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*Original article*

# Echocardiographic assessment and reference values of clinically healthy white storks (*Ciconia ciconia*) using transcoelomic approach

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## Abstract

This study aimed to determine species-specific echocardiographic parameters in rehabilitated and clinically healthy white storks (*Ciconia ciconia*) using transcoelomic echocardiography, providing essential reference values for avian cardiovascular assessment. Thirty-seven white storks admitted to the Istanbul University-Cerrahpasa Veterinary Faculty Rehabilitation Center were evaluated after a 3-month observation period. Only clinically healthy individuals were included. Echocardiographic measurements were performed using a transcoelomic approach without anesthesia. Cardiac parameters, including interventricular septal thickness (IVSd, IVSs), left ventricular dimensions (LVIDd, LVIDs, LVPWd, LVPWs), functional indices (fractional shortening, ejection fraction), and Doppler-derived hemodynamic values, were recorded and analyzed statistically. All individuals exhibited normal cardiac morphology and hemodynamics. No significant differences were observed between male and female storks ($p > 0.05$). The ejection fraction ranged from 23% to 97%, and interventricular septal thickness in diastole (IVSd) was between 0.27 and 0.86 cm. Fractional shortening varied from 14% to 75%. Hemodynamic measurements, including mitral valve inflow velocities and aortic flow parameters, were comparable to values reported in other large avian species. No pathological jet flow or valvular regurgitation was detected via color Doppler imaging. This study establishes the first echocardiographic reference ranges for white storks, offering valuable insights into avian cardiac physiology. The findings contribute to wildlife rehabilitation, providing a diagnostic baseline for assessing cardiovascular health in migratory birds. Future studies incorporating larger sample sizes and age-based comparisons will enhance our understanding of species-specific cardiac adaptations.

**Keywords:** cardiac reference values, echocardiography, transcoelomic, white stork

## Introduction

Storks (*Ciconia ciconia* Linnaeus 1758) are long-distance migratory birds commonly found in aquatic and terrestrial ecosystems. Populations breeding in Europe spend the winter across a vast geographical range from Central Africa to South Africa, with Turkey serving as a strategic migratory corridor and an essential transit area for this species (Dolata 2006, Göcek et al. 2010, Süel 2019). Storks inhabit diverse habitats such as open fields, extensive plains, forest edges, and wetlands (Hancock et al. 1992).

The avian cardiovascular system is highly adapted to meet elevated metabolic demands, and the heart--to-body mass ratio is larger in birds than in mammals (King andMcLelland 1984, Smith et al. 2000). The size and function of the heart vary depending on the species' physiological needs. For instance, in long-distance migratory birds such as the barnacle goose (*Branta leucopsis*), a significant increase in heart mass has been observed before migration (Hall et al. 1987). Additionally, heart rate in birds varies with activity level, metabolic demands, and environmental factors (King andcMcLelland 1984, Pees and Krautwald-Junghanns 2009).

Diagnosing cardiovascular diseases in free-ranging birds is challenging due to the limitations of physical examination and the lack of sufficiently developed cardiac imaging techniques (Beaufrère et al. 2010). Radiography is a widely used diagnostic tool for detecting cardiomegaly, and reference values for heart size measurements have been established for various avian species (Broek and Darke 1987, Buchanan 2000). Several studies have reported cardiac measurements for psittacines (Straub et al. 2002), raptors (Lumeij et al. 2011, Mirshahi et al. 2016), and waterbirds (Locke et al. 2020); however, a systematic evaluation in storks has not yet been conducted.

Echocardiography is one of the most crucial non--invasive imaging techniques to assess cardiac morphology and function. While this technique is widely utilized in mammals, its application in birds presents challenges due to the acoustic window limitations imposed by the sternum and air sacs (Beaufrère et al. 2010). Nevertheless, transcoelomic echocardiography has overcome these obstacles, providing a reliable method for cardiac assessment in birds (Pees et al. 2005).

Studies focusing on cardiac parameters in storks are minimal. A previous study assessed heart size in storks using radiographic measurements and reported that the heart width-to-thoracic width ratio was 59.16% (Gunay et al. 2022). However, since radiography provides limited soft tissue detail, echocardiographic measurements allow for a more comprehensive evaluation of cardiac function in storks.

This study aimed to determine fundamental echocardiographic parameters and establish species-specific reference ranges for clinically healthy storks admitted to a rehabilitation center after experiencing migration-related exhaustion. The findings of this study are expected to serve as an essential reference for future wildlife rehabilitation efforts and the diagnosis of cardiac diseases in birds.

## Materials and Methods

### Ethical statement

According to the decision of the Istanbul University-Cerrahpasa Local Ethics Committee for Animal Experiments (decision number 2025/23, dated 07 March 2025), this observational study, which involved only non-invasive clinical evaluations, did not require ethical approval under national regulations.

### Study population and selection criteria

This study was conducted in 2024 at the Istanbul University-Cerrahpaşa Veterinary Faculty Rehabilitation Center on storks admitted after experiencing fatigue and injuries during migration. A total of 37 storks were included in the study after being monitored in the rehabilitation center for 3 months. Only individuals who exhibited normal behavior, regular feeding, and water intake based on general health examinations were selected who exhibited normal behavior, regular feeding, and water intake were selected based on general health examinations.

### Clinical examination and echocardiographic assessment

Before the study, all birds underwent a comprehensive health evaluation, and only clinically healthy individuals were included. Echocardiographic assessments were performed without anesthesia using manual restraint. Following previous studies in avian species, the transcoelomic echocardiography technique was applied (Perrin et al., 2019).

Measurements were performed using an ultrasound device (Mindray Vetus 9, Shenzhen Mindray Animal Technology Co. Ltd, Shenzhen, China) with a specialized cardiac probe. The storks were positioned in dorsal recumbency, and the ultrasound probe was placed ventromedially just caudal to the sternum. By directing the probe cranially, standard longitudinal, horizontal, and vertical heart views were acquired. This setup enabled the measurement of several cardiac

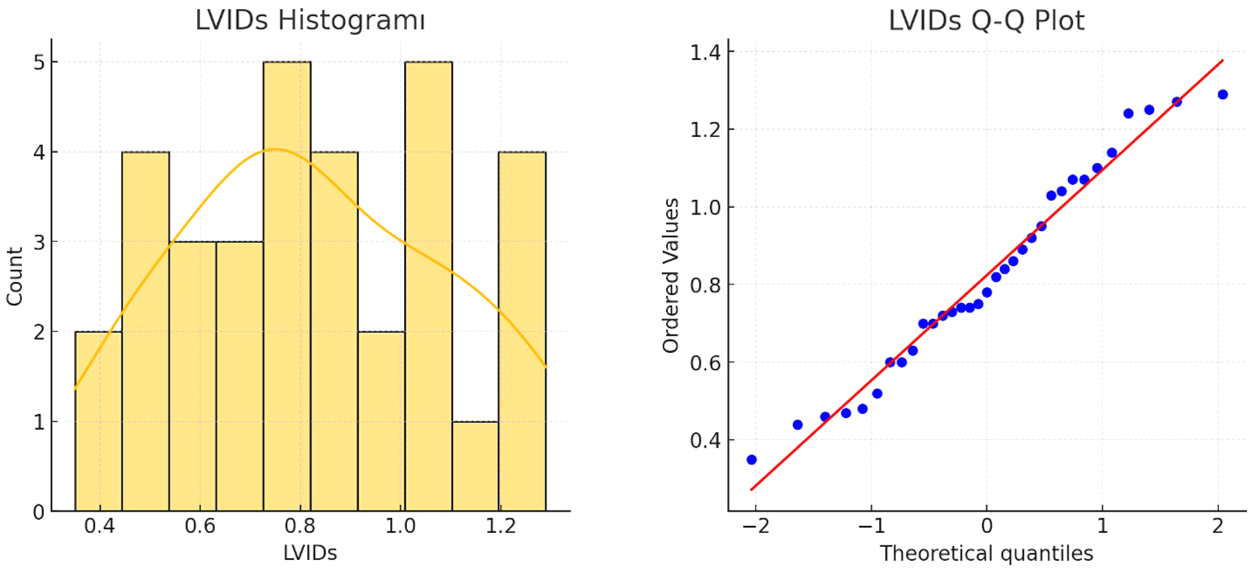


Fig. 1. Histogram and Q-Q plot of left ventricular internal diameter in systole (LVIDs) in white storks (*Ciconia ciconia*). The histogram illustrates the distribution of LVIDs values, showing a near-normal distribution. The Q-Q plot further supports this observation, as the data points closely follow the diagonal reference line, indicating that the LVIDs values exhibit normality.

parameters, interventricular septal thickness in diastole (IVSd) and systole (IVSs), left ventricular internal diameter in diastole (LVIDd) and systole (LVIDs), left ventricular posterior wall thickness in diastole (LVPWd) and systole (LVPWs), IVSd/LVPWd and IVSs/LVPWs ratios, fractional shortening (FS), ejection fraction (EF), end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), left ventricular length and area in diastole (LVLd, LVAd) and systole (LVLs, LVAs), ejection fraction calculated from A4C views (EF(A4C)), mitral valve early and atrial filling velocities (MV E Vel, MV A Vel), MV E/A ratio, mitral valve filling pressure gradients (MV E PG, MV A PG), aortic outflow maximum velocity (AV Vmax) and pressure gradient (AV PGmax), pulmonary venous maximum velocity (PV Vmax), and pulmonary venous pressure gradient (PV Pgmaks). Each parameter was measured three times, and the average values were recorded.

### Data processing and statistical analysis

Before analysis, the data were checked and formatted. Non-numeric characters were corrected, and missing data entries were handled appropriately. Outlier analysis was conducted using the Z-score method, where values outside ±2.5 standard deviations were excluded. The distribution of the data was visually assessed using boxplots and histograms.

To determine whether the data followed a normal distribution, Shapiro-Wilk and Kolmogorov-Smirnov tests were applied, and histogram and Q-Q plot analyses were used for visual assessment (Figure 1). Measures of central tendency and dispersion (mean, median, standard deviation, minimum and maximum values) were calculated. Reference ranges were established using either the 95% confidence interval (mean ± 1.96 × standard deviation) for normally distributed parameters or the 2.5th-97.5th percentile range for non-normally distributed parameters.

## Results

This study systematically evaluated cardiac parameters in 37 rehabilitated and clinically healthy white storks (Ciconia ciconia) using transcoelomic echocardiography. Echocardiographic measurements were performed from the left side using a transcoelomic approach. The acoustic window was optimal in all individuals, and all parameters were successfully recorded (Table 1).

Two-dimensional echocardiographic views of the left ventricle and outflow tract, as well as Doppler imaging of mitral and aortic flows, were obtained to assess cardiac morphology and hemodynamics (Figs. 2 and 3).

The body weight of the individuals ranged from 2.01 to 3.78 kg, with both sexes included in the analysis. A comparison of echocardiographic parameters between male and female storks revealed no statistically significant differences ($p>0.05$). The interventricular septal thickness in diastole (IVSd) ranged from 0.27 to 0.86 cm, while the interventricular septal thickness in systole (IVSs) varied between 0.39 and 1.02 cm. The left ventricular internal diameter in diastole (LVIDd) was measured between 1.11 and 1.90 cm, and the systolic diameter (LVIDs) ranged from 0.31

Table 1. Echocardiographic parameters of white storks (*Ciconia ciconia*).

| Parameters | Confidence interval | Median | Mean | SD |
|---|---|---|---|---|
| HR (bpm) | 122-141 | 131 | 131.6 | 24.5 |
| Weight (kg) | 2. - 3.8 | 2.9 | 2.9 | 0.45 |
| IVSd | 0.28-0.86 | 0.45 | 0.48 | 0.14 |
| LVIDd (cm) | 1.1-1.9 | 1.49 | 1.47 | 0.25 |
| LVPWd (cm) | 0.17-0.72 | 0.43 | 0.45 | 0.14 |
| IVSs (cm) | 0.39-1.02 | 0.68 | 0.7 | 0.16 |
| LVIDs (cm) | 0.31-1.32 | 0.75 | 0.82 | 0.26 |
| LVPWs (cm) | 0.22-0.95 | 0.57 | 0.59 | 0.19 |
| IVSd/LVPWd | 0.57-1.7 | 1.13 | 1.14 | 0.29 |
| IVSs/LVPWs | 0.65-1.75 | 1.26 | 1.2 | 0.28 |
| FS % | 14-75 | 47.73 | 44.75 | 15.61 |
| EF % | 22-97 | 79.4 | 74.05 | 20.32 |
| EDV(teichholz) (ml) | 3.10-11.1 | 5.7 | 5.99 | 240 |
| ESV(teichholz) (ml) | 0.19-3.91 | 1.25 | 1.52 | 1.16 |
| SV (teichholz) (ml) | 0.35-8.9 | 4.16 | 4.64 | 2.19 |
| LVLd(A2C) (cm) | 2.39-4.2 | 3.3 | 3.3 | 0,47 |
| LVAd(A2C) ($cm^2$) | 2.5-5.9 | 4.13 | 4.23 | 0.88 |
| EDV(A2C) (ml) | 1.8-7.7 | 4.71 | 4.77 | 1.53 |
| SV(A2C) (ml) | 0.96-5.7 | 3.14 | 3.33 | 1.21 |
| LVLs(A2C) (cm) | 1.4-3.3 | 2.38 | 2.4 | 0.50 |
| LVAs(A2C) ($cm^2$) | 0.96-3.12 | 1.98 | 2.04 | 0.55 |
| ESV(A2C) (ml) | 0.62-3.2 | 1.4 | 1.54 | 0.70 |
| EF(A2C) | 0.48-0.88 | 0.72 | 0.69 | 0.10 |
| MV E Vel (cm/s) | 40.2-96 | 55.54 | 58.67 | 15.08 |
| MV A Vel (cm/s) | 15-76 | 42.75 | 45.73 | 15.69 |
| MV E/A | 0.73-2.6 | 1.34 | 1.42 | 0.56 |
| MV E PG (mmHg) | 0.65-3.69 | 1.23 | 1.47 | 0.82 |
| MV A PG (mmHg) | 0.21-2.13 | 0.73 | 0.93 | 0.61 |
| AV Vmaks (m/s) | 0.141-1.327 | 0.86 | 0.86 | 0.30 |
| AV PGmax (mmHg) | 0.30-7 | 2.96 | 3.28 | 1.82 |
| PV Vmax(m/s) | 0.4-1.3 | 0.96 | 0.89 | 0.25 |
| PV Pgmaks (mmHg) | 0.38-6.5 | 3.68 | 3.42 | 1.55 |

to 1.32 cm. The left ventricular posterior wall thickness in diastole (LVPWd) varied between 0.17 and 0.72 cm, and the systolic thickness (LVPWs) was 0.22-0.95 cm.

The functional parameters assessed included fractional shortening (FS) values between 14% and 75% and ejection fraction (EF) ranging from 23% to 97%. The end-diastolic volume (EDV) measured using the Teichholz method ranged from 3 to 11 ml, while the end-systolic volume (ESV) varied between 0.19 and 3.91 ml. The stroke volume (SV) measured by the Teichholz method was determined to be 0.35-8.93 ml. In the apical two-chamber view, the left ventricular diastolic length (LVLd) was 2.38-4.21 cm, while the diastolic area (LVAd) varied between 2.50 and 5.96 $cm^2$. The end-diastolic volume (EDV A4C) ranged from 1.78 to 7.76 ml, and the end-systolic volume (ESV A4C) varied between 0.62 and 3.19 ml. The stroke volume (SV A4C) was measured between 0.96 and 5.70 ml, while the ejection fraction in the A4C view (EF A4C) ranged from 0.49 to 0.88.

The early mitral valve inflow velocity (MV E Vel) ranged from 40 to 96 cm/s, while the atrial mitral inflow velocity (MV A Vel) was between 15 and 76 cm/s. The MV E/A ratio varied between 0.73 and 2.63. The early mitral valve pressure gradient (MV E PG) was found to be 0.65-3.69 mmHg, and the atrial mitral valve pressure gradient (MV A PG) ranged from 0.21 to 2.13 mmHg.

The peak aortic velocity (AV Vmax) varied between 0.14 and 1.33 m/s, with a peak aortic pressure gradient

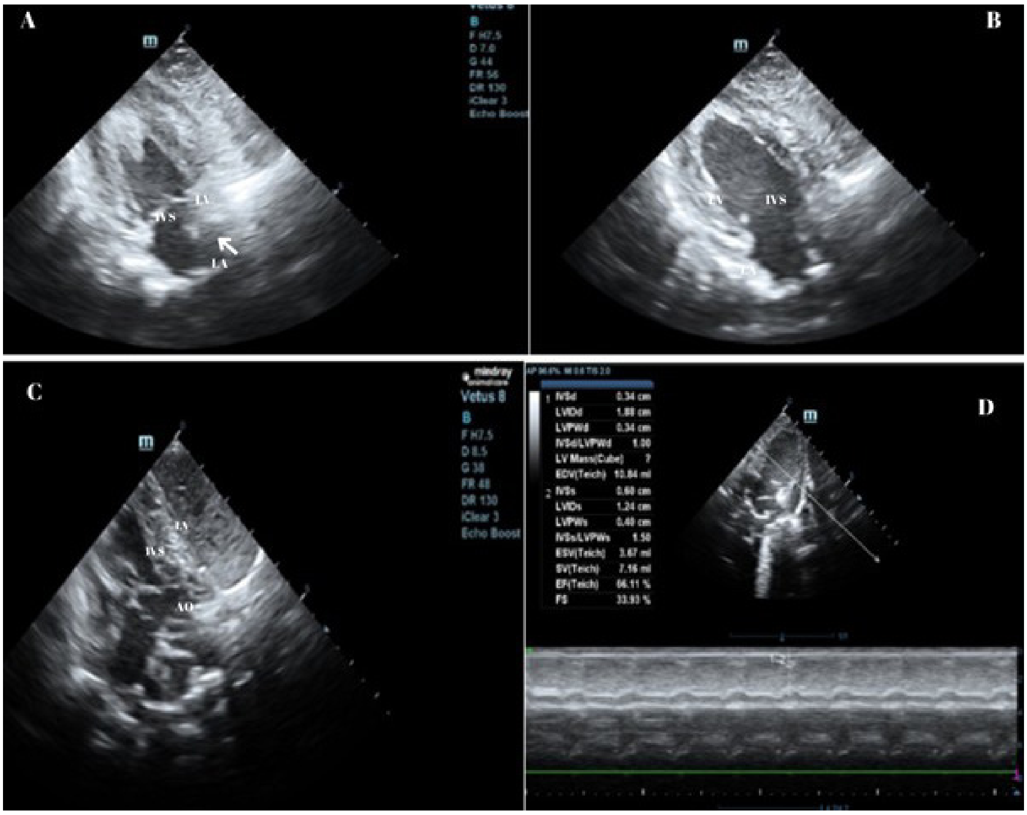


Fig. 2. Two-dimensional echocardiographic view of the left ventricle and outflow tract of Ciconia Ciconia. A: systolic phase. B: diastolic phase. C: Aortic long axis view. D: M-mode imaging. M-mode image of the LV displays dimensions of the ventricular walls, LV cavity, and cardiac function measurements. LV indicates left ventricle, IVS: Interventricular septum, AO: aortic outflow tract, LA: left atrium, arrow: mitral valves.

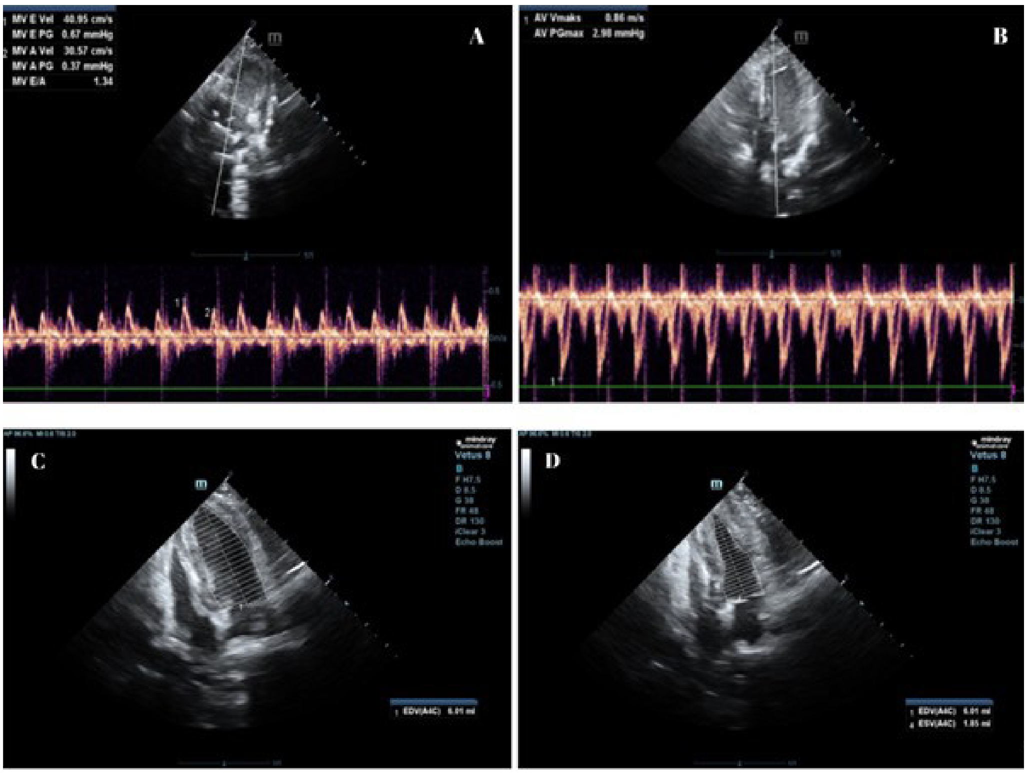


Fig. 3. A: Spectral Doppler image demonstrating inflow through the mitral valve during diastole. B: Aortic flow spectrum obtained from a left parasternal long axis. C, D: The echocardiographic images provided illustrate the application of the modified Simpson's rule (biplane disc summation approach) for estimating left ventricular (LV) volumes.

(AV PGmax) ranging from 0.30 to 6.99 mmHg. The pulmonary venous maximum velocity (PV Vmax) was measured at 0.41-1.38 m/s, while the pulmonary venous peak pressure gradient (PV PGmax) was found to be 0.39-6.46 mmHg.

All individuals exhibited normal morphological and hemodynamic cardiac structures. Intracardiac flow dynamics were assessed using color Doppler imaging, and no pathological jet flow or regurgitation was detected.

This table presents echocardiographic measurements of 37 healthy white storks. Values are shown as reference ranges determined using either 95% confidence intervals (for normally distributed parameters) or the 5-97.5 percentile range (for non-normally distributed parameters), along with mean, median, and standard deviation.

## Discussion

This study evaluated cardiac parameters in 37 rehabilitated and clinically healthy white storks (*Ciconia ciconia*) using transcoelomic echocardiography, and species-specific reference ranges were established. The echocardiographic evaluation of the cardiovascular system in wild birds has been reported in limited studies. Determining cardiac parameters in large migratory bird species is essential for understanding their normal physiological values.

The transcoelomic echocardiography technique has been used in various avian groups, including *Psittacines* (Pees et al. 2005), raptors (Straub et al. 2004), and waterfowl (Beaufrère et al. 2012). This technique is considered an ideal imaging method for large bird species as it minimizes acoustic interference caused by the sternum and air sacs (Fitzgerald and Beaufrère 2016). In our study, the transcoelomic approach provided optimal image quality in all individuals.

The results of this study demonstrated similarities with previous echocardiographic research on *Eudocimus ruber* (scarlet ibis). Perrin et al. (2019) reported an ejection fraction range of 60-80% in scarlet ibis, which falls within the broader 22-97% range determined in our study. Scarlet ibis's left ventricular diastolic length (3.1-3.9 cm) closely corresponds to the 2.38-4.21 cm range measured in white storks. However, the early mitral valve inflow velocity (MV E Vel) in white storks (40-96 cm/s) was slightly higher, likely due to interspecies anatomical and hemodynamic differences.

Interventricular septal thickness in diastole (IVSd) in white storks (0.27-0.86 cm) exceeded the 0.26-0.47 cm range reported for scarlet ibis, while left ventricular posterior wall thickness in diastole (LVPWd) was also slightly greater in white storks (0.17-0.72 cm) compared to scarlet ibis (0.25-0.50 cm). These variations may reflect differences in body size and cardiovascular adaptations among species. Similarly, studies on *Ephippiorhynchus senegalensis* (saddle-billed storks) have commonly reported mitral regurgitation (Garces Torres et al. 2023). In contrast, no pathological valvular regurgitation was detected in our study's rehabilitated white storks, indicating good overall cardiac health. Furthermore, left atrium length values in white storks (1.22-1.92 cm) were comparable to those in saddle-billed storks (1.1-2.0 cm), suggesting that certain cardiac traits may be conserved among large *Ciconiiformes*.

However, white storks exhibited lower interventricular septal thickness in diastole (0.27-0.86 cm) and left ventricular posterior wall thickness in diastole (0.17-0.72 cm) than saddle-billed storks (0.42-0.69 cm and 0.31-0.87 cm, respectively). These findings suggest that while both species belong to the same order, saddle-billed storks may have relatively more developed myocardial structures, possibly due to differences in body size and flight adaptations.

Moreover, fractional shortening (FS) values in white storks (14-75%) were within the range observed in saddle-billed storks (31-54%), although some white storks exhibited higher FS values. This variation may be attributed to species-specific hemodynamic demands or individual variability in cardiac function.

In our study, the left ventricular systolic length ranged from 2.02 to 2.89 cm, while the diastolic length was between 3.01 and 3.92 cm. These values were significantly larger than those reported for *Psittacus Erithacus* (African grey parrots), where Pees et al. (2004) documented a range of 1.65-2.57 cm. In diurnal raptors, the left ventricular systolic length has been reported to range from 0.91 to 2.03 cm, while the diastolic length varies between 1.1 and 2.18 cm. The significantly greater values in white storks suggest that migratory species possess larger ventricular dimensions to support sustained aerobic activity during long-distance flights.

The ejection fraction (EF) in white storks (23-97%) was higher than that reported for psittacine species (Pees et al. 2006) and within the range found in diurnal raptors (18.2-36.2%) (Straub et al. 2004). This elevated EF may be an adaptation to migratory endurance, ensuring efficient oxygen transport and cardiovascular performance under prolonged exertion. Similarly, fractional shortening (FS) values in white storks (14-75%) overlapped with the 18.2-36.2% range found in diurnal raptors but extended beyond the maximum recorded for raptors. This variation may reflect species-specific differences in myocardial contractility and stroke volume regulation, which could be further influenced by body size and metabolic demands (Pees et al. 2006).

Interventricular septal thickness in diastole (IVSd) in white storks (0.27-0.86 cm) was within the 0.09-0.29 cm range reported in diurnal raptors (Straub et al., 2004). However, the systolic interventricular septal thickness (IVSs) in white storks (0.39-1.02 cm) was slightly higher than the 0.07-0.31 cm range observed in raptors, suggesting a relative increase in septal thickening during contraction, possibly due to enhanced myocardial function in a migratory species. These findings indicate that while white storks and raptors share certain cardiovascular features, the specific demands of long-distance migration may result in larger cardiac structures and higher functional efficiency in storks compared to raptors.

The interventricular septal thickness in diastole ranged from 0.27 to 0.86 cm, broader than the 0.19-0.31 cm range reported for psittacine birds by Pees et al. (2004). This difference may be due to white storks' larger body size and higher cardiac output demands. Similarly, the aortic diameter in white storks (0.93-1.44 cm) was larger than in psittacine species, reflecting cardiovascular adaptations necessary for sustaining prolonged flight in migratory birds.

When compared to diurnal raptors, the interventricular septal thickness in diastole in white storks (0.27-0.86 cm) was within the 0.09-0.29 cm range reported for raptors (Straub et al. 2004). However, systolic interventricular septal thickness (0.39-1.02 cm) in white storks was slightly greater than the 0.07-0.31 cm range observed in raptors, suggesting increased myocardial contractility in storks, likely due to their endurance-based flight requirements.

Additionally, the left ventricular posterior wall thickness in diastole (LVPWd) in white storks (0.17-0.72 cm) was lower than the 0.31-0.87 cm range reported in saddle-billed storks (*Ephippiorhynchus senegalensis*) (Garces Torres et al. 2023). This suggests that while both species belong to *Ciconiiformes*, saddle-billed storks may have relatively thicker myocardial walls, possibly due to differences in mass-specific metabolic rates or flight mechanics.

The left ventricular diastolic inflow velocity (LV Diastolic Inflow) and systolic aortic outflow velocity (Systolic Aortic Outflow) values obtained via transcoelomic echocardiography show certain similarities and differences when compared to previous Doppler echocardiographic studies in raptors. In a study by Straub et al. (2004) on raptors, the reported LV diastolic inflow velocity ranged between 0.12 and 0.38 m/s, while the systolic aortic outflow velocity ranged from 0.81 to 1.43 m/s. The AV Vmax (0.14-1.33 m/s) value obtained in this study aligns with the reported values for Harris's hawks (*Parabuteo unicinctus*, 0.75-1.43 m/s) and common buzzards (*Buteo buteo*, 1.04-1.68 m/s) but is higher than those recorded for smaller-bodied parrot species (e.g., *Psittacus erithacus*, 0.63-1.15 m/s) and *Ara* spp. (0.55-1.07 m/s). These differences may reflect variations in cardiac workload and metabolic rate between diurnal raptors and migratory storks.

Additionally, pulmonary venous maximum velocity (PV Vmax) in white storks (0.41-1.38 m/s) was slightly higher than the 0.13-0.29 m/s range reported for falcons (*Falco spp.*) and similar to values observed in Harris's hawks (0.13-0.27 m/s) (Straub et al. 2004). These results suggest that white storks exhibit hemodynamic characteristics comparable to those of diurnal raptors, likely as an adaptation to sustained high-energy flight.

Pees et al. (2006) assessed similar cardiac parameters using Doppler echocardiography in various avian species and emphasized the influence of stress factors on cardiac function. Straub (2003) highlighted that handling-induced stress could affect spectral Doppler echocardiography results in this context. Future studies are recommended to confirm that stress factors do not significantly affect the values obtained in this study.

Long-distance migratory birds are generally characterized by larger ventricles and higher ejection fractions than non-migratory species, as demonstrated in comparative studies of exotic pet birds (Pees et al. 2006). This physiological adaptation allows for increased aerobic efficiency during sustained flight. Previous studies have shown that the heart mass of migratory birds significantly increases before migration. Hall et al. (1987) reported pre-migratory cardiac hypertrophy in Branta leucopsis (barnacle goose), which was directly associated with improved flight performance. This study's high cardiac function parameters support existing literature indicating that migratory species undergo physiological adaptations to sustain prolonged aerobic activity.

Cardiac troponin I (cTnI) has been evaluated as a biochemical marker in *Eudocimus ruber* (Perrin et al. 2019), though considerable interspecies variation in cTnI levels has been noted. While cTnI measurements were not performed in this study, future investigations incorporating this parameter could provide a more comprehensive assessment of cardiac health in migratory birds.

The white storks in this study were observed in a rehabilitation center for 2-3 months, and only clinically healthy individuals were selected. This selection criterion ensures that the reference values obtained represent healthy individuals. However, the study is limited by its sample size and the lack of age-based comparisons. Future studies incorporating larger sample sizes and analyzing different age groups and sex-based differences would contribute to a more detailed understanding of species-specific normative cardiac values.

A study conducted by Bartyzel et al. (2003) on *Columba palumbus* (wood pigeon) compared cardiac morphology between males and females and found no statistically significant sex-based differences. Similarly, our study found no significant differences in echocardiographic parameters between male and female white storks. This suggests this species may have minimal sex-related physiological differences in cardiac morphology.

In conclusion, this study provides the first comprehensive dataset on echocardiographic parameters in rehabilitated and clinically healthy white storks. The findings contribute to the objective evaluation of the cardiovascular system in wildlife rehabilitation and may serve as a reference for future veterinary clinical applications.

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