.* infection, did not show significant associations with FAR development risk in this analysis. The factors 'presence of raw in puppyhood diet' and 'predominantly fed puppyhood diet' showed a non-significant tendency for lower risk of FAR development. Associated p-values were 0,13 and 0,15 respectively. Furthermore, the factor 'share of raw in puppyhood diet' showed a non-significant association with FAR development. As can be seen in the plot in Figure 5, according to the used data, feeding for 30-60% raw increased risk when compared to feeding <30% raw and feeding 60-90% or more than 90% decreased the risk. Associated p-values were 0,638 (30-60%), 0.158 (60-90%) and 0.141 (>90%) respectively.

5 Discussion and conclusion

This discussion will cover potential bias hazards of this study and discuss the results and their validity. Future perspectives in the field of Nutrition and FAR will be discussed as well.

5.1 Bias

Starting with an important part of this discussion: susceptibility for bias. The organization of this study is sensitive to information bias, selection bias and confounding bias. All types of bias affect the study's validity negatively. The biases and examples of them in this thesis are explained in the following sections.

5.1.1 Selection bias

Selection bias can occur if certain types of owners are more likely to participate in the study than others. For example, if owners who have experienced health issues with their pets are more motivated to participate, the prevalence of reported health issues may be overestimated. Similarly, if the survey is distributed primarily through certain channels (e.g., social media), it may attract a specific demographic of owners, potentially skewing the results. Efforts were made to reach a diverse range of participants through various recruitment channels, though the largest proportion of responders answered through the same channel (social media, Facebook).

Examples

A suspect for selection bias was raised after the evaluation of the data that was retrieved from the survey. The high amount of labradoodles in the study group might suggest that Labradoodle owners may have specific health concerns regarding FAR such as allergies or preferences regarding their dog's diet.

5.1.2 Information bias

Information bias can arise due to discrepancies between owner-reported data and objective measurements or medical records. Owners may inaccurately recall or report information about their pet's health status, diet, or other factors, leading to misclassification bias. Additionally, owners may interpret questions differently or provide socially desirable responses, particularly if they perceive certain answers as more favourable or more shameful.

Mitigation of information bias was attempted by designing clear and unambiguous survey questions, providing examples or definitions where necessary, and validating owner-reported data against objective measures whenever possible. If answers continued to be ambiguous, they were removed from the dataset.

Examples

Examination and cleaning procedures performed on the data revealed information bias. A few examples were owners who believed they were feeding their dogs a raw meat-based diet but were in fact not. Upon researching the brand name of the diet, it turned out they were actually providing a cold-pressed diet. Not for all dogs the brand name was available and the validity of these answers could not be checked. This lead to an increase in risk of information bias. This discrepancy highlights the information gap that exists in the dog owner population and is worth considering when investigating dietary practices in the future. For (future) veterinarian it also highlights the importance of education and communication on pet nutrition.

5.1.3 Confounding bias

Confounding bias can occur if factors that influence both the exposure (e.g., puppyhood diet) and the outcome (e.g., food allergies) are not adequately controlled for in the analysis. For example, if certain breeds are more likely to be fed a particular type of diet and are also predisposed to food allergies, failing to account for breed differences could lead to a biased estimate of the association between diet and allergies. In 2002, a breed predisposition was found for Labrador Retrievers and German Shepherd (Chesney, 2002). However, since there is no recent evidence for specific examples of breed predispositions, adequate control could not be achieved in our analysis.

5.2 Findings

In conclusion, does feeding a raw meat based diet, when compared to feeding a heat-processed diet, to dogs of <6 months reduce chances of developing FAR in the first 2 years of their life? Not significantly. Significant differences were found however for risk of adverse food reaction development between males and females. Animals that had been fed any quantity of raw meat in their puppyhood diet had a borderline significant result but appeared to have a lower risk of developing a FAR. Conclusion can be drawn from non-significant results as well: contrary

to belief in the pet owner community, *Giardia spp.* did not seem to have any influence on FAR development. In the same way it is also not of significance to its risk of food adverse development if a dog performs pica behaviour and if owners provide supplements for their dog. Also insignificant were castration status, crossbreed status and quantity of raw meat fed. In the survival curves, these trends were also present; significant differences were found for survival probability between the sexes, non-significant results were seen in the remainder of the tested variables. For a subjective difference was seen in odds ratios and survival proportions for a part of the tested variables, further research is warranted. Suggested research topics will be discussed in 'Future perspectives'.

5.3 Future perspectives

5.3.1 Protective quality of raw meat based diets

In the opinion of the writer, the results of this study considering raw meat in the puppyhood diet, though insignificant, warrant further research into this topic. Mostly, a clinical study with a randomized control trial (RCT) set up on the effect of a fully raw meat based diet would be interesting. This study might be helpful to assess the possibly protecting effects of raw meat against FAR that, though insignificantly, was seen in this study. It could also prove to exclude the existence of any effect of raw meat in the puppyhood on FAR in a more significant matter thereby lay the foundation for solid, evidence-based advice on puppy nutrition. This study however, is hard and costly to perform due to the large amount of time needed for follow up. For this reason, a case-controlled study in a clinical environment is a good first step to more reliable research on this topic.

5.3.2 *Giardia spp.*

For a very insignificant effect of *Giardia spp.* infection history on FAR development was found in this study, a RCT study would be the next step as to exclude the existence of any influence of *Giardia spp.* infections on FAR development.

For new topics to study, the influence on FAR development of the exact contents of the puppyhood diet could be studied. Study variables could be related to different types of animal proteins (i.e. chicken, horse, beef, etc.) used, use of add-ons (multivitamins and minerals) and use of fruits and vegetables in the diet.

Overall, insignificant results of this study are not to be discarded for a non-significant trend was seen of the influence of raw meat puppyhood diets on FAR development. This trend should be researched further. A confirmative study in a randomized control trial set up on the effect of a *Giardia spp.* infection and treatment of this infection on FAR development is also warranted.

5.4 Moderation

Indeed, while the results of the study suggest that raw meat could potentially offer some form of protection against the development of FAR, it's essential to consider the drawbacks associated with feeding a 100% raw meat based diet. As outlined in the introduction and background sections of this thesis, there are several disadvantages to this diet form. Including, but not limited to bacterial contamination, nutritional imbalances, and the potential for spreading of antimicrobial resistance.

Therefore, it is evident to this writer that in the best-case scenario, if a risk for FAR is identified in a dog, the use of raw meat as a preventative measure would likely take the form of an add-on to an otherwise heat-processed, complete diet. This approach would allow pet owners to balance any potential benefits of raw meat with the risks associated with a solely raw diet. However; not all risks can be mitigated by this approach. For example, adding raw meat in an otherwise complete wet food or kibble diet is helpful in challenging nutritional imbalances but is not of influence on the risk of contamination and infection of guardians with antibiotic resistance microbes.

6 Acknowledgements

This author declares no conflict of interest was present. AI was used in this thesis to find synonyms for words frequently used, help with structuring sentences, creating tables and detecting errors in spelling.

References

Aguilera, A. C., Dagher, I. A. & Kloepfer, K. M. (2020). Role of the microbiome in allergic disease development. *Current allergy and asthma reports*, *20*, 1–10.

Baker, M. G. & Nowak-Wegrzyn, A. (2020). Food allergy prevention: current evidence. *Current Opinion in Clinical Nutrition & Metabolic Care*, *23*(3), 196–202.

Beacom, E. (2024). *Tony's chocolonely: Sustainable, ethical business and supply chain*. SAGE Publications: SAGE Business Cases Originals.

Bhagat, R., Sheikh, A. A., Wazir, V., Mishra, A. & Maibam, U. (2017). Food allergy in canines: A review. *J. Entomol. Zool. Stud*, *5*(6), 1522–1525.

Biancardi, P., Papini, R., Giuliani, G. & Cardini, G. (2004). Prevalence of giardia antigen in stool samples from dogs and cats. *Revue de médecine vétérinaire*, *155*(8-9), 417–421.

Butowski, C., Moon, C., Thomas, D., Young, W. & Bermingham, E. (2022). The effects of raw-meat diets on the gastrointestinal microbiota of the cat and dog: A review. *New Zealand Veterinary Journal*, *70*(1), 1–9.

Carlotti, D. N., Remy, I. & Prost, C. (1990). Food allergy in dogs and cats. a review and report of 43 cases. *Veterinary Dermatology*, *1*(2), 55–62.

Chesney, C. (2002). Food sensitivity in the dog: a quantitative study. *Journal of Small Animal Practice*, *43*(5), 203–207.

Covacin, C., Aucoin, D., Elliot, A. & Thompson, R. (2011). Genotypic characterisation of giardia from domestic dogs in the usa. *Veterinary parasitology*, *177*(1-2), 28–32.

Craig, J. (2019). Food intolerance in dogs and cats. *Journal of Small Animal Practice*, *60*(2), 77–85.

Empert-Gallegos, A., Hill, S. & Yam, P. S. (2020). Insights into dog owner perspectives on risks, benefits, and nutritional value of raw diets compared to commercial cooked diets. *PeerJ*, *8*, e10383.

Fitzsimmons, C. M., Falcone, F. H. & Dunne, D. W. (2014). Helminth allergens, parasite-specific ige, and its protective role in human immunity. *Frontiers in immunology*, *5*, 61.

Freeman, L. M., Chandler, M. L., Hamper, B. A. & Weeth, L. P. (2013). Current knowledge about the risks and benefits of raw meat–based diets for dogs and cats. *Journal of the American Veterinary Medical Association*, *243*(11), 1549–1558.

Jackson, H. A. (2023). Food allergy in dogs and cats; current perspectives on etiology, diagnosis, and management. *Journal of the American Veterinary Medical Association*, *261*(S1), S23–S29.

Knight, A. (2023). The relative benefits for environmental sustainability of vegan diets for dogs, cats and people. *Plos one*, *18*(10), e0291791.

Knight, A., Huang, E., Rai, N. & Brown, H. (2022). Vegan versus meat-based dog food: Guardian-reported indicators of health. *PLoS One*, *17*(4), e0265662.

Łuszczki, E., Boakye, F., Zielińska, M., Dereń, K., Bartosiewicz, A., Oleksy, Ł. & Stolarczyk, A. (2023). Vegan diet: nutritional components, implementation, and effects on adults' health. *Frontiers in Nutrition*, *10*.

Michel, K. E., Willoughby, K. N., Abood, S. K., Fascetti, A. J., Fleeman, L. M., Freeman, L. M., ... Van Doren, J. R. (2008). Attitudes of pet owners toward pet foods and feeding management of cats and dogs. *Journal of the American Veterinary Medical Association*, *233*(11), 1699–1703.

Mueller, R. S. & Olivry, T. (2017). Critically appraised topic on adverse food reactions of companion animals (4): can we diagnose adverse food reactions in dogs and cats with in

vivo or in vitro tests? *BMC veterinary research*, *13*, 1–5.

Olivry, T. & Mueller, R. S. (2020). Critically appraised topic on adverse food reactions of companion animals (9): time to flare of cutaneous signs after a dietary challenge in dogs and cats with food allergies. *BMC veterinary research*, *16*, 1–4.

Painter, M. R., Tapp, T. & Painter, J. E. (2019). Use of the health belief model to identify factors associated with owner adherence to elimination diet trial recommendations in dogs. *Journal of the American Veterinary Medical Association*, *255*(4), 446–453.

Rook, G. A. (2009). Review series on helminths, immune modulation and the hygiene hypothesis: the broader implications of the hygiene hypothesis. *Immunology*, *126*(1), 3–11.

Schmidt, M., Unterer, S., Suchodolski, J. S., Honneffer, J. B., Guard, B. C., Lidbury, J. A., ... Kölle, P. (2018). The fecal microbiome and metabolome differs between dogs fed bones and raw food (barf) diets and dogs fed commercial diets. *PloS one*, *13*(8), e0201279.

Sicherer, S. H. & Sampson, H. A. (2018). Food allergy: a review and update on epidemiology, pathogenesis, diagnosis, prevention, and management. *Journal of Allergy and Clinical Immunology*, *141*(1), 41–58.

Skubic-Vengust, B. (2003). *Association between giardia infection and pruritic skin disease in dogs* (Unpublished doctoral dissertation). University of Guelph.

Stiemsma, L. T. & Michels, K. B. (2018). The role of the microbiome in the developmental origins of health and disease. *Pediatrics*, *141*(4).

Strachan, D. P. (1989). Hay fever, hygiene, and household size. *BMJ: British Medical Journal*, *299*(6710), 1259.

Swanson, K. S., Carter, R. A., Yount, T. P., Aretz, J. & Buff, P. R. (2013). Nutritional sustainability of pet foods. *Advances in nutrition*, *4*(2), 141–150.

van Bree, F. P., Bokken, G. C., Mineur, R., Franssen, F., Opsteegh, M., van der Giessen, J. W., ... Overgaauw, P. A. (2018). Zoonotic bacteria and parasites found in raw meat-based diets for cats and dogs. *Veterinary Record*, *182*(2), 50–50.

Yazdanbakhsh, M. & Matricardi, P. M. (2004). Parasites and the hygiene hypothesis: regulating the immune system? *Clinical reviews in allergy & immunology*, *26*, 15–23.

Yu, W., Freeland, D. M. H. & Nadeau, K. C. (2016). Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nature Reviews Immunology*, *16*(12), 751–765.

A Survival curves

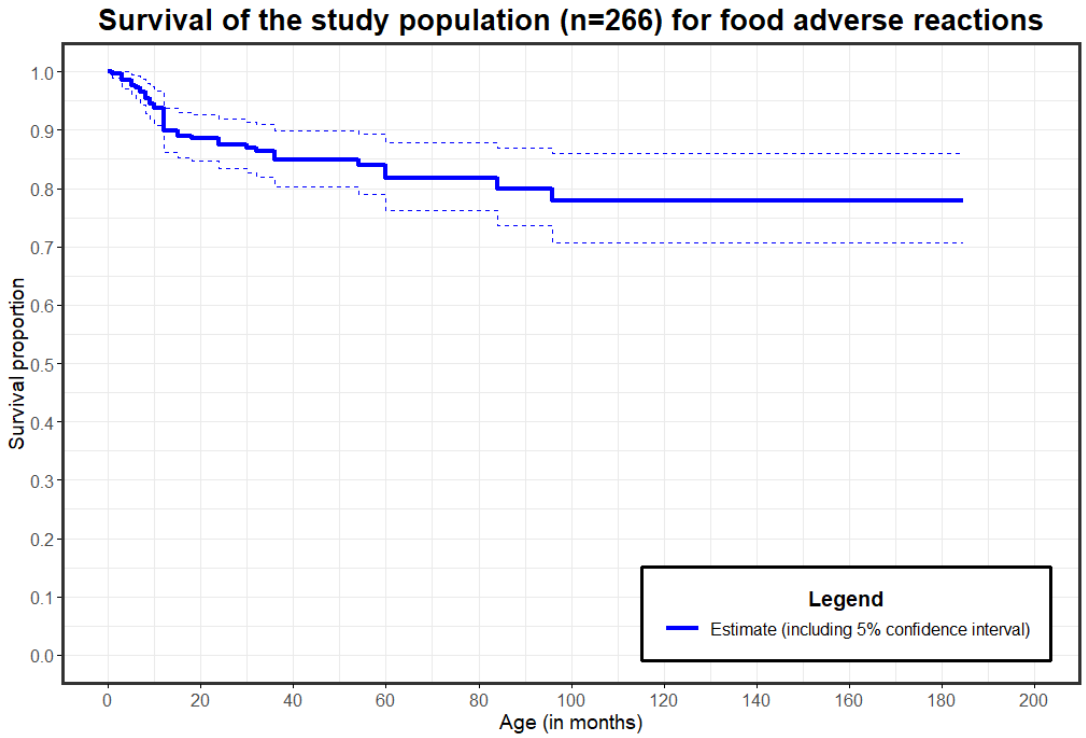

Figure 3: *Overall survival of dogs. This figure shows the probability of remaining food allergy-free. The median survival time, as well as the confidence intervals at 2.5% and 97.5%, are presented.*

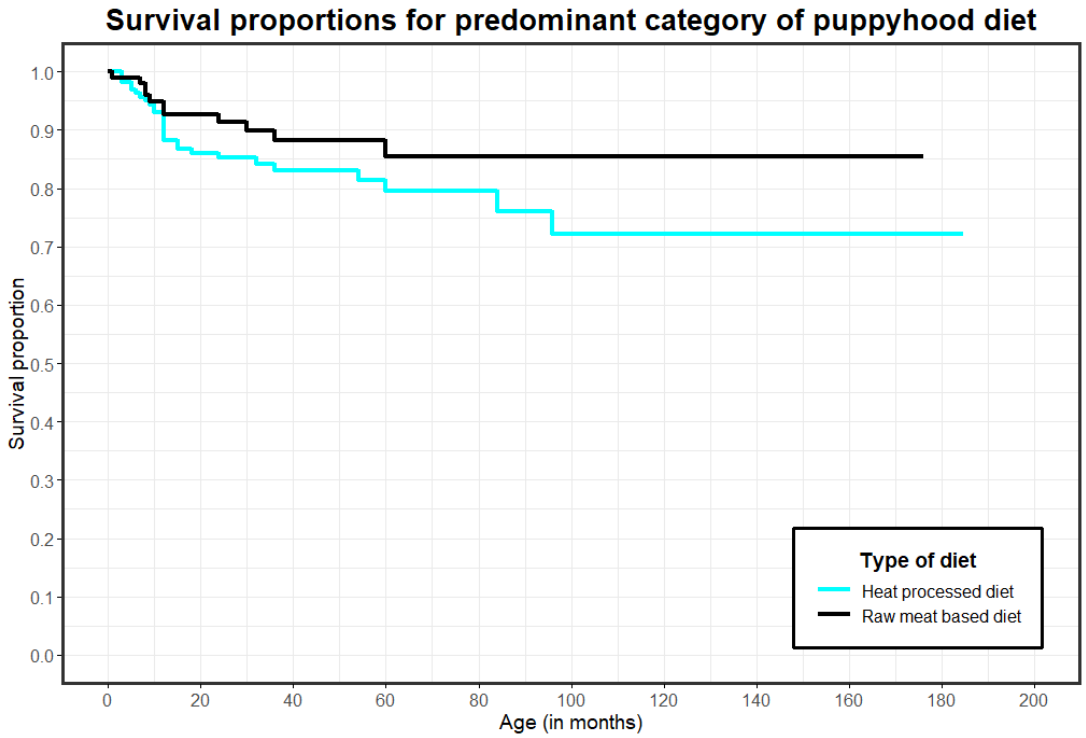

Figure 4: *Survival proportions for dogs fed a raw meat based puppyhood diet versus heat processed puppyhood diet. Feeding at least 50% of the puppyhood diet raw has a non- significant impact on the development of food allergies (log-rank test, $p = 0.15$). Dogs fed with a raw meat-based diet showed a tendency for a lower probability of developing FAR over time. $n = 263$.*

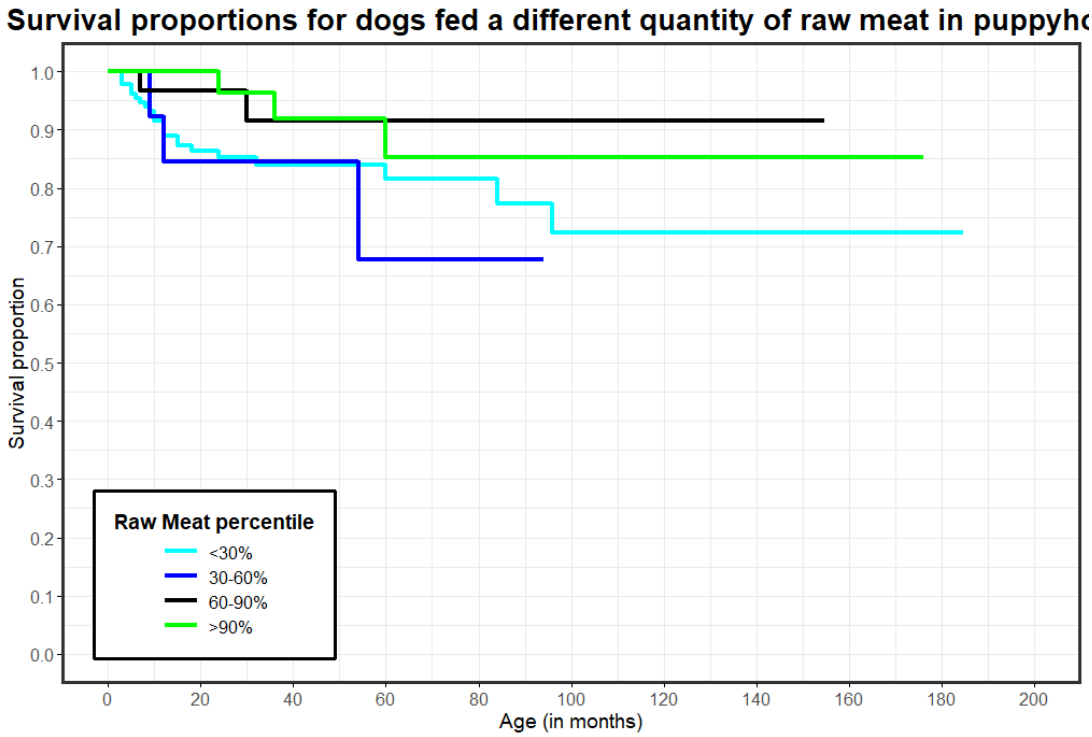

Figure 5: *Survival plot based on the percentage of raw meat in the puppyhood diet. 4 categories were made: dogs eating less than 30%, between 30% and 60%, between 60% and 90% and more than 90% of their diet raw. The largest proportion of survivors was found in the group of dogs that had eaten between 60% and 90% of the diet raw.* $n = 213$.

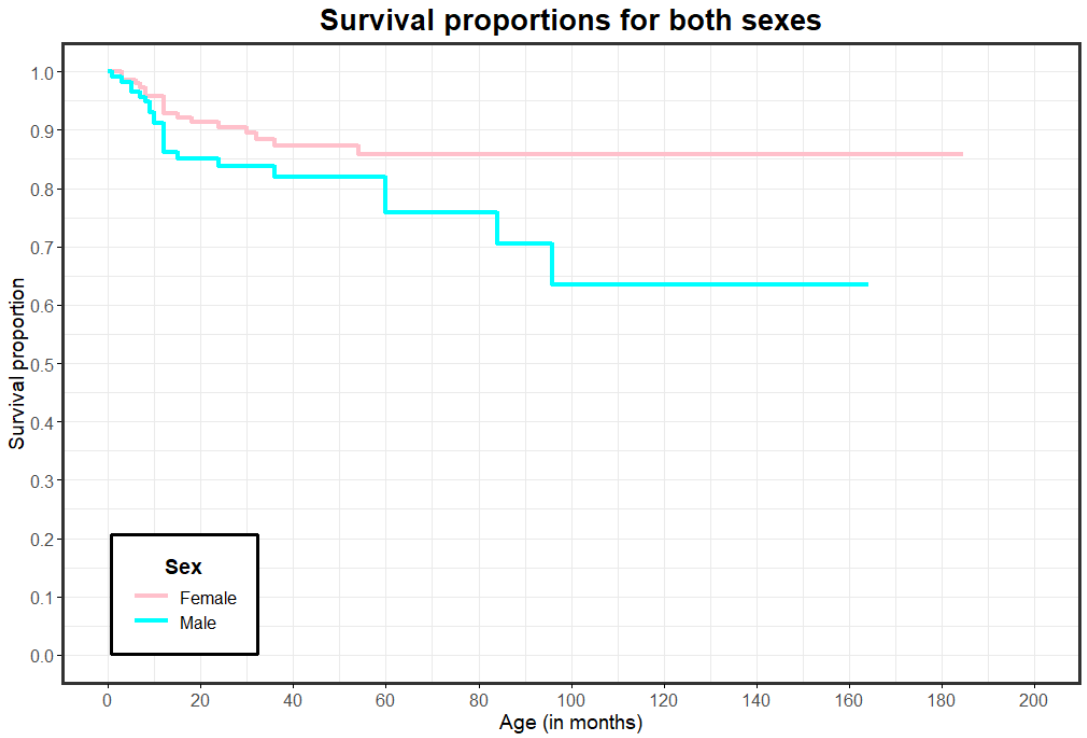

Figure 6: *Survival proportions for males and females. Overall, males had a lower survival proportion than females at a significance level of 5%.* $n = 263$.

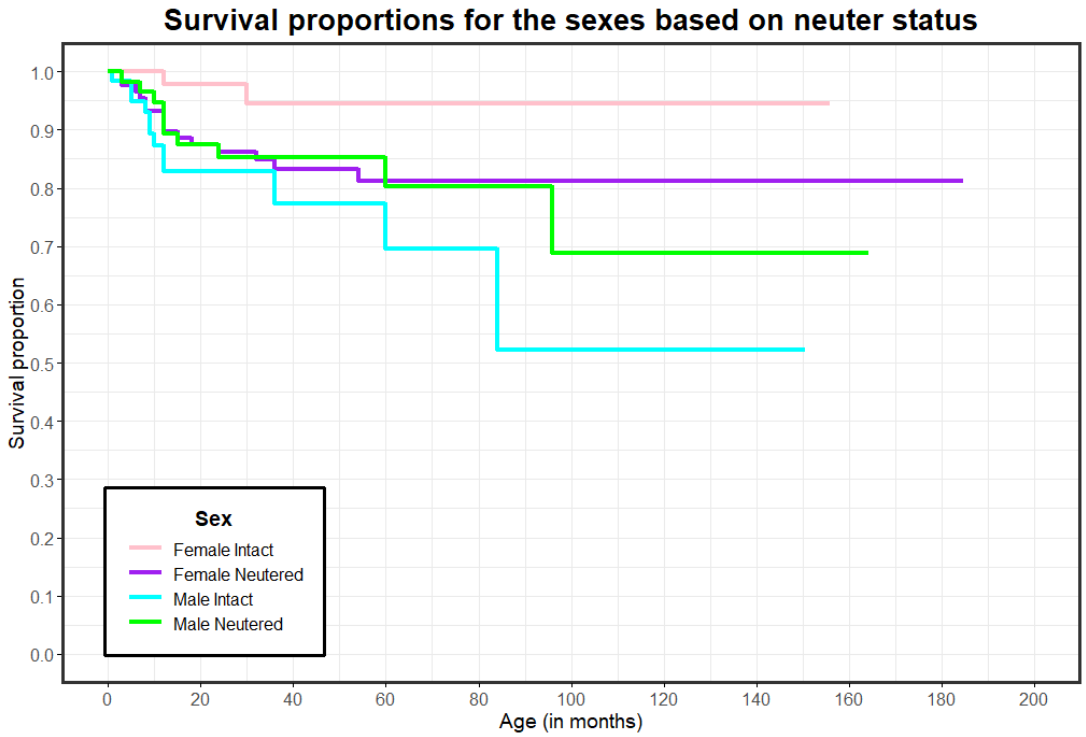

Figure 7: *Survival curves for sex and neutering status. These plots indicate that intact males have the lowers survival rate and intact females the highest proportion. This could mean that neutering effects differ between the sexes, however: results were not significant.* $n = 263$.

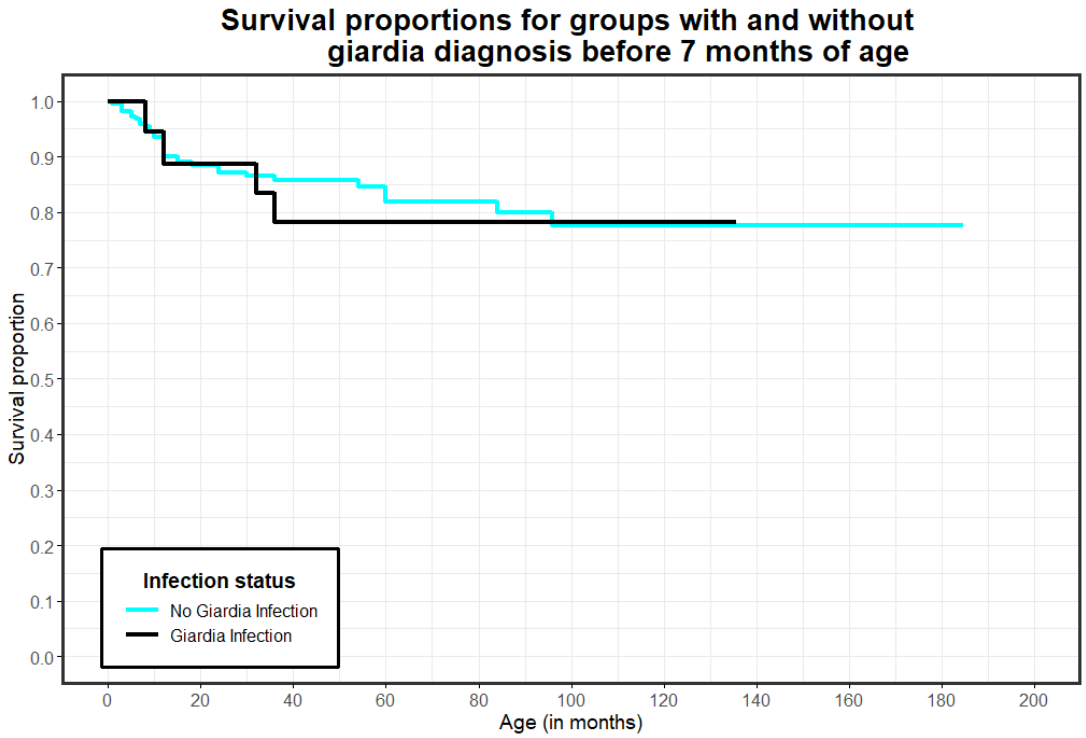

Figure 8: *Survival proportions for dogs with and without Giardia spp. diagnosis before 7 months of age. As can be seen Giardia spp. infections were not of influence on risk of FAR development with a p-value of 0.823.* $n = 260$.

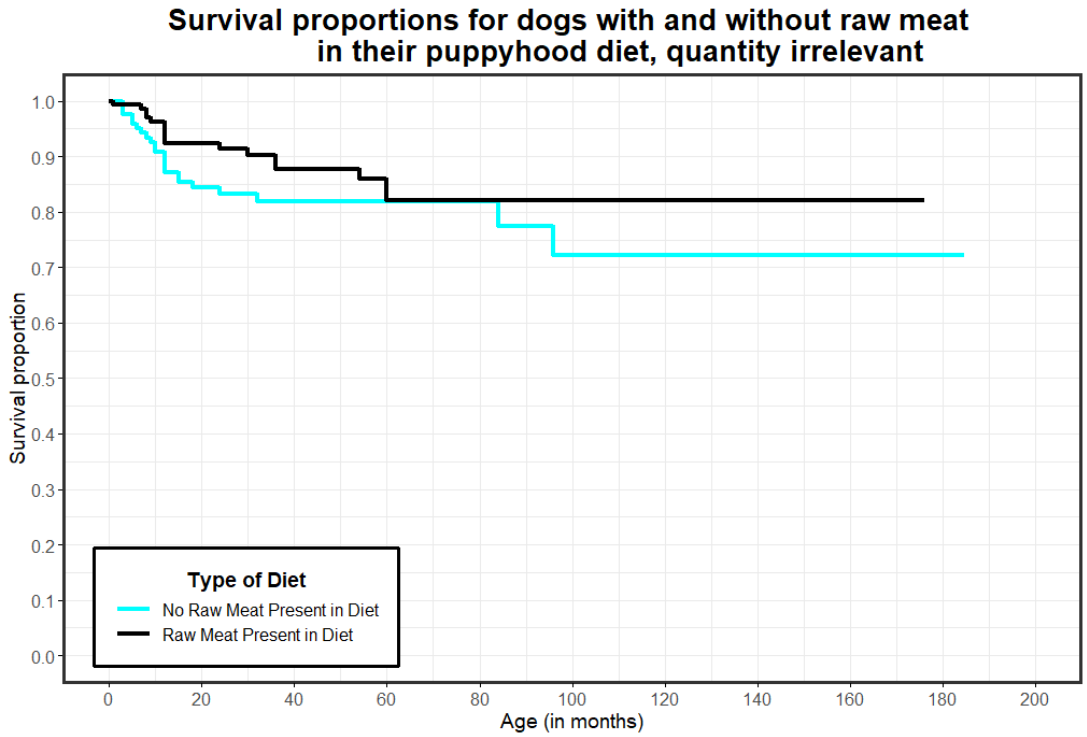

Figure 9: *Survival proportions for dogs with and without raw meat in their puppyhood diet. Quantity of raw meat was irrelevant for this variable. As can be seen, the same trend as in Figure 3 is also present in this plot. Dogs with raw meat in their diet have a higher survival proportion for FAR development.* $n = 263$.

B Tables

Table 9: *Obtained variables from the questionnaire including their measurement unit and data type. Data types are subcategorized in quantitative (discrete, continues) and qualitative (nominal, ordinal).*

Name	Measurement unit	Data type
Age	Days	Quantitative, discrete
Breed	Character	Qualitative, nominal
crossbreed	Character (Yes/No)	Qualitative, nominal
Sex	Character (Female/Male)	Qualitative, nominal
Castration status	Character (Yes/No)	Qualitative, nominal
Raw meat in puppydiet	Character (Yes/No)	Qualitative, nominal
Main diet in puppyhood	Character (raw fed diet, heat processed diet)	Qualitative, nominal
Share of raw in puppyhood diet	Percentage (0, 1-30, 30-60, >60)	Qualitative, ordinal
Giardia spp.	Character (Yes/No)	Qualitative, nominal
Pica	Character (Yes/No)	Qualitative, nominal
Degree of Pica	Character (0-1, 2-4, 5-7, 8-9, >10)	Qualitative, ordinal
Supplements	Character (Yes/No)	Qualitative, nominal
Veterinarian visits	Visits in last 12 months	Quantitative, discrete
Medication use	Days	Quantitative, discrete
Presence of chronic disease	Character (Yes/No)	Qualitative, nominal
FAR	Character (Yes/No)	Qualitative, nominal
Age of diagnosis FAR	Months	Quantitative, discrete
Atopic Dermatitis	Character (Yes/No)	Qualitative, nominal

Table 10: *Proportion of the study group ($n = 284$) per health score (1-10).*

Health rating	1	2	3	4	5	6	7	8	9	10
Proportion	0.007	0.004	0.007	0.014	0.014	0.046	0.102	0.204	0.232	0.370