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Effects of Birth Season, Breed, Sex, and Sire Family on Cardiac Morphology Determined in Pigs (*Sus scrofa domestica*) by Use of Echocardiography

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Purpose: **Echocardiography played an important role in the screening and diagnosis of hypertrophic cardiomyopathy. In the study reported here, we attempted to evaluate the effects of birth season, breed, sex, and sire family on cardiac morphology determined in pigs by use of echocardiography.**

Methods: **A total of 411 pigs (mean body weight and age of 105.7 ± 10.6 kg and 214.4 ± 25.5 days, respectively) with different genetic backgrounds (Landrace, Yorkshire, and their two-way crossbred) were studied. Cardiac morphologic measurements included thickness of left ventricle and interventricular septum at end-systolic and end-diastolic phases. Meanwhile, the statistical model included the following effects: birth season, breed, sex, interaction between breed and sex, sire family, body weight, and age.**

Results: **Mean cardiac morphologic measurements were as follows: thickness of the interventricular septum at end-systolic and end-diastolic phases was 1.74 and 1.14 cm, respectively; and thickness of the left ventricular free wall at end-systolic and end-diastolic phases was 1.81 and 0.98 cm, respectively. Medium positive correlations existed among the cardiac morphologic measurements $r = 0.31$ to 0.53; $P < 0.001$). Pigs born in spring had significantly ($P < 0.05$) lower cardiac thickness at the end systolic phase than did pigs born in other seasons, and Landrace pigs had higher cardiac morphologic measurements than did Yorkshire and two-way crossbred pigs. Additionally, thickness of interventricular septum at the end-diastolic phase in male pigs was significantly higher than that in female pigs ($P < 0.05$). Cardiac morphologic measurements for the sire family were significantly ($P < 0.05$) different, and contributed 77.2 to 87.9% of the total variation, suggesting that genetic variation in cardiac morphology might exist in pigs.**

Conclusions: **Cardiac morphology of pigs might be influenced by genetic background. The effects of birth season, breed, sire family, and sex should be adjusted when using pigs as an animal model for comparative cardiovascular studies.**

Physiologic, anatomic, and biochemical natures of the cardiovascular system of humans and pigs are extremely similar, and the ready availability of pigs makes them a suitable animal model for human cardiovascular research (1-2). Hypertrophic cardiomyopathy (HCM) is characterized by the thickened interventricular septum and/or left ventricular free wall, and other causes do not exist for the increased cardiac mass (3). An HCM animal model has substantial potential for providing insight into the pathogenesis of this disease, which as been found to be naturally acquired in pigs, and has gross and histopathologic features similar to those in humans (4-6). Previous studies have indicated that HCM is moderately heritable in pigs (7); thus, we began an investigation to establish a pig model for HCM in humans (8).

Echocardiography (ECHO) is a non-invasive instrument for cardiovascular research and clinical diagnosis (9), and for assessing cardiac function (10). Echocardiography has played an important role in the screening and diagnosis of HCM in humans (11, 12). Despite numerous limitations in the use of ECHO to diagnose HCM (13), genetic screening on the basis of gene mutations is now available (14-16). Charron and co-workers (17) reported that ECHO has excellent specificity and low sensitivity in diagnosing familial HCM in adults. Since ECHO is easier and cheaper than genetic screening, it can be used in primary screening for HCM.

Morphologic and pathologic measurements of the heart from sacrificed pigs can be used to characterize porcine HCM (18). However, Mellor and Love (19) reported that some effects, such as age and body weight, could confound disease risk factors; thus, these effects should be considered in the diagnosis. Breed, sex, and sire family have been found to influence variation of heart measurements after sacrifice of pigs at market body weight (20), and correcting factors have been established for influences on these measurements (21). However, it is important to obtain cardiac measurements from still living pigs; thus, ECHO has been reported and established in pigs (22). When applying the technique to the clinical diagnosis of HCM in pigs, it is crucial to understand the contribution of factors that influence variation in cardiac morphology. In the study reported here, we attempted to evaluate the effects of birth season, breed, sex, and sire family on cardiac morphologic measurements in pigs to establish a reference for use in clinical diagnosis and comparative cardiovascular studies.

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Materials and Methods

Experimental animals and their feeding and management. A total of 411 pigs from 33 sire families were studied. Mean ± SD body weight and age were 105.7 ± 10.6 kg and 214.4 ± 25.5 days, respectively. Meanwhile, the genetic background of pigs included purebred Landrace, purebred Yorkshire, and a two-way crossbreed of Landrace and Yorkshire, numbering 277, 35, and 99, respectively. Numbers of females and males in the sample were 219 and 192, respectively. Birth season of the pigs was classified as spring (March, April, and May), summer (June, July, and August), autumn (September, October, and November), or winter (December, January, and February).

All experimental pigs were raised in pens in the same house with natural lighting environment at a traditional breeding farm. Progenies of the same sire were not necessarily raised in the same pen because more than one litter of progenies of one sire were used. The pigs were managed under the same husbandry conditions, consumption of the same feed, and exposure to the same disease conditions. The pigs were fed a diet containing 17.5% crude protein, with 3,050 kcal of metabolizable energy/kg. Feed and water were provided ad libitum. Range of room temperature and relative humidity was typically 15 to 34°C and 50 to 90%, respectively. Humane care and use of the animals were according to guidelines established by the National Science Council, Republic of China (23).

Echocardiographic measurements. Echocardiography was performed in pigs reaching market body weight (typically around 100 kg in Taiwan) and was carried out following the method of Wang and co-workers (22). Briefly, atropine (0.05 mg/kg of body weight, s.c.) and azerperone (0.4 mg/kg, s.c.) were administered to all pigs as pre-anesthetic agents, followed by thiamylal sodium (10 mg/kg, i.v.) for general anesthesia; then, pigs were positioned in left lateral recumbency.

Echocardiography was conducted, using a commercially available instrument (SONOS-100, Hewlett Packard, Andover, Mass.) and a 2.5-MHz transducer. Two-dimensional and M-mode echocardiographic images were recorded. To achieve parasternal short- and long-axis views, the pigs were imaged in the right lateral decubitus position with the ultrasound beam directed cephalad and to the right of the sternum, generally at the third or fourth intercostal space. Using this approach, we aimed to achieve, as closely as possible, the standard cross-sectional planes described in humans (24). Parameters were measured, using the M-mode echocardiogram (average of measurements from three to five consecutive heart cycles) and included thickness of the left ventricular free wall and interventricular septum at end-systolic and end-diastolic phases. Data from pigs suspected to be infected with disease were excluded in further analysis.

Statistical analysis. Effects of birth season (spring, summer, autumn, and winter), breed, sex, interaction between breed and sex, sire family, body weight, and age on cardiac morphologic measurements were analyzed by use of analysis of variance with the general linear models procedure (PROC GLM) in the Statistical Analysis System (SAS) package (25). Residual correlations among body weight, age, and cardiac morphologic measurements were determined as Pearson correlation coefficients after correcting the main effects (season, breed, sire family, sex, and interaction between breed and sex) and were calculated by use of the correlation procedure (PROC CORR) of SAS. Determination coefficients (the percentage of sum of squares of an variable to the overall sum of squares) of the main effects also were calculated. Finally, differences in cardiac morphologic measurements among seasons of birth, breeds, and sire families and between sexes were analyzed on the basis of age- and body weight-corrected data, using the least-squares means method (25).

Results

Statistics of cardiac morphologic measurements in pigs. We used ECHO to determine cardiac morphologic measurements in 411 pigs that had reached market body weight. Table 1 lists the basic statistics of body weight, age, and cardiac morphologic measurements. Mean body weight and age were 105.7 kg and 214.4 days, respectively. Thickness of the interventricular septum at the end-systolic and end-diastolic phases was 1.74 and 1.14 cm, respectively, and thickness of the left ventricular free wall was 1.81 and 0.98 cm, respectively. Coefficients of variation indicated that variation in cardiac morphology exceeded 17%, whereas variations of thickness at the end-diastolic phase exceeded those at the end-systolic phase.

Correlation coefficients among cardiac morphologic measurements. Residual correlation coefficients among body weight, age, and cardiac morphologic measurements were calculated after correcting for effects of birth season, breed, sire family, sex, and interaction between breed and sex and are listed in Table 2. Body weight and age had a medium to high positive correlation with cardiac morphologic measurements (r = 0.31 to 0.99; $P < 0.001$), whereas a medium positive correlation existed among cardiac morphologic measurements. The correlation coefficients (r = 0.31 to 0.53; $P < 0.001$) indicated that thickness of the left ventricle increased with that of the septum.

Determination coefficients (R^2) of the main effects on cardiac morphologic measurements. Possible factors influencing cardiac morphologic measurements were evaluated, and Table 3 lists the R^2 values for the major influences on cardiac morphologic measurements. The contributions of various effects

Table 1. Statistics of cardiac morphologic measurements in pigs (n = 411)

Trait	Mean	Range	SD	Coefficient of variation (%)
Body weight (kg)	105.70	85.5–130.0	10.60	10.0
Age (d)	214.40	136.0–269.0	25.50	11.9
IVSd (cm)	1.14	0.56–2.17	0.24	21.1
LVFWd (cm)	0.98	0.40–2.41	0.25	25.5
IVSs (cm)	1.74	0.99–3.20	0.30	17.2
LVFWs (cm)	1.81	0.97–2.90	0.32	17.7

IVSd = thickness of the interventricular septum during end diastole; IVSs = thickness of the interventricular septum during end systole; LVFWd = thickness of the left ventricular free wall during end diastole; LVFWs: thickness of the left ventricular free wall during end systole.

Table 2. Residual correlations (r)† among cardiac morphologic measurements in pigs (n = 411)

Trait	BW	Age	IVSd	LVFWd	IVSs	LVFWs
BW	1					
Age	0.47***	1				
IVSd	0.99***	0.47***	1			
LVFWd	0.47***	0.99***	0.47***	1		
IVSs	0.53***	0.31***	0.53***	0.31***	1	
LVFWs	0.35***	0.35***	0.35***	0.42***	0.33***	1

†Residual correlations were Pearson correlation coefficients after correcting the effects of season, breed, sire family, sex, and interaction between breed and sex.
*** $P < 0.001$.
BW = body weight.
See Table 1 for key.

to the variation in cardiac morphologic measurements were as follows. Birth season contributed 1.9 to 10.7% of the total variation in cardiac morphologic measurements, and breed contributed 3.3 to 13.1%. However, sire family had the most significant effect on cardiac morphologic measurements ($P < 0.001$) and contributed 77.2 to 87.9% of the total variation (Table 3). Finally, other effects contributed < 5% of the total variation in cardiac morphologic measurements.

Effect of birth season on cardiac morphologic measurements. Table 4 lists the fixed effect of birth season on cardiac morphologic measurements. A significant seasonal effect on cardiac thickness was not evident at the end-diastolic phase. However, pigs born in spring had lower cardiac thickness at the end-systolic phase than did pigs born in other seasons ($P < 0.05$).

Effect of breed on cardiac morphologic measurements. Table 5 presents the effect of breed on cardiac morphologic measurements, which differed significantly among pigs with different genetic backgrounds. Landrace pigs had significantly thicker cardiac morphologic measurements than did Yorkshire and two-way crossbred Landrace and Yorkshire pigs ($P < 0.05$).

Effect of sire family on cardiac morphologic measurements. There were significant differences in cardiac morphologic measurements among sire families ($P < 0.001$, data not shown). Ranges of least- squares means of cardiac morphologic measurements in the sire families were: thickness of the interventricular septum at the end-systolic and end-diastolic phases was1.24 ± 0.22 to 1.94 ± 0.10 cm and 0.71 ± 0.16 to 1.33 ± 0.12 cm, respectively, and thickness of the left ventricular free wall at the end-systolic and end-diastolic phases was 1.30 ± 0.22 to 2.10 ± 0.16 cm and 0.66 ± 0.13 to 1.21 ± 0.15 cm, respectively. Cardiac morphologic measurements of an individual sire family generally experienced the same trend (i.e., a sire family with high left ventricular free wall thickness also had high interventricular septum thickness).

Effect of sex on cardiac morphologic measurements. Table 6 shows the least-squares means of cardiac morphologic measurements in male and female pigs. The only notable difference between the sexes lay in thickness of the interventricular septum at the end-diastolic phase, which was significantly higher in male than female pigs ($P < 0.05$).

Table 3. Determination coefficients (R^2)* of the main effects on echocardiographic measurements# in pigs

Effect	IVSd	LVFWd	IVSs	LVFWs
Season of birth	1.9	2.6	5.5	10.7
Breed	3.3	3.4	13.1	7.6
Sire	87.9	87.9	77.4	77.2
Sex	4.9	1.9	0.9	0.1
Breed × sex	1.4	1.8	0.0	0.0
BW	0.5	0.7	0.7	4.1
Age	0.2	3.7	2.4	0.4

*Determination coefficients were the percentage of sum of squares of an effect on the overall sum of squares.
See Tables 1 and 2 for key.

Table 4. Least-squares means of cardiac morphologic measurements for pigs born in different seasons

Trait	Autumn	Spring	Summer	Winter
No. of pigs	105	66	164	76
IVSd (cm)	1.08±0.04	1.04±0.05	1.09±0.05	1.06±0.05
LVFWd (cm)	0.93±0.05	0.90±0.05	0.94±0.05	0.98±0.05
IVSs (cm)	$1.63±0.06^{a,b}$	$1.52±0.07^{a}$	$1.62±0.06^{b}$	$1.65±0.06^{b}$
LVFWs (cm)	$1.75±0.06^{b}$	$1.59±0.07^{a}$	$1.76±0.06^{b}$	$1.81±0.06^{b}$

^{a,b} Means in the same row with different superscript differ significantly ($P < 0.05$).
See Table 1 for key.

Discussion

Echocardiography is an important non-invasive method for describing and detecting heart disease in humans (26-28), and has been applied to livestock (22, 29-31). Few previous investigations evaluated the factors that influence echocardiographically determined cardiac morphologic measurements in livestock species, yet it is important to predict cardiac morphologic measurements when using pigs as animal models. The factors influencing cardiac morphologic measurements in pigs were characterized, and the results suggested that birth season, breed, sire family, and sex could be the major influences on variation of cardiac morphologic measurements in pigs. These results offered baseline echocardiographic data in market weight of Landrace, Yorkshire, and their crossbred pigs in a tropical environment under open housing conditions, and for the first time, we identified the factors that influence the variation of cardiac morphologic measurements in pigs.

Mellor and Love (19) mentioned age and body weight as potential confounding factors in disease. The pigs of this study had narrow age and body weight ranges. Though age and body weight were found to contribute < 5% of the total variation of cardiac morphologic measurements (Table 3), a medium to high residual correlation existed between age or body weight and cardiac morphologic measurements (Table 2). The residual correlation coefficients suggested that age and body weight may be associated with cardiac morphologic measurements; thus, effects of age and body weight must be appropriately corrected. Genetic background and sex also were found to significantly influence body weight and age (data not shown); thus, effects of age and body weight were adjusted for comparing cardiac morphologic measurements.

Birth season was found to significantly affect cardiac morphology at the end-systolic phase (Table 4). Alaku and co-workers (32) reported that month of birth could influence heart growth in pigs, and results of our previous study suggested that heart measurements at necropsy were lower for pigs born in spring than for pigs born in other seasons (21). Results of the study reported here also indicated that pigs born in spring had lower echocardio-

Table 5. Least-squares means of the cardiac morphologic measurements of pigs with different genetic backgrounds*

Trait	Landrace	Yorkshire	LY
No. of pigs	277	35	99
IVSd (cm)	$1.18±0.03^{x}$	$0.92±0.13^{y}$	$1.10±0.04^{x,y}$
LVFWd (cm)	$1.02±0.03^{x}$	$0.88±0.14^{x,y}$	$0.91±0.05^{y}$
IVSs (cm)	$1.85±0.04^{b}$	$1.37±0.17^{a}$	$1.60±0.06^{a}$
LVFWs (cm)	$1.89±0.04^{b}$	$1.65±0.17^{a,b}$	$1.65±0.06^{a}$

^{a,b}Means in the same row with different superscripts differ significantly ($P < 0.05$).
^{x,y}Means in the same row with different superscripts differ significantly ($P < 0.1$).
LY = Two-way crossbred Landrace and Yorkshire pigs.
See Table 1 for key.

Table 6. Least-squares means of cardiac morphologic measurements for sex of pigs

Trait	Female	Male
No. of pigs	219	0192
IVSd (cm)	$1.03±0.04^{a}$	$1.10±0.04^{b}$
LVFWd (cm)	0.91±0.04	0.96±0.04
IVSs (cm)	1.58±0.05	1.63±0.06
LVFWs (cm)	1.72±0.06	1.73±0.06

$^{a, b}$Means in the same row with different superscripts differ significantly ($P < 0.05$).
See table 1 for key.

graphically determined cardiac morphologic measurements (Table 4). This phenomenon might be caused by the fact that pigs born in spring grew mostly in summer; thus, heart growth may be retarded by heat stress associated with Taiwan's subtropical climate. Owing to the aforementioned phenomenon, season of birth should be corrected for comparison of cardiac morphologic measurements in pigs born in different seasons.

Genetic background significantly influenced cardiac morphologic measurements (Table 5). Landrace pigs had significantly higher left ventricle and ventricular septum thickness than did Yorkshire and two-way crossbred Landrace and Yorkshire pigs. Echocardiography has been documented to accurately identify ventricular septal defect morphology in newborn Yucatan micropigs (31). Further characterization exploring the correlations between echocardiographic cardiac measurements and anatomic heart measurements in market weight domestic pigs will be necessary. Our results may imply that Landrace pigs have larger heart mass and heart weight at necropsy (20, 33).

Sex contributed only a low percentage of the variation of cardiac morphologic measurements (Table 3). However, male pigs had a significantly higher ventricular septal measurement at the end-diastolic phase than did female pigs (Table 6). This observation was consistent with previous investigations (20). Earlier studies suggested that sex might be the major influence on heart weight (33-35). The significant sex effect indicated that proper adjustment for such effect might be helpful for comparison of ventricular septum thickness at the end-diastolic phase.

Sire family had a significant effect on cardiac morphologic measurements and contributed over 75% of the variation in cardiac morphologic measurements (Table 3). Results of our previous study also indicate that sire family contributed 56 to 79% of total variation in heart measurements at necropsy (20). Both sets of results suggest that genetic variations may exist in cardiac morphologic measurements of market weight pigs.

As Fananapazir and Epstein (13) reported, limitations and future enhancements of the present approach, incorporating new parameters and techniques for heart disease screening, heart volume and mass estimation, are necessary. This study focused on pigs at approximately market weight, and the results are only applicable to pigs of similar body weight. Further study involving a time series follow-up of cardiac morphologic measurements in pigs at various developmental stages is urgently needed to develop clinical applications. The real biological value of the significant alterations of echocardiographically determined cardiac morphologic measurement of pigs also needs further exploration.

In conclusion, echocardiographically determined cardiac morphologic measurements of pigs may be influenced by genetics. Consequently, effects of birth season, breed, sire family, sex, body weight, and age should be properly adjusted when using pigs as animal models for comparative cardiovascular studies. However, correcting factors should be developed for these effects to allow further practical applications.

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