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Technical White Paper

Approach to further investigation of the hypothesised relationship between grain-free diets and acquired dilated cardiomyopathy in dogs

Ausvet and One Health Scientific Solutions

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20 October 2020

Disclaimer

The purpose of this white paper is to provide general information about key issues relating to observational approaches to investigation of the hypothesised relationship between diet and acquired dilated cardiomyopathy in canines. Design of a particular observational study requires specific consideration of many factors relating to the study population. Private companies, organisations or individuals intending to undertake observational studies are advised to seek expert advice, to ensure the study design is appropriate for the particular circumstances.

One Health Scientific Solutions and Ausvet were contracted by Champion Petfoods to independently review the literature regarding the hypothesised association between grain-free diets and dilated cardiomyopathy from an epidemiological perspective, and provide recommendations for further investigation.

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Summary

Amongst the veterinary community, concerns have been raised of an association between grain-free diets and acquired dilated cardiomyopathy (DCM) in dogs. These concerns prompted investigation by the US Food and Drug Administration (FDA), announced in July 2018.

While observations by veterinary medicine clinicians have driven identification of important causal factors of veterinary diseases, it is essential that these observations are subject to rigorous scientific investigation before conclusions are drawn. This approach is the cornerstone of evidence-based medicine, contributes to understanding of the pathophysiology of disease, and informs objective decision making at the regulatory level. By these standards, the hypothesis that grain-free diets are associated with acquired DCM in dogs has not yet been adequately explored and requires further investigation in accordance with well-published and accepted epidemiological principles that enable robust inference.

A carefully designed case control study with multidisciplinary input is an appropriate next step in investigating whether grain-free diets are associated with acquired DCM in canines, and if so, the possible causal mechanisms involved. Case control studies are relatively cheap and easy to implement compared to randomised controlled trials; they are an appropriate choice for investigation of diseases that are relatively rare; they allow for concurrent investigation of multiple causal hypotheses, enabling broad investigation of the occurrence of acquired DCM in breeds of dog not previously known to be at risk; and they are informative to the design of efficient and appropriately-targeted randomised controlled trials.

Ausvet and One Health Scientific Solutions are keen to promote evidence-based medicine in the investigation of this hypothesised association. This document outlines important points for consideration in the design of a case-control study, contextualised to the investigation of the hypothesised association between grain-free diets and acquired DCM in canines. Key considerations covered include:

1) a structured approach to identification of the potential for confounding;
2) strategy to control for confounding;
3) the required sample size for a sufficiently powerful study;
4) whether the utilisation of clinical records is a possible and valid approach;
5) methods for recruitment of cases;
6) methods for recruitment of representative controls;
7) whether there is a need for screening controls for preclinical DCM;
8) approaches to masking (blinding) staff and pet owners involved in the study;
9) the possible impacts of recall bias on study findings;
10) efficient data collation; and
11) the appropriate approach to data analysis.

Contents

1. Background

Amongst the veterinary community, concerns have been raised of an association between grain-free diets and acquired dilated cardiomyopathy (DCM) in dogs. These concerns prompted investigation by the US Food and Drug Administration (FDA), announced in July 2018.[1]

While observations by veterinary medicine clinicians have driven identification of important causal factors of veterinary diseases, it is essential that these observations are subject to rigorous scientific investigation before conclusions are drawn. This approach is the cornerstone of evidence-based medicine, contributes to understanding of the pathophysiology of disease, and informs objective decision making at the regulatory level.

To identify whether a causal relationship between grain-free diets and canine DCM exists and, if so, the particular causal mechanisms involved, cross-disciplinary and structured epidemiological investigation is required. Thus far, few research studies have investigated this hypothesised association, and they tend to lack epidemiological rigour, leaving study inferences regarding causality highly susceptible to chance influences and various sources of bias. Whilst analytical inferences have been made (and cited) in some studies, we find that these cannot be justified based on the quality of evidence presented; this was also the conclusion of a recent published review of the literature.[2] We suggest some key points for consideration in establishing whether such an association exists and, if so, whether it represents a causal relationship.

2. Understanding disease causality

Assessments of causality are made considering the results of multiple studies, in view of causal criteria such as the Bradford Hill criteria or Evans' rules. Examples of these criteria include evidence of temporal sequence (cause preceding effect), the consistency of an association across multiple studies undertaken in different populations and circumstances, and biological gradient (dose-response: the risk of the outcome increasing with increasing exposure to the cause).[3,4,5]

In assessments of disease causality, the value of evidence contributed by research varies between different types of study. Case reports and case series reports are essential sources of hypothesis generation, but do not contribute evidence that a particular factor is associated with or causes disease. For example, a clinician may observe that many patients referred and diagnosed with acquired DCM are fed grain-free diets; but it is possible that the choice of a grain-free diet may reflect the socio-economic status of owners utilising referral services whilst being unrelated to the occurrence of DCM. Analytic observational studies (including case-control studies and cohort studies) are often the next step in testing hypotheses arising from clinical observations, and can provide some degree of evidence to consider against certain causal criteria, if the study is well designed and executed. Of individual studies, a well-designed randomised controlled trial will provide the best-quality evidence; and overall, meta-analysis of the findings of multiple randomised controlled trials investigating the same hypothesis provides the best possible level of evidence as to the role of a particular factor in disease causality.[6]

Currently, published literature investigating the hypothesised association between grain-free diets and canine DCM is dominated by case studies and case-series reports. A well-designed analytic observational study is an appropriate next step in investigating this hypothesis. Analytic observational studies progress clinical observations by rigorously testing for an association between a potential cause (in this case, diet) and outcome (in this case, DCM), including exploration of hypothesised causal pathways (e.g. if diet is associated with DCM, investigating what specific aspect(s) of diet are associated with DCM). Analytic observational studies can also provide evidence for consideration against some causal criteria in making causal inferences. Additionally, they allow for concurrent exploration of additional causal hypotheses: in the case of the hypothesised association between diet and DCM, an analytic observational study thus provides a good opportunity to investigate in detail the apparent occurrence of DCM in breeds not previously identified as being at high risk for the condition. Whilst randomised controlled trials are the gold standard for evaluation of causality, they are more difficult to implement and are less flexible in investigating multiple hypothesised causal factors concurrently, and so are often pursued subsequent to well-designed observational studies that find an association between hypothesised cause(s) and effect.[7] Analytic observational study results can also be informative to the design of appropriately targeted and efficient randomised controlled trials.[8]

3. Appropriate analytic observational study designs to investigate a hypothesised association between diet and canine DCM

An appropriate analytic observational study design needs to be both capable of investigating the research question at hand and logistically feasible. For investigating the hypothesised association between grain-free diets and acquired DCM, a case-control study is considered the most appropriate analytic observational study design. Briefly, in a case-control study, the dietary history of dogs with DCM is compared to the dietary history of a representative control group of dogs without DCM. If there are considerable differences between exposure to grain-free diets in cases compared to controls, it provides evidence of an association between diet and the occurrence of DCM (assuming the study is well designed and executed). Conversely, if there is not a substantial difference in dietary history between cases and controls, it suggests that diet type is not associated with the disease.

Case-control studies are an appropriate choice in this case, given that the outcome under consideration (acquired DCM) is relatively rare, and the lag time between exposure to a grain-free diet and development of acquired DCM (if the relationship is causal) may be relatively long. Additionally, case-control studies are a very efficient design for investigation of multiple factors hypothesised to be associated with canine DCM, meaning that the association with dietary history can be explored alongside additional hypotheses.[9] This may include testing for an association between DCM and specific factors that may be related to grain-free diets (such as bioavailable taurine and L-carnitine), to explore possible mechanisms of any association between DCM and diet in detail; alongside hypotheses that may be unrelated to diet (for example, testing for exposure to certain infections between cases and controls).

Other analytic observational study designs present logistical difficulties in investigating this research question. Cohort studies involve following cohorts of dogs fed grain-free diets and dogs not fed grain-free diets over time, to compare the occurrence of acquired DCM (this is different from a randomised controlled trial, in that dogs are not randomly allocated to receive a particular diet; they are on the diet through the owners' choice or circumstance). Cohort studies are likely to require unfeasibly large sample sizes to attain adequate statistical power, given that the occurrence of acquired DCM appears to be relatively rare, irrespective of whether or not grain-free diets are fed to the dog. Additionally, prospective cohort studies (as opposed to historical cohort studies) would require considerable resources to follow up study subjects over time, and the follow up period may be relatively long, which may be cost prohibitive.

4. Key considerations in undertaking a valid case-control study investigating the hypothesised relationship between diet and acquired dilated cardiomyopathy

4.1 Consider the causal framework of the hypothesised association to identify potential confounding

Confounding is an important potential source of bias in observational research (as opposed to randomised controlled trials). Confounding is a statistical phenomenon, where a statistical association is observed between two factors, which is actually causally attributable to a third (confounding) factor. As an example, observations were made of an association between drinking coffee and the occurrence of pancreatic cancer in humans. Further investigation of this association indicated that it was partially or fully explained by confounding, particularly confounding by smoking: coffee drinkers are more likely to be smokers, with the smoking causing a relatively higher risk of pancreatic cancer observed in coffee drinkers, rather than the consumption of coffee.[10] Thus, in this case, drinking coffee is associated with pancreatic cancer, but it is not causing the cancer.

In view of concerns about confounding, the first step in design of an observational study investigating disease causality is structured consideration of the hypothesised relationship between a potential cause and outcome. This can involve synthesis of known or plausible relationships between a hypothesised cause (in this case, grain-free diets) and outcome (in this case, acquired DCM), and potential biasing pathways, into a directed acyclic graph (e.g. Figure 1). Directed acyclic graphs facilitate identification of potential biasing pathways between the potential cause and outcome, which may confound an observed relationship. This information can then be used to design a study—including approach to data collection and analysis—that enables appropriate control of confounding. Controlling for potential confounders cannot be undertaken in an ad hoc fashion when considering disease causality: restriction of the study by suggested confounders in an attempt to control for confounding can unnecessarily limit the external validity of study findings;[11] and inclusion of inappropriate variables in a statistical model can induce bias in results and invalidate study findings.[12, 13, 14]

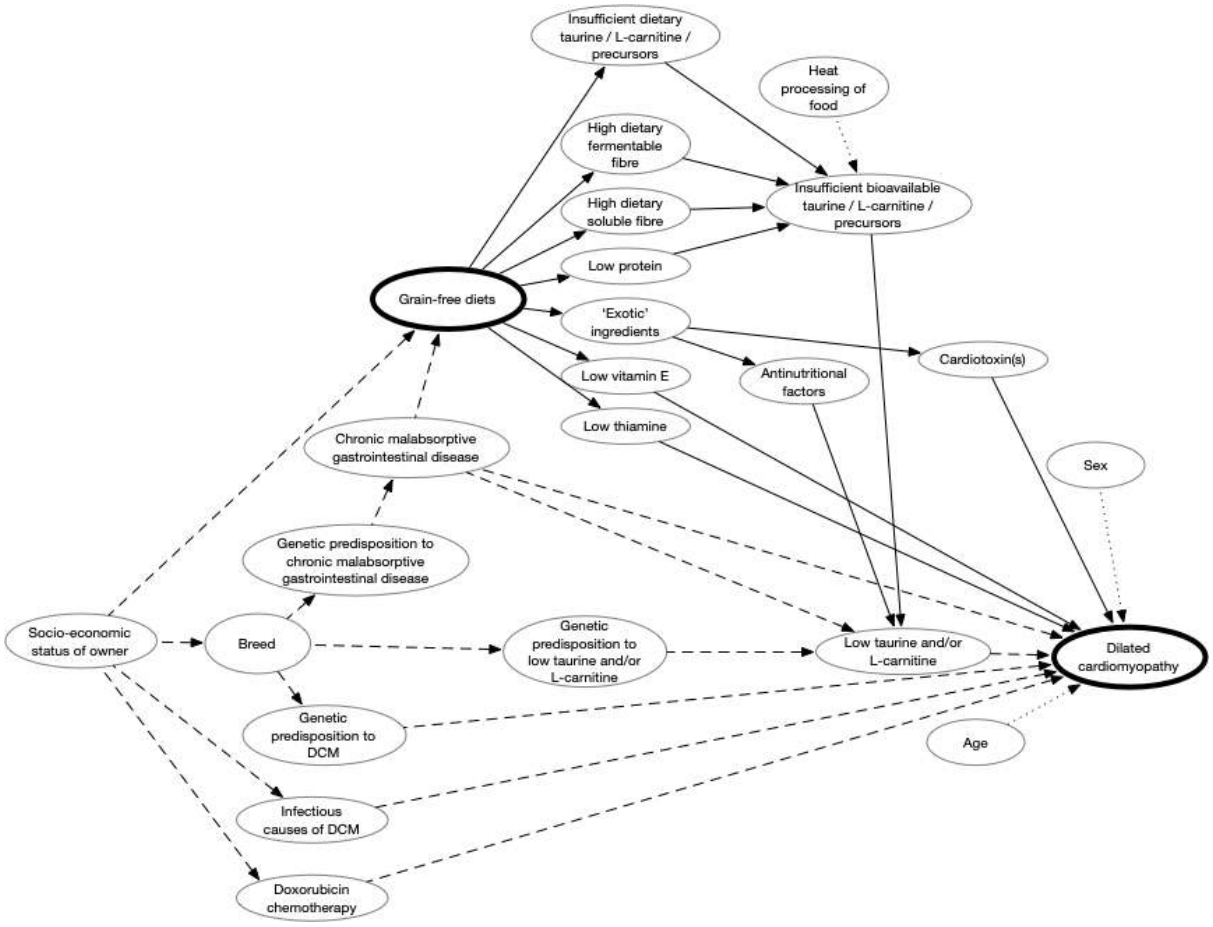

Figure 1: Directed acyclic graph considering the hypothesised association between grain-free diets and acquired DCM in dogs.

Grain-free diets (the exposure-of-interest) and dilated cardiomyopathy (DCM; the outcome-of-interest) are highlighted; plain edges (paths between the nodes) indicate potential causal paths between grain-free diets and DCM; dashed edges (paths between the nodes) indicate potential biasing paths between the grain-free diets and DCM; dotted edges (paths between the nodes) indicate non-biasing paths.

Construction of a directed acyclic graph that considered the suggested association between grain-free diets and acquired DCM was undertaken, informed by the findings of a literature review investigating causal factors or risk factors for canine DCM, with a few additional considerations (Figure 1). Socio-economic status was included, given the plausible association between socio-economic status of the owner and breed of dog, choice of diet for their dog, access to doxorubicin chemotherapy and exposure to infections linked to DCM. Risk factors for DCM identified in non-canine animal models of disease were also included, unless the quality of evidence was very low. Chronic malabsorptive gastrointestinal disease (and directly related nodes) were associated with grain-free diets, based on generalised media suggesting that some pet owners specifically chose grain-free diets due to concerns about 'food sensitivities' and 'allergies' that may reflect undiagnosed malabsorptive disease (e.g.[15]): this node may therefore represent a biasing pathway. 'Exotic' ingredients common to grain-free diets was included as a node, based on hypotheses regarding the presence of cardiotoxins and/or constituents that have antinutritional effects.[16]

Using the directed acyclic graph and the software DAGitty v2.3[17], biasing pathways were systematically identified, with determination of appropriate combinations of covariates to control for confounding bias. The options were: 1) chronic malabsorptive gastrointestinal disease and socio-economic status of owner; 2) chronic malabsorptive gastrointestinal disease, doxorubicin chemotherapy, infectious causes of DCM and breed; or 3) chronic malabsorptive gastrointestinal disease, doxorubicin chemotherapy, infectious causes of DCM, genetic predisposition to low taurine/ L-carnitine and genetic predisposition to DCM.

For clarity, a confounding factor is associated with the outcome (in this case, DCM) and also independently associated with the causal factor (grain-free diets)—i.e. not on the causal pathway of an association between grain-free diets and DCM. In the directed acyclic graph (Figure 1), dietary factors such as high dietary soluble fibre are not confounders, as they are on hypothesised causal pathways between grain-free diets and DCM. In contrast, breed is identified as a potential confounder, given its potential association with grain-free diets (through socio-economic status of the owner and chronic malabsorptive gastrointestinal disease) and independent associations with DCM. It is not necessary to control for all variables on all biasing pathways to control for confounding: control of sufficient variables to block all biasing pathways is the appropriate approach.[14] For complex directed acyclic graphs, use of software such as DAGitty[17] is a valuable aide to determining the appropriate variables required to control for confounding.

Of note, directed acyclic graphs considering a relationship between a hypothesised cause and effect are dynamic: they can and should be refined as evidence accumulates. For example, if further research demonstrates that one or more hypothesised causal pathways in Figure 1 are implausible, they should be removed from consideration in further studies.

4.2 Plan the approach to control of confounding

Control or minimisation of potential confounding can be achieved by study design and in analysing data.[18]

Restriction, through the use of inclusion and exclusion criteria, is a strategy that can be employed in study design to minimise the risk of confounding. Restriction criteria used to control for confounding apply equally to cases and controls. For example, a study could be restricted to one particular breed of dog, to avoid confounding by breed (Figure 1). However, restriction can come at a cost of limiting study external validity[11]: considering the example of breed, study findings may not reflect findings in unrelated breeds of dog if breed-specific differences exist.

Multivariable approaches to data analysis are generally the most flexible approach to controlling for confounding. However, they require a reasonably balanced data set in regard to potential confounders, and accurate data on these, to adequately control for confounding.[19] Considering the different sets of factors that

could be used to control for confounding of the suggested relationship, factors such as 'chronic malabsorptive gastrointestinal disease' and 'socio-economic status of owner' may be relatively easy to measure with a reasonable degree of accuracy, through review of medical records or questioning of the owner for medical history, and through statistical bureau proxies for socio-economic status using the owner's address, ZIP Code or similar. In contrast, accurately measuring 'genetic predisposition to low taurine/ L-carnitine' and 'genetic predisposition to DCM' would be virtually impossible at this stage, and so these are not realistic options to control for confounding.

Matching control dogs to cases in respect to hypothesised confounders (e.g. breed) may be employed to improve the precision of study results when controlling for confounding in a multivariable analysis. However, matching must be employed judiciously, and paired with an appropriate approach to data analysis, as use of matching can induce confounding in study results, where confounding would not otherwise occur. Matching can be logistically challenging to implement, dependent on the matching criteria; though on the other hand, well-considered matching criteria can provide a useful set of criteria for identification of controls for recruitment to a study.[20]

4.3 Calculate the required sample size for a sufficiently powerful study

An appropriate sample size in a case-control study is essential for statistical power: if a sample size is too small, the probability of the study statistically detecting small-to-medium differences in DCM risk between dogs fed grain-free diets and dogs fed grain-based diets will be relatively low, even if these differences are truly present.

The appropriate sample size will vary somewhat with study design. Study design influences on required sample size include whether the study subjects are to be recruited from one or multiple sample sources (e.g. veterinary hospitals), whether matching is used, the number of controls recruited per case, and the number of covariates intended for inclusion in a multivariable statistical analysis. It will also vary depending on how common feeding grain-free diets is in the study base (population from which the study subjects are recruited), and the magnitude of difference in risk of DCM that the study is designed to be able to detect statistically.[20] Additionally, consideration of potential misclassification of cases and/or controls (inadvertently including dogs with DCM in the control group, and/or inadvertently including dogs without DCM in the case group) may be appropriate (Sections 4.5 and 4.7).[22]

As a basic orientation to an adequate sample size, and to indicate the scale by which required sample size can vary according to pertinent factors, basic sample size estimates are presented (Table 1). These were derived using Epi Info 7[23], using the Fleiss method, assuming study requirements of 95% confidence and 80% power,

and assuming a study design involving one sample source (e.g. one veterinary hospital), no matching of controls to cases, and no misclassification of cases and controls.

Table 1 Example of required sample sizes for a case-control study to detect a difference in risk of DCM in dogs fed grain-free diets vs. dogs not fed grain free diets, assuming 95% confidence, 80% power and no misclassification.

Ratio of controls to cases	Assumption of the % control dogs in the study that are fed a grain-free diet	Magnitude of difference in DCM risk that the study is capable of detecting*	No. cases of DCM required	No. controls required	Total no. dogs required
1	10%	Odds ratio = 2	283	283	566
		Odds ratio = 4	58	58	116
2	10%	Odds ratio = 2	205	410	615
		Odds ratio = 4	41	82	123
2	20%	Odds ratio = 2	126	252	378
		Odds ratio = 4	28	56	84
2	30%	Odds ratio = 2	105	209	314
		Odds ratio = 4	26	51	77

* Given assumptions of 95% confidence and 80% power

The use of multiple control dogs per case can help minimise the required case sample size (Table 1), which is particularly beneficial when cases are rare—though there is generally no further benefit beyond four controls per case.[24] If feeding of grain-free diets is relatively common amongst the study base, the study will require fewer dogs, compared to undertaking the study amongst a population where grain-free diets are relatively rare (Table 1). If the study is designed to detect relatively small differences in the risk of DCM between dogs fed grain-free and grain-based diets, it will require a larger sample size than a study that is focussed on detection of larger differences in risk (Table 1).

If grain-free diets are relatively rare amongst the study base, the sample size required for a case-control study of sufficient power will be unfeasibly large. However, this issue may be able to be overcome in a case-control study the utilises existing data, if sufficient data are available (Section 4.4).

4.4 Utilise clinical records?

Case-control study designs that use medical records to identify cases and controls for investigation of disease risk factors are possible. For example, Renwick et al.[25] used extensive digital records available through veterinary general practices in a case control study. However, the validity of such investigations is heavily dependent on having access to adequate data regarding the research question at hand. Given that these studies are generally limited to data collected at the time of a consultation or clinical investigation (i.e. without an

intention of contributing to structured research), they are prone to various sources of bias—most particularly due to missing data, and also sources of information bias.[26,27] Depending on the research question, there may be an opportunity to contact pet owners to gain further information on certain risk factors (e.g. diet) and other important data that are missing from clinical records retrospectively. However, this method of data collection is particularly prone to recall bias (Section 4.9). Case-control studies that collect 'new' data in a tailored approach are preferable to those using clinical records, as all required data can be collected in a systematic way that limits the potential for bias. However, tailored studies are considerably more time-consuming and expensive to undertake.

4.5 Recruitment of cases

A practical mode of recruitment of cases and controls is through veterinary hospitals. If possible, all cases of acquired DCM presenting to veterinary hospital(s) during the study time period should be involved in the study (dependent on owner consent), to minimise the risk of selection bias in the case series. Alternatively, if it is predicted that there will be more cases than required over the timeframe of the study, a random sample of cases could be selected for participation.[18]

For recruitment of cases, a case definition needs to be clearly defined.[18] Case definition criteria should allow a case definition that is as accurate as possible, whilst also considering logistical feasibility. Excessively detailed criteria should be avoided, as they are generally not necessary, can increase the cost of the study exponentially, and may lead to a set of cases that is not representative of acquired DCM in general. For example, in the context of a population study, specifying a case definition of 'diagnosis of acquired DCM by a veterinary cardiologist, after examination including m-mode echocardiography', is likely to be highly accurate; and dictating more specific criteria as part of the case definition may be problematic (for example, due to the numerous echocardiographic parameters used in the diagnosis of canine DCM, and their breed-specific variations).[28] In a population study, a limited degree of misclassification (i.e. dogs unaffected by DCM being incorrectly identified as cases, and/or dogs affected by DCM incorrectly identified as controls) will typically bias findings to the null (assuming observers involved in identifying cases are masked to the dog's exposure status (diet), Section 4.8): the study will be less likely to identify a difference in DCM occurrence between diet groups if there is one, whilst any identified differences between the groups remain valid.[29] This bias may be relatively easily compensated for by a modest increase in overall sample size[22], which may compare favourably to the expense and limitations associated with more complicated case definitions.

The use of incident cases (for example, recruiting cases as they present to a veterinary hospital for the first time for DCM) is strongly preferable to the use of prevalent cases (recruiting cases of acquired DCM that have already been diagnosed). There are a raft of potential biases that may be associated with the use of prevalent cases. For example, dog owners may have changed the diet of their dog as a result of the diagnosis

of DCM, and not accurately remember what the dog was being fed prior to diagnosis. Additionally, if type of diet is a factor that is associated with survival of dogs with acquired DCM after diagnosis, that diet type may be identified to be more common amongst prevalent cases of acquired DCM, without it being a causal factor for the disease. The risk of these types of bias are minimised by recruiting cases at the time of diagnosis.[30]

4.6 Recruitment of representative controls

For a case-control study to be valid, it is essential that the controls selected are representative of the population from which the cases arose.[18] For example, where cases are dogs with DCM recruited through a specialist veterinary services, the population from which the cases arose are pet dogs who, if they acquired DCM, would have access to the specialist veterinary services involved in recruitment of cases. A suitable control group may therefore be selected from amongst other dogs admitted to the specialist veterinary services without DCM: both these groups of dogs have gone through the hard-to-define selection process in obtaining specialist veterinary service care (which is likely to include socio-economic status of the owner, and other factors such as owner attitudes and geographical location).[31] Of additional benefit, hospital-based controls are often the most convenient option in gathering sufficiently detailed data.[32]

However, if hospital-based controls are used, it is important that they are carefully recruited. It is essential that dogs being treated for conditions known or possibly related to grain-free diets are not recruited to the study, as this may misrepresent the diet choice amongst the control group, compared to the broader study base that the control group should represent.[24] For example, if the control group contained a considerable number of dogs being treated for chronic gastrointestinal or skin complaints that motivated owners to choose a grain-free diet, grain-free diets may be relatively more common in the study control group than in dogs unaffected by DCM in the study base, potentially biasing study findings.

Recruiting control dogs from sources such as the pets of veterinary hospital staff or kennels of breeding dogs may introduce considerable bias into a case control study, and is not a valid approach. For example, veterinary staff are likely to differ from the broader population from which the cases arose, in terms of their knowledge of veterinary medicine, veterinary nutrition and associated FDA warnings. This may influence their likelihood of feeding grain-free diets. Thus, where veterinary staff dogs are overrepresented in the control group compared to the case group, it leaves study findings prone to bias in such a way that study findings will be invalid. An exclusion criterion around staff pets is an appropriate guard against such bias.

In recruiting control dogs, any inclusion or exclusion criteria applied to cases to control for confounding (for example, inclusion or exclusion of specified breed(s)) must also be applied to control dogs; with no additional confounding-related inclusion or exclusion criteria imposed on control dogs compared to cases.[33]

4.7 Screen controls for preclinical DCM?

Screening control dogs for indicators of preclinical DCM (for example, by echocardiogram) could be undertaken to minimise the likelihood of the control group containing dogs in the latent stage of acquired DCM.

However, this may not be necessary or justified from a resource point of view. Given that acquired DCM is relatively rare in the broader canine population, it may be more efficient to accept the possibility of a limited degree of misclassification of controls (inclusion of cases of undetected DCM in the control group). Statistically, such misclassification of controls would bias assessments of an association between diet and DCM to the null (meaning that the study has less power: it is less likely to detect a difference in DCM occurrence between diet groups, if such a difference exists, whilst any identified differences remain valid).[29] This loss of power may be relatively efficiently compensated for through a modest increase in sample size[22]; and small increases in sample size may compare favourably to the expense, ethics and logistical issues associated with running expensive screening tests on a large number of control dogs, who may not otherwise be subject to such test(s). Additionally, in the case of screening for preclinical DCM, the value of undertaking screening is compromised by the lack of unambiguous diagnostic criteria to use in identification of preclinical DCM, compounded by breed-specific variation.[28]

In contrast, if a study is restricted to particular breed(s) that are at high risk of DCM, or a large proportion of the population from which the cases arose is expected to be high-risk breeds, the use of screening may be appropriate, as the probability of a dog with preclinical DCM being recruited to the control group may be considerably higher. Exclusion criteria based around identification of preclinical DCM would need to be clearly defined and consistently applied across control dogs, though with appropriate consideration of breed-specific differences in the detection of preclinical DCM.

4.8 Masking (blinding) staff and pet owners

Observers involved in tests that are used to classify a dog as affected or unaffected by DCM should be masked to that dog's exposure status (dietary history), to guard against observer bias influencing study findings.[18] For example, a cardiologist or radiologist interpreting an echocardiogram should not be aware of the dog's dietary history; and if an ultrasonographer is involved in obtaining images, they too should be masked. After information required for the case-control study is collected, observers can be unmasked. Unmasking is expected to be possible at the time of diagnosis for cases and after screening (if used) for controls. This would enable clinical veterinarians to proceed in patient management without any restrictions on the information available to them, and so would not be expected to compromise patient care.

If an observer is used to collect information from the dog owners regarding the dog's dietary history and confounding variables, that observer should be masked as to whether the owner is of a case or control dog. Similarly, if dog owners are providing information to the study through a questionnaire or interview, it is ideal to keep them masked to the study hypothesis, including whether or not their dog is a case or control, until all relevant information has been collected, to minimise the likelihood of recall bias (Section 4.9).[18] For example, depending on the specific study design, it may be possible to convey to the owners the aims of the research in suitably generic terms (without being misleading) at the time of recruitment, and provide further detail once all information has been collected. However, if owners have pre-existing knowledge of links made between diet and canine DCM, there is no particular strategy that will mitigate that potential for recall bias (Section 4.9).

4.9 Beware recall bias

Recall bias can be particularly problematic in case-control studies.[34] If dog owners are aware of the hypothesised association between grain-free diets and DCM, and their pet is referred to a veterinary cardiologist for investigation of cardiac disease (or specifically following a preliminary diagnosis of DCM made by a veterinary generalist), they may recall the dietary history of their dog in a different way to owners of dogs that are not being investigated for cardiac disease. For example, owners of cases may be more particular in their recall of the type(s) of diet previously fed to their dog. Obtaining dietary history from an independent source such as veterinary generalist clinical records would avoid this bias, but such information may not be readily available or sufficiently thorough for all study participants. Where an owner does not have pre-existing knowledge of the study hypothesis, masking the owner to the study hypothesis under investigation and whether or not their dog is a case or control (Section 4.8) will minimise the likelihood of recall bias.

Asking each owner if they are aware of the suggested association between grain-free diets and DCM, *after all other information has been collected*, may help gauge the potential for such bias influencing study findings.

4.10 Collate data efficiently

The approach to management of study data should be decided early on the study design, in parallel with the determination of data requirements and intended statistical approach to analysis (Section 4.11). Employment of a secure database (with regular backup) is the optimal approach for large studies, enabling storage and mobilisation of large amounts of data for timely analysis with relative ease.[35] Collating multiple spreadsheets and manually entering data can markedly increase the resources required for data analysis, and increase the risk of errors in the final dataset.

4.11 Analyse data appropriately

In considering the approach to analysis when designing an analytic observational study, control of confounding, the appropriate approach in view of matching (if used) and data hierarchies are important considerations. Data hierarchies may exist, for example, if multiple veterinary hospitals are used to collect data, or if multiple dogs from the same household or kennels contribute to the study.[36]

5. Conclusion

Observations made by veterinary clinicians are the foundation of investigating and understanding mechanisms of causality of veterinary diseases. Nevertheless, it is essential that these observations are subject to rigorous scientific investigation before conclusions are drawn. By these standards, the hypothesis that grain-free diets are associated with acquired DCM in dogs has not yet been adequately explored and requires further investigation in accordance with well-published and accepted epidemiological principles that enable robust inference. Such investigation is readily achievable, and this document outlines some key points for consideration.

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