

# Anatomical comparison of the heart and the thoracic vessels in calves and humans - Xenograft prospects.

## Comparaison anatomique du cœur et des vaisseaux thoraciques entre le Veau et l'Homme - Perspectives de la xénogreffe.

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### KEY WORDS

Heart;  
Thoracic vessels;  
Aorta;  
Pulmonary trunk;  
Cranial *vena cava*;  
Caudal *vena cava*;  
Xenograft

### Abstract

*Objectives.* – To analyse Calf cardiovascular thoracic system in terms of measurements and proportions, and evaluate its relevancy as a study model for humans.

*Methods.* – Twenty Calf carcasses, of variable age and size, were dissected. Four of them were previously injected with latex and contrast product, and then were scanned, so that cardiovascular structures were enlightened in scan images. The other calves were simply put down. Variables were measured on every carcass.

*Results.* – Calf cardiac and thoracic volumes are correlated. Cardiac length is the most linked parameter to other thorax, heart and vessel variables. Cardiac cavities do not seem to reflect whole heart proportions. Regardless of the size difference between a calf and a man, the main differences in both species are heart shape and cardiac ostium diameters.

*Conclusion.* – As far as anatomy is concerned, Calf cardiovascular thoracic structures can be compared to human ones to some extends.

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## MOTS-CLÉS

Cœur ;  
Vaisseaux  
thoraciques ;  
Aorte ;  
Tronc pulmonaire ;  
Veine cave crâniale ;  
Veine cave caudale ;  
Xénogreffe

## Résumé

*Objectifs.* – Analyser le système cardio-vasculaire thoracique du Veau en termes de mesures et de proportions, et évaluer sa pertinence comme modèle d'étude pour l'Homme.

*Méthodes.* – Vings carcasses de veau, d'âge et de gabarit variables, sont disséquées. Quatre d'entre elles sont préalablement injectées avec du latex et du produit de contraste, puis passées au scanner, de façon à faire ressortir les structures cardio-vasculaires sur les images scanner. Les autres sont simplement euthanasiés. Des variables sont mesurées sur chacune des carcasses.

*Résultats.* – Les volumes du cœur et du thorax du Veau sont corrélés. La longueur cardiaque est liée au plus grand nombre d'autres variables du thorax, du cœur et des vaisseaux. Les cavités cardiaques ne semblent pas refléter les proportions du cœur entier. En s'affranchissant de la différence de taille entre un veau et un être humain, les différences majeures entre les deux espèces sont la forme du cœur et le diamètre des ostiums cardiaques.

*Conclusion.* – D'un point de vue anatomie, les structures cardio-vasculaires thoraciques du Veau peuvent être comparées à celles de l'Homme jusqu'à un certain niveau.

## Introduction

If xenotransplantation tests were performed much earlier than one would believe, with xenotransfusions and bone grafts from the 17th century onwards [1, 2], it was not until 1968 that the first heart xenograft was tried [3, 4]. In parallel, cardiac allograft surgery techniques have been well-mastered from the mid 60's, but attempts have become more and more frequent thanks to immunosuppressive therapy progress [5-7].

However, the shortage of human organs for grafts (especially hearts) has driven scientists to turn towards artificial machinery and animal source [8-10]. Nowadays, the use of pig cardiac structures for xenotransplants is indeed a well-known issue, particularly based upon comparison of porcine and human anatomy [11-14]. Since bovine quantitative features are poorly-documented, this study aims at comparing Calf and human structures so as to evaluate the Calf cardiovascular model relevancy for human surgical procedures. A qualitative comparison has already been carried out, revealing the following statements: despite a same general layout in 4 contractile cavities (2 atria and 2 ventricles), Calf and human heart shapes are to be distinguished: due to the form of the thorax, one is flattened laterally whereas the other is flattened ventrodorsally. Thoracic cardiovascular structures in calves and humans are roughly similar as well, although supra-aortic vessels (brachio-cephalic trunk, common carotid arteries, subclavian arteries) and superior *vena cava* roots (brachio-cephalic, jugular and subclavian veins) are organized differently [15].

## Materials and methods

*Animals.* – 20 calves, 11 males and 9 females, were dissected for the study. Two breeds were used: 12 Prim'Holstein and 8 Charolais, aged from 53 to 262 days and weighing 70 to 154 kg. All of them came from various farms around Oniris Veterinary School.

*Anesthesia, injections and scanner.* – All husbandry and experimental procedures were approved by the institutional ethics committee and the French Ministry of Agriculture for ONIRIS (N° A 44.274). 4 calves were anesthetized intravenously with a protocol composed of

xylazine (ROMPUN®) 0.1 mg/kg and ketamine (IMALGENE®) 4 mg/kg. Heparin was also injected. Following a cutaneous incision on the upper neck region, the left external jugular vein and the left common carotid artery of each calf were dissected. The carotid catheter was first used for external bleeding, whereas a pump was linked to the jugular catheter then back to the carotid catheter. Thanks to this pump, the first calf was injected with latex, whereas a mix of latex and contrast agent was used for the 3 others, since the scanner images were not satisfying enough with latex only. In any case, the product was injected until it was seen in the femoral vein (approximately 4 liters). The injected calves had then a scan at Image Et, Mordelles. The 16 other calves were put down with pentobarbital (DOLETHAL®) 5 mL/kg. All calves were finally frozen until their dissection at Oniris.

*Dissection and measurements.* – Before any dissection issue, the following parameters were measured on every calf carcass: **thoracic perimeter** (TP), **thoracic height** (TH) and **thoracic width** (TW) (caudal to the shoulder), and **distance from the upper part of the scapular spine to the ischiatic tuberosity** ( $d_{SS-IT}$ ). The volume of the smallest rectangular parallelepiped containing the trunk was also calculated (trunk volume). The skin and most superficial muscles of the left thoracic wall were first removed, prior to the left anterior limb, the second to thirteenth ribs (the **first rib length** 1RL was measured), plevra, left pulmonary lobes and pericardium. The following cardiovascular structures were then dissected and exposed: left azygos vein (LAV) and distribution, descending aorta and collateral branches, ascending aorta, aortic arch, brachiocephalic trunk (BCT), pulmonary trunk (PT), cranial (CrVC) and caudal (CdVC) *vena cava*. Moreover, the main vessels were cut upon their entry in the heart to remove it from the thoracic cavity. Next, the same steps were performed on the right side of the carcass.

The heart was first **weighed** (CWe). **Cardiac length** (CL), from the middle of the aortic ostium to the apex, sagittal diameter of the cardiac base (**width** CWi), transversal diameter of the cardiac base (**height** CH) and **cardiac perimeter** (CP) (at the coronary groove level) were measured. The volume of the smallest rectangular parallelepiped containing the heart was also calculated (heart volume). Therefore, a transversal section was performed on the third of the cardiac height from the apex,

revealing the ventricular walls. **Marginal wall thickness of the left (LVWT) and right (RVWT) ventricles, septum thickness (ST), LV and RV width (LVW, RVW) and height (LVH, RVH)** were measured. Each cardiac cavity was finally opened, and the following elements were measured too: **LV and RV length (LVL, RVL), CrVC and CdVC internal perimeter (CrVCP, CdVCP), coronary sinus internal perimeter (CSP), aortic (A1), PT (PT1), tricuspid and mitral ostia internal perimeter (TriP, MitP).**

Other measurements were also made on thoracic vessels: **aortic internal perimeters**, caudal to the *ductus arteriosus* (A2) and cranial to the diaphragm (A3), **aortic length (AoL)**, from the aortic valves to the BCT origin, **PT internal perimeter**, caudal to the *ductus arteriosus* (PT2), **BCT internal perimeter (BCTP)** and **thoracic CdVC length (CdVCL)**. Thus, 34 different measures were obtained on each calf.

*Statistical methods.* – In order to find relationships among the 34 variables, a correlation matrix was built using the R® program.

A Shapiro-Wilk test was performed on R® so as to identify the normal variables. Indeed, the final aim was to work with a better representativity of the actual calf population, multiplying the strength of the dissected calves. On the one hand, for normal variables, a simulation program was run to obtain 1000 values for each variable, and the corresponding descriptive statistics illustrated by a box plot. On the other hand, the same steps were used for non normal variable, except that a bootstrap program was run. In any case, from a 20 strength for each variable, a 1000 strength is virtually obtained, as if 1000 calves had been dissected. Then, descriptive statistics were done on every variable, using the 1000 values for each : minimum, maximum, 25% quantile, 50% quantile, 75% quantile, interquartile range (IQR), mean and standard deviation (sd).

## Results

*Correlation matrix (bivariate analysis).* – The results of the bivariate analysis are obtained (table 1). According to this table, some facts can be emphasised. Since trunk volume was calculated from TH, TW and  $d_{SS-IT}$  and is linked to TP, it can be said that carcass size and age are linked, which seems logical so far. Among all cardiac measurements, cardiac length is correlated to the biggest amount of other variables, including thoracic statements, which might explain why heart volume and trunk volume share a high correlation rate. However, the lack of correlation among cardiac variables is more surprising and suggests heterogeneity in the cardiac shape. In the same way, cardiac cavity proportions do not seem to be linked to cardiac size. In contrast, A1 is highly-correlated to a wide range of thoracic and cardiac variables: according to these results, knowing A1 can give a first idea of carcass and heart proportions. Moreover, there is good homogeneity in aortic diameter, as A2 shares a high correlation rate with A1 and A3; whereas the pulmonary trunk is irregular, at least on its measured portion. Finally, surprisingly enough once again, there is no link between ventricular proportions and the diameter of big arteries.

**Table 1:** Correlation ratios among different measured variables

**Tableau 1:** Ratios de corrélation entre les différentes variables mesurées

Variable	r > 0,7
<b>Age</b>	Weight, TP*, TH, 1RL, CWe*, CL, LVW, A1*, AoL*, CrVCP, CdVCP
<b>Weight</b>	Age, TP*, TH*, $d_{SS-IT}$ *, CWi*, CL, A1*
<b>TP</b>	Age*, Weight*, TH*, TW, $d_{SS-IT}$ , 1RL, CWe, CL*, CWi*, LVH, A1*, CrVCP
<b>TH</b>	Age, Weight*, TP*, 1RL, CWi, CL*, A1
<b>TW</b>	TP, LVH
<b><math>d_{SS-IT}</math></b>	Weight*, TP, CWi, A1
<b>1RL</b>	Age, TP, TH, CWe, CL, A1
<b>Trunk volume</b>	Trunk volume*
<b>CWe</b>	Age*, TP, 1RL, A1, A2*, CdVCP, CSP
<b>CP</b>	A1
<b>CH</b>	/
<b>CWi</b>	Weight*, TP*, TH, $d_{SS-IT}$ , CL*, A1
<b>CL</b>	Age, Weight, TP*, TH*, 1RL, CWi*, A1*, A2*
<b>ST</b>	/
<b>Heart volume</b>	Trunk volume*
<b>LVH</b>	TP, TW, CrVCP
<b>LVW</b>	Age, SCP
<b>LVL</b>	CdVCP
<b>LVWT</b>	/
<b>RVH</b>	/
<b>RVW</b>	/
<b>RVL</b>	/
<b>RVWT</b>	/
<b>TriP</b>	A1*
<b>MitP</b>	/
<b>A1</b>	Age*, Weight*, TP*, TH, $d_{SS-IT}$ , 1RL, CWe, CP, CWi, CL*, TriP*, A2*, CSP
<b>A2</b>	CWe*, CL*, A1*, A3*
<b>A3</b>	A2*
<b>AoL</b>	Age*
<b>PT1</b>	/
<b>PT2</b>	/
<b>CrVCP</b>	Age, TP, LVH
<b>CdVCP</b>	Age, CWe, LVL
<b>CdVCL</b>	/
<b>BCTP</b>	/
<b>CSP</b>	CWe, LVW, A1

\*r > 0,8

*Descriptive statistics (univariate analysis).* – Considering the previous correlations, the available measured variables in human anatomy and knowing that some measurements are not used in cardiac transplants, the results of descriptive statistics of important variables for surgery are obtained (table 2). They were formerly submitted to a simulation or bootstrap program, according to their normality, to obtain 1000 values for each variable. Furthermore, cardiac length mean and standard deviation is taken as a reference variable, according to which every other mean is given a factor so that it can be compared to human values (cf. discussion), freeing itself from size difference between these two species.

**Table 2:** Descriptive statistics and factors of variables of interest

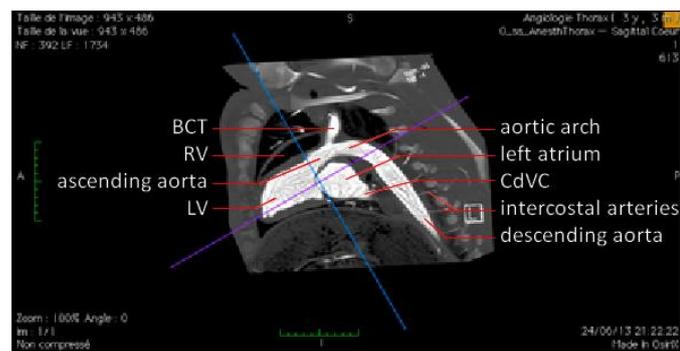
**Tableau 2 :** Statistiques descriptives et facteurs des variables d'intérêt

	0% quantile	25% quantile	50% quantile	75% quantile	100% quantile	mean	sd**	mean factor	sd factor
<b>Age</b>	15.32	106.61	151.88	195.63	417.94	154.15	66.65	/	/
<b>Weight</b>	70.00	79.95	93.69	115.09	154.00	99.62	25.95	/	/
<b>TH</b>	21.34	41.29	45.58	50.60	66.87	46.09	7.48	2.33	3.12
<b>TW</b>	6.66	18.04	20.67	23.20	31.90	20.52	3.92	1.17	1.63
<b>CWe</b>	105.72	391.17	474.42	555.61	876.77	473.69	125.89		
<b>CP</b>	17.80	25.14	28.95	31.10	34.10	27.85	4.68	1.99	1.95
<b>CH</b>	4.14	7.33	8.33	9.14	12.37	8.17	1.39	0.46	0.58
<b>CWi*</b>	10.90	12.27	12.75	13.55	15.80	13.02	1.25	0.89	0.52
<b>CL</b>	10.71	15.85	17.41	19.06	25.06	17.52	2.39	1.00	1.00
<b>ST</b>	0.11	1.22	1.41	1.64	2.32	1.41	0.32	0.08	0.13
<b>LVWT</b>	0.16	1.15	1.33	1.52	2.49	1.34	0.29	0.11	0.12
<b>RVWT*</b>	0.40	0.77	0.85	0.90	1.50	0.85	0.29	0.04	0.12
<b>TriP</b>	5.98	11.68	12.89	14.25	19.62	12.95	1.93	0.73	0.80
<b>MitP</b>	3.33	8.28	9.42	10.59	15.91	9.50	1.78	0.54	0.74
<b>A1</b>	6.04	8.10	8.70	9.35	11.61	8.73	0.91	0.49	0.38
<b>PT1</b>	4.48	7.84	8.75	9.70	13.51	8.71	1.40	0.49	3.36
<b>CrVCP</b>	3.78	5.87	6.48	7.06	9.63	6.49	0.88	0.37	0.37
<b>CdVCP</b>	5.40	6.04	6.82	8.57	10.20	7.33	1.71	0.41	0.71
<b>CSP</b>	0.01	1.18	1.53	1.93	3.08	1.53	0.54	0.08	0.22

\* Gross values because of aberrational values

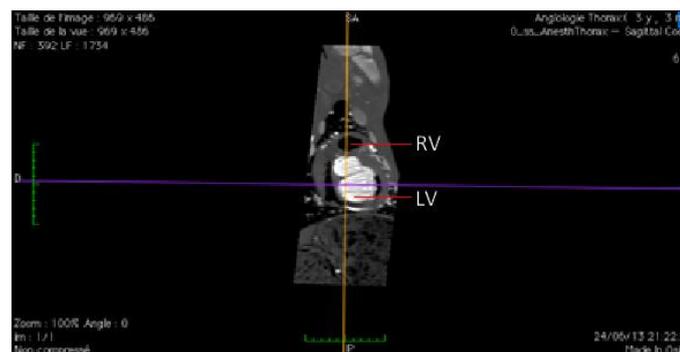
\*\* standard deviation

**Scanner images.** – To illustrate these dissections and measurements, a scan was performed on the 4 calves which had already been injected with latex and contrast product. Scan images were obtained (figures 1, 2, 3). Thanks to OSIRIX® program, some reconstructed images can be shown (figure 4) where cardiovascular structures are enlightened. Some cardiovascular structures were actually not full of contrast product, but it gives a first overview about scan perspective for comparison.



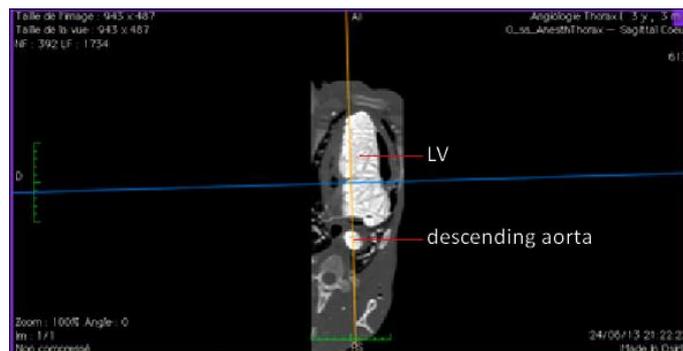
**Figure 1:** Scan image of sagittal thoracic plane

**Figure 1 :** Image scanner du plan thoracique sagittal



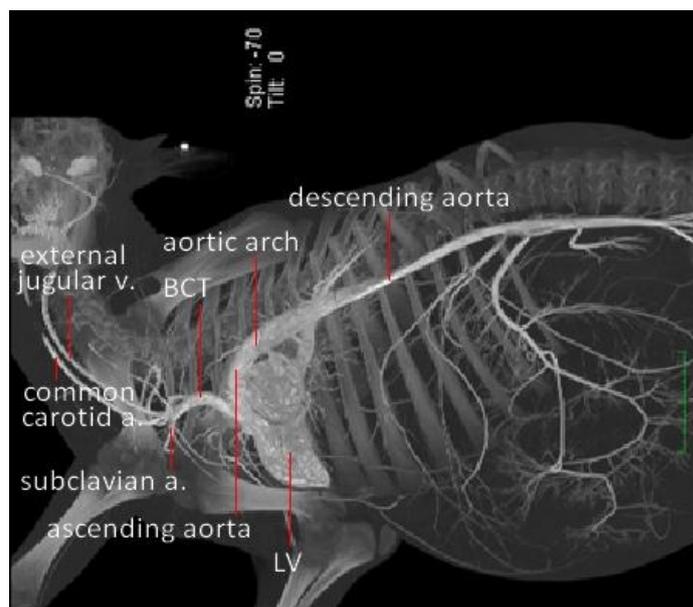
**Figure 2:** Scan image of frontal thoracic plane

**Figure 2 :** Image scanner du plan thoracique frontal



**Figure 3:** Scan image of transversal thoracic plane

**Figure 3 :** Image scanner du plan thoracique transversal



**Figure 4:** Scan image of cardiovascular structures reconstruction

**Figure 4 :** Image scanner de la reconstruction des structures cardiovasculaires

## Discussion

The number of organ transplant requests has been steadily increasing over the last decade, from 12,512 people in 2006 to 21,464 in 2015, though allograft surgeries have also greatly multiplied over this 9-year period of time [16]. To overcome this deficiency, alternatives have been found. Indeed, the CARMAT® company is developing a whole artificial heart made from biomaterial and transplanted in four different patient so far, as part of clinical testing [8-10]. On the other hand, animal resources are also being studied, especially porcine cardiovascular structures. This study proposes to evaluate the Calf thoracic cardiovascular model.

First of all, some points are to be discussed regarding our material and methods. Some of the links among measured variables appeared surprising, such as the lack of correlation between cardiac size and ventricle size. This might be explained by the small batch used (20 calves altogether), and the big variability in terms of age and basic size. Besides, variables were measured on animals which had not necessarily been euthanized under the same conditions. Also, latex was only injected into 4 calves out of 20 and its influence on vessel diameter or heart cavity capacity must be taken into account. For practical reasons, carcasses were frozen then thawed at least once: it would have been more relevant to dissect all of them in a row, without any freezing period. Finally, heart and trunk volumes were updated: actually, the volume of the smallest rectangular parallelepipeds was evaluate, since it can be calculated more easily. It would also have been more relevant to measure thoracic volume rather than trunk volume.

Equivalent means in human anatomy can be found in the bibliography. Indeed, for instance, human cardiac length is said to reach 11 cm on average (table 3) [16-21]. Similarly to bovine variables, a factor was given to every variable according to the human cardiac length, in order to get rid of size difference between species: one can only compare like with like. It would also have been relevant and more precise to measure these variables in scanner images obtained for calves, and to compare them to human

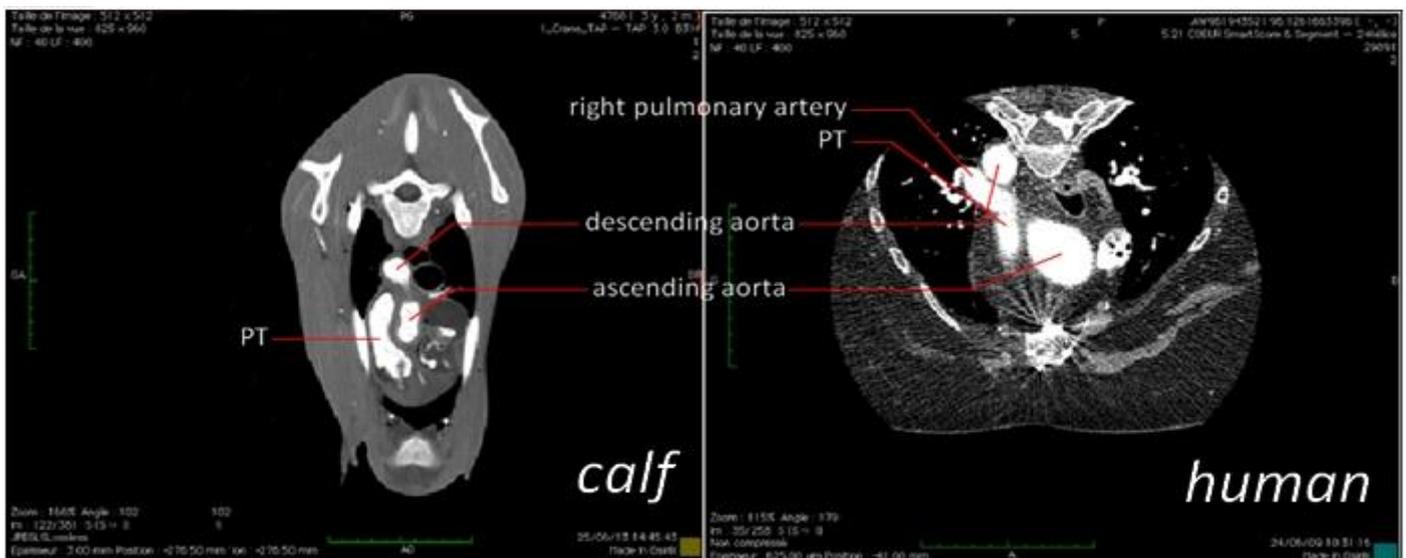
values that CARMAT® society has at its disposal. However, this would have implied to scan all of our 20 carcasses, which is practically unworkable. From calf mean and standard deviation factors for each variable, a confidence interval (CI) was built, with a 5% probability level ( $\alpha$ ).

**Table 3:** Confidence intervals of variables of interest factors

**Tableau 3 :** Intervalles de confiance des facteurs des variables d'intérêt

	CI ( $\alpha = 0.05$ )	Lower bound	Upper bound	Humane mean factor	Belongs to CI?
TH	0.26388	2.06	2.59	2.18	Yes
TW	0.10151	1.06	1.27	2.54	Higher
CP	0.12127	1.86	2.11	2.10	Yes
CH	0.03605	0.43	0.50	0.90	Higher
CWi	0.07948	0.81	0.97	0.72	Lower
CL	0.06198	0.93	1.06	1.00	Yes
ST	0.01979	0.06	0.10	0.10	Yes
LVWT	0.03476	0.08	0.15	0.13	Yes
RVWT	0.05362	-0.01	0.10	0.04	Yes
TriP	0.25005	0.48	0.98	1.01	Higher
MitP	0.04631	0.49	0.58	0.90	Higher
A1	0.02376	0.47	0.52	0.64	Higher
PT1	0.20880	0.28	0.70	0.64	Yes
CrVCP	0.06300	0.30	0.43	0.57	Higher
CdVCP	0.04441	0.37	0.46	0.85	Higher
CSP	0.01401	0.07	0.10	0.24	Higher

So, quantitative comparisons can be done from these results. The first striking statement is that a Calf heart seems to be wider in proportions, but has a greater height than a human heart, with a similar perimeter. This goes hand in hand with qualitative comparison [15]. The explanation has to be found in the thoracic shape, which is flattened laterally in calves whereas flattened ventrodorsally in humans.



**Figure 5:** Scan image of compared cardiovascular structures, transversal plane

**Figure 5 :** Image scanner des structures cardiovasculaires comparées, plan transversal

As for ST, LVWT, RVWT and PT1, they are equivalent in both species. Unlike PT, A1 is greater in humans, with a wider mitral ostium when human tricuspid ostium is hardly wider. CrVCP, CdVCP and CSP are greater in humans than in calves too. It might be explained by the vessel orientation in both species. Some of them, like the aorta, are vertically oriented in humans, whereas it is more horizontal in calves. Nonetheless, this has to be studied in the light of hemodynamic statements (pressure and capacity, among others), which was ignored in this study.

As a result, this method can be applied to compare cardiovascular measurements between a calf and a man. Human scan images were given by CARMAT® [22]. According to the previous statements, but using only cardiac proportions (vessel proportions were not known), the proportionally closest calf was found. These scan images were compared in figure 5. It appeared that, as described in bibliography [15], the bovine thorax seems higher and less wide than the human thorax. This is also the case for the heart. As for the major thoracic vessels, even if they seem proportionally bigger in human thorax, human measurements altogether must be known to conclude the comparison with calf vessels.

## Conclusion

The study of Calf cardiovascular thoracic structures, in the light of knowledge in the human model, has shown that some anatomical aspects in both species are close enough to be compared to some extent. Indeed, besides a circulatory system with a similar general layout shown by scan images, many cardiac proportions remain similar regardless of the size difference between these species and are linked to thoracic template. The main differences that seem statistically significant are the heart global shape, differently flattened according to thoracic aspect, as well as a disparity between cardiac ostium diameters, greater in the human than in the bovine heart. These statements are established without taking into account many other factors, especially physiology, hemodynamics and compatibility between donor and recipient, which plays a major role in the transplant success.

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