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Methods and normal values for echocardiography in adult dairy cattle☆

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Abstract *Objective:* The objective of the study was to report normal ultrasonographic appearance and intra-cardiac dimensions in two dairy breeds and to calculate cardiac output (CO) using echocardiography.
Background: Intra-cardiac dimensions, time indices and CO estimation have not previously been reported in adult cattle.
Animals, materials and methods: Echocardiograms were obtained from healthy adult dairy cows (10 Jersey (J) and 12 Holstein Friesians (HF)) in the body weight range of 400 to 700 kg. Standard echocardiographic images were obtained from the left and right hemithoraces. Velocity time integrals were obtained in order to calculate CO using pulsed wave Doppler of aortic flow in the J cows. Measurements obtained included pulmonary artery and aortic diameters, left and right ventricular diameters (and calculated fractional shortening and left ventricular ejection fraction), left atrial size and time indices assessing valve function.
Results: HF cows had significantly ($p < 0.05$) larger pulmonary artery and aortic diameters, larger left atrial diameters and left ventricular internal diameters during diastole, but these were not different when corrected for body weight. Left and right ventricular dimensions, adjusted for body weight, were significantly larger ($p = 0.02$ and $p = 0.035$ respectively) in J cows when compared to HF cows. No differences were noted in the time indices between the two groups. No significant differences were noted in intra-operator variability and the only significant difference in inter-operator variability was in measurement of the pulmonary artery ($p = 0.03$; ICC $= 0.63$).
Conclusions: It is possible to obtain repeatable, reliable echocardiograms in order that meaningful intra-cardiac dimensions can be obtained in adult dairy cattle.

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Introduction

Echocardiography is a non-invasive, straightforward method for assessment of the bovine heart. It is a technique that has been utilized frequently in small animals and horses for evaluation of changes in wall thickness, chamber size and valvular appearance and function. For measurements to be accurate and repeatable, images must be taken from correctly orientated imaging planes.[1] Although a qualitative description of the technique and appearance of echocardiograms in adult cattle has been reported,[2–6] to the authors' knowledge a report assessing cardiac dimensions, outside of calves[7–10] and juvenile animals, has not.

The purpose of this paper is to report normal cardiac chamber appearance and quantitative dimensions in adult Jersey (J) and Holstein-Friesian (HF) cows based on techniques adapted from echocardiographic studies in horses.[11–14]

Animals, materials and methods

The work was carried out at the Royal Veterinary College, London. UK.

Study animals

Eleven adult non-pregnant J cows and twelve adult non-pregnant HF cows were recruited. These animals were the property of the Royal Veterinary College Farm. All cows received a full clinical examination and were deemed healthy. Cows were excluded from the study if a cardiac murmur was detected on auscultation or if they were tachycardic (>85 beats per minute). Any cows with structural valvular disease identified on echocardiographic analysis were excluded from the study. This study was approved by the Royal Veterinary College Ethics and Welfare Committee.

Study protocol

This was a prospective experimental study. Cows were weighed and scored for body condition using a 5-point scale[15] prior to echocardiographic examination. Age was obtained from the animals' passports. The cows were restrained in either stocks or a crush and allowed to acclimatize to this environment for 10 min. A small area on the left and right hemithorax just behind the elbows was clipped and surgical spirit was used to clean the skin. Ultrasound coupling gel[c] was then applied and allowed to soak into the skin for a minimum of 5 min. An electrocardiogram (ECG) was acquired, with the positive and negative leads being placed in the right and left axillae and the ground lead attached to the skin cranial to the scapula.

Echocardiographic protocol

All echocardiographic examinations were performed in the standing unsedated animal. Six two-dimensional (2-D) parasternal images were obtained from the right and three 2-D parasternal images from the left as described for horses by Reef.[14] Additionally, three M-mode images were obtained from the right side and colour-flow Doppler was performed on all valves. Pulsed wave spectral Doppler was performed to assess flow in the left ventricular outflow tract (LVOT) of the J cows from the left side in order to calculate cardiac output. A phased-array probe was used at a frequency of 2.5 MHz attached to either a Vingmed System V[d] or MyLab 30[e] ultrasound machine. Coupling gel was applied to the transducer, and this was applied to the skin approximately 5–10 cm dorsal to the olecranon in the 3rd and 4th right intercostal spaces and the 4th and 5th left intercostal spaces. Depth and gain controls were adjusted to optimize the image. The images were obtained in the following order: right parasternal cranial long-axis view of the right ventricular outflow tract (RVOT), right parasternal caudal long-axis view of the LVOT and the right parasternal long-axis view of the left ventricle (LV). Right parasternal short-axis views and M-mode images were then obtained of the LV, mitral valve (MV) and aortic valve (AV). A left parasternal caudal long-axis view of the MV and left atrium (LA) was obtained followed by the left parasternal cranial long-axis view of the AV and LV and the left parasternal cranial long-axis view of the RVOT. Spectral Doppler of the LVOT was performed from an adapted view of the left parasternal cranial long-axis view such that blood flow was parallel with the ultrasound beam. The intercostal space and probe orientation used to obtain each image was recorded at the end of each examination.

Data collection and statistical methods

All images were stored as raw image data on optical disk and analysed at a later date using dedicated software.[d] A minimum of three cardiac

[c] Aquasonic gel 100, Parker Labs Inc., Fairfield, NJ, USA.

[d] Vingmed system V and Echopac software, GE Ultrasound, Bedford, UK.

[e] MyLab 30, Esoate, **Universal Medical Systems Inc.**, Bedford Hills, NY, USA.

cycles were saved. Any noteworthy findings were recorded at the end of each echocardiographic examination and on review of the images. Measurements were performed using the electronic callipers within the analysis system using the leading-edge to leading-edge method technique.[16] These measurements were done in triplicate by one author (G.H.) on three separate occasions. Measurements were also performed by another author (T.P.) on one occasion. Mean $\pm$ standard deviation (SD) were calculated from the three measurements of each observer. Measurements recorded from 2-D images included pulmonary artery diameter in diastole (PA), aortic diameter in diastole (Ao), aortic sinus diameter in diastole (AoS), left atrial diameter in systole (LAD)e and cross-sectional diameter of the aorta in diastole (Ao-cs). Measurements recorded from M-mode measurements included right ventricular diameter in both systole and diastole (RVDs and RVDd), inter-ventricular septum thickness (IVSs and IVSd), left ventricular diameter (LVDs and LVDd) and thickness of the left ventricular free wall (FWs and FWd). Other M-mode measurements recorded included ejection time (ET) and pre-ejection period (PEP) and E-point to septal separation (EPSS). Calculated measurements included the ratio of the pulmonary artery diameter to the aortic diameter (PA:Ao), fractional shortening (FS) ($100 * [LVDd - LVDs]/LVDd$) and left ventricular ejection fraction (LVEF). The latter was calculated with the integrated calculation program ($LVEF = [(A - B) * 100/A]$) where $A = (7 * D_1^3)/(2.4 * D_1)$ and $B = (7 * D_2^3)/(2.4 * D_2)$ with D_1 being the left ventricular diastolic diameter (cm) and D_2 being the left ventricular systolic diameter (cm). A mean of three velocity time integrals (VTI) were obtained from a pulsed wave Doppler image of the LVOT and used to calculate stroke volume ($CO = VTI \times (Ao\text{-}cs/2)^2 \times \pi \times HR$). Heart rate was recorded from the ECG that was attached to the ultrasound machine.

Data are presented as the mean $\pm$ SD. Measurements between breeds were compared using a Student's *t*-test. Data was then corrected for body weight; each individual measurement was divided by the individual cows' body weight. Inter-operator variability was analysed using a repeated measures analysis of variance (ANOVA) and intra-operator variability was analysed using a Student's *t*-test and Spearman correlation coefficients. Statistical analyses were performed using a statistical package.[f] Intra-class correlation coefficients were performed to assess repeatability and reproducibility using an internet-based statistical package.[g]

Results

Animals

Eleven non-pregnant, non-lactating J cows and 12 non-pregnant, lactating HF cows were used in this study. The J cows were aged 74 ± 13 months and weighed 458 ± 56 kg with a range of body condition scores from 2.5 to 4.0. The HF cows were aged 66 ± 12 months and weighed 636 ± 55 kg with a range of body condition scores from 1.75 to 2.5. The HF cows were 5 to 31 days into their lactation (median = 12 days). No abnormalities were detected on clinical examination of the cardiothoracic systems of any of the animals. The heart rates during the examination were 71.0 ± 6.1 beats per minute (BPM) and 72.3 ± 4.2 BPM in the J cows and HF cows, respectively. One J cow was excluded at the end of the echocardiographic examination because of pulmonary valve prolapse with moderate pulmonary valve regurgitation. Thus 10 J cow cardiac dimensions and 12 HF cow cardiac dimensions were included.

Image generation and appearance

Nine 2-D images (short and long-axis) and three M-modes were obtained in all 22 cows. In the right parasternal cranial long-axis view (Fig. 1), images were obtained of the tricuspid valve (TV), RVOT, pulmonary valve (PV) and pulmonary outflow tract. This image was obtained in 20 of the 22 animals with the probe placed in the 3rd right intercostal space and in 2 of the J cows in the 4th right intercostal space with the probe angled cranially with 0° transducer rotation. A cross-sectional image of the right coronary artery in the centre of the screen was used as a landmark. In the right parasternal caudal long-axis view of the LVOT (Fig. 2) images of the right ventricle (RV), LV, AV and LVOT were seen in all animals. Images of the TV were visible in 70% of the J cows and 80% of the HF cows. The *ossa chordis* was visible as a sub-aortic hyperechoic thin shadowing area in 40% of the J cows, but in only 10% of the HF cows. Images were obtained with the probe placed in the 4th intercostal space with the probe directed laterally across the thoracic wall and the transducer rotated between 0° and 30°. In the right parasternal

[f] SPSS for Windows 14.0, Chicago, IL, USA.

[g] Statistical toolbox of the Chinese University of Hong Kong. Accessed at: http://department.obg.cuhk.edu.hk.

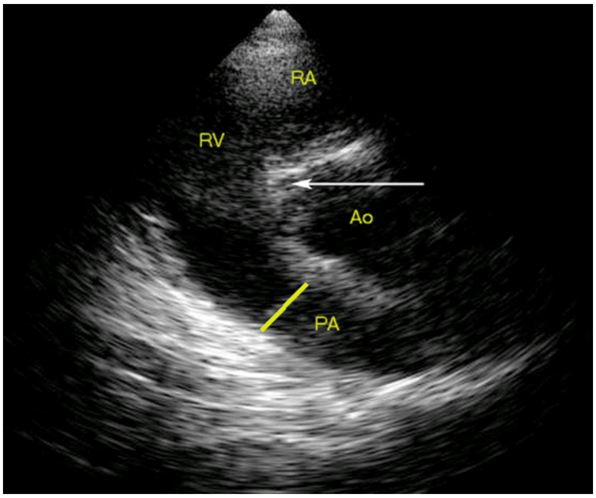

Figure 1 This is a right parasternal cranial long-axis view of the right ventricular outflow tract (RVOT). The pulmonary valve can be seen in the far field (ventrally) between the labels RV (right ventricle) and PA (pulmonary artery). The line shown displays the position where the diameter of the pulmonary artery was measured. Other visible structures labelled are the right atrium (RA) and aorta (Ao). The arrow is pointing to one of the coronary arteries, a notable landmark for obtaining repeatable views of the RVOT.

long-axis view of the LV, images were obtained of the RV, TV, LV and MV in all animals. A standard view including the entire LA and right atrium (RA) could not be obtained, although a portion of

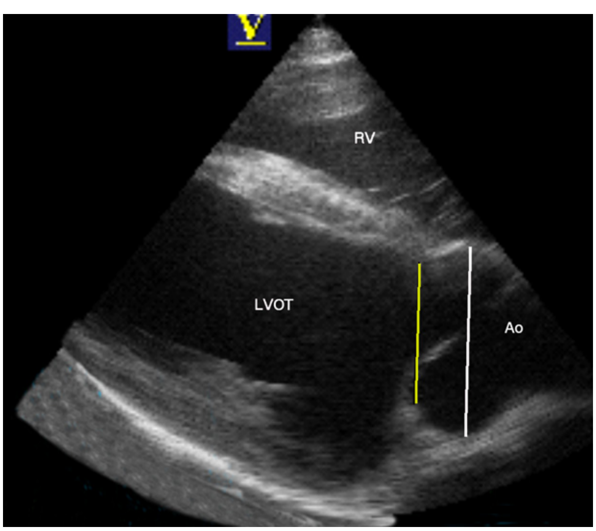

Figure 2 This is a right parasternal caudal long-axis view of the left ventricular outflow tract (LVOT). The right ventricle (RV) can be visualised in the near field and the LVOT and aorta (Ao) in the far field. The aortic valve can be seen to the right of the image. The yellow line shows where the aortic diameter was measured and the white line where the diameter of the aortic sinus was measured.

each of these chambers was seen in every cow. The *ossa chordis* cast a shadow in 100% of the J cows, but was only visible in 30% of the HF cows. Images were obtained with the probe placed in the 4th right intercostal space angled slightly caudally with 0° rotation of the transducer.

The right parasternal caudal short-axis view of the LV revealed the LV and RV in all animals. Papillary muscles were visible in all views and symmetrical views were obtained in 80% of the J cows and 92% of HF cows. A symmetrical view was defined as being able to see both papillary muscles on the left and right sides of the image with equal thickness of the left ventricular wall and similar area of internal diameter on either side of a virtual line drawn through the point of the left ventricle. M-mode images of the LV were obtained by positioning the probe in the 4th right intercostal space approximately 10 cm above the olecranon with the transducer rotated 90°. The right parasternal caudal short-axis view of the MV revealed the RV and the MV in all animals. Symmetrical views were obtained in 90% of J cows and 92% of HF cows. In order to obtain these images, the probe was placed approximately 8 cm above the olecranon and angled caudo-dorsally in the 4th right intercostal space with the transducer rotated 90°. This was required to avoid images of the pleural surface obscuring the heart. The right parasternal caudal short-axis view of the AV revealed views of the aorta, the MV, the RV and TV in all animals. To obtain this image, the probe was placed in the 4th right intercostal space approximately 6 cm above the olecranon and angled dorsally with the transducer rotated 90°. This position improved image quality and allowed visualization and measurement of the AV, but the pleural surface was visible in 80% of images saved.

Three parasternal long axis images were obtained from the left side of the animal. The left parasternal caudal long axis image (Fig. 3) allowed imaging of the LV, MV, LA and right auricular appendage in all animals. The landmark used for the correct view was the presence of the right auricular appendage as a spherical hypoechoic structure in the far field. As from the right, the *ossa chordis* was visualized above the MV in 100% of J cows and 30% of HF cows. To obtain this view, the probe was placed in the 5th left intercostal space in 80% of the cows and in the 4th left intercostal space in the remaining 20%, 10 cm above the olecranon and angled caudally with 0° transducer rotation.

The left parasternal cranial long-axis view (Fig. 4) was obtained in all cows and allowed imaging of the LV, AV and LVOT. This view was obtained

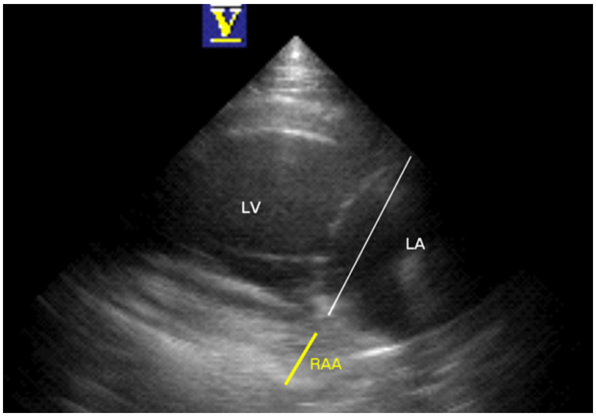

Figure 3 This is a left parasternal caudal long-axis view of the mitral valve, which is in the centre of the image. The left atrium (LA) is to the right of the image and the left ventricle (LV) to the left. The white line represents the position that the LA was measured. The yellow line shows the diameter of the right auricular appendage (RAA), which is used as a landmark for obtaining this view consistently.

with the probe placed as for the left caudal long-axis view, but with the probe angled in a craniodorsal direction with the transducer rotated 0 to -30°. All images were obtained from the 4th left intercostal space. The left parasternal cranial long-axis view of the RVOT was obtained with the probe in the 3rd left intercostal space in 80% of the cows and 4th left intercostal space in 20% of the cows. The probe was angled cranially and slightly dorsally, with 0° transducer rotation. In this view it was possible to visualise the RA, TV, RVOT, pulmonary artery and aorta in all cows.

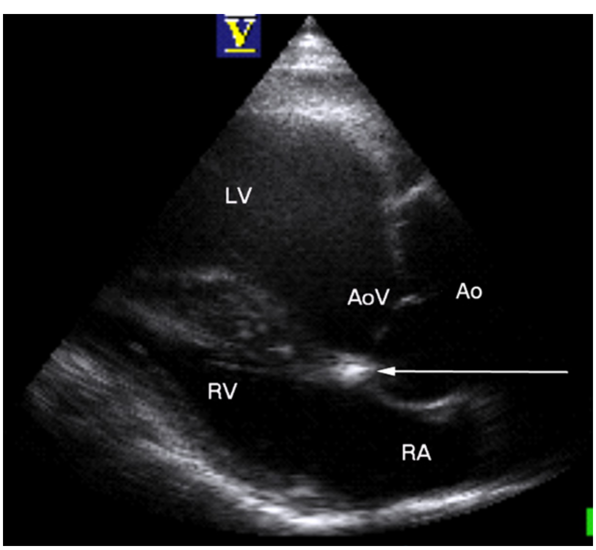

Figure 4 This is a left parasternal cranial long-axis view of the left ventricle (LV), aorta (Ao), and aortic valve (AoV). The right atrium (RA) and right ventricle (RV) can be seen in the far field. The arrow is pointing to the *ossa chordis*.

The images required some physical strength to obtain sufficient contact and probe placement. The images from the left hemithorax were easier to obtain than those from the right, as they did not require probe insertion as far beneath the triceps muscle. No problems were encountered regarding intercostal space width and image generation. Lung interference was a minor problem with the short-axis views of the MV and AV and required the probe to be moved ventrally down the chest wall and angled dorsally.

Cardiac dimensions

Cardiac dimensions and intra-class correlation coefficients are recorded in Table 1. No significant differences were found between repeated measures performed by one operator. There was excellent repeatability between operators for all measurements except for PA diameter.

Discussion

This study has demonstrated that reliable and repeatable cardiac images and measurements of internal cardiac structures can be obtained from adult dairy cattle. Knowledge of the normal appearance and cardiac dimensions should improve identification, quantification and assessment of cardiac disease in this population and may allow cardiac conditions to be recognised and treated earlier in the course of disease.

Echocardiography is a practical technique in cattle, although as previously reported,[2] physical strength was required to achieve some of the images. Most of the views were obtained in the same intercostal spaces and with similar probe positions as was found by Braun et al.[2] The most technically challenging views to obtain were the short-axis views due to difficulties in obtaining symmetrical images and poor visualization due to the hyperechogenicity of the pleural surface. Some differences in probe placement were noted compared to a previous bovine study;[2] the right parasternal short-axis view of the LV was obtained with the probe higher up the thoracic wall and the majority of the right parasternal long-axis views of the LV were obtained in the fifth left intercostal space. These differences might explain why the images were different from those described by Long et al.[13] and Reef[14] in horses. In addition, the left parasternal cranial long-axis view of the

Table 1 Echocardiographic measurements and repeatability (ICC, intraclass correlation coefficient for intra-operator repeatability) in normal cattle

Parameter	ICC	Jersey cows				Holstein-Friesian cows			
		N	Mean ± SD	Min	Max	*N*	Mean ± SD	Min	Max
PA (cm)*	0.62	10	4.2 ± 0.27	3.31	4.84	12	5.5 ± 0.8	4.2	6.1
Ao (cm)*	0.92	10	5 ± 0.26	4.7	5.38	12	6.4 ± 0.62	5.0	7.1
PA:Ao		10	0.85 ± 0.07	0.8	0.98	12	0.86 ± 0.09	0.74	0.95
AoS (cm)*	0.96	10	5.7 ± 0.34	5.01	6.13	12	7.94 ± 0.56	7.02	9.03
Ao-cs (cm)*	0.87	10	5.15 ± 0.31	4.5	5.5	12	7.05 ± 1.17	6.4	7.8
LAD (cm)*	0.96	10	10.9 ± 0.5	10.4	11.9	12	12 ± 1.2	10.6	12.7
ET (ms)	0.99	10	0.26 ± 0.08	0.21	0.36	12	0.21 ± 0.1	0.04	0.38
PEP (ms)	0.99	10	0.07 ± 0.03	0.04	0.1	12	0.09 ± 0.1	0.01	0.21
EPSS (cm)	0.99	9	0.45 ± 0.48	0	1.08	11	0.62 ± 0.23	0.2	0.9
RVDd (cm)$^+$	0.86	10	2.45 ± 0.53	1.72	3.38	12	2.27 ± 0.76	0.78	3.2
RVDs (cm)$^+$	0.87	10	1.32 ± 0.63	0.54	2.09	12	1.14 ± 0.43	0.20	1.8
IVSd (cm)	0.99	8	2 ± 0.4	1.4	2.7	11	2.2 ± 0.51	1.7	3.2
IVSs (cm)$^+$	0.95	8	3.6 ± 0.5	2.7	4.3	11	3.4 ± 0.5	2.8	4.0
LVDd (cm)*	0.99	8	7.7 ± 0.7	6.3	8.6	11	8.7 ± 1.0	7	10.3
LVDs (cm)$^+$	0.98	8	4.2 ± 0.53	3.2	4.98	11	4.2 ± 0.8	3.1	5.9
FWd (cm)	0.98	8	1.2 ± 0.3	0.64	1.67	11	1.5 ± 0.4	0.9	2.2
FWs (cm)	0.96	8	1.5 ± 0.5	0.92	2.79	11	1.4 ± 0.5	0.8	1.6
FS (%)	0.98	8	44.7 ± 8.3	33.3	50.9	11	46.5 ± 9.5	28	55
LVEF	0.99	8	0.85 ± 0.09	0.74	0.94	11	0.79 ± 0.15	0.61	0.89
HR (bpm)		10	71.0 ± 6.0	62	78	11	72.3 ± 4.5	66	79
VTI (cm)		10	30.4 ± 3.41	32.2	40.1				
CO (L/min)		10	56.5 ± 8.44	46.9	64.7				
CI (ml/kg/min)		10	123.2 ± 5.7	116.3	132.9				

N = number of measurements used in analysis (*shows significant breed differences and $^+$shows significant differences when corrected for bodyweight; $p < 0.05$).

Abbreviations: PA, pulmonary artery diameter in diastole; Ao, aortic diameter in diastole; PA:Ao, ratio of PA and Ao; AoS, aortic sinus diameter in diastole; Ao-cs, aortic cross sectional diameter in diastole; LAD, left atrial diameter in systole; ET, ejection time; PEP, pre-ejection period; EPSS, E-point to septal separation; RVDd, right ventricular diameter in diastole; RVDs, right ventricular diameter in systole; IVSd, interventricular septal diameter in diatole; IVSs, interventricular septal diameter in systole; LVDd, left ventricular diameter in diastole; LVDs, left ventricular diameter in systole; FWd, left ventricular free wall diameter in diastole; FWs, left ventricular free wall diameter in systole; FS, fractional shortening; LVEF, left ventricular ejection fraction; HR, heart rate; VTI, velocity time integral; CO, cardiac output; CI, cardiac index.

LVOT was orientated differently from that reported by Long et al.[13] and Reef.[14]

All cattle, particularly the HF cows tolerated this procedure well. The cows objected most to placement of the ECG clips. Many of the animals were unwilling to stand with the limb moved cranially, but were much more tolerant of the limb being abducted. It has been reported previously in both horses and cattle that intercostal space width was a limiting factor in the quality of the images obtained;[2,16] in the current study this was not a problem and most likely relates to the small size of the phased-array head of the transducers. Unlike previous reports,[2,17] the operators had no difficulty obtaining the right sided cranial view of the RVOT, but agree that a similar image is technically easier to obtain from the left.

Echocardiographic descriptions of the *ossa chordis* have not been previously reported. The fact that this structure was seen less frequently in the HF cows compared with J cows may be a breed difference, but the authors propose it is more likely due to the demands of lactation on the body calcium store. There is increased bone and calcium resorption in the first 14 days after parturition and higher yielding dairy cows mobilize calcium more actively than their low yielding counterparts.[18,19]

To the authors' knowledge, a full set of internal cardiac dimensions in adult dairy cattle have not been previously published. One study[20] investigated some of the internal dimensions of 51 cattle although their body weight was not reported in the abstract.[2] The largest difference between this previous study and the current study are the reported dimensions of the RV diameter. In other species, this measurement has been shown to be extremely angle and technique dependent.[21]

In studies carried out in horses, no association has been demonstrated between internal cardiac dimensions and body weight.[13,21] Despite similar absolute values, J cows had relatively larger LV diameters and thicker interventricular septae during systole than HF cows when corrected for body weight. The RV in both systole and diastole was relatively larger in the J cows compared to the HF cows. The reason for this finding is unknown. It may relate to the J cows being of higher BCS. In obese humans it has been shown that eccentric hypertrophy of the left ventricle occurs, with increased wall thickness and decreased fractional shortening;[22,23] these changes are often associated with hypertension. However the J cows and HF cows are found to have similar indirect blood pressure measurements (Veasey R, Fishwick J, Hallowell G, unpublished data). The relatively large RV diameters in the J cows compared to the HF cows may have been due to difficulties in assessment of RV size since there are no landmarks available to ensure standard images are produced.

Cardiac output was calculated from the spectral wave Doppler trace of aortic flow, and despite possible pitfalls, several studies have demonstrated a reasonable correlation between this method and the thermodilution method in the horse.[24] Cardiac output values obtained in this study were similar to those reported in anesthetized adult J cows using a thermodilution technique.[25] Ideally CO measurements obtained in this study should have been compared with measurements from an alternative technique.

Intra-operator variability was deemed acceptable for all measurements. The measurements were performed on three separate occasions from the same images. Ideally measurements should have been performed on three separate sets of images and is a limitation of the study. Inter-operator variability was also good except for measurements of PA. Due to this structure being at a depth of approximately 20–24 cm in the cows, images of the PA were not as clear compared to structures in the near field and this may explain the significant differences seen in measurements between operators.

Finally, one intrinsic problem of the study was that it was not possible to confirm that these animals had normal cardiac structure at postmortem examination. At 6-month follow-up all cows used in this study are still alive and have been clinically well. Ideally, the two breeds of cow should have been matched for age and stage of lactation, and this is a disadvantage of the study.

Conclusion

The data presented in this paper demonstrate that it is possible to obtain good-quality echocardiograms in adult cows and provide normal cardiac dimensions to compare against diseased animals. This study is thus relevant for the diagnosis of early manifestations of cardiac disease in adult dairy cattle. The technique is relatively straightforward but does require some physical strength.

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