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Original Study

Echocardiographic and Radiographic Parameters in 20 Lesser Flamingos (*Phoeniconaias minor*)

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Abstract: The establishment of reference cardiologic parameters has gained importance in recent years due to an increased recognition of the role cardiovascular disease plays in avian morbidity and mortality. However, baseline cardiac parameters for lesser flamingos (*Phoeniconaias minor*) have yet to be fully evaluated. This study was performed to evaluate and generate baseline cardiac measurements for lesser flamingos. Echocardiographs and radiographs were opportunistically performed on 23 unsedated lesser flamingos (13 males, 10 females). All birds were manually restrained for cardiac evaluation, and echocardiographic dimensions, functional indices, and radiographic images were collected. Cardiocoelomic width ratios were determined via radiographic measurements when positioning was deemed adequate. Reference materials were established for aortic velocity, left ventricular diastolic length, left ventricular systolic length, left ventricular width in end-diastole, left ventricular width in end-systole, fractional shortening percentage, cardiac silhouette width, thoracic width, and cardiocoelomic ratio. The mean $\pm$ SD was calculated for each parameter: aortic velocity (0.8 0.27 m/s), left ventricular diastolic length (25.2 4.20 mm), left ventricular systolic length (17.8 4.64 mm), left ventricular width in end-diastole (8.9 15.4 mm), left ventricular width in end-systole (6.8 1.61 mm), fractional shortening percentage (29.4 9.11%), cardiac silhouette width (34.8 2.69 mm), thoracic width (67.3 4.91 mm), and cardiocoelomic ratio (51.7 4.02%). No significant associations (all $P > 0.05$) were noted for the echocardiographic dimensions, functional indices, or cardiocoelomic width ratios by age or sex. However, significant associations (all $P < 0.05$) were observed with body weight. Cardiac pathology was detected in 3 individuals; thus, these birds were excluded from the final results (n = 20 animals; 12 males, 8 females). While this study provides baseline parameters for lesser flamingos, owing to the limited number of individuals, further cardiac evaluation of this population is warranted.

Key words: Avian, cardiology, echocardiography, lesser flamingo, *Phoeniconaias minor*, radiography

INTRODUCTION

Flamingos are commonly managed in zoological collections and are a highly adapted species that can thrive in aquatic habitats with harsh salinity and/or alkalinity aquatic habitats while occupying a unique feeding niche.[1,2] Endemic to the Rift Valley lakes of East Africa, the lesser flamingo (*Phoeniconaias minor*) is the smallest of the Old World Phoenicopteriformes species.[2] The International Union for Conservation of Nature lists lesser flamingos as near threatened, with populations noted to have declined because of sustained anthropogenic effects such as pollution, habitat alteration, and the wild bird trade.[3] The incidence of cardiovascular disease in the wild population and its potential contribution to population decline is unknown. However, cardiovascular diseases are commonly noted in zoological avian species as a contributing factor to the decline in the collection population.[4–9]

The high efficiency of the avian heart, coupled with the stress sensitivity of most avian species, prompts cardiac diseases to play an outsized role in morbidity and mortality.[7,8,10–14] Determination of normal structural cardiac parameters in species housed at zoological institutions can provide foundational knowledge to improve their overall veterinary management. This knowledge could then be used as an important tool

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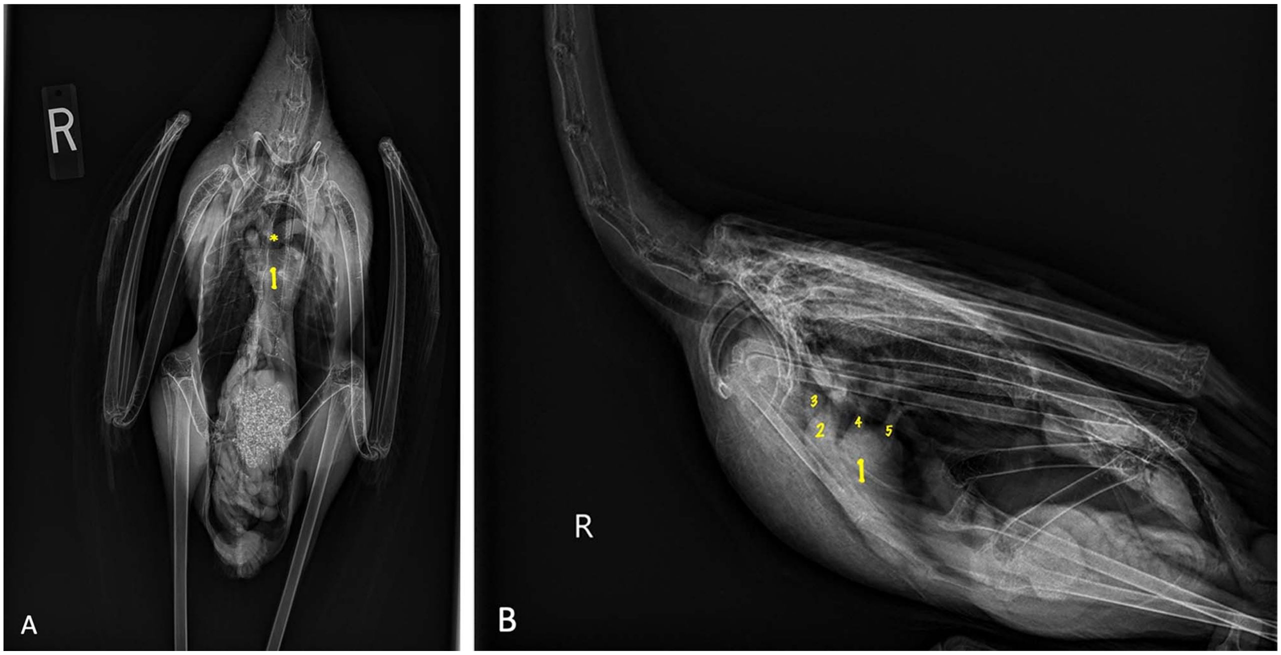

Figure 1. Radiographic images of a healthy lesser flamingo (*Phoeniconaias minor*). (A) Ventrodorsal view: the heart (1), aorta (*), and other large vessels may be noted as dense structures at the base of the heart. Cardiac silhouette measurements were performed via the ventrodorsal view. Note the importance of correct positioning with superimposition of the keel and spine. (B) Right lateral view: the heart (1), brachiocephalic trunk (2), aorta (3), and pulmonary arteries (4–5) can be evaluated; however, compared with other avian species, the cardiac silhouette is not easily assessed, and additional measurements were not performed on this view.

in the health assessments of both free-ranging and managed-care populations of various threatened species. Various techniques have been used to investigate cardiac parameters in many avian species, with population-level parameters being highly variable.[5–7,9,13,15,16] However, this information has not been fully evaluated within the lesser flamingo population.

Radiography is a commonly performed imaging modality used in avian medicine.[6,11,14,15,17,18] Radiographic examination is not a specific diagnostic test for cardiovascular disease; however, it can often reveal information about alterations to heart size and shape that may indicate the need for echocardiographic evaluation.[6–8,14,17] The "hourglass" shape, commonly referenced in avian radiology, consists of the apex of the heart and the overlay of the liver noted within the ventrodorsal view (Fig 1A).[6,14,17] This view also provides visualization of the aorta and other large vessels, noted primarily as dense structures at the base of the heart. In the lateral view, visualization of the brachiocephalic trunk, the aorta, and parts of the pulmonary arteries can be assessed (Fig 1B).[8,14] The ability to obtain measurements of the length and width of the cardiac silhouette on radiographic images has moderate limitations. This is partly because of the superimposition of the apex of the heart and liver in the lateral and ventrodorsal views.[6,8,14,15,17] To obtain accurate measurements for any given species, precise positioning of the individual with correct superimposition of the keel and the spine is necessary.

Echocardiography is a useful noninvasive morphological and functional cardiac assessment technique in avian medicine.[5,9,11–13,16,19,20] The presence of air sacs and the keel bone present a major obstacle when attempting to establish a suitable acoustic window if using a parasternal approach.[12,13] By using the ventromedian approach, 2 horizontal longitudinal views provide visibility of the chambers, the interventricular septum, and the valves of the heart.[12,14,16] The ventral midline approach has been described and used to obtain normal 2-dimensional echocardiographic parameters for many avian species, including the grey parrot (*Psittacus erithacus*), yellow-crowned Amazon parrot (*Amazona ochrocephala*), and white cockatoo (*Cacatua alba*).[11,14,16] This approach allows for the diagnosis of common cardiac pathologies, such as hydropericardium, concentric and eccentric hypertrophy ± dilatation of the right ventricle, and concentric hypertrophy of the muscular right atrioventricular valve, which would not be overtly seen with radiographic evaluation only.[8,16]

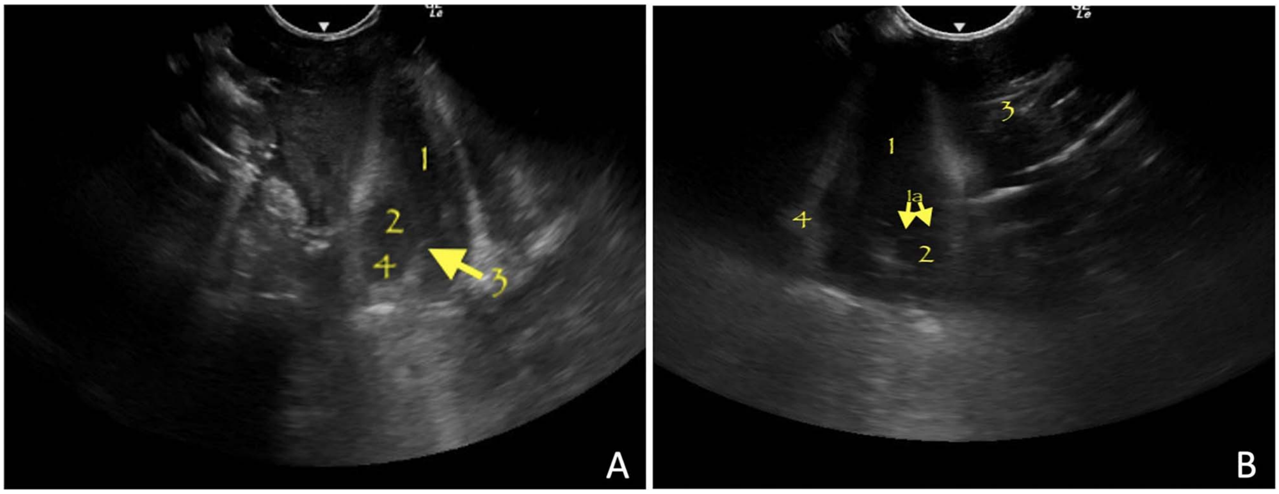

Figure 2. (A) Horizontal "4-chamber" view via echocardiographic examination in a lesser flamingo (*Phoeniconaias minor*) using the ventromedian approach. The heart appears embedded in liver tissue with the larger left ventricle (1), smaller right ventricle (2), and corresponding aortic root (3) and right atrium (4) highlighted. (B) Vertical "2-chamber" view. The left ventricle (1) with corresponding AV-valve (1a), left atrium (2), liver (3), and sternum (4) are highlighted.

Cardiac parameters can vary depending on subtle anatomical or conformational differences between avian species. These variations may require adjustment in the echocardiographic approaches or potential radiographic measurements for each species.[5-9,11,12,14,16-18,20] Thus, obtaining baseline cardiac parameters for individual species provides invaluable improvements in antemortem diagnosis for avian cardiovascular diseases.[4,8,11–13] This study aimed to establish reference echocardiographic dimensions, functional indices, and cardiac silhouette measurements for apparently healthy lesser flamingos from a North American institution. We hypothesized that previously established echocardiographic and radiographic techniques could be used to diagnose antemortem cardiovascular disease in this species.

MATERIALS AND METHODS

Echocardiographic dimensions, functional indices, and cardiocoelomic width ratios were measured in 23 apparently healthy lesser flamingos (Supplemental Tables 1 and 2). The study population consisted of 13 adult males and 10 females, and their body weights ranged from 1.2–2.1 kg. All individuals were wild-born with estimated ages ranging from 13–24 years old. Each bird was manually restrained by animal care staff during the examinations. Any individuals that exhibited signs of potential stress during the procedure (eg, postural weakness, reduced consciousness, progressive agitation, exercise intolerance) were immediately released and excluded from the study population.

Each flamingo was held upright during echocardiographic measurements to reduce the influence of restraint on the cardiovascular system. A water-soluble ultrasound coupling gel (Medline Industries, Northfield, IL, USA) was applied before echocardiographic examination. No individuals within this study received any sedatives or tranquilizers. The echocardiographic examinations were performed by the same board-certified cardiologist using a wide-band microconvex array probe at a frequency between 4 and 10 MHz and a maximum depth of 8 cm (GE LOGIQe; Universal Diagnostics Solutions, Inc, Vista, CA, USA). The echocardiographic examinations were performed following previously established techniques adapted for avian species.[8,12,13,20] The probe was placed in a small area directly ventromedial to the sternum to obtain an adequate cardiac window. The transducer beam plane was directed craniodorsally, with the heart visualized by using the liver as an acoustic window. The heart was visualized in 2 longitudinal views perpendicular to each other as follows: the vertical view ("2-chamber view") and the horizontal view ("4-chamber view"). In the horizontal view, both ventricles, the interventricular septum, the atria, and the aortic root could be visualized (Fig 2A).[5,12,16,20] In the vertical view, the left ventricular and atrial chambers could be visualized (Fig 2B). When necessary, the direction of the probe was adjusted to optimize visualization. No electrocardiogram tracing was performed during these examinations.

Using B-Mode (2-dimensional echocardiography) at a frame rate of 100 frames per second, the following measurements were recorded for each individual flamingo: aortic velocity (AoV), left ventricular diastolic length (LVLd), left ventricular systolic length (LVLs), left ventricular width in end-diastole (LVWd), left ventricular width in end-systole (LVWs), and fractional shortening percentage (FS%) (Supplemental Fig 1). The fractional shortening percentage, or ventricle contractility, was calculated by using the formula FS [%] = (LVWd – LVWs)/LVWd × 100%. In this formula, LVWd represents the left ventricular internal or transverse diameter at end-diastole, and LVWs represent the left ventricular internal or transverse diameter at end-systole. The same operator performed all echocardiographic examinations and calculations. These measurements and calculations were evaluated 3 times during separate heart cycles, and the average of all values was recorded. All measurements were made within the end-diastolic and end-systolic echocardiographic phases. End-systolic was determined when the echocardiogram frame showed the smallest volume of the left ventricle or the frame right before the mitral valve was observed open. End-diastolic was determined when the echocardiogram frame showed the largest volume of the left ventricle or the frame right after the mitral valve was observed closed.

Whole-body radiographic images (ventrodorsal and right lateral views) were obtained on all individuals via manual restraint. Cardiocoelomic width ratio was measured on the ventrodorsal radiographic views after confirmation of correct superimposition of the keel and the spine. Radiographic measurements were only obtained from views that had positioning deemed to be adequate. The width of the cardiac silhouette (CSW) and thoracic width (TW) measurements were performed at the maximum width of the cardiac silhouette (Fig 3).[6,7,11,15,17,18] The ratio of the width of the cardiac silhouette to the width of the thorax (CSW:TW) was calculated as follows: (CSW/TW) × 100; this provided the cardiocoelomic width ratios.[11,15,17,18] A digital imaging and communications in medicine medical image viewer (Vet Rocket LLC, Santa Clara, CA, USA) was used to view the radiographic images and make the abovementioned measurements.

Descriptive statistics (mean, SD, range) were calculated for the echocardiographic dimensions, functional indices, and the cardiocoelomic width ratios using commercial software (Microsoft Excel; Microsoft, Redmond, WA, USA). All additional statistical analysis testing was performed using standard software (Prism; GraphPad Software, Boston, MA, USA). The Shapiro-Wilk test was used to investigate the distributions of the data. The nonparametric Mann-Whitney U test was used to compare the different measures between sexes, ages (< or >18 years old), and weights (< or > 1.5 kg). Differences between measurements were considered significant when the $P < 0.05$.

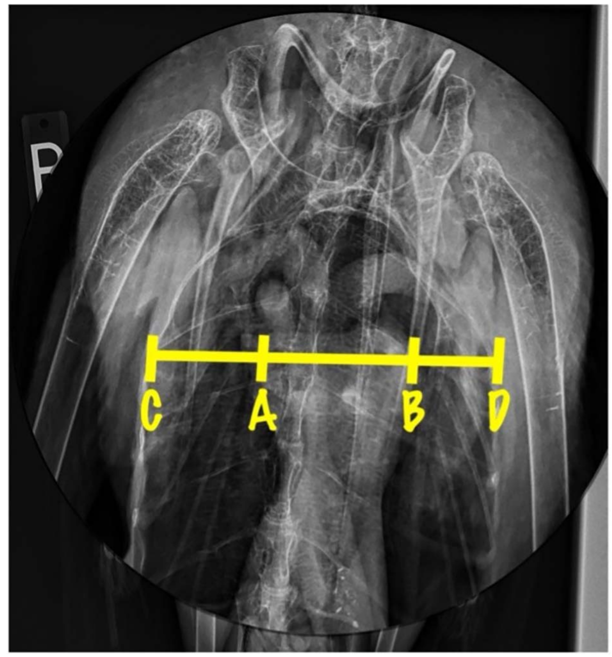

Figure 3. A ventrodorsal radiographic projection of the thoracic region of a lesser flamingo (*Phoeniconaias minor*) illustrating cardiocoelomic width ratio measurements. The horizontal line is placed across the thoracic cavity where the cardiac width is at maximum. The 2 innermost markers (A to B) represent the measured width of the cardiac silhouette. The 2 outermost markers (C to D) represent the width of the thorax at the level of the maximum width of the cardiac silhouette.

RESULTS

The original population for this study consisted of 23 lesser flamingos; however, 3 individuals (n = 1 male, n = 2 females) were found to have cardiac pathology on radiographic or echocardiographic testing. Cardiac pathologies among these individuals included mild cardiomegaly, mild pericardial effusion, and a suspected myxoma or cardiac mass on the atria. One individual was noted to have a corresponding grade II-III/VI heart murmur and a moderate respiratory whistle under manual restraint. Although none of these individuals exhibited historic clinical signs prior to these opportunistic examinations, their cardiac data were excluded from the final study results. Thus, the final study population consisted of 20 (n = 12 males; n = 8 females) healthy lesser flamingos. Moreover, 9 flamingos were < 18 years old and <1.5 kg, and

Table 1. Echocardiographic and radiographic parameters measured for 20 lesser flamingos (*Phoeniconaias minor*).

Parameters	Units	N	Range	Mean	SD	Normality
Weight	kg	20	1.2–2.1	1.6	0.23	Yes
LV length, diastole	mm	20	19.6–34.7	25.2	4.20	Yes
LV width, diastole	mm	20	7.0–12.9	8.9	1.54	Yes
LV length, systole	mm	20	11.6–30.6	17.8	4.64	Yes
LV width, systole	mm	20	4.7–10.7	6.8	1.61	Yes
AoV	m/s	20	0.3–1.3	0.8	0.27	Yes
FS	%	20	12.0–50.0	29.4	9.11	Yes
CSW	mm	20	28.1–39.4	34.8	2.69	Yes
TW	mm	20	58.9–75.0	67.3	4.91	Yes
CSW:TW	%	20	43.3–57.3	51.7	4.02	Yes

Abbreviations: LV, left ventricle; AoV, aortic velocity; FS, fractional shortening; CSW, cardiac silhouette width; TW, thoracic width; CSW:TW, cardiocoelomic ratio.

11 were > 18 years old and > 1.5 kg. The 18 years of age and 1.5 kg cut-offs were based on the median age and weight of our study population.

Cardiac parameters, including AoV, LVLd, LVLs, LVWd, LVWs, FS%, CSW, TW, and CSW:TW, were consistently measurable for all individuals within the study population. There were no statistically significant differences in the echocardiographic dimensions, functional indices, or radiographic parameters based on sex or age; however, there were statistically significant differences noted based on weight ($P < 0.001$). Echocardiographic dimensions, functional indices, and cardiocoelomic width ratios not found to differ by weight are reported in Table 1.

DISCUSSION

This is the first known report of reference values for radiographic and echocardiographic parameters for lesser flamingos. The establishment of this species' reference echocardiographic appearance and cardiac dimensions can help to facilitate the identification and early diagnosis of cardiac diseases in the population.

Results of the present study suggest that the cardiac silhouette of healthy lesser flamingos, when measured on the ventrodorsal radiographic view, should be between 43% and 58% of the width of the thorax. In previous studies with medium-sized psittacine birds (200–500 g), the width of the cardiac silhouette in relation to the thoracic width was noted to be between 51% and 61%.[6,11,14,17] In Canada geese (*Branta canadensis*), studies showed the width of the cardiac silhouette to be between 47% and 57% of the thoracic width.[11] Thus, cardiocoelomic width ratios (CSW: TW) obtained within this study fall within the comparable ranges of other species.[11,14,15,17,18,21]

Echocardiographic evaluations performed in a recent study within the closely related American flamingo (*Phoenicopterus ruber*) provided a unique opportunity to compare and validate the current study results.[22] Not all parameters could be directly compared because of inconsistent reporting between the 2 studies; however, LVLd, LVWd, and AoV cardiac parameters were comparable between the 2 species with minor variations. Potential reasons for the minor parameter differences include species variability in size, sex ratio, and age makeup of the study populations.[1] Thus, larger future cross-sectional or longitudinal echocardiographic studies may be beneficial to further refine and establish robust baseline cardiac parameters for these species.

Differences in cardiac parameters by body weight (< or > 1.5 kg) were found in the lesser flamingos from this study population. The relationship between body weight and echocardiographic dimensions was mentioned by Krautwald-Junghanns et al[12] in a study performed in psittacine birds and pigeons, where statistical significance was noted between body weight and echocardiographic variables. The trend for radiographic measurements to increase with lower body weights were mentioned by Straub et al[7] for multiple psittacine species. Findings within this study were consistent with those results and provide additional validation of our findings.

Three individuals were excluded from the study because cardiac pathologies were noted on their radiographic or echocardiographic images. Despite all 3 being minor pathologies, cardiac parameters for these 3 individuals remained within the range of values measured in the clinically normal flamingos. Because no overt clinical signs were observed in these 3 individuals before their diagnostic evaluation, their cardiac pathologies would not have been identified without testing. These results raise additional questions regarding the extent of cardiac pathology required to alter the values of these cardiac parameters in this species. However, diagnosing these occult cardiac pathologies emphasizes the utility of performing routine echocardiographic and radiographic imaging evaluations in this species.

Owing to the small sample size of this present study, true echocardiographic and radiographic reference ranges could not be established. Thus, further evaluation with a larger study population is warranted. Although the number of birds included in this study was limited, the normal echocardiographic parameters and cardiocoelomic width ratios obtained are a useful supplement to the scientific community because these values have not yet been established for lesser flamingos. This study demonstrated that it is possible to obtain good quality echocardiograms in adult lesser flamingos using standard techniques and establishes a baseline for cardiac evaluation within this population.

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