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Parameters related to diagnosing hypertrophic cardiomyopathy in cats

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ABSTRACT

Background: The initial diagnostic markers are important for general practitioners to identify cats suspected of having cardiac disease, particularly hypertrophic cardiomyopathy (HCM).

Aim: The aim of this study is to investigate the indicators that suggest feline cardiac disease, especially HCM.

Methods: This is a retrospective study, using the data from 354 cats, to identify various clinical parameters that indicate the presence of cardiac disease in cats in order to develop a model to predict the likelihood of HCM in cats. Among all the parameters gathered, heart sound and LA size are the most significant in predicting the likelihood of HCM in cats.

Results: After undergoing statistical analysis, we created a formula that could help screen cats with HCM and normal cats before further diagnosis, such as echocardiography. The formula $Y_1 = -3.637 + 2.448$ (LA size) +2.683 (murmur) +1.274 (gallop) is the fittest model with an area under curve from the ROC analysis of 0.889. A new set of data was used to validate the model. This predictive model has 40% accuracy but correctly predicts 90% of the truly normal cats, making this model beneficial in helping veterinarians exclude truly normal cats from cats suspected of having HCM.

Conclusion: The model may assist in distinguishing normal cats from those suspected of having HCM. Further diagnosis with echocardiography remains the gold standard for the final diagnosis of cardiac diseases in cats.

Keywords: Cardiac disease, Diagnosis, Heart sound, Hypertrophic cardiomyopathy, Left atrial size.

Introduction

Cardiovascular disease in cats is a severe condition affecting about 10%–15% of the cat population (Laudhittirut *et al.,* 2020). Abnormalities in feline cardiac muscles can lead to conditions such as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and restrictive cardiomyopathy (RCM) (Kittleson and Côté, 2021c). The true causes of feline cardiovascular disease are often difficult to pinpoint. Different breeds of cats are more susceptible to various heart conditions. For example, American Shorthair, Maine Coon, Persian, Siamese, Ragdoll, and Sphynx are predisposed to HCM (Khor, 2013). Furthermore, some cats may develop severe and progressive cardiac disease (Kittleson and Côté, 2021a).

Feline cardiac disease can be classified into four stages (Fuentes *et al.,* 2020): Stage A: Cats with risk factors for cardiac disease but have not yet developed the condition. Stage B: Cats with subclinical feline cardiomyopathies, which can be divided into two sub-stages, including Stage B1: Cats with minimal to no atrial enlargement. Cats in this stage may have a low risk of developing congestive heart failure and arterial thromboembolism. Stage B2: Cats with moderate to marked atrial enlargement, with a high risk of developing congestive heart failure and arterial thromboembolism. Stage C: Cats with congestive heart failure and/or arterial thromboembolism. Cats that are treated and are in stable condition are still classified at this stage. Stage D: Cats with congestive heart failure that does not respond to treatment or worsens.

Diagnosing feline cardiac disease can be challenging. Physical findings, such as abnormal heart sounds (heart murmurs, gallop, and arrhythmias), heart rate, respiratory rate, color of mucous membrane, and capillary refilling times, may not always be conclusive in identifying cats with cardiac disease (Dickson et al., 2018). Cats with progressive cardiac disease may have left atrial enlargement, pleural effusion, or pulmonary edema, which can be observed through radiography. Additional diagnostic tests include measuring cardiac biomarkers such as NT-proBNP (Häggström et al., 2015). Echocardiography is needed for the definitive diagnosis of cardiac disease in cats. However, some

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cats may not tolerate it well due to the requirement for intense handling during the examination. Additionally, performing echocardiography requires specialists and expensive ultrasound machines, which may be challenging for general practitioners to access (Chobsuk *et al.,* 2021). Therefore, utilizing alternative diagnostic markers is essential for a more comprehensive assessment of feline cardiac disease.

The objective of this study was to investigate the markers derived from physical findings, blood-based cardiac biomarkers, and radiographic findings to identify cardiac disease in cats. The goal of this study is to provide general practitioners with initial diagnostic markers for identifying cats suspected of having cardiac disease before referring them to cardiology specialists for further investigation and disease confirmation.

Materials and Methods

A retrospective study with a cross-sectional design was conducted. The medical records from 2018 to 2022 of the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand, were retrieved for analysis.

The study included data from 386 cats. Among these, 354 cats were recruited to establish the model, while data from the remaining 32 cats were used to assess the accuracy of the model.

All cats underwent echocardiography to obtain the definitive diagnosis, and the results of a physical examination, electrocardiography, radiography, and the NT-proBNP point-of-care test were recorded. Cats diagnosed with unremarkable changes in the heart by echocardiography were classified as normal. Cats diagnosed with cardiac diseases and HCM were categorized as such, while those diagnosed with cardiac abnormalities other than HCM were classified under the category of cats with cardiac diseases.

The diagnosis of cardiac diseases was confirmed through echocardiography using an ultrasound machine equipped with 2–4 MHz and 4–12 MHz multi-frequency phased array transducers, which were assessed by a single operator. Results from a feline NT-proBNP point-of-care test kit (SNAP® Feline proBNP, IDEXX, Maine, United States) were recorded as normal or abnormal. The vertebral heart score (VHS) was measured from radiographic images. To assess both cardiac size and pleural cavity on the same image, imaging-specialized veterinarians subjectively assessed the size of the left atrium (LA) and the presence of pleural effusion using right lateral thoracic radiography. The LA was recorded as either normal or enlarged, while pleural effusion was noted as either absent or present. Physical examination findings including heart sound, lung sound, heart rate, respiratory rate, temperature, color of mucous membrane, and capillary refilling time, were retrieved from electronic medical records.

Twelve parameters were analyzed, including heart sound (normal, murmur, and gallop), NT-proBNP result (normal and abnormal), heart rate with cut-off values >200 bpm, lung sound (normal, increased, decreased, and crackle), temperatures with cut-off values <99.5 F, respiratory rate with cut-off values >80 bpm, the color of mucous membrane (pale pink, pink), capillary refilling time (1 second, 2 second, <2 second, and >2 second), VHS with cut-off values <8, subjective radiographic assessment of left atrial size (normal and enlargement), subjective radiographic assessment of the presence of pleural effusion (absence and presence) (Payne et al., 2013).

Statistical analysis

The study variables included NT-proBNP, heart sound, heart rate, lung sound, respiratory rate, temperature, VHS, color of mucous membrane, capillary refilling time, size of the LA, and the presence of pleural effusion. Statistical analysis was conducted using SPSS software, version 22.0 (IBM Corp., Amonk, NY, USA). Descriptive statistical analysis was performed for cat characteristics. Continuous data were tested for normality using the Kolmogorov-Smirnov test. Non-distributed data were presented as the median and interquartile range. Differences in general characteristics were compared with the Mann-Whitney U test. To select the candidate criteria, a univariable logistic regression model was performed between normal cats and cats with cardiac diseases (HCM with other cardiac diseases) and normal cats and cats with HCM. Any associations with a p-value less than 0.2 were subsequently included in the multivariable model, which was performed using the forward elimination method. A significance level of p-value less than or equal to 0.05 was considered significant. The receiver operating characteristic (ROC) curve was also utilized to determine the model with the highest sensitivity.

Ethical approval

Not needed for this study.

Results

Data from 354 cats were collected, comprising males (n = 193), females (n = 152), and unknown (n = 9). The breeds included Domestic Shorthair (n = 209), Persian (n = 65), Scottish Fold (n = 34), Maine Coon (n = 14), American Shorthair (n = 8), Exotic Shorthair (n = 4), British Shorthair (n = 3), Mixed breed (n = 3), Sphinx (n = 2), Ragdoll (n = 2), Khao Manee (n = 2), Munchkin (n = 2), Bengal (n = 2), Siamese (n = 1), Kinkalow (n = 1), American curl (n = 1), and European Shorthair (n = 1). Of these, 238 cats were normal, while 116 cats had cardiac diseases, including HCM (n = 81) and other cardiac diseases (n = 35), such as arrhythmogenic right ventricular cardiomyopathy (n = 5), unclassified cardiomyopathy (n = 2), RCM (n = 3), DCM (n = 2), tetralogy of Fallot (n = 2), atrial septal defect (n = 2), patent ductus arteriosus (n = 1), peritoneopericardial diaphragmatic hernia (n = 8), tricuspid regurgitation (n = 2), pulmonic stenosis (n = 2), aortic stenosis (n = 1), hyperthyroid (n = 3), and systemic hypertension (n =

Table 1. The characteristics of cats included into the study.

Parameters	**Normal (*n* = 238)**	**HCM (*n* = 81)**	**Cardiac diseases (*n* = 116)**
Age (year)	5.00 (2.00–9.75)	7.00 (3.00–10.00)	5.50 (2.00–10.00)
Weight (kg)	3.87 (3.185–5.00)	4.50 (3.50–5.0175)*	4.02 (3.06–4.90)
Sex	M (*n* = 77)	M (*n* = 23)	M (*n* = 16)
	Mc (*n* = 49)	Mc (*n* = 26)	Mc (*n* = 2)
	F (*n* = 66)	F (*n* = 17)	F (*n* = 11)
	Fs (*n* = 38)	Fs (*n* = 15)	Fs (*n* = 5)
	Unknown (*n* = 9)		Unknown (*n* = 1)
Breed	DSH (*n* = 146)	DSH (*n* = 44)	DSH (*n* = 19)
	Persian (*n* = 35)	Persian (*n* = 24)	Persian (*n* = 6)
	Scottish (*n* = 23)	Scottish (*n* = 6)	Scottish (*n* = 5)
	KhaoManee (*n* = 1)	KhaoManee (*n* = 1)	Mainecoon (*n* = 1)
	Mainecoon (*n* = 10)	Mainecoon (*n* = 3)	American shorthair (*n* = 1)
	Sphinx (*n* = 1)	Sphinx (*n* = 1)	Exotic shorthair (*n* = 1)
	American shorthair (*n* = 6)	American shorthair (*n* = 1)	Munchkin (*n* = 2)
	American curl (*n* = 1)	Mixed (*n* = 1)	
	European shorthair (*n* = 1)		
	Exotic shorthair (*n* = 3)		
	British shorthair (*n* = 3)		
	Ragdoll (*n* = 2)		
	Siamese (*n* = 1)		
	Kinkalow (*n* = 1)		
	Mixed (*n* = 2)		
	Bengal (*n* = 2)		

* indicates the significant difference between the normal and HCM groups assessed by the Mann-Whitney U test (p = 0.004). DSH, domestic shorthair; HCM, hypertrophic cardiomyopathy; M, male; Mc, castrated male; F, female; Fs, spayed female. Data are reported as median within 25th to 75th Interquartile range (IQR). The cardiac diseases column defines both cats diagnosed as hypertrophic cardiomyopathy (HCM) and those with other cardiac diseases.

2). The age, weight, and breeds of normal cats and cats with HCM and cardiac diseases are shown in Table 1.

Univariable logistic regression analyses for evaluating the association between potential indicators of cardiac diseases and HCM are shown in Tables 2 and 3, respectively.

The multivariable logistic regression including parameters with a p-value < 0.2 for evaluating the association between potential indicators and cardiac diseases (Table 4) and HCM (Tables 4 and 5) was conducted.

Because of the similarity in results between cats with cardiac diseases and HCM and the majority of the cat population studied, which consisted mainly of cats with HCM, the model was specifically designed to predict the presence of HCM in cats.

The multivariable logistic regression results for predicting HCM were then converted into the formula to find the fittest model. The formula is as follows:

$Y_1 = -3.637 + 2.448(\textit{LA size}) + 2.683(\textit{murmur}) + 1.274(\textit{gallop})$.

Additionally, ROC analysis was performed to analyze the performance of the model. The area under the curve, sensitivity, specificity, positive predictive value, and negative predictive value of each model are presented in Table 6. The ROC curves of each model are shown in Figure 1.

Discussion

The purpose of this study is to determine the clinical parameters indicating the presence of feline cardiac disease and HCM and to develop models for predicting the probability of cardiac diseases and HCM in cats.

Table 2. The univariable logistic regression analysis for evaluating the association between potential indicators and cardiac diseases in cats.

Variable	Category	Normal (*n* = 238)	Cardiac diseases (*n* = 116)	Odd ratios (95% CI)	*p*-value
Heart sounds	Normal (*n* = 184)	155	29		<0.001
	Murmur (*n* = 70)	34	36	5.659 (3.063–10.457)	<0.001
	Gallop (*n* = 24)	12	12	5.345 (2.188–13.055)	0.011
NT-proBNP	Normal (*n* = 28)	25	3		
	Abnormal (*n* = 72)	37	35	7.883 (2.184–28.455)	0.002
HR > 200	Yes (*n* = 60)	37	23	1.779 (0.910–3.479)	0.092
	No (*n* = 112)	83	29		
Lung sounds	Normal (*n* = 181)	137	44		0.028
	Increased (*n* = 79)	52	27	1.617 (0,909–2.875)	0.102
	Decreased (*n* = 14)	13	1	0.240 (0.030–1.883)	0.174
	Crackle (*n* = 8)	3	5	5.189 (1.192–22.595)	0.028
Temperature <99.5	Yes (*n* = 41)	29	12	1.328 (0.618–2.855)	0.467
	No (*n* = 160)	122	38		
RR >80	Yes (*n* = 11)	5	6	2.605 (0.751–9.030)	0.131
	No (*n* = 130)	89	41		
MM color	Pale pink mm (*n* = 72)	53	19	0.853 (0.465–1.565)	0.608
	Pink mm (*n* = 196)	138	58		
CRT	>2 sec (*n* = 2)	2	0	0.000	0.999
	1 second, 2 seconds, <2 seconds (*n* = 178)	122	56		
VHS >8	Yes (*n* = 136)	66	70	4.582 (2.644–7.940)	<0.001
	No (*n* = 133)	108	25		
LA size enlargement	Yes (*n* = 66)	23	43	8.468 (4.083–17.564)	<0.001
	No (*n* = 94)	77	17		
Pleural effusion	Yes (*n* = 60)	46	14	0.812 (0.189–3.479)	0.779
	No (*n* = 11)	8	3		

CRT, capillary refilling time; HR, heart rate; LA, left atrial; MM, mucous membrane; NT-proBNP, N-terminal pro B type natriuretic peptides; RR, respiratory rate; VHS, vertebral heart score. The n of each parameter may be lower than the total number of normal and cardiac diseases due to missing data. *P*-value less than 0.05 indicates statistical significance.

Additionally, the accuracy of the model was determined using data from thirty-two cats through a blind testing technique.

According to the age, weight, sex, and breed of each group of cats, the Mann-Whitney U test was conducted to differentiate age and weight between normal cats and cats with cardiac diseases and normal cats and cats with HCM. The results indicated that the normal cats had significantly lower weight than cats with HCM. In a prior study, the weight relationship between cats with HCM and normal cats revealed that the former had considerably higher body weights than the latter (Nakagawa *et al.,* 2022). Another study showed that HCM is a disease that occurs in cats aged between 8 months to 16 years, with the mean of onset around 6.5 years (Kraus *et al.,* 1999; Nakagawa *et al.,* 2022). Due to the wide age range of HCM, the difference in age between normal cats and cats with HCM was not found in our study. Male cats are more likely to be diagnosed with HCM when they are older, according to epidemiological research (Kittleson and Côté E, 2021b).

Maine Coon and Ragdoll breeds are shown to have a predisposition to develop HCM due to their genetic mutation (Szarková *et al.,* 2022). In this study, we observed that, in addition to Maine Coon and Ragdoll, Persian cats were the second most common breed to develop HCM after domestic shorthair cats. This finding can be attributed to the popularity of Persian cats in Thailand, where the study was conducted.

Table 3. The univariable logistic regression analysis for evaluating the association between potential indicators and hypertrophic cardiomyopathies in cats.

Variable	Category	Normal (*n* = 238)	HCM (*n* = 81)	Odd ratios (95% CI)	*p*-value
Heart sounds	Normal (*n* = 169)	155	14		<0.001
	Murmur (*n* = 61)	34	27	8.792 (4.176–18.513)	<0.001
	Gallop (*n* = 21)	12	9	8.304 (2.986–23.092)	<0.001
NT-proBNP	Abnormal (*n* = 63)	37	26	17.568 (2.237–137.938)	0.006
	Normal (*n* = 26)	25	1		
HR >200	Yes (*n* = 54)	37	17	2.383 (1.087–5.225)	0.03
	No (*n* = 99)	83	16		
Lung sounds	Normal (*n* = 163)	137	26		
	Increased (*n* = 72)	52		2.027 (1.043–3.939)	0.037
	Decreased (*n* = 14)	13	20 1 4	0.405 (0.051–3.234)	0.394
	Crackle (*n* = 7)	3		7.026 (1.484–33.252)	0.014
Temperature < 99.5	Yes (*n* = 36)	29	7	1.472 (0.569–3.812)	0.425
	No (*n* = 142)	122	20		
RR > 80	Yes (*n* = 9)	5	4	2.373 (0.598–9.418)	0.219
	No (*n* = 120)	89	31		
MM color	Pale pink mm (*n* = 69)	53	16	1.19 (0.609–2.328)	1.19
	Pink mm (*n* = 173)	138	35		
CRT	>2 seconds (*n* = 2)	2	0	0	0.999
	1 second, 2 seconds, <2 seconds (*n* = 158)	122	36		
VHS > 8	Yes (*n* = 117)	66	51	4.909 (2.618–9.204)	<0.001
	No (*n* = 125)	108	17		
LA size enlargement	Yes (*n* = 57)	23	34	11.383 (4.889–26.499)	<0.001
	No (*n* = 87)	77	10		
Pleural effusion	Yes (*n* = 57)	46	11	0.957 (0.178–5.149)	0.959
	No (*n* = 10)	8	2		

CRT, capillary refilling time; HCM, hypertrophic cardiomyopathy; HR, heart rate; LA, left atrial; MM, mucous membrane; NT-proBNP, N-terminal pro B type natriuretic peptides; RR, respiratory rate; VHS, vertebral heart score. The n of each parameter may be lower than the total number of normal and cardiac diseases due to missing data. P-value less than 0.05 indicates statistical significance.

The univariable logistic regressions revealed that the potential indicators for predicting cardiac diseases and HCM were mostly the same including heart sound, heart rate >200 beats/min, NT-proBNP, lung sound, VHS>8, and LA size. However, the respiratory rate >80 breath/min was found to be significantly associated only with cardiac diseases.

The statistically significant parameters from univariable logistic regression were then utilized in a multivariable logistic regression analysis. The forward elimination technique was employed to create the predictive model for HCM. The objective of prediction models is to accurately estimate the probability of disease occurrence. Receiver operating characteristic analysis was performed to assess the model's performance by comparing the area under curve (AUC) among different models. The higher AUC value indicates better performance in distinguishing between positive and negative cases. For a perfectly fitted model, the AUC is close to 1. In this study, The fittest model was the one created using heart sound and LA size, which yields an AUC value of 0.889.

As the results of the multivariable logistic regression, the equation of the fittest model is $Y_1 = -3.637 + 2.448$*(LA size)* $+2.683$*(murmur)* $+1.274$*(gallop)*. The value of *LA size* is filled in as 0 or 1, indicating no enlargement and enlargement present, respectively. Similarly, 0 or 1 is assigned to *murmur* representing no murmur sound or murmur sound present, and 0 or 1 in *gallop* indicates the absence or presence of gallop sound, respectively. These values can be inserted into the parentheses accordingly. The cutoff of this model is 0.34. Values (Y_1) over 0.34 are considered to have a higher probability of having HCM, while values under 0.34 have a higher probability of being normal. The sensitivity and specificity of this model are moderate (75.9% and 85.9%, respectively).

Overfitting and generalizability of the model are constant concerns; therefore, prediction models should always undergo validation. When models are tested and fitted on the same set of data, overfitting can lead to an exaggeration of the model's performance. Thus, validation becomes necessary, an external validation is performed by testing the model with the new set of data.

To test the model, data from 32 cats diagnosed with echocardiography were retrieved. The results, including LA size and heart sound, were collected and then filled into the formula using a blind technique. The results show that the model correctly predicted 40.63% of the cases. For further clarification, the model accurately predicted 9 out of 10 normal cats, or 90%, and correctly identified cats with HCM 4 out of 22, or 18.18%. The prediction demonstrated higher accuracy in terms of predicting true negatives rather than true positives, indicating a high sensitivity in the prediction. Therefore, the model mentioned above can be effectively used to exclude cats without HCM.

This study suggests that heart sound and LA size could be effective indicators for differentiating between cats with HCM and normal cats. Heart murmur or gallop sounds are manifestations of cardiac auscultation abnormalities in most cats with subclinical cardiomyopathy. However, it is important to note that the presence of a detectable murmur or gallop alone does not always indicate the existence of underlying cardiac disease (Ferasin *et al.,* 2022). The dynamic ventricular outflow tract obstruction, which can cause a murmur, is a benign flow disruption that can occur in a cat with a normal heart (Kittleson and Côté E, 2021a). Enlargement of LA size, which occurs due to

Table 4. The multivariable logistic regression analysis for evaluating the association between potential indicators and cardiac diseases.

Parameters	Odd ratios (95% CI)	*p*-value
Heart sounds		
Normal	1.000	-
Murmur	6.649 (2.186–20.227)	**<0.001**
Gallop	2.391 (0.619–9.241)	0.206
VHS>8	1.708 (0.563–5.183)	0.345
LA size	7.028 (2.477–19.941)	**<0.001**

LA size, left atrial size assessed by radiography; VHS, vertebral heart score.

The bold values indicate statistical significance (*p*-value <0.05).

Table 5. The multivariable logistic regression analysis for evaluating the association between potential indicators and HCM in cats.

Parameters	Odd ratios (95% CI)	*p*-value
Heart sound		
Normal	1.000	-
Murmur	14.628 (3.682–58.111)	**<0.001**
Gallop	3.575 (0.704–18.140)	0.124
VHS>8	1.503 (0.376–6.003)	0.564
LA size	11.565 (3.072–43.539)	**<0.001**

LA size, left atrial size assessed by radiography; VHS, vertebral heart score.

The bold values indicate statistical significance (*p*-value <0.05).

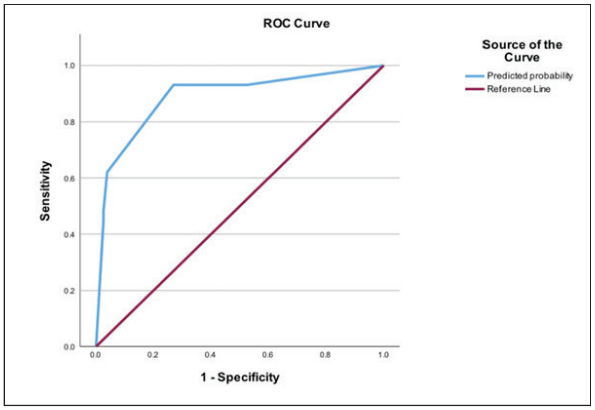

Fig. 1. The receiver operating characteristic (ROC) curve of the model in predicting cats with HCM.

Table 6. The area under the curve, sensitivity, specificity, positive predictive value, negative predictive value and model accuracy for predicting HCM in cats.

Model	n	AUC	Sensitivity	Specificity	PPV	NPV	Model accuracy
Heart sound and LA size assessed by radiography	107	0.889	75.9	85.9	93.6	65.5	40%

AUC, area under the curve, PPV, positive predictive value, NPV, negative predictive value.

higher than normal LA pressure, can be caused by left ventricular dysfunction. Cats that develop moderate to severe LA enlargement are at risk of developing left heart failure (Kittleson and Côté E, 2021b).

The limitations of the study included missing information on several criteria, which made the statistical analysis more complicated. Additionally, cats with other diseases, such as hyperthyroidism and systemic hypertension, may exhibit pathological changes in heart structures. Therefore, veterinarians should consider running additional tests, such as total T4 or free T4, to rule out other diseases before leaning towards a diagnosis of cardiac diseases. Moreover, using a single view of radiographic images may miss pleural effusion in some cases which can be better seen on a ventrodorsal view. However, we decided to use only the right lateral view as it is an easier way to reveal both cardiac size and the pleural cavity in a single image. In addition, because the heart and lung sounds were retrieved from the medical records, the variations in individual veterinary assessments may influence the result. Lastly, since peritoneopericardial diagphargmatic hernia and secondary HCM caused by hyperthyroidism and systemic hypertension, which are not primary cardiac diseases, were included in the study, these conditions could influence the results of the present study.

Conclusion

This research aims to investigate whether parameters obtained from initial examinations, such as physical examination and radiography, can predict the likelihood of cardiac disease in cats. The study found that heart sound and LA size were assessed by radiography associated with HCM. Therefore, heart sound and LA size remain important parameters for veterinarians to consider when assessing the likelihood of HCM in cats. However, since echocardiography can assess both heart structure and function, it is considered the gold standard for diagnosing heart diseases in cats. Cats with any type of cardiac disease mostly share the same clinical signs. Therefore, every cat suspected of having heart disease based on physical examination and radiographic findings should undergo further investigation with echocardiography for a final diagnosis of the type of heart disease. The findings from this study may contribute to improving decision-making regarding which cats should undergo further diagnosis using echocardiography.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Data availability

All data are available in the published manuscript.

Authors' contributions

Tantitamtaworn, Adisaisakundet, Chairit and Choksomngam: Data collection, data analysis, and writing the first draft; Hunprasit and Jeamsripong: supervision of statistical analysis; Surachetpon: Supervision, data validation, editing. All authors read and approved the final manuscript.

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