scientific reports

OPEN

Transthoracic echocardiography for evaluating cardiac parameters in growing piglets

Amna Qasim[1], Eli Contorno[2], Herra Javed[2], Anshaal Furrukh[2], Louis Steen[2], Ahmed Zaghw[3], Joanna Murphy[4], Simon Chung[5,6], Krittika Joshi[1] & T. Konrad Rajab[2,7]✉

Large animal model research is a vital step in the pre-clinical testing of new therapeutic interventions in congenital heart disease to ensure that they are safe and effective before clinical trials are begun. Although swine hearts are an excellent model for the study of cardiovascular disease due to their anatomical and physiological similarities with human hearts, there are differences in body habitus and internal organ positioning that necessitate a deviation from standard human echocardiography protocols. Previously published data on swine echocardiography has focused on mature animals and a standardized protocol for serial valvar assessment is lacking. The aim of this study was to create a protocol for a complete sedated piglet transthoracic echocardiogram. The protocol is designed for the evaluation of ventricular size and function and detailed evaluation of all cardiac valves. Additionally, we demonstrate implementation of this protocol by reporting normative growth trends of semilunar valves and left ventricular dimensions in piglets studied in our research lab. The goal of this standardized protocol is to allow for further cardiovascular research on growing piglets, particularly in the field of partial heart transplantation. Standardized views for valve measurements enable reproducibility across observers and across serial follow-up to reliably assess hemodynamic function and growth over time.

Keywords Congenital heart disease, Cardiac imaging, Animal echocardiography, Partial heart transplantation, Pig, Translational research, Pre-clinical

Abbreviations

PHT	Partial heart transplantation
TTE	Transthoracic echocardiography
GAMM	Generalized additive mixed model

Large animal research is a major component of preclinical testing and validation of new surgical techniques, medications and devices for the treatment of cardiovascular disease[1–4]. Swine are an excellent model for the study of cardiovascular disease due to their rapid growth, and anatomical and physiological similarities to human hearts and have thus become the favored large animal model for the study of cardiovascular disease[5]. Echocardiography is a non-invasive cardiac imaging modality that is easily accessible and has multiple advantages including real-time imaging, ease of transportation and lower cost compared to other modalities such as computed tomography or magnetic resonance imaging.

While the anatomy and physiology of the heart in swine is very similar to the human heart, the positioning of the heart relative to the other structures differs significantly between swine and humans. These differences are primarily due to the quadruped stance of swine as well as the unusual sternal structure of humans compared to other mammals. While mammals generally have laterally compressed thoraces, the human thorax is dorsoventrally compressed[6]. Due to these thoracic structural differences, the commonly used echocardiography views of humans are not optimal use in swine models. Echocardiography in the piglet has been shown to be feasible but there is currently no standardized transthoracic echocardiography (TTE) protocol[7]. Standardized

[1]Department of Cardiology - Arkansas Children's Hospital, Little Rock, AR, USA. [2]Department of Cardiovascular Surgery - Arkansas Children's Hospital, Little Rock, AR, USA. [3]Department of Cardiovascular Anesthesia, Arkansas Children's Hospital, Little Rock, AR, USA. [4]Department of Ultrasound, Arkansas Children's Hospital, Little Rock, AR, USA. [5]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR, USA. [6]Arkansas Children's Research Institute, Little Rock, AR, USA. [7]University of Arkansas for Medical Sciences, 1 Children's Way, 72202 Little Rock, AR, USA. ✉email: tkrajab@uams.edu

landmarks are important to ensure reproducibility of echocardiographic measures. Our group is studying partial heart transplantation (PHT) using piglet models[1,2,8,9], which involves monitoring of transplanted semilunar valves for growth and function over time, with piglet TTE being the most important surveillance technique for the transplanted valves. Normative data on semilunar valve growth rate over time in healthy piglets has not been reported in the literature and is important for studying and extrapolating the effects of therapies such as valve transplants in translational research.

The goal of this study was to: (1) create a standardized piglet TTE protocol with defined areas on the piglet's chest wall to evaluate and measure semilunar valves; (2) report normal semilunar valve and left ventricular dimension growth rates in piglets.

Methods

Standardized piglet TTE protocol

This study used purpose-bred Yorkshire pigs (8–15 kg, 5–8 weeks old), obtained from the National Swine Resource and Research Center (Columbia, MO) and Oak Hill Genetics (Ewing, IL). All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Arkansas Medical Sciences (Protocol # IPROTO202300000160) and conformed to standard Office of Laboratory Animal Welfare on the housing and medical care of swine used in research. This study was conducted in compliance with relevant guidelines and regulations and is reported in accordance with the ARRIVE guidelines.

Our standardized protocol (Table 1; Figs. 1, 2, 3 and 4) was developed by a trained pediatric cardiologist and pediatric cardiac sonographer. Piglets were scanned under anesthesia using 5 MHz and 6 MHz probes on the Phillips EPIQ 7 ultrasound machine [Epiq7c Circular Edition] to obtain views like those acquired in human echocardiography. Landmarks were recorded for obtaining the views.

The details of housing animals in our facility between echocardiograms as well as anesthesia techniques are included in Supplemental material.

Study population and data collection

Serial TTEs were performed on eight piglets under anesthesia using our standardized echocardiography protocol (Table 1), each evaluated between 4 and 8 times from the approximate age of 4 weeks to 5 months at intervals of 1–2 weeks. Additional echocardiographic evaluation was performed for preoperative piglets (Table 2). Every session was performed under general anesthesia. At each imaging session, measurements of the aortic and pulmonary valve annuli were recorded in millimeters. The aortic valve was measured in end-systole from the parasternal long axis view (Fig. 4b), and the pulmonary valve was measured in end-systole and diastole from the parasternal short axis view (Fig. 3g). Ventricular cavity dimensions and wall thickness measurements were obtained using M-mode from the parasternal short axis view (Fig. 3b and c). Ventricular ejection fraction and shortening fraction were calculated using M-mode measurements. Simultaneously, age (in days), weight (in kilograms), and length (in centimeters), were collected on the day of the echocardiogram.

Statistical analysis

To characterize how valve size changes with growth, generalized additive mixed models (GAMM) were employed. To explore the association individually, we fit a model for each combination of two valves' size (aortic and pulmonary valves measured in centimeters) and cardiac dimensions (left ventricular end diastolic dimension [LVEDD], left ventricular end systolic dimension [LVESD] and left ventricular posterior wall dimension in systole [PWs]) as response and the three predictors (age, weight and length), resulting in a total of 15 models. For each predictor X, each response Y was modeled as:

$$Y = \beta_0 + f(X) + Zu + \epsilon$$

where β_0 is the intercept, f represents a penalized cubic regression spline function, Z is the known desgin matrix for the random effect, u is the random intercept subject to each piglet, and ϵ is the error term.

In addition, a multivariable GAMM was also fitted to account for the combined effects of age, weight and length to each valve size and cardiac dimensions, which takes the following form:

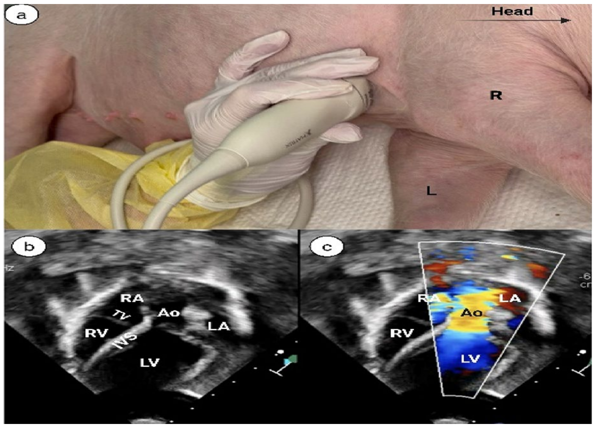
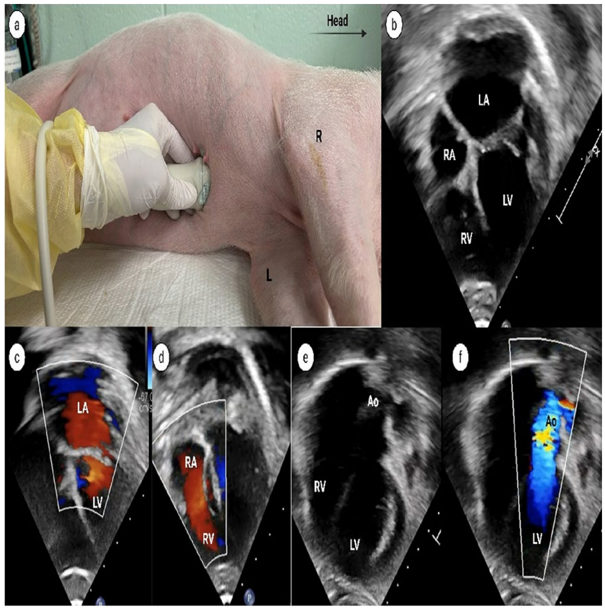
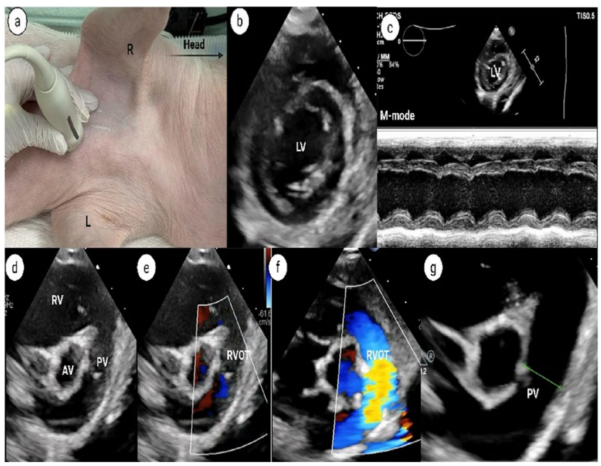
$$Y = \beta_0 + \sum_{i=1}^{3} f_i(X_i) + Zu + \epsilon$$

where X_i is age, weight and length respectively and f_i is the corresponding penalized cubic spline function.

The use of penalized cubic spline relaxes the assumption on the actual relationship between our responses and predictors. The upper limits of knots are set to be 10 by default and the actual number of knots that reflects the flexibility is determined by the penalization process. Model-based marginal prediction and its 95% confidence band were used to visualize the growth for each combination of valves and predictors. Predictions were computed over the observed range of each predictor, from its minimum to maximum values in the data. P-values < 0.05 were considered statistically significant. For model fitting, we used the mgcv package in R. All data were analyzed using R version of 4.4.3.

Results

A total of 59 readings from 8 piglets were included in the analysis, with a median of 8 readings per piglet (minimum: 4, maximum: 10). The overall ejection fraction has a mean of 53.8% (95% CI: [49.0%, 58.8%]) and the overall shortening fraction has a mean of 27.6% (95% CI: [24.6%, 30.6%]). The growth trends generated for

Method	Representative figures
Subcostal view	
(Fig. 1)	
How to obtain:	
Sub-xyphoid, notch at 4–5 o'clock. Piglet laying supine or left lateral decubitus (Fig. 1a)	
• Evaluate biventricular size and function, look for effusion (Fig. 1b)	
• Tilt anteriorly to evaluate aortic valve by 1b, color & spectral Doppler (Fig. 1c)	
Apical 4 chamber view	
(Fig. 2)	
How to obtain:	
Right mid-axillary line, fifth inter-costal space, notch at 4–5 o'clock. Piglet laying left lateral decubitus (Fig. 2a)	
• Evaluate biventricular size and function, look for effusion (Fig. 2b)	
• Assess mitral and tricuspid valves in 4 chamber type view by 2D, color Doppler and spectral Doppler (Fig. 2c and d)	
• Attempt to get a TR jet if possible	
• Tilt anteriorly to evaluate aortic valve by 2D, color & spectral Doppler (Fig. 2e and f)	
Parasternal short axis view	
(Fig. 3)	
How to obtain:	
Right mid-axillary line, second to third intercostal space, notch at 1–2 o'clock, pointed to left shoulder (Fig. 3a)	
• Evaluate ventricular size and function (Fig. 3b and c)	
• Tilt anteriorly to evaluate pulmonary valve by 2D, color & spectral Doppler (Fig. 3d-f)	
• Measure pulmonary valve annulus in systole and diastole (Fig. 3g)	
Continued	

Method	Representative figures
Parasternal long axis view	
(Fig. 4)	
How to obtain:	
Right mid-axillary line, second to third intercostal space, notch at 10–11 o'clock, pointed to right shoulder (Fig. 4a)	
• Evaluate aortic valve and measure annulus size in systole (Fig. 4b)	
• Evaluate aortic and mitral valve by color Doppler (Fig. 4c)	
• Tilt probe anteriorly to evaluate pulmonary valve (Fig. 4d and e)	

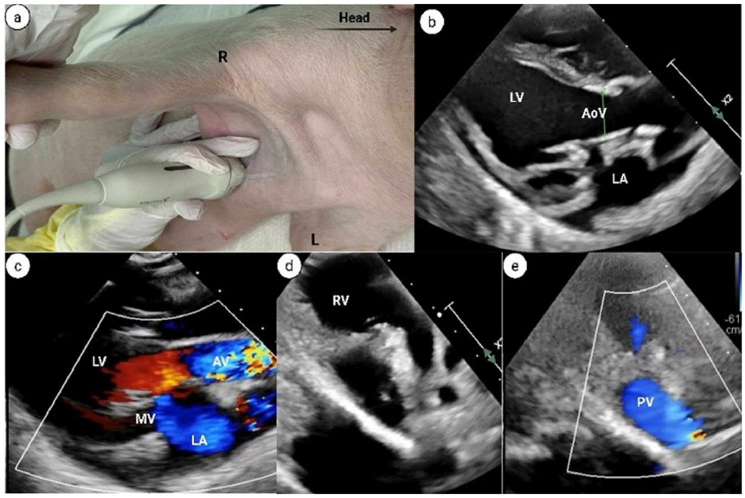


Table 1. Standardized piglet transthoracic echocardiography protocol [RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle; aov: aortic valve; PV: pulmonary valve; MPA: main pulmonary artery; R: right; L: left].

valve size against age, weight and length are shown in Fig. 7, and those for cardiac dimensions are shown in Fig. 8. Full model summaries are provided in the Supplementary Material.

Univariate analysis showed age was the strongest variable predictor (adjusted $R^2 = 0.848$, Fig. 7A) and length was the poorest predictor (adjusted $R^2 = 0.723$, Fig. 7C) for pulmonary valve size. For aortic valve size, length was the strongest predictor (adjusted $R^2 = 0.892$, Fig. 7F) and age was the poorest predictor (adjusted $R^2 = 0.876$, Fig. 7D). All terms in the univariate models were significant. For cardiac dimensions, weight was the best predictor for LVEDD (adjusted $R^2 = 0.819$, Fig. 8B) and PWs (adjusted $R^2 = 0.709$, Fig. 8H), whereas age was the best predictor for LVESD (adjusted $R^2 = 0.638$, Fig. 8D).

The multivariate GAMM with age, weight and length outperform all the univariate models for both valve sizes and cardiac dimensions. The adjusted R^2 values were 0.877 for the pulmonary valve and 0.918 for the aortic valve, and 0.877, 0.918, and 0.918 for LVEDD, LVESD, and PWs, respectively. Weight was not statistically significant in the multivariable model for either the pulmonary or aortic valve when all three growth characteristics were included as predictors. For cardiac dimensions, weight was the only predictor significantly associated with LVEDD and PWs, whereas none of the growth characteristics were significantly associated with LVESD in the full model.

Discussion

This is the first description of a standardized piglet echocardiography protocol for the assessment of porcine semilunar valve growth over time as well as the first report of normative porcine semilunar valve growth trends. Piglet echocardiography has been reported to be feasible[7] and has been used to assess ventricular function and pulmonary hypertension in porcine models[10–1213]. There were no complications in the 59 serial sedated echocardiograms performed for this study in control piglets. We have reported our emergency protocol for potential emergencies in the supplementary materials.

We found that to obtain views like parasternal short and long axis, we had to scan the piglet from the right side of the chest and have reported the best way to acquire the images in this protocol. We have used this protocol successfully to generate serial comprehensive echocardiographic assessments of dozens of piglets undergoing partial heart transplantation as well as several controls[8,9]. Preoperative assessment for presence of patent ductus arteriosus and intracardiac shunts (using a bubble study) is helpful for procedural planning prior to cardiac surgery.

Secondly, data on serial semilunar valve evaluation and normative semilunar valve growth trends was lacking prior to this study and would be important for studying cardiovascular models like partial heart transplantation in the piglet[1–4]. This study provides a useful protocol as well as normative data for all researchers conducting long term studies utilizing porcine donors as it allows for accurate and reproducible evaluation of the valves that are being studied.

Additionally, while prior work has reported feasibility of ventricular function assessment in piglets[7,12] our data is the first to report serial interventricular dimensions over time and ventricular ejection fraction over time in piglets.

While this is a small sample, the repeated measurements over time allowed us to develop normal growth curves. This is important in advancing the piglet model specifically for pediatric cardiovascular disease as it will allow researchers interested in a variety of diseases and treatments to better estimate the valve growth and identify outliers within their cohort. This advancement could also prove useful to future xenotransplantation efforts as

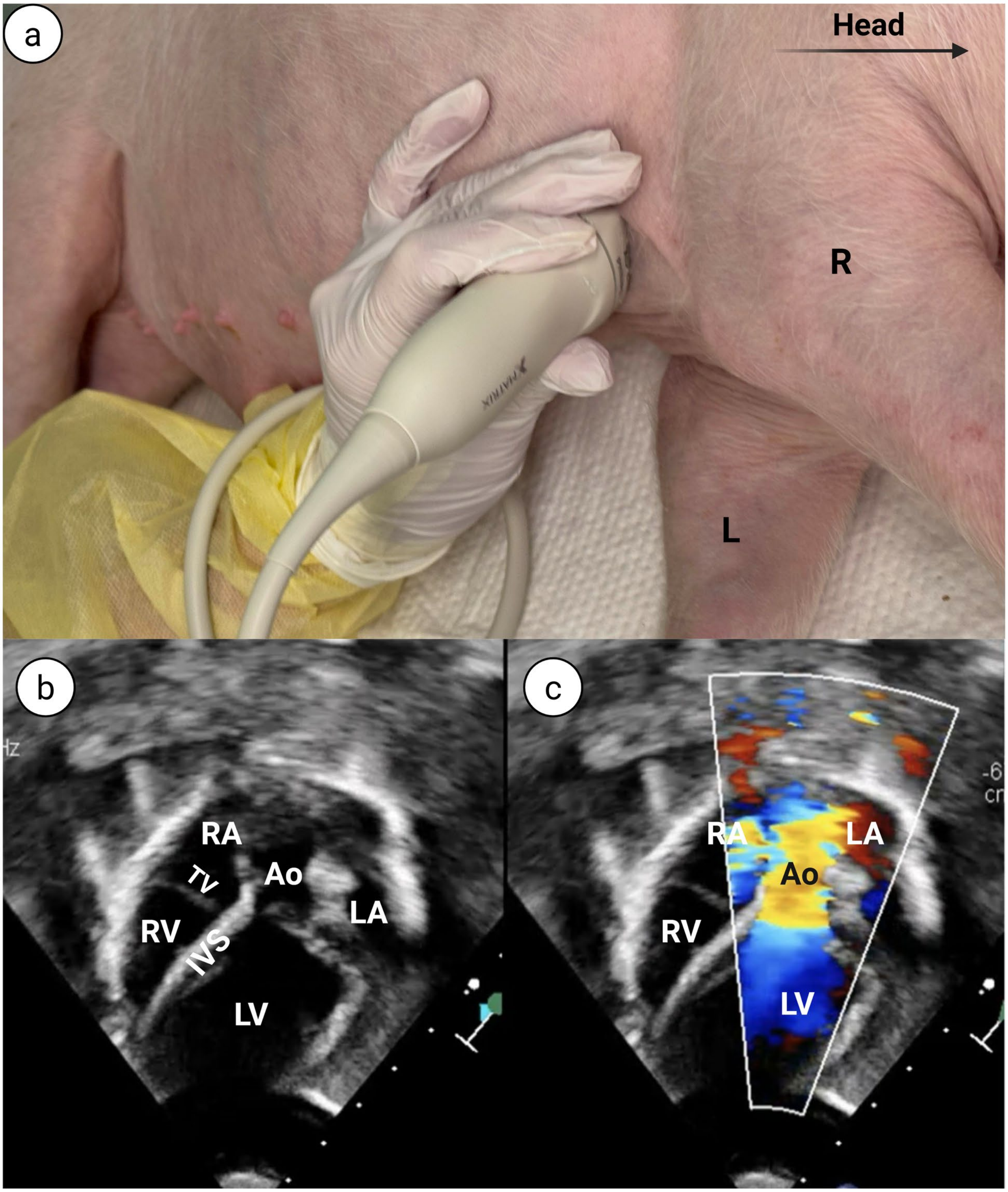


Fig. 1. Subcostal view.

data on the growth and size of piglet cardiac features in relation to weight, age and body size will be useful for selecting donors with appropriate sizes for the desired recipient. Our data show that age is the strongest predictor of pulmonary valve growth and length is the strongest predictor of aortic valve growth in control piglets. In our experience, in piglets undergoing cardiac surgery that have poor post-operative growth, correlation with weight is the most useful. These growth trends serve as a comparison for our future experimental models.

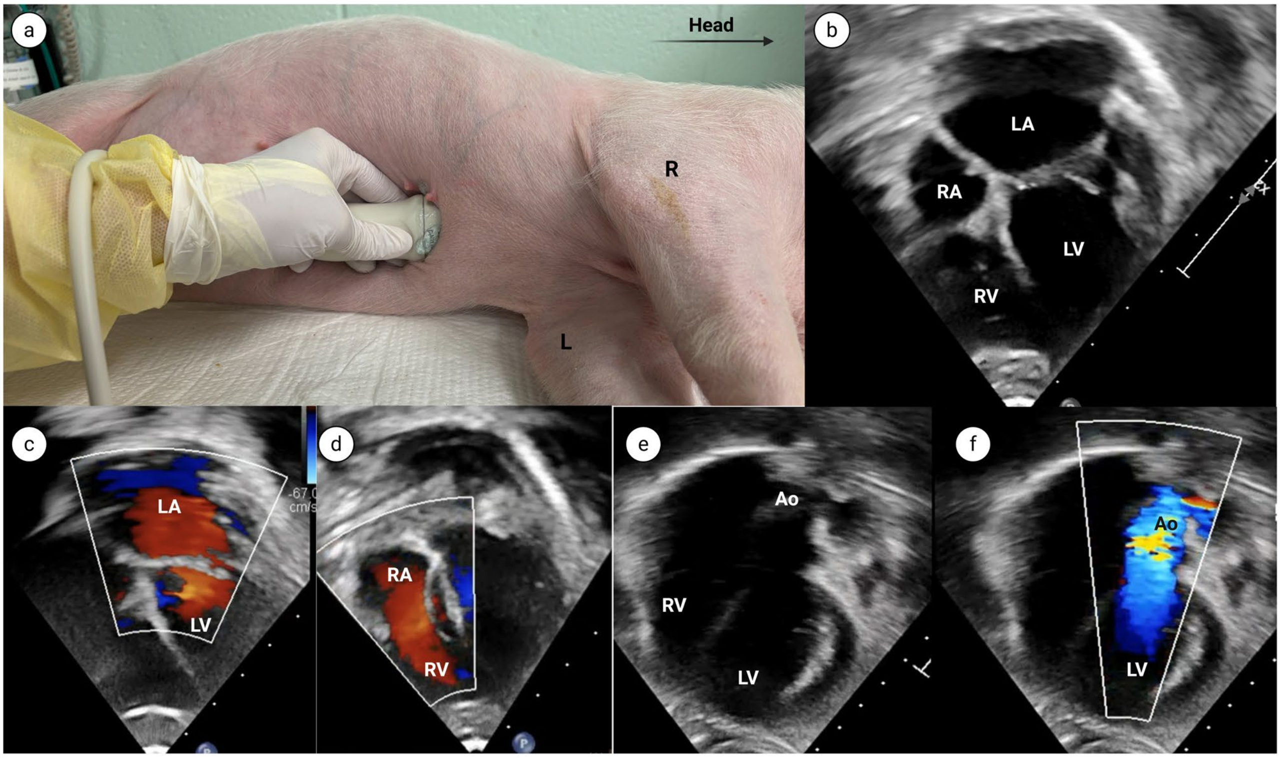


Fig. 2. Apical 4 chamber view.

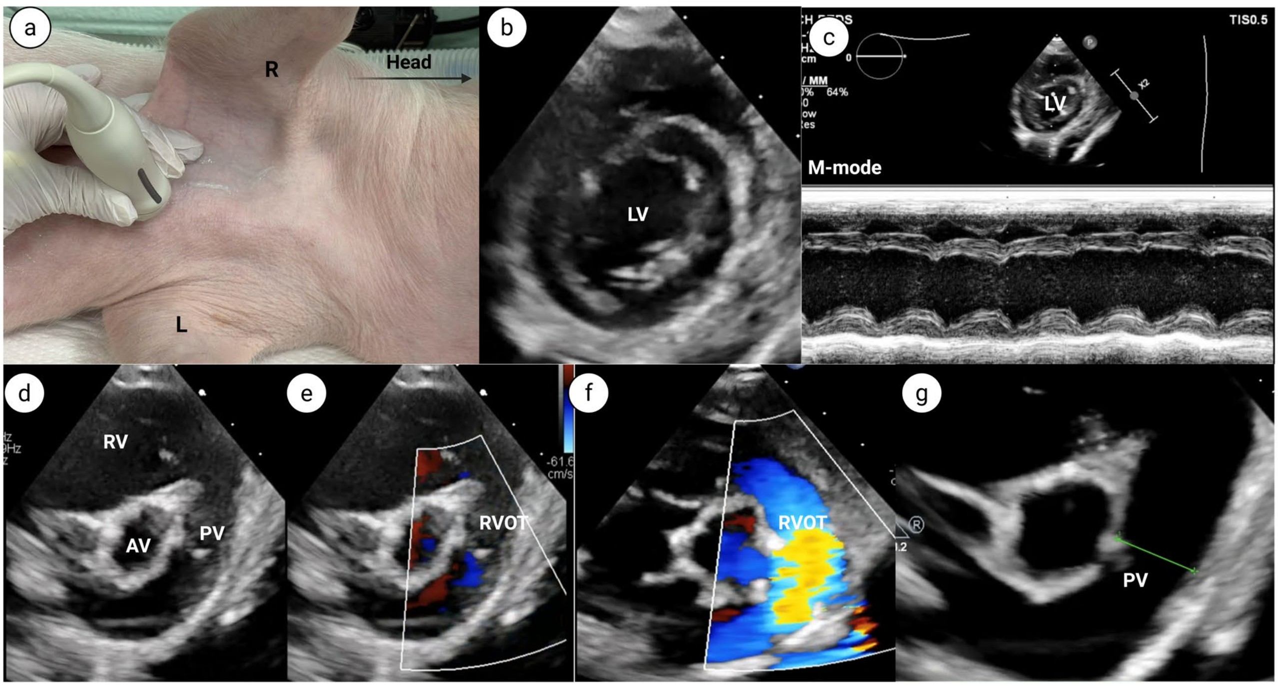


Fig. 3. Parasternal long axis (PLAX) view.

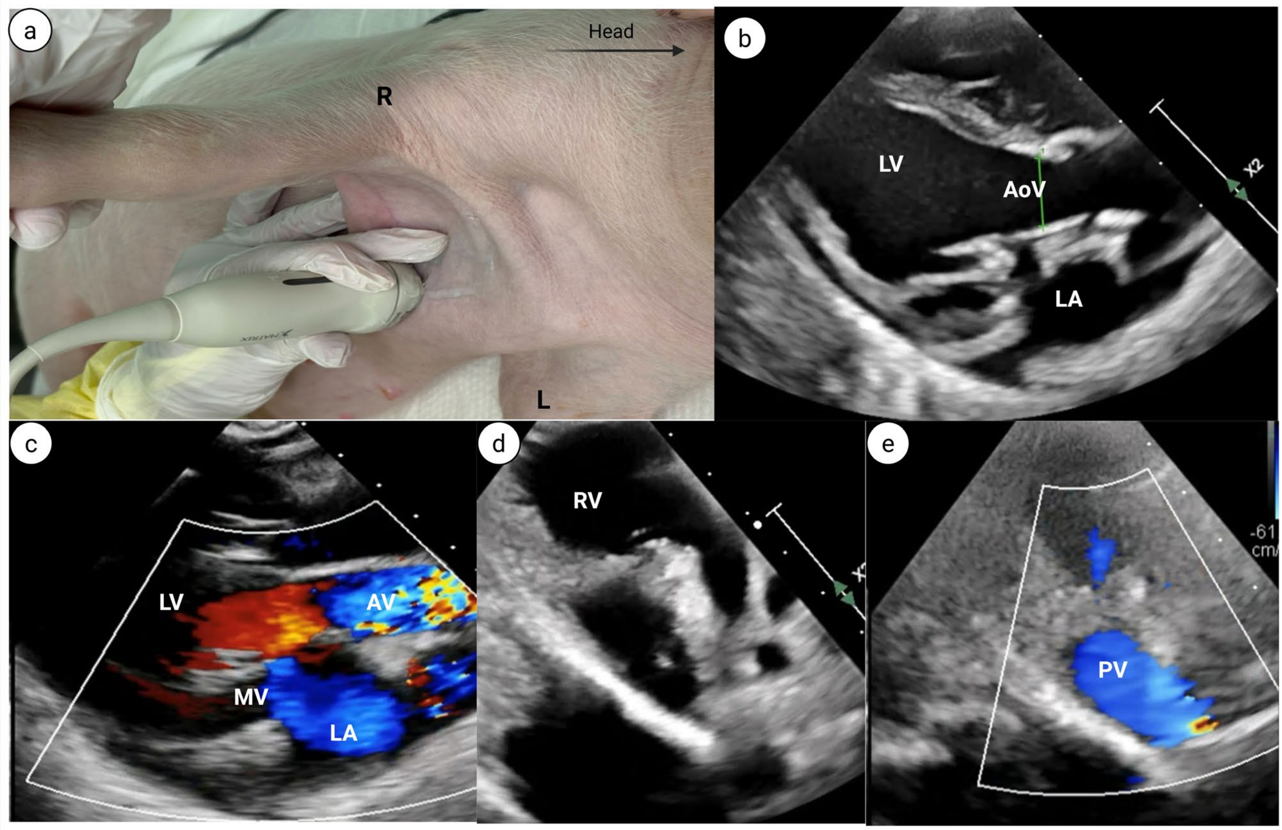


Fig. 4. Parasternal short axis (PSAX) view.

Conclusions

We report our standardized piglet echocardiography protocol and normative semilunar valve and left ventricular growth trends by age, weight and length.

Institutional review board statement

The animal study protocol was conducted in accordance with the Institutional Animal Care and Use Committee of the University of Arkansas Medical Sciences (Protocol # IPROTO202300000160) and conformed to standard Office of Laboratory Animal Welfare on the housing and medical care of swine used in research. The study is reported in accordance with ARRIVE guidelines.

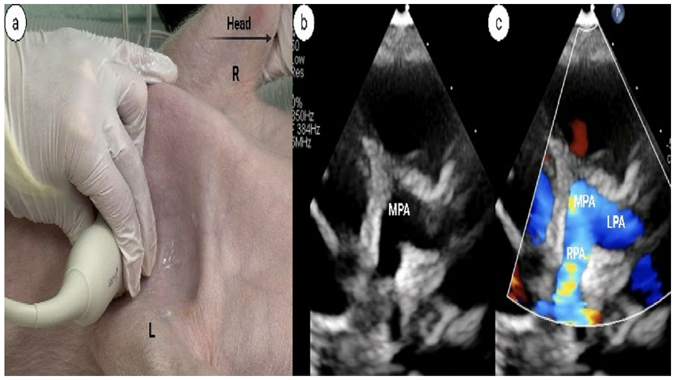
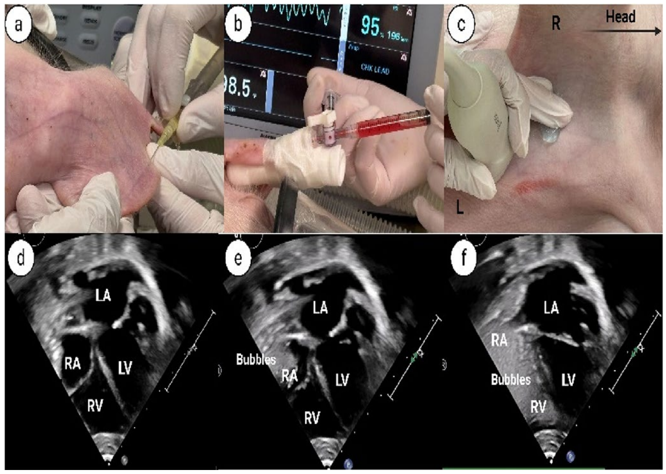
Method	Representative figures
PDA view (Fig. 5a-c)	
How to obtain: Supine position, probe positioned in the left axillary region, notch 1–3'oclock (Fig. 5a) • Evaluate branch pulmonary arteries by 2 d and color Doppler to look for patent ductus arteriosus (Fig. 5b-c)	
Bubble Study (Fig. 6a-f)	
How to obtain: Agitated saline contrast injected from peripheral IV by certified veterinarian and Apical 4 chamber view obtained as described in Table 1 (Fig. 6a-c) • Evidence for bubbles crossing to left side in the first five cardiac cycles noted after right sided opacification (Fig. 6d-f) for evidence of intracardiac shunting.	

Table 2. Additional echocardiographic evaluation prior to cardiac surgery [RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle; aov: aortic valve; PV: pulmonary valve; MPA: main pulmonary artery; R: right; L: left].

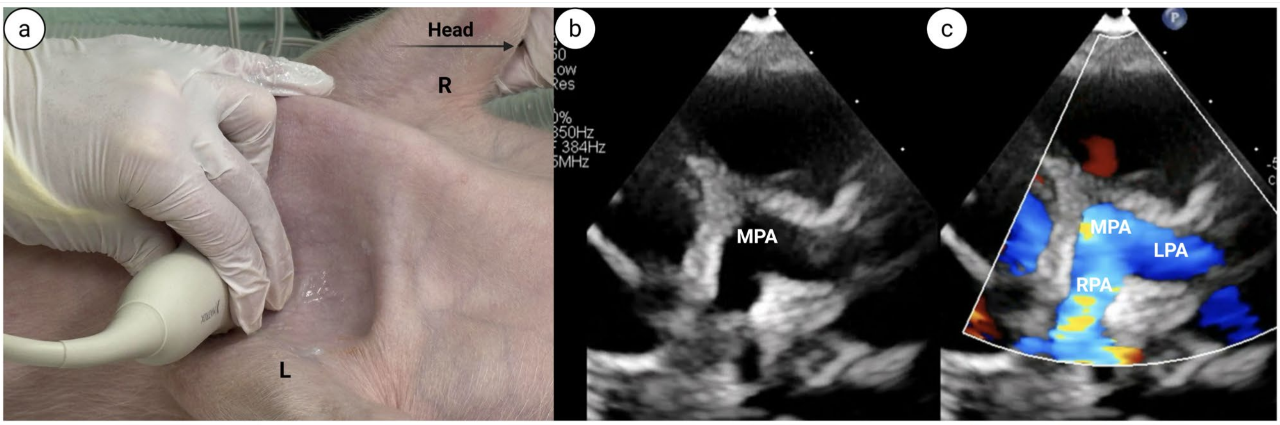


Fig. 5. Patent ductus arteriosus (PDA) view.

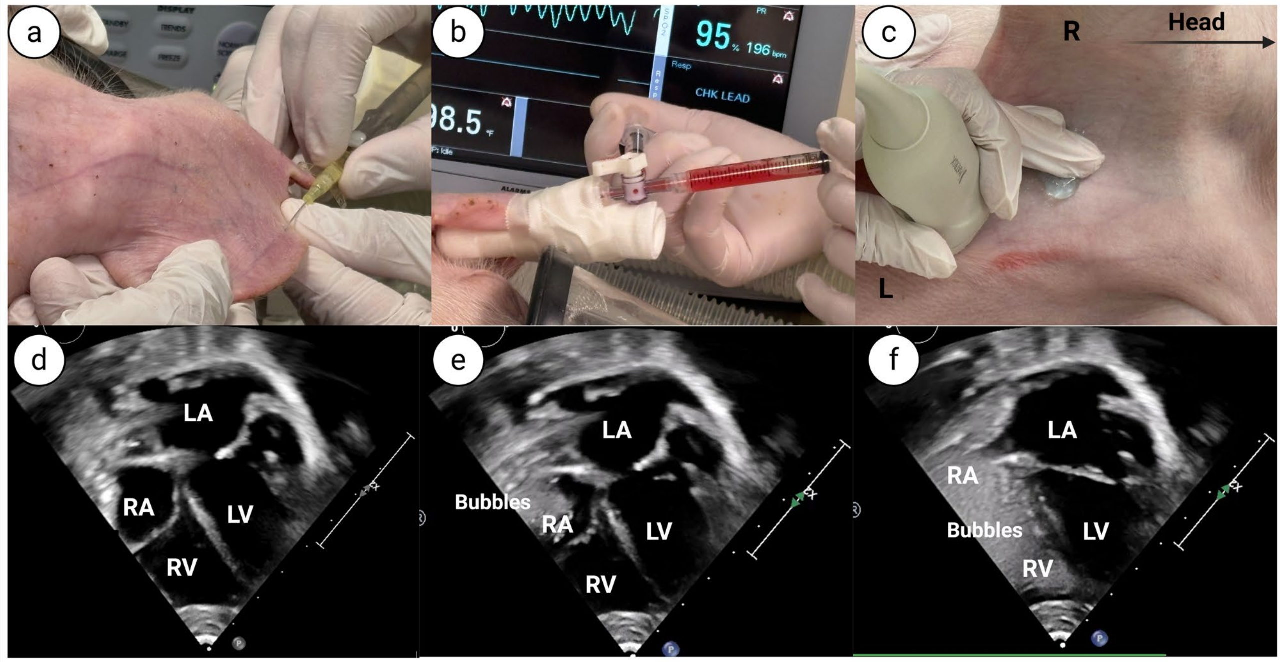


Fig. 6. Bubble study.

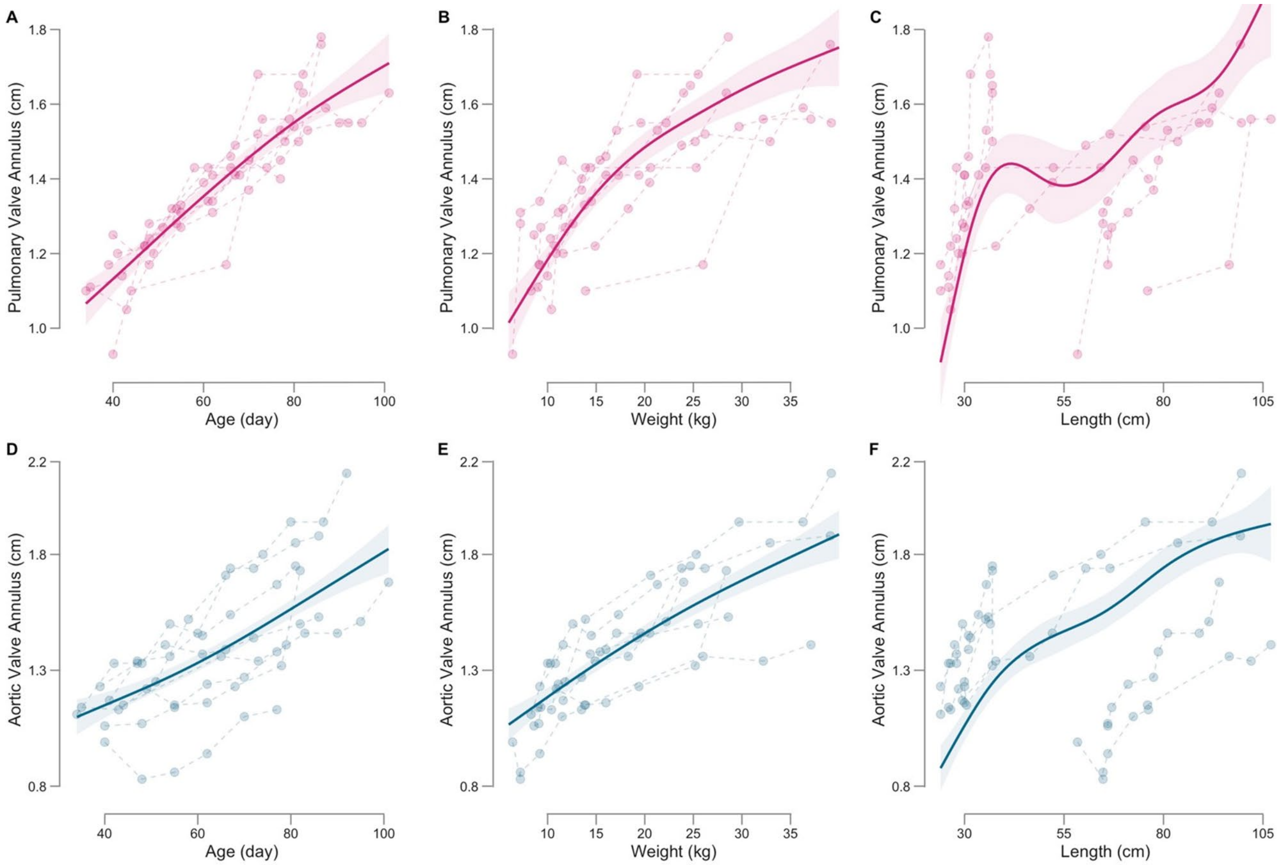


Fig. 7. Generalized additive mixed models for pulmonary and aortic valve size in relation to age, weight and length. Solid lines denote marginal predictions from the generative additive mixed models. Pink and blue shaded areas denote 95% confidence interval. Circle points represent echocardiographic measurements, with dotted lines linking repeated observations from the same piglet to show within-subject trajectories.

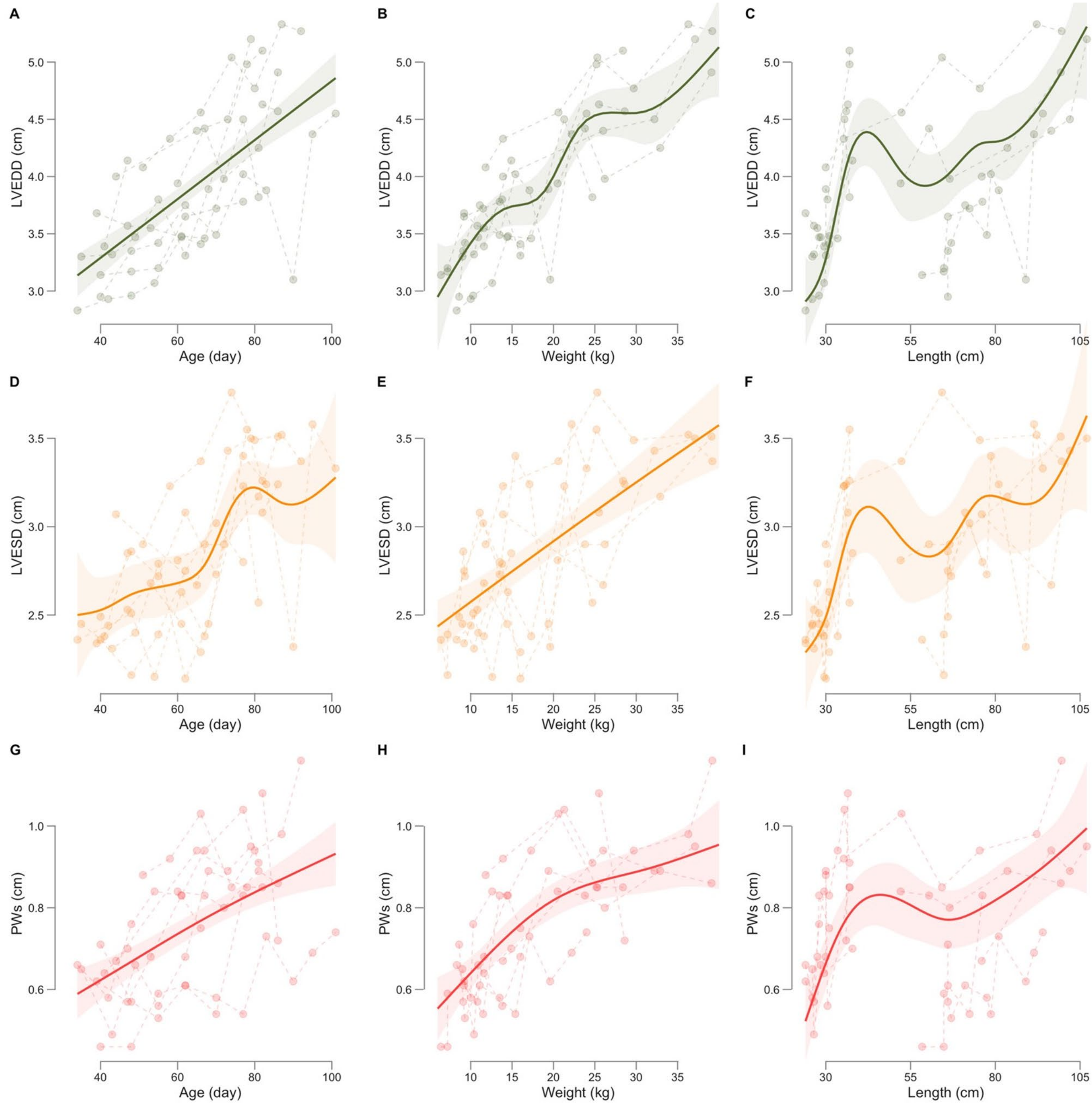


Fig. 8. Generalized additive mixed models for left ventricular end diastolic dimension (LVEDD), left ventricular end systolic dimension (LVESD) and left ventricular posterior wall dimension in systole (PWs) in relation to age, weight and length. Solid lines denote marginal predictions from the generative additive mixed models. Green, orange and red shaded areas denote 95% confidence interval. Circle points represent echocardiographic measurements, with dotted lines linking repeated observations from the same piglet to show within-subject trajectories.

Data availability

The data presented in this study is available upon request from the corresponding author.

Received: 20 June 2025; Accepted: 26 September 2025
Published online: 03 November 2025

References

1. Yerger, J., Hill, M. A., Fitzgerald, D. C. & Rajab, T. K. Animal models for partial heart transplantation. *Pediatr. Transpl.* **28** (4), e14788. https://doi.org/10.1111/petr.14788 (2024).

2. Medina, C. K. et al. Survival after partial heart transplantation in a piglet model. *Sci. Rep.* **14**, 12318. https://doi.org/10.1038/s41598-024-63072-1 (2024).
3. Harrison, D. K., Waldrip, Z. J., Burdine, L., Shalin, S. C. & Burdine, M. S. DNA-PKcs Inhibition extends allogeneic skin graft survival. *Transplantation* **105** (3), 540–549. https://doi.org/10.1097/TP.0000000000003442 (2021).
4. Bourque, K. et al. Design rationale and preclinical evaluation of the heartmate 3 left ventricular assist system for hemocompatibility. *ASAIO J.* **62** (4), 375–383. https://doi.org/10.1097/MAT.0000000000000388 (2016).
5. Crisóstomo, V. et al. Common swine models of cardiovascular disease for research and training. *Lab. Anim. (NY)*. **45** (2), 67–74. https://doi.org/10.1038/laban.935 (2016).
6. Lelovas, P. P., Kostomitsopoulos, N. G. & Xanthos, T. T. A comparative anatomic and physiologic overview of the Porcine heart. *J. Am. Assoc. Lab. Anim. Sci.* **53** (5), 432–438 (2014).
7. Schwarz, S., Kalbitz, M., Hummler, H. D. & Mendler, M. R. Transthoracic echocardiography of the neonatal laboratory piglet. *Front. Pediatr.* **7**, 318. https://doi.org/10.3389/fped.2019.00318 (2019).
8. Javed, H., Qasim, A., Chung, S., Henrich-Lobo, R. & Rajab, T. K. Partial heart transplants require immunosuppression in growing piglets. *Transplantation Published Online June*. **17** https://doi.org/10.1097/TP.0000000000005456 (2025).
9. Kang, L. et al. Partial heart transplant growth and hemodynamic function in piglets. *JACC Basic. Transl Sci.* **10** (4), 502–504. https://doi.org/10.1016/j.jacbts.2024.10.015 (2024).
10. Guihaire, J. et al. Right ventricular reserve in a piglet model of chronic pulmonary hypertension. *Eur. Respir J.* **45** (3), 709–717. https://doi.org/10.1183/09031936.00081314 (2015).
11. Abdullah, M. et al. Superiority of 3-dimensional versus 2-dimensional echocardiography for left ventricular volume assessment in small piglet hearts. *J. Am. Soc. Echocardiogr*. **13** (10), 918–923. https://doi.org/10.1067/mje.2000.106792 (2000).
12. Linden, K. et al. Comparison of stroke volumes assessed by three-dimensional echocardiography and transpulmonary thermodilution in a pediatric animal model. *J. Clin. Monit. Comput.* **31** (2), 353–360. https://doi.org/10.1007/s10877-016-9843-7 (2017).
13. Lunde, S., Smerup, M., Hasenkam, J. M. & Sloth, E. A model for left ventricular hypertrophy enabling non-invasive assessment of cardiac function. *Scand. Cardiovasc. J.* **43** (4), 267–272. https://doi.org/10.1080/14017430802647389 (2009).

Acknowledgements
The authors acknowledge the excellent care of the pigs provided by Arkansas Children Research Institute (ACRI) staff, with special thanks to David Irby, Haley Scheen, John Lowery and Melaney Gee.

Author contributions
All authors have read and agreed to the published version of the manuscript.A.Q: Conception, Study Design, Original Draft, Statistics, Images & Tables. E.C: Writing - Original Draft. H.J: Data Acquisition, Writing - Original Draft, Editing and Reviewing. A.F: Data Acquisition, Editing and Reviewing. L.S: Data Acquisition, Editing and Reviewing. A.Z: Reviewing and Editing. J.M: Conception, Data Acquisition, Editing and Reviewing. S.C: Statistics, Images & Tables, Editing and Reviewing. K.J: Reviewing and Editing. T.K.R: Supervision, Conception, Study Design, Writing - Reviewing and Editing.

Funding
T.K.R.'s research on partial heart transplantation is supported by the National Institutes of Health/National Heart, Lung, and Blood Institute grant R41 HL169059, the American Association for Thoracic Surgery, the Brett Boyer Foundation, the Saving tiny Heart Society, the Emerson Rose Heart Foundation, philanthropy from senator Campbell and the Arkansas Children's Research Institute.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-22176-y.

Correspondence and requests for materials should be addressed to T.K.R.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.