Dilated cardiomyopathy (DCM) is considered a predominantly inherited disease in dogs. Recent reports suggest an increased incidence of DCM in atypical breeds eating grain-free and/or legume-rich diets. Emerging communications have noted that there is an apparent increase in the DCM incidence rate in the United States (US). However, little data regarding the incidence of DCM are currently available. To address the gap in the literature, this project examines the DCM incidence rate, over time, by retrospective polling of veterinary cardiologists across the US. Further, grain-free brick-and-mortar pet food market share data from 2011 to 2019 were presented. Fourteen US hospitals, out of 88 that were contacted to participate, provided all cardiology canine cases and of those, which specifically were diagnosed as DCM cases, for as many years as were available (1–20 years, average 8.1 years). This included a total of 68,297 canine patients evaluated by a cardiologist. Of the hospitals that participated, three provided age and breed data. A Poisson regression analysis revealed a statistically significant positive trend in the Mixed Breed group (P = 0.025, RC = 0.082), indicating that this group of dogs had an increased trend of DCM diagnoses over the past 15 years. However, there was no correlation (P = 0.16) for the Mixed Breed group and grain-free pet food sales data. The average incidence rate of DCM, amongst referral cases seen in the participating hospitals, was 3.90% (range 2.53–5.65%), while grain-free diet sales increased from 2011 to 2019. Nationally, the data did not support a significant change in percent DCM over time, from 2000 to 2019. There was no significant correlation between the national DCM incidence rate or the individual breed groups (P > 0.05) in relation to the grain-free pet food sales. However, additional studies are necessary to understand whether regional factors contribute to increased DCM incidence rates within smaller cohorts.

**Keywords:** canine, dilated cardiomyopathy, grain-free, incidence, legume-rich, pet food
INTRODUCTION

Dilated cardiomyopathy (DCM) is the second most common acquired cardiac disease and the most common cardiomyopathy in the dog; and is an important cause of canine morbidity and mortality (Buchanan, 1999; Sisson et al., 2000; Dutton and Lopez-Alvarez, 2018). Dilated cardiomyopathy results in decreased systolic function typically followed by chamber dilation, often progressing to congestive heart failure, arrhythmias, and sudden death (Dukes-McEwan et al., 2003; Suboc, 2021). While historically considered a predominantly inherited disease common to specific breeds, such as the Doberman pinscher (Petric et al., 2002), Great Dane (Meurs et al., 2001), and Irish wolfhound (Vollmar, 2000), more recent studies have identified heritable forms of DCM in additional breeds, such as the toy Manchester terrier (Legge et al., 2013), Welsh springer spaniel (Yost et al., 2019) as well as standard and giant schnauzers (Harmon et al., 2017; Leach et al., 2021).

While several reports have investigated the incidence of DCM over the years, many questions remain. The Veterinary Medical Database reported a DCM incidence rate of 0.5% among all dogs seen at referral clinics from 1986 to 1991, as reported by veterinary referral teaching hospitals (Dutton and Lopez-Alvarez, 2018). More recently, a DCM incidence rate of 0.4% was reported among all dogs evaluated at a United States veterinary teaching hospital from 1995 to 2010 (Bellumori et al., 2013). While these studies are helpful to understand the overall incidence of DCM, they do not report annual incidence rates of DCM over time. Moreover, the annual incidence of DCM diagnosed by veterinary cardiologists across the United States has not been reported. Additionally, a study (Dukes-McEwan et al., 2003), recommends that a diagnosis of DCM only comes from a veterinary cardiologist, which could potentially be more accurate (Hillman et al., 2020).

The question of DCM incidence over time is particularly interesting, given recent reports in the veterinary community of a proposed link between certain diets and the development of DCM in atypical breeds (those breeds without a known hereditary predisposition) (Freeman et al., 2018; US FDA, 2018, 2019a,b). Recent literature has focused on a potential link between grain-free diets and the development of DCM in dogs (Adin et al., 2019). In some of these reported cases, the dogs were fed grain-free diets, which typically have a higher inclusion rate of pulse ingredients (US FDA, 2018). Pulse ingredients may be included in grain-free diets as a non-grain carbohydrate source (Quilliam et al., 2021), which can aid in the processing of pet food. Additionally, pulses are known for their high levels of protein (Quilliam et al., 2021), which can also be used as complementary protein sources (Tulbek et al., 2020), along with animal-based protein (Singh, 2017; Mansilla et al., 2019), to assist in achieving the desired protein percentage. In DCM cases submitted to the FDA, a subset of dogs had improved cardiac function after changing to a grain-inclusive and/or non-pulse-rich diet and veterinary care, with or without dietary supplementation of taurine, leading investigators to suspect a dietary factor in the development of DCM (Fascetti et al., 2003; Kaplan et al., 2018; Adin et al., 2019; US FDA, 2019a,b; Walker et al., 2021). Considering these reports, the overall frequency that DCM has been diagnosed within the US, over the past decade, is important to understand.

The study aimed to analyze the annual incidence of DCM diagnosed by veterinary cardiologists over time, in addition to the age and breed distribution of DCM patients. Additionally, this study compiled brick-and-mortar grain-free diet sales data from 2011 to 2019, then overlayed it with annual incidence to analyze correlation.

MATERIALS AND METHODS

Cardiology Services

A retrospective study design was utilized to evaluate the reported incidence of DCM from 2000 to 2019, diagnosed by veterinary cardiologists across the United States. All accredited colleges of veterinary medicine in the US were contacted, as well as private referral hospitals that were identified by internet queries. According to vetspecialists.com, in 2020, 37 states have roughly 256 board-certified veterinary cardiologists, in the United States. From the list of cardiologists, only those who were actively seeing patients were considered, which pertained to 88 referral institutions. For chain veterinary clinics, the individual company was contacted. Eighty-eight veterinary referral hospitals with board-certified or residency-trained veterinary cardiologists were contacted to represent different geographic areas throughout the United States. These referral hospitals included veterinary teaching hospitals at accredited colleges of veterinary medicine, as well as private veterinary referral practices. Of those contacted, 14 hospitals agreed to participate in the DCM survey; out of those who responded, three hospitals agreed to provide breed and age data. The participating cardiology services were provided with a DCM incidence survey and asked to report how DCM was diagnosed, the diagnostic criteria used in their hospital, number of cardiologists in the practice, number of cardiologists residents in the practice, and search terminology used to identify DCM cases in their databases. A DCM case was included based on the criteria of diagnosis by a board-certified veterinary cardiologist or supervised cardiology resident.

Data Collection

The participating cardiology services provided the number of initial canine cardiomyopathy cases seen per year; as well as the number of those cases that had an initial diagnosis of DCM. The cardiology services provided information about how many residents and board-certified cardiologists were in their hospitals for the years given and their search data criteria and keywords. Recheck appointments of the same patient were excluded. Data were presented in line graphs to provide an overall summary.

Breed Distribution

Breed distribution data were provided by three cardiology services (the University of Missouri, Columbia, Missouri; Garden State Veterinary Specialists, Tinton Fall, NJ; Red Bank Veterinary Hospitals, Tinton Falls, NJ). A total of 68 breeds were identified from 2004 to 2019 across the three hospitals. Breeds were divided into five groups: Inherited Breeds (breeds with a reported inherited predisposition to DCM); Small Breeds (<13.6 kg); Mixed Breeds; Retrievers (Golden Retrievers,
Labradors, Chesapeake Bay Retrievers), and Other Breeds. Groups were plotted as line graphs for each hospital to evaluate trends over time.

**Age Distribution**
Age distribution data were provided by Red Bank Veterinary Hospital, Tinton Falls, NJ, from 2005 to 2020. Data were evaluated by comparing the average age of DCM patients upon initial diagnosis to other canine cardiac patients and other canine patients evaluated by all services. Further, the percentage of DCM patients diagnosed at age 0–6 years and ≥7 years was evaluated over time. Age distribution data were also provided by the University of Missouri veterinary teaching hospital from 2004 to 2019. The age data were only provided for dogs with a diagnosis of DCM in this referral hospital.

**Brick-and-Mortar Pet Food Sales Data**
Growth in annual grain-free pet food sales, in the United States, were provided by the Nielsen Company for the years 2011–2019. Nielsen Company's definition at the time of data collection was solely based on label claims/declarations and did not account for ancient grain diets. Sales numbers for 2011–2015 (The Nielsen Company, 2020a) represented sales in only pet specialty retail stores. Sales numbers from 2016 to 2019 represented sales in brick-and-mortar food, drug, mass, convenience, and pet specialty stores (The Nielsen Company, 2020b). Excluded in these sales numbers are online sales due to capabilities for tracking online sales were not well-established at the time. In addition, sales through farm and feed stores, veterinary clinics, hardware stores, and other stores selling pet food are not noted above (The Nielsen Company, 2020a,b). Sales data was unavailable for these excluded channel outlets during the study time. It is possible that the percentage of grain-free versus other diets could have been different in these excluded channels compared to brick-and-mortar pet specialty, food-drug-mass, and convenience stores. Only sales data for grain-free pet foods was evaluated as grain-inclusive pet foods are not being investigated as being associated with DCM (Freeman et al., 2018; US FDA., 2018, 2019a,b). These data were shared with permission.

**Statistical Analysis**
The data represented a retrospective survey from cardiology referral institutes to acquire information on the trend of DCM incidence across years, among states. The DCM incidence data were analyzed using a mixed model random coefficient regression analysis in Statistical Analysis Software (SAS Institute Inc. 2012. SAS/STAT® 9.4 User's Guide, Second Edition. Cary, NC: SAS Institute Inc.) and expressed as the percent of DCM cases concerning the number of referrals for each year at each state site. Regression analyses were performed by grouping states together to determine the overall DCM response trend and the statistical significance of the trend across years. The best-fitted regression model assumed a common slope (response trend) allowing for differing intercepts among states. The response measure used for all statistical analyses was the percent of DCM cases reported for the number of referrals made at each state's cardiology referral institutions for each year surveyed. The percent DCM cases reported provided a convenient, reliable, and interpretable response measure for analysis. The regression analysis included all states' results, except for Hawaii, which only had one year of data. A correlation analysis was conducted for the annual average DCM incidence against the grain-free pet food sales data, for the years that sales data was available. Significance was set at $P < 0.05$.

Dog breeds were grouped by kind and groups were evaluated using a Poisson regression to analyze breed distributions. The number of dogs in each of the five classes (Inherited, Small Breed, Retrievers, Other, or Mixed Breed) were modeled as a Poisson distributed response variable in a generalized linear model. The year of each observation was modeled as a random effect, controlling for the total number of dogs seen by the cardiology service in each year and the cardiology referral institutions (Red Bank, Garden State, or Missouri). The primary interest was the regression coefficient, its confidence interval, and $P$-value. Significance was set at $P < 0.05$.

**RESULTS**

**Cardiology Services’ Survey Responses**
Geographically, each region of the United States was represented by at least one participating veterinary cardiology service. Out of the states that have a board-certified cardiologist, the state participant rate ($n = 13$) was 35.1%. Of the 88 cardiology services contacted, 74 cardiology services declined. Veterinary medical records are only required to be kept for 3–7 years, depending on the specific state's law, and therefore, was a limitation to the amount of data retrospectively collected. Fourteen services agreed to provide information from the DCM incidence survey. The services that declined to participate did so for various reasons including, but not limited to, being unable to adequately search their medical record systems, not having the resources to do such a search, or for unknown reasons.

Incidence rates were based on 68,297 cardiology patients seen at a referral hospital and not the entire dog population. From these participating services, one provided 20 years of data, two provided 14–15 years of data, four provided between 8 and 11 years of data, and the remaining seven services provided 5 years or less of data (Supplementary Table 1). Some of the reporting hospitals contributed fewer years of data due to their medical record search limitations or they did not have a board-certified cardiologist on staff for a long enough period to provide data.

While cardiologists reported a variety of methods used in diagnosing DCM, all reported using echocardiographically derived breed-specific reference intervals and allometrically scaled prediction intervals (Cornell et al., 2004) for left ventricular internal diameter in diastole and left ventricular internal diameter in systole, fractional shortening, and breed predisposition as benchmarks for diagnosis, as well as the active exclusion of other congenital and acquired cardiac diseases that can lead to cardiac chamber dilation and systolic dysfunction, such as left-to-right cardiac shunts and degenerative valve disease (Wess, 2021). Other parameters, such as normalized left ventricular internal diameter (Visser et al., 2019), left ventricular volume indices, left ventricular ejection fraction, and
left ventricular sphericity index (Dukes-McEwan et al., 2003) also were used by some cardiologists when considering a diagnosis of DCM. All database searches included a diagnosis of “DCM”, or “Dilated Cardiomyopathy.”

From the 14 cardiology services, across the United States, one site, Ethos-Hawaii, provided only one year of data and was excluded from the analysis, as one year is insufficient to establish a trend. For these 13 veterinary hospitals, the annual average of DCM was 3.90% (range 2.53–5.65% ± 0.79%) (Figure 1).

Geographically, the four regions reported incidence rate as follows: West – 3.63%; Midwest – 4.6%; South – 3.9%; and Northeast – 5.02%. The Northeast region had the highest reported incidence rate compared to the other regions, with New Hampshire, despite being able to provide only three years of data (2017–2019) had the highest incidence rate and an average of 11.61% (range 9.52–14.51%). Conversely, the western region had the lowest incidence rate. Washington state provided 20 years of data (2000–2019) and had an average incidence rate of 3.19% (range 1.26–5.86%).

A mixed model random coefficient regression analysis of all sites that reported more than 1 year of data (n = 13) revealed no significant upward or downward trend in the DCM incidence rate (P = 0.9815, Slope = −0.0009) (Figure 2). The trend line, of the small cohort of participating institutes, does not support a change in DCM incidence over time; however, the rate for the entire dog population, during a similar timeframe, in the US is unknown. It can be argued that this percentage is a ratio of two random variables, DCM cases reported for the number of case referrals, but the added complexity to the statistical analysis to address this concern is not warranted.

**Breed and Age Distribution**

Three cardiology services [Missouri and New Jersey (n = 2)] provided additional data on annual breed predisposition (Figures 3A–C). Breeds were identified phenotypically and not genetically tested, as the retrospective collection only allowed for a review of the medical records. In all sites, breeds with a known inherited predisposition for DCM comprised most cases diagnosed with DCM, annually. A Poisson regression analysis revealed positive trends for the Small and Other Breed groups over the past 15 years; however, these trends were not statistically significant [Small Breed, n = 28, P = 0.055, regression coefficient (RC) = 0.114; Other Breeds, n = 167, P = 0.053, RC = 0.015]. The Inherited group showed a slight negative trend, which was not significant (n = 672, P = 0.134, RC = −0.017). A statistically significant positive trend was observed in the Mixed Breed group (n = 71, P = 0.025, RC = 0.082), indicating that this group of dogs had an increased trend of DCM diagnoses over the past 15 years (Figure 3D). There was no significant correlation (P > 0.05) when comparing the grain-free pet food sales from 2011-2019 to the Small Breed, Other Breed, and Mixed Breed groups. The Retriever group (n = 110) and Not Specified group (n = 28) were not significantly significant.

One service additionally provided annual age distribution (Red Bank - Tinton Falls, NJ) among DCM patients upon initial diagnosis, compared to all cardiology patients, and all patients annually evaluated by all services at the hospital (Figure 4). An overall upward trend in age was noted among the three groups represented: All Cardiology Patients, DCM Patients, and All Canine Patients, over the past 15 years. Dilated cardiomyopathy patients were consistently younger than other canine cardiology patients but older than the general canine hospital population at the time of diagnosis. This trend was stable over time, although the age of diagnosis increased within each of the three groups over time. The age of DCM patients was divided into two groups: 0–6 years, and ≥7 years from 2005 to 2020. Across 15 years, most patients (60% or more) were diagnosed with DCM at age 7 years or older. More specifically, in 2005, 67% of DCM patients were 7 years or older, while 33% were diagnosed at 6 years or younger. The age gap increased in 2020, with 75% diagnosed at 7 years or more and 25% diagnosed between 0–6 years of age, suggesting that dogs with DCM are living longer before diagnosis, according to the database. A second referral hospital (University of Missouri) provided age data on dogs that had a diagnosis of DCM. However, the age of all patients and all cardiac patients could not be gathered. There was no appreciable trend in the age in the dataset over the 16 years, with the average age being 6.8 years (range 5–11 years ± 1.62).

**Annual Grain-Free Pet Food Sales**

The Nielsen Company, 2020a provided data on the growth of annual grain-free pet food sales from 2011 to 2019 (Figure 5) and the percent market share of grain-free dry dog food from 2016 to 2019 (The Nielsen Company, 2020b). From 2011 to 2019, there was not a significant correlation (P = 0.62; r² = −0.18) observed between DCM incidence rate and grain-free pet food sales. Grain-free pet food sales reached $900 million in 2011, the first year that grain-free pet food sales were recorded. From 2011 to 2015, grain-free sales data were collected from brick-and-mortar pet specialty retail outlets only. Albeit a small portion of all pet food retailers, this demonstrated a three-fold increase in sales, from $900 million to $2.7 billion. From 2016 to 2019, the sales data additionally reflected grocery, drug, mass merchandise, and convenience stores (along with pet specialty store sales), in accordance with the increasing popularity of grain-free diets during this time. Grain-free pet food sales had a two-fold increase, reaching $5.4 billion in 2019 (Figure 6). Moreover, total grain-free pet food increased from 21.5 to 29% of the market share from 2016 to 2019, while grain-free dog food increased from 31.9 to 43% of all kibble diets sold during this time. Sales data did not include information about grain-free pet foods sold by veterinary clinics, farm and feed stores, and electronic commerce. Nor did it include data from privately-held companies that did not report their annual sales, which could lead to a gross underestimate of total sales from 2011 to 2019.

**DISCUSSION**

This retrospective study provided a dataset that shed insight on the overall incidence of DCM as diagnosed by 14 veterinary cardiology services across the United States. Although 88 veterinary referral practices were contacted, it is unknown how many veterinary practices there are with board-certified veterinary cardiologists actively seeing patients in the US.
It is estimated that there are about 256 board-certified veterinary cardiologists in the US, but some referral practices may have multiple cardiologists, some may have none and some cardiologists may service multiple referral locations. A definitive diagnosis of DCM should only be made via echocardiographic evaluation by a board-certified veterinary cardiologist (Sisson et al., 2000, p 874–895); therefore, general veterinary practices were not queried for this study. Variation in DCM incidence rates was observed at cardiology services across all regions. This variation in DCM incidence should be expected due to multiple regional variables, including differences in diagnostic echocardiographic criteria among cardiologists, breed distributions across states, pet owners’ ability to seek referral veterinary care (Way, 2017; AVMA, 2018), regional infectious diseases that may result in myocarditis, and other systemic diseases that can be associated with DCM. Regional trends in different diets and/or nutritional preferences for dogs could not be evaluated. Despite the state-by-state variation in annual DCM incidence, the overall trend did not support a significant increase in DCM incidence over time. Important to note, the average incidence rate reported is considerably higher than what has been previously reported (Bellumori et al., 2013; Dutton and Lopez-Alvarez, 2018). However, this study’s reported incidence rate is a proportion of cardiology cases seen by the participating...
cardiology services, rather than the whole dog population or within all canine referral hospital cases. Reasonably, this would be an explanation for the difference from previously reported incidence rates.

Moreover, a statistically significant increase in DCM incidences was observed for the Mixed Breed group and while not statistically significant, the Small Breed group had a positive trend, which is in line with recent reports suggesting an uptick in the incidence of DCM among atypical breeds (Freeman et al., 2018; US FDA., 2018, 2019a,b). This dataset, while not depicting a universal upward trend in DCM incidence, may not be sufficiently powered to identify changes in DCM incidence over time among small cohorts of dogs or within certain breeds or categories of dogs. Small breed dogs are not typically predisposed to DCM, and there has been an increase of small breed dogs in the pet population since 1999. Small breed dogs have been consistently on the increase while big and medium-breed dogs are declining (Ferdman, 2015). There was no significant correlation between grain-free pet food sales and Small Breed and Mixed Breed group. While this small cohort suggests an uptick, it is not correlated to grain-free pet food sales from 2011–2019. This may be a potential explanation for increasing diagnosis of DCM in this cohort. Moreover, these dogs were not genetically tested to rule out any potential inherited breeds or genetic mutations; nor was the diet history, medical care, concurrent illnesses, or possible predisposing medical conditions known about these dogs. Further studies should genetically test mixed breed and atypical dogs diagnosed with DCM to potentially rule out a genetic component as well as, larger studies performed to further investigate if DCM is increasing in mixed and small breed dogs nationwide or regionally. A slight decrease in DCM seen in inherited breeds in the data from the three hospitals that provided breed data may be due to improved breeding practices (Baker, 2020) and deselection of breeding practices.
individual dogs with a history of DCM. Furthermore, from 2015 to 2020 the percentage of purebred dogs in the US decreased from 62 to 56%, while during the same timeframe mixed breed dogs increased from 47 to 51% (The Human Society of the United States, 2022). Between 2015 and 2022 dogs adopted from shelters fluctuated, 37% (2015–2016), 28% (2017–2018), 44% (2017–2018), and 36% (2019–2020); and based on a 2015 study (Strand, 2015), <5% of dogs in a shelter were purebreds. Dogs adopted from breeders have decreased from 34 to 19% (The Human Society of the United States, 2022).

Interestingly, there was a slight increase in DCM incidence diagnosis among the participating hospitals from 2008 to 2009 and 2018 to 2019. The Heart Vet in New York provided incidence data starting in 2009, the reported incidence rate in 2009 was the highest from all the participating hospitals during that timeframe. This could potentially be due to a regional effect on DCM. Additionally, the 2018-2019 increase may be due to increased interest in cardiology evaluations among veterinarians and pet owners due to published articles during that time (Freeman et al., 2018; US FDA., 2018, 2019a,b).

Conditions, such as degenerative valve disease and left-to-right cardiac shunts, can cause cardiac remodeling similar to DCM with characteristics of chamber dilation and systolic dysfunction (Machen and Sleeper, 2014); however, the definitive diagnosis of DCM requires active exclusions of these diseases.
with echocardiography. Furthermore, concurrent diseases (cardiac and otherwise) can be associated with the DCM phenotype, while not investigated in the present study, future studies should consider this to better understand all contributing factors to DCM.

This investigation did not observe a correlation between the rise in brick-and-mortar grain-free pet food sales and the average incidence rate of DCM, considering the cohort of institutions that participated and the breeds of their cardiology referral cases across the United States. Furthermore, the DCM incidence data received from participating referral hospitals did not support a concurrent overall increase in cases of DCM from 2000 to 2019.

Limitations
The authors understand there are many limitations when conducting a retrospective study based on referral case numbers of multiple independent institutions. Some of those included collecting sufficient information from different geographic regions, collecting multiple years of data from each institution to understand the trend of DCM diagnoses for that hospital, and the varying criteria for the diagnosis of DCM for each referral hospital.

Due to the retrospective design, there were limitations within the collected data. The data presented in this study represented a small number of the total cardiologists currently in the United States, as many cardiology services were unable or unwilling to participate. Depending on the clinic, veterinary medicine records are not updated or as detailed as they should be. Additionally, the transition from paper records to electronic databases could result in missing records (Zeltman, 2017). Additionally, according to the American Veterinary Medical Association (AVMA, 2019), most states’ laws typically require veterinarians to keep records of their patients 3–7 years after their last treatment/examination. Therefore, it is possible, that the dataset does not represent the trends observed by all veterinary cardiologists. Additionally, based on the cardiology services that participated, Ethos—Illinois was unable to provide data for 2018, which could affect the incidence trend. Moreover, data regarding grain-free pet food sales were fragmented and underestimated compared to the actual sales as companies that are not publicly traded are not required to report this information and data do not exist for some sales channels. Online sales were also unable to be tracked, which could lead to further underestimated sales as online purchases are becoming more popular. To further the understanding of the incidence rate of DCM in the entire dog population, future studies should take into consideration regional variations and breed differences.

Finally, while some upward and downward trends were noted in the breed groups that were diagnosed with DCM over time, the nature of this dataset did not allow for the evaluation of potential reasons for the increase. While this information is important to report, its overall significance is unknown.

Conclusion
Limitations notwithstanding, this information comprises the most complete dataset to date, describing the incidence of DCM diagnosed by veterinary cardiologists over time. Additionally, previously unknown data regarding the growth of grain-free pet food sales from 2011 to 2019 are provided. The data revealed a static overall trend in DCM incidence over time, while an increase in mixed breed DCM incidence was noted in the practices participating in this study. The data reported here were collected from a comparatively small proportion of cardiology practices and may not be representative of the entirety of the country. A five-fold increase in grain-free pet food sales was demonstrated from 2011 to 2019. Future studies are necessary to understand whether a correlation exists between different nutrients within pet foods and the development of DCM in dogs.

DATA AVAILABILITY STATEMENT
The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.
ETHICS STATEMENT

Ethical review and approval was not required for the study of animals/human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/participants or patients/participants legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

BWQ and AK designed research and analyzed the data. BWQ, SBL, and SG collected the data. SDC and AK designed tables and figures. SDC, BWQ, and SBL wrote the paper. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2022.846227/full#supplementary-material

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