

Two-dimensional and M-mode echocardiographic parameters in Ghezel sheep

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Abstract

Echocardiography is a non-invasive method of cardiac evaluation in most species. Echocardiographic reference parameters are poorly documented in sheep and not documented in Ghezel sheep. The purpose of this study was to determine reference ranges of normal echocardiographic parameters using two-dimensional and M-mode techniques in Ghezel sheep. In 15 healthy female Ghezel sheep aged between 15 and 18 months, echocardiography was performed in standing position from left and right parasternal approach focused at 3rd - 5th intercostal spaces using a 2.50 - 5.00 MHz phased array transducer. The following parameters were measured in two-dimensional echocardiography: left atrial diameter (LAD), mitral valve annulus (MVA), aortic sinus (AoS), aortic valve (AoV), pulmonary sinus (PuS), and pulmonary valve (PuV); and in M-mode echocardiography: interventricular septum (IVS), left ventricular internal diameter (LVID), left ventricular free wall (LVFW), right ventricle free wall (RVFW), right ventricle internal diameter (RVID). Calculated variables included the ratios LAD/Ao and Pu/AoS, and the fractional shortening (FS), end diastolic volume (EDV), end systolic volume (ESV), ejection fraction (EF), stroke volume (SV) and cardiac output (CO) of the left ventricle. In conclusion, echocardiographic parameters could be reliably assessed in Ghezel sheep and our study provided some normal echocardiographic reference ranges that might be useful in proper identification, visualization, and measurements of cardiac structures. Such findings could be useful to assess and to diagnose the specific heart diseases in sheep practice and also for experimental studies in sheep as an animal model used for research purposes in cardiovascular studies of human.

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Introduction

Echocardiography is considered as an important, rapid, harmless and non-invasive diagnostic method for research and clinical purposes, as widely used for functional and structural evaluation of heart chambers in animals.¹⁻³

Several papers have examined cardiac dimensions and time indices in lambs.^{4,5} Others have examined cardiac echocardiography from different aspects of performance in sheep.⁶ Published studies showed a wide range of cardiac dimensions and measurements at different stages of the reproductive cycle in goats.⁷⁻⁹ Echocardiography is a practical technique in sheep and goats, although a small

acoustic window makes it challenging to achieve some of the images in certain animals.^{7,8}

Sheep are infrequently clinically diagnosed with cardiac abnormalities due to silent or atypical clinical signs, relatively resistant to cardiac diseases, incomplete or delay in presentation, incomplete evaluation for these disorders and absence of normal values of the cardiac specification. In this regard, endocarditis due to different pathogens and toxins is one of the most important cardiac pathologies in many species that could be also seen in sheep,¹⁰ however, the rarity for reports may be for the above reasons. Also, due to the shortfalls in the cardiovascular examination, congenital cardiac abnormalities

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such as ventricular septal defects, tetralogy of Fallot, patent ductus arteriosus which is seen and diagnosed frequently in human, horses and small animals, have been very rarely diagnosed and reported in sheep.¹¹⁻¹³

Among large animals, sheep are considered as a suitable animal model because of its docility, slow growth and easy housing for long-term follow up. Furthermore, dimensional and functional parameters of the ovine heart have remarkable similarities to the human heart.¹⁴⁻¹⁷

The Ghezel sheep is one of the most common Iranian native fat-tailed and medium-sized breeds which is originated in northwestern Iran and northeastern Turkey. It is a multipurpose sheep, raised mainly for milk and meat production.^{18,19} Most studies of echocardiography in sheep have been focused on M-mode and pulse-wave echocardiography.^{5,17,19-21} In this study, two-dimensional echocardiography values have been investigated in both systolic and diastolic times. To the best of our knowledge, echocardiographic values in healthy Ghezel sheep have not been published so far. Therefore, the purpose of this study was to document reference ranges of normal echocardiographic measurements and cardiac function using two-dimensional (2D) and M-mode techniques in Ghezel sheep.

Materials and Methods

Eighteen clinically normal female Ghezel sheep were selected for the study. To confirm the health of the sheep, a thorough clinical examination was performed by cardiopulmonary auscultation, a base-apex electrocardiogram, hematologic and biochemical tests including complete blood count (CBC), concentrations of serum total protein and fibrinogen and troponin I. Color Doppler echocardiography was also used to investigate the possibility of valvular abnormalities. Finally, 15 sheep aged between 15 and 18 months (16.40 ± 1.18 months) and weighing 37.00 - 54.00 kg (42.69 ± 4.73 kg) were included for final echocardiographic assessments. Before starting echocardiography, each sheep was placed for 5 to 10 min in an individual boxes to reduce stress and anxiety and another sheep was accompanying each of the studied sheep to reduce restlessness during echocardiography.

In all cases, echocardiography was done in the standing position and none of the sheep were sedated during the study. Ultrasound gel was placed on the transducer and then applied to 3rd to 5th left and right intercostal spaces. Echocardiographic examinations were performed with a digital color Doppler ultrasound system (Q9 Vet; Chison Medical Technologies Co., Jiangsu, China) using a 2.50-5.00 MHz phased array transducer. Suitable images and Cine memories were stored on the machine hard disk for

further investigation and off-line analysis to increase the accuracy of measurements. Each parameter was measured three times during cardiac cycles with a distance of three heartbeats and then averaged. In the present study based on Boon²² end-diastole measurements were recorded when the mitral valve was closed and the left ventricle was at its highest size. End-systole measurements were recorded before the mitral valve opening and the left ventricle was at its lowest size.

Two-dimensional echocardiography was performed from the following views to evaluate related parameters. 1- Right parasternal long-axis four-chamber view (Fig. 1A): left atrial diameter in end-diastole (LADd) and end-systole (LADs), mitral valve annulus in end-diastole (MVAd) and end-systole (MVAs). Left ventricular functional parameters in B-mode, including end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), and stroke volume (SV) were calculated using modified Simpson's method built-in machine software (Fig. 1B). 2- Right parasternal long-axis left ventricular outflow tract (Fig. 1C): end-diastolic aortic sinus (AoS) and end-diastolic aortic valve (AoV), just before the aortic valve opens and end-diastolic ratio of left atrial diameter to aortic sinus (LADd/AoS). 3- Right parasternal short-axis view optimized for: chordae tendineae to obtain the ventricular M-mode image, at level of aorta (Fig. 1D) to measure ratio of the left atrium diameter (LAD) to the aortic valve (Ao) in closed status, at level of the main pulmonary artery to measure pulmonary artery sinus (PuS) and pulmonary artery valve (PuV) in closed status. M-mode echocardiographic parameters were measured from two views: 1- Right parasternal short-axis to assess interventricular septal thickness at end-diastole (IVSd) and end-systole (IVSs), left ventricular internal diameter at end-diastole (LVIDd) and end-systole (LVIDs), left ventricular free wall thickness at end-diastole (LVFWs) and end-systole (LVFWd), (Fig. 2A). 2- Left parasternal short-axis to assess right ventricular internal diameter at end-diastole (RVIDd), right ventricular free wall thickness at end-diastole (RVFWd) and end-systole (RVFWs) (Fig. 2B). Left ventricular functional parameters in M-mode, including fractional shortening (FS), end-diastolic volume (EDV) and end-systolic volume (ESV), ejection fraction (EF) and stroke volume (SV) were calculated by using the device software (Fig. 2A). All measurements and calculations of parameters were done according to previously described guidelines.^{8,22-24}

Statistical analysis. Descriptive statistics were presented in terms of mean \pm standard deviation (SD), range, and 95.00% confidence intervals (CI). Evaluation relationship between weight and echocardiographic parameters was performed using Pearson correlation.

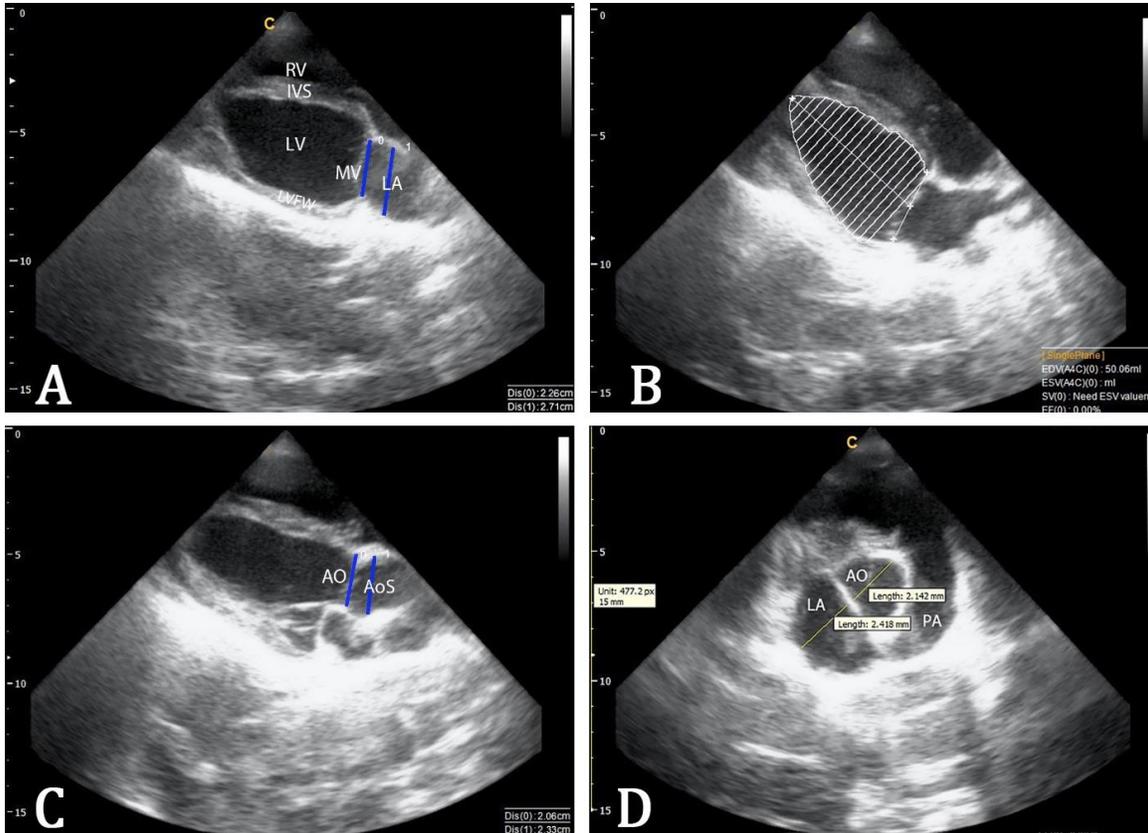


Fig. 1. Two-dimensional B-mode echocardiographic views in Ghezel sheep: **A)** Right parasternal long-axis four-chamber view, mitral annulus diameter measurement (0) and left atrial diameter measurement (1) at end-diastole; **B)** Right parasternal long-axis four-chamber view, left ventricular end-diastolic volume measured by modified Simpson's method; **C)** Right parasternal long-axis outflow view of the left ventricle, measurement of the aortic valves (0) and aortic sinus diameter (1); **D)** Right parasternal short-axis view at the level of the aorta, measurement of the aorta and left atrium are shown. LV: Left ventricle; RV: Right ventricle; IVS: Interventricular septum; LVFW: Left ventricular free wall; MV: Mitral valve; LA: Left atrium; AO: Aortic valves; AoS: Aortic sinus; PA: Pulmonary artery.

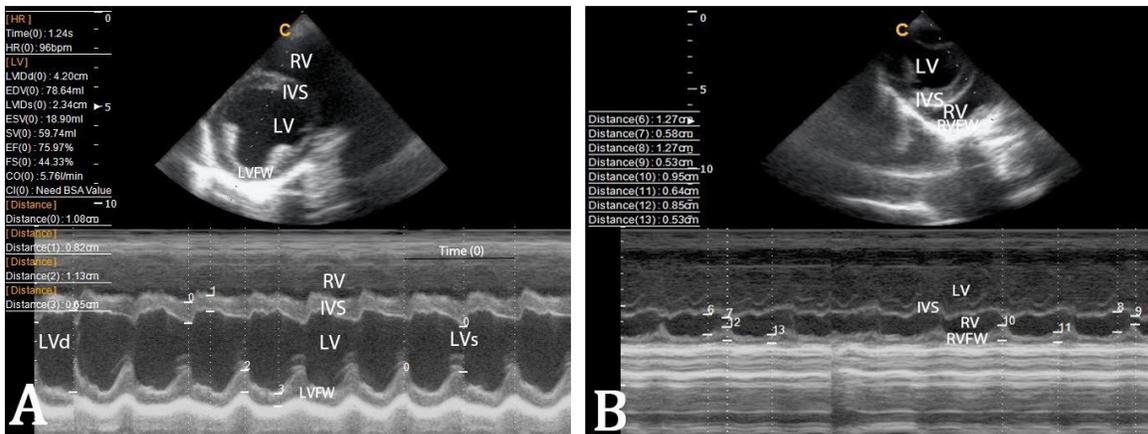


Fig. 2. M-mode echocardiographic views in Ghezel sheep. **A)** Right parasternal short-axis at the level of chordae tendineae, heart rate (HR) M-mode calculation (time 0), left ventricular internal diameter measurement at end-diastole (LVD) and at end-systole (LVs), Left ventricular functional parameters M-mode calculation (LV), interventricular septum diameter measurement in systole (Distance 0) and in diastole (Distance 1), left ventricle free wall diameter measurement in systole (Distance 2) and in diastole (Distance 3); **B)** Left parasternal short-axis, right ventricular internal diameter measurement in diastole (Distances 6 and 8) and in systole (Distances 7 and 9); right ventricle free wall diameter measurement in systole (Distances 10 and 12) and in diastole (Distances 11 and 13) are shown. LV: Left ventricle; RV: Right ventricle; IVS: Interventricular septum; LVFW: Left ventricular free wall; RVFW: Right ventricular free wall.

Results

None of the studied sheep showed abnormal heart sounds and tachycardia at the beginning of the study and during the echocardiography procedure. All sheep showed normal sinus rhythm in the electrocardiographic examination. The mean heart rate during the study was 89.52 ± 12.04 . Almost the investigated sheep were in the same age and weights so the Pearson correlation was not surprising and some measurements were corrected with

weights. Measurement of left atrium diameter was more difficult at end-systole than end-diastole. The right ventricular outflow tract for measurement of the pulmonary artery valve and sinus was not identifiable from the right cranial parasternal position in any of the examined sheep. Right parasternal short-axis view optimized for main pulmonary was a good alternative view for measurement of the pulmonary artery valve and sinus. All 2D and M-mode echocardiographic measurements are summarized in Tables 1 and 2, respectively.

Table 1. Mean \pm SD, range and 95.00% confidence interval of the two-dimensional echocardiographic parameters in Ghezel sheep.

Parameters	Mean \pm SD	Range	95.00% Confidence interval	
			Lower bound	Upper bound
AoS (cm)	2.54 \pm 0.14	2.33-2.85	2.46	2.62
AoV (cm)	2.07 \pm 0.12	1.95-2.39	2.00	2.13
PuS (cm)	2.19 \pm 0.13	1.93-2.45	2.11	2.26
PuV (cm)	1.81 \pm 0.11	1.61-1.95	1.74	1.86
MADd (cm)	2.50 \pm 0.16	2.21-2.74	2.41	2.59
MADs (cm)	2.52 \pm 0.36	1.90-3.06	2.32	2.72
LADd (cm)	3.47 \pm 0.28	2.70-3.91	3.32	3.62
LADs (cm)	4.46 \pm 0.46	3.44-5.36	4.20	4.71
EF (%)	78.23 \pm 4.91	67.38-85.62	75.51	80.95
SV (mL per beat)	41.65 \pm 9.21	29.92-65.33	36.55	46.75
EDV (mL)	54.80 \pm 8.97	47.67-82.58	49.83	59.76
ESV (mL)	12.33 \pm 3.04	8.53-18.09	10.64	14.01
LADd/AoS (Long axis)	1.37 \pm 0.11	1.20-1.51	1.31	1.43
LAD/AO (Short axis)	1.19 \pm 0.08	1.06-1.34	1.14	1.23
PuS/AoS	0.86 \pm 0.53	0.75-0.96	0.83	0.89

AoS: Aortic sinus diameter; AoV: Aortic valves diameter; PuS: Pulmonary artery sinus diameter; PuV: Pulmonary artery valve diameter; MADd: Mitral annulus diameter at end-diastole; MADs: Mitral annulus diameter at end-systole; LADd: Left atrial diameter at end-diastole; LADs: Left atrial diameter at end-systole; EF: Ejection fraction; SV: Stroke volume; EDV: End-diastolic volume; ESV: End-systolic volume; LADd/AoS: Ratio of left atrial diameter to aortic sinus diameter in diastole; LAD/AO: Ratio of left atrial diameter to aortic valve diameter in end-diastole; PuS/AoS: Ratio of pulmonary artery sinus diameter to aortic sinus diameter in diastole.

Table 2. Mean \pm SD, range and 95.00% confidence interval of the M-mode echocardiographic parameters in Ghezel sheep.

Parameters	Mean \pm SD	Range	95.00% Confidence interval	
			Lower bound	Upper bound
RVIDd (cm)	1.50 \pm 0.32	0.92-1.97	1.38	1.80
RVFWs (cm)	1.06 \pm 0.22	0.68-1.27	0.88	1.24
RVFWd (cm)	0.59 \pm 0.09	0.41-0.69	0.51	0.67
IVSs (cm)	1.40 \pm 0.18	1.20-1.85	1.22	1.41
IVSd (cm)	0.81 \pm 0.14	0.65-1.13	0.69	0.86
LVIDd (cm)	3.74 \pm 0.30	3.29-4.30	3.57	4.06
LVIDs (cm)	1.76 \pm 0.27	1.31-2.35	1.71	2.09
LVFWd (cm)	0.74 \pm 0.12	0.61-0.96	0.62	0.79
LVFWs (cm)	1.40 \pm 0.27	1.13-2.05	1.16	1.37
FS (%)	53.62 \pm 5.09	44.88-60.76	46.95	55.68
CO (L per min)	4.77 \pm 1.06	3.32-6.45	4.39	6.07
EDV (mL)	60.38 \pm 11.39	43.87-83.23	53.89	73.20
ESV (mL)	9.58 \pm 3.95	4.33-19.43	7.85	14.74
EF (%)	80.24 \pm 12.21	45.48-90.56	66.07	89.07
SV (mL per beat)	55.13 \pm 14.87	38.94-87.03	45.62	68.97
HR (bpm)	89.52 \pm 12.04	67.00-112.00	-	-

RVIDd: Right ventricular internal diameter at end-diastole; RVFWs: Right ventricular free wall thickness at end-systole; RVFWd: Right ventricular free wall thickness at end-diastole; IVSs: Interventricular septal thickness at end-systole; IVSd: Interventricular septal thickness at end-diastole; LVIDd: Left ventricular internal diameter at end-diastole; LVIDs: Left ventricular internal diameter at end-systole; LVFWd: Left ventricular free wall thickness at end-diastole; LVFWs: Left ventricular free wall thickness at end-systole; FS: Fractional shortening; CO: Cardiac output; EDV: End-diastolic volume; ESV: End-systolic volume; EF: Ejection fraction; SV: Stroke volume; HR: Heart rate.

Discussion

It has been reported in many species that echocardiographic measurements may be affected by breed, sex, age, and body weight.²⁶⁻²⁸ Therefore, in this study, to eliminate these confounding factors, echocardiographic findings were limited to female Ghezel sheep with a specific age and weight range. Pearson correlation method showed a significant positive correlation between bodyweight and end-diastolic volume of the left ventricle in two-dimensional echocardiography (Pearson's $r = 0.555$, $p = 0.032$) also between bodyweight and left ventricular free wall thickness in systole in M-mode echocardiography (Pearson's $r = 0.558$, $p = 0.031$). The lack of correlation between weights with more measurements could be due to the number of samples and low weight difference between them.⁷ Because the examined sheep were in the same age, none of the echocardiographic measurements were indexed to age. Acorda and Pajas reported that there was a meaningful correlation between most echocardiographic values/physiologic factors and bodyweights/age.⁹

The results of this study showed that 2D and M-mode echocardiography could be performed in sheep without prescribing any sedative drugs and in standing position. In the present study in agreement with Boon²² end-diastolic and end-systolic phases for 2D-echocardiography measurements were identified based on the largest left ventricular size at mitral valve closure and the lowest left ventricular size before the mitral valve opening, respectively. Some other studies used ECG to calculate these variables.^{14,21}

Cardiac internal diameters by two-dimensional echocardiography have been evaluated in few studies in sheep.^{21,22,25} In Tables 3 and 4, differences of values of the present study with others are demonstrated in which the differences were attributed to the weight, breed, sex, age and at level of transducer.^{21,26-28} In the study of Acorda and Pajas,⁹ standard approaches for evaluation the cardiac values was not used.²⁹

Measurements of the left atrium at end-systole in right parasternal long-axis was more difficult to obtain than end-diastole, because the anterior wall of the left atrium interfered with the pleura and was less visible by creating the acoustic shadowing artifact, and therefore required more precision and a change in the position of the transducer and the angle beam width. Similar findings have been reported by Leroux *et al.*⁸ According to our literature review, the size of the left atrium at end-diastole has not been determined in other studies previously in sheep.

The right ventricular outflow tract for measurement of the pulmonary artery valve and sinus has not been viewed from the right parasternal cranial position in sheep the same as other studies.^{7,8} Because sheep seem to be reluctant to move the forelimb in a forward and upward position, therefore, the transverse aspect at the level of the pulmonary artery from the right parasternal position was an appropriate alternative view. Leroux *et al.* measured the pulmonary artery valve and sinus in the Saanen goats at right parasternal cranial long-axis view.⁸

Right Parasternal short axis view at the level of the aorta and pulmonary artery were the main challenges of the present study. Because the pleura was an obstacle to

Table 3. Mean values of two-dimensional echocardiography parameter in the current study compared to other studies.^{9,21,22,25}

Variables	Current study	Philippine sheep ⁹	Sheep ²¹	Various breeds of sheep ²²	Lori-Bakhtiari female sheep ²⁵
BW (kg)	42.69 ± 4.73	14.20 ± 3.70	74.00 ± 13.00	74.00 ± 11.00	NR
Age	16.40 ± 1.18 M	22.70 ± 20.63 M	2.00-4.00 Y	2.00-5.00 Y	1.00 Y
AoS (cm)	2.54 ± 0.14	NR	3.20 ± 0.31	NR	2.02 ± 0.03
AoV (cm)	2.07 ± 0.12	1.53 ± 0.17	2.74 ± 0.25	3.29 ± 0.33	2.03 ± 0.00
PuS (cm)	2.19 ± 0.13	NR	NR	NR	NR
PuV (cm)	1.81 ± 0.11	NR	2.38 ± 0.21	NR	NR
MADd (cm)	2.50 ± 0.16	NR	NR	NR	NR
MADs (cm)	2.52 ± 0.36	NR	NR	NR	NR
LADd (cm)	3.47 ± 0.28	NR	NR	NR	NR
LADs (cm)	4.46 ± 0.46	1.42 ± 0.17	4.59 ± 0.88	3.02 ± 0.35	1.58 ± 0.03
EF (%)	78.23 ± 4.91	76.00 ± 7.71	79.90 ± 4.90	NR	61.81 ± 3.26
SV (mL per beat)	41.65 ± 9.21	NR	NR	NR	NR
EDV (mL)	54.80 ± 8.97	NR	NR	NR	NR
ESV (cm)	12.33 ± 3.04	NR	NR	NR	NR
LADd/AoS (long axis)	1.37 ± 0.11	NR	NR	NR	NR
LAD/AO (short axis)	1.19 ± 0.08	0.90 ± 0.02	NR	0.92 ± 0.10	NR
PuS/AoS	0.86 ± 0.05	NR	0.89 ± 0.06	NR	NR

AoS: Aortic sinus diameter; AoV: Aortic valves diameter; PuS: Pulmonary artery sinus diameter; PuV: Pulmonary artery valve diameter; MADd: Mitral annulus diameter at end-diastole; MADs: Mitral annulus diameter at end-systole; LADd: Left atrial diameter at end-diastole; LADs: Left atrial diameter at end-systole; EF: Ejection fraction; SV: Stroke volume; EDV: End-diastolic volume; ESV: End-systolic volume; LADd/AoS: Ratio of left atrial diameter to aortic sinus diameter in diastole; LAD/AO: Ratio of left atrial diameter to aortic valve diameter in diastole; PuS/AoS: Ratio of pulmonary artery sinus diameter to aortic sinus diameter in diastole. NR: Not reported; M: Months; Y: Years.

Table 4. Mean values of M-mode echocardiography parameter in the current study compared to other ones.^{9,21,22,25}

Variables	Current study	Philippine sheep ⁹	Sheep ²¹	Various breed of sheep ²²	Lori-Bakhtiari female sheep ²⁵
RVIDd (cm)	1.50 ± 0.32	0.83 ± 0.26	1.42 ± 0.36	2.03 ± 0.56	1.44 ± 0.02
RVFWs (cm)	1.06 ± 0.22	0.26 ± 0.11	NR	0.94 ± 0.22	NR
RVFWd (cm)	0.59 ± 0.09	0.17 ± 0.06	NR	0.51 ± 0.11	NR
IVSs (cm)	1.40 ± 0.18	0.94 ± 0.12	1.55 ± 0.18	1.41 ± 0.22	1.40 ± 0.04
IVSd (cm)	0.81 ± 0.14	0.66 ± 0.09	1.19 ± 0.15	0.94 ± 0.17	0.88 ± 0.03
LVIDs (cm)	1.76 ± 0.27	0.91 ± 0.13	2.62 ± 0.35	3.23 ± 0.46	1.28 ± 0.02
LVIDd (cm)	3.74 ± 0.30	1.78 ± 0.07	4.42 ± 0.54	5.17 ± 0.74	2.94 ± 0.57
LVFWs (cm)	1.40 ± 0.27	0.43 ± 0.08	1.50 ± 0.28	1.53 ± 0.11	1.66 ± 0.03
LVFWd (cm)	0.74 ± 0.12	0.23 ± 0.04	0.99 ± 0.14	0.89 ± 0.20	0.93 ± 0.00
FS (%)	53.62 ± 5.09	42.10 ± 5.62	40.20 ± 4.80	37.20 ± 5.70	57.32 ± 0.64
CO (L per min)	4.77 ± 1.06	NR	NR	NR	NR
EDV (mL)	60.38 ± 11.39	NR	NR	NR	NR
ESV (mL)	9.58 ± 3.95	NR	NR	NR	NR
EF (%)	80.24 ± 12.21	76.00 ± 7.71	76.90 ± 4.90	NR	61.81 ± 3.26
SV (mL per beat)	55.13 ± 14.87	NR	NR	NR	NR
HR (bpm)	89.52 ± 12.04	NR	84.3 ± 5.40	96.1 ± 21.6	87.90 ± 3.83

RVIDd: Right ventricular internal diameter at end-diastole; RVFWs: Right ventricular free wall thickness at end-systole; RVFWd: Right ventricular free wall thickness at end-diastole; IVSs: Interventricular septal thickness at end-systole; IVSd: Interventricular septal thickness at end-diastole; LVIDs: Left ventricular internal diameter at end-systole; LVIDd: Left ventricular internal diameter at end-diastole; LVFWs: Left ventricular free wall thickness at end-systole; LVFWd: Left ventricular free wall thickness at end - diastole; FS: Fractional shortening; CO: Cardiac output; EDV: End-diastolic volume; ESV: End-systolic volume; EF: Ejection fraction; SV: Stroke volume; HR: Heart rate.

heart imaging and we had to place the transducer as far as possible perpendicular to the part of the acoustic window, and then angle of the beam slightly was up warded (the base of heart). This problem has also been addressed in another study.²¹

In the M-mode echocardiographic study, the largest left ventricular chamber volume before the ventricular contraction was used to visualize the diastolic end, and the least its size was used to illustrate the systolic end.⁸ In an M-mode echocardiography study of human that measured diastolic endpoints through ECG, the results showed that there was very little difference between the measurements with the highest left ventricular cavity size.⁸

The transverse axis of the right ventricle from the right parasternal imaging planes for M-mode measurement was hardly visible since it was covered with air shadows of the lungs, the right ventricle in the vicinity of the image so created the near field artifact and also crescent-shape of the right ventricle. Instead, the left parasternal short axis view for M-mode imaging was selected as alternative.

The recommended method for calculating left ventricular end-systolic and end-diastolic volumes in veterinary medicine is the modified Simpson's method from the right parasternal long axis four-chamber view, which was used in the current study (Fig. 1B).²⁴ Modified Simpson's method in sheep as an animal model, have been used in other studies.³⁰⁻³²

Ejection fraction is the gold standard for assessing the overall performance of the left ventricle. The best way to calculate the ejection fraction in human models is to use bi-plane methods in 2D images that are simultaneously calculated along with end-systolic and end-diastolic volumes.³³ In the present study, the ejection fraction and

stroke volume in the 2D images were calculated using Simpson's method and in M-mode images by the left ventricle menu, using the device built-in software.

In conclusion, the findings of this study could be used to evaluate cardiovascular disease in sheep, as well as an animal model for translating results for other animals and as human being.

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The study was conducted in the Veterinary Hospital of the Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Conflict of interest

The authors have declared no competing interests; none of the authors have any conflict of interest or relation with any third part that bias the publication of this report.

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