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kathleen@dcpmail.org <kathleen@dcpmail.org> 19 de mayo de 2018, 15:15
Para: marieladassis@gmail.com
Cc: aquaticmammals@gmail.com

Dear Mariela Dassis,

Mariela - you will see both reviewers were pleased with your revision. Shane is traveling and I am overseeing his manuscripts. Please send your revised manuscript and any responses to reviewers to me directly by email (see my address below). If you can get me a revision within 3-4 weeks, I can include your manuscript in the next issue of the journal (to publish on July 15). Thanks! Cheers Kathleen

Based on the comments by reviewers and our reading of the manuscript, the editors of Aquatic Mammals are pleased to accept your manuscript "Echocardiographic Left Ventricular Structure and Function in healthy non-sedated Southern Sea Lions (Otaria flavecens)" (2018-0939) for publication in the next issue of the journal, pending appropriate revisions.

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Echocardiographic Left Ventricular Structure and Function in healthy non-sedated Southern Sea Lions (*Otaria flavecens*)

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Running head: Echocardiography in Southern Sea Lions

Abstract

The goal of this study was to test transthoracic echocardiography as method to characterize heart morphology and function in the Southern Sea lion (SSL) for health evaluation. Four clinically healthy captive SSLs (mean weight 110.0 ± 17.5 kg) were trained to be examined by transthoracic echocardiography at Mar del Plata Aquarium (Mar del Plata, Argentina). Two-dimensional guided M-mode images were obtained using a portable cardiovascular ultrasound system equipped with a 1.5-3.5 MHz convex 3S phased-array transducer. The mean left ventricular internal dimension at end-diastole was 73 ± 5.8 mm, the mean interventricular septum thickness and posterior wall thickness at end-diastole were 9 ± 1.1 mm and 8.9 ± 2 mm, respectively. Fractional shortening and ejection fraction were 44.6 ± 1.7% and 74.4 ± 1.7%, respectively. The left atrial diameter-to-aortic root index was 0.92 ± 0.03. The most suitable position for obtaining good quality images was the left lateral recumbency (with slight inclination to 45°), with the probe placed on the left side of the thorax, ventrally just near the sternum, at the level of the caudal portion of the left pectoral fin. The best acoustic window in relation to the breathing cycle occurred between inspirations. We successfully demonstrated that the *in vivo* structure and function of the SSL heart can be safely and effectively evaluated with the use of transthoracic echocardiography in captive trained animals. These data have clinical and research implications for evaluating diseases of the cardiopulmonary system in pinnipeds.

Keywords: Echocardiography, Trained behavior, Pinnipeds, Heart anatomy, Heart disease

Introduction

Cardiac abnormalities and diseases have been reported postmortem in marine mammals such as northern elephant seals (*Mirounga angustirostris*), pygmy sperm whale (*Kogia breviceps*), bottlenose dolphins (*Tursiops truncatus*), northern fur seals (*Callorhinus ursinus*), dwarf sperm whales (*Kogia sima*), Florida manatees (*Trichechus manatus latirostris*) and Southern sea otters (*Enhydra lutris*; Trupkiewicz et al., 1997; Kreuder et al., 2003; Bossart et al., 2007; Powell et al., 2009; Spraker & Lander, 2010; Gerlach et al., 2013). Only few studies have achieved the *in vivo* diagnosis of heart abnormalities in marine mammals through echocardiography, such as ventricular septal defects found in a harbor porpoise (*Phocoena phocoena*; Szatmári et al., 2016) and California sea lions (*Zalophus californianus*; Dennison et al., 2011a) or the foramen ovale and ductus arteriosus patency found in neonatal harbor seals (*Phoca vitulina*) (Dennison et al., 2011b).

Echocardiography is a useful technique for screening and routine follow-up of cardiac diseases. However, its use as a diagnosic tool requires knowledge of echocardiographic techniques and reference values for each species (Pereira & Pizzi, 2012). This information is not currently available for most of marine mammals, the only exception at this time being trained bottlenose dolphins (Chetboul et al., 2012; Miedler et al., 2015).

The southern sea lion (SSL) occurs throughout the coastal waters of South America from Peru to southern Brazil (Bastida & Rodríguez, 2003). Although its life history, ecology and physiology have been broadly studied, there are just few studies of its cardiovascular anatomy and physiology. The electrocardiogram of anaesthetized SSLs has been recently characterized (Dassis et al., 2016), but it did not include any evaluation of heart disease. The objective of this study was to test transthoracic echocardiography as method to characterize heart morphology and function in sea lions for health evaluation that can lead to improved veterinary care and animal welfare (Brando et al., 2010; Poser et al., 2011).

Materials and methods

Four healthy captive SSL (Table 1) were trained to allow veterinary examination at Mar del Plata Aquarium (Mar del Plata, Argentina). Animals were trained using standard operant conditioning with positive reinforcement (International Marine Animal Trainer's Association [IMATA], 2004; Brando et al., 2010) and following international and local ethical standards for wild animal manipulation (Institutional Committee for Care and Use of Laboratory Animals, http://www.mdp.edu.ar/exactas/index.php/cicual). Animals were considered healthy on the basis of clinical examination performed by veterinarian as well as behavior, food intake, and body mass.

Transthoracic echocardiographic examinations were performed indoors using a portable cardiovascular ultrasound system (Sonoscape A6V® with a 1.5-3.5 MHz convex 3S phased-array transducer, Providian Medical Equipment, Ohio, USA). Second harmonic tissue imaging was used to obtain optimal two-dimensional guided M-mode images. The video images were then analyzed off line for M mode and two-dimensional measurements. Each variable was assessed twice during the cardiac cycle for which the endocardial borders were considered well defined. Measured and calculated values were expressed as a mean ± SD. Heart rate (HR) was determined from M mode measurements by calculating the time interval between the beginning of one cardiac cycle to the beginning of the next.

To obtain the best images, animals were examined from three body positions: left and right lateral recumbency (sensu strictu and intermediate positions with slight inclination to 45°), dorsal recumbency, and upright standing position over a two steps training platform. Differences in the transducer position and orientation were also tested for each body position. Due to the apneustic respiratory pattern of SSL, the acoustic window was also evaluated in relation to the different phases of the breathing cycle. Once the optimum procedure for image acquisition was determined (*see results*), a total of twelve echocardiographic examinations were performed on three different days over a three-week period. All the echocardiographic variables presented in Table 1 result from the average of 3 different echocardiographic examinations in each animal.

Results

All echocardiographic images obtained were interpretable and all variables could be calculated (Fig. 1; Table 1). The mean HR was 75 ± 11.1 bpm. The left atrial diameter-to-aortic root index was 0.92 ± 0.03 and all animals presented larger dimensions of aortic root compared to the left atrium.

The most suitable position for obtaining good quality two-dimensional M-mode images was left lateral recumbency (sensu strictu and intermediate positions with slight inclination to 45°) with the probe placed on the left side of the thorax, ventrally just near the sternum at the level of the caudal portion of the left pectoral fin (Figure 2).

The acoustic window determined in relation to the breathing cycle was short (ca. 3-5 sec) and located between the end of the expiration and the beginning of the next inspiration. During this period, the quality of the heart images obtained was noticeably better because chest movement was minimal and there was less air in the lungs.

Discussion

These are the first published values for left ventricular structure and function in SSLs and are comparable with other marine mammals such as dolphins (Sklansky et al., 2006; Chetboul et al., 2012; Miedler et al., 2015) and manatees (Gerlach et al., 2013, 2015) and similar to those reported in some terrestrial mammals such as dairy cattle (Hallowell et al., 2007), sheep (Locatelli et al., 2011), gorillas (Murphy et al., 2011), grizzly bears (Nelson et al., 2003) and humans (Lang et al., 2015).

In contrast to the cardiac anatomy in humans, all SSL of this study showed larger dimensions of the aorta with respect to the left atrium. This agrees with data reported for other aquatic mammals of a slightly dilated segment in the ascending aorta called the aortic bulb, which dampens changes in blood pressure during the bradycardia associated with diving (Chetboul et al., 2012; Kirkwood & Goldsworthy, 2013; Guimaraes et al., 2014).

The average resting HR (75 ± 11.1 bpm) was similar to the value (73 ± 14 bpm) previously reported for this species, which was estimated from 300 wild SSLs resting on land (De León, 2016). This indicates a little or no stress response to handling and examination, which enhances the reliability of the echocardiographic results. Successful training techniques that enable health examinations without restraint or sedation have greatly enhanced the daily care and husbandry of marine mammals (Brando et al., 2010).

Based on our results, potential heart abnormalities could be preliminarily evaluated enabling a cardiac disease diagnosis. In this sense, a previous left atrial enlargement suggested by an ECG with a notched p wave of unusually high amplitude and a deviated positive electrical axis found in one free ranging SSL female (Dassis et al., 2016) could be confirmed by echocardiography. With regards to the animals in our study, the lack of previous values for this species prevents the accurate identification of heart abnormalities or pathologies. However, no evident heart abnormality was suggested by any of the images obtained.

Although the standard guidelines developed for dog and cat echocardiography can be extended to other mammalian species, differences in anatomical and physiological parameters in marine mammals (such as heart size, heart and respiratory rate) can have a marked influence in the ability to carry out an optimal echocardiographic technique (Pereira & Pizzi, 2012). For example, respiratory cycle of the SSL consists of a rapid inspiration (ca. 2-3 sec.) followed by a long pause (between 5 and 80 sec.) and a rapid expiration (ca. 3-4 sec.), with no evident pause before next inspiration (Lyamin et al., 2002; De León, 2016; Fahlman & Madigan, 2016). Therefore, different phases of the breathing cycle involve differences in chest movement and the amount of air filling the lungs, conditions that affected the quality of heart images and determined a very short acoustic window at the end of the expiration.

Our study has established guidelines for the appropriate echocardiographic examination in this species including the type of transducer, transducer position, the animal position and the acoustic window in relation to the breathing cycle. We suggested that the optimum procedure to obtain successive images therefore involve two steps. First, the transducer operator should localize the probe in the correct position over the animal's chest (Figure 2) and second, the operator should wait for the exhalation to have the best acoustic window, a procedure that can be repeated as many times as necessary.

One limitation for this study was that all of the animals were female. Sexual dimorphism is pronounced in SSL, and this will influence the mean values for cardiac morphology. However, the large size and aggressiveness of adult males makes echocardiographic examination difficult without sedation. Although we focused on measurements of the left heart chambers and aorta, other cardiac structures were correctly identified as pulmonary artery, right ventricle and interatrial septum, but without obtaining reliable quantitative measurements. The use of cardiac ultrasound to evaluate non-sedated aquatic mammals has been limited by some anatomophysiological conditions to obtain optimal acoustic window such as lung interposition, large sternum and respiratory pattern (Sklansky et al., 2006; Chetboul et al., 2012; Miedler et al., 2015).

This study showed that *in vivo* left heart structure and function can be safely and effectively evaluated by transthoracic echocardiography in trained and non-sedated SSLs. Results reported here provide a baseline for future echocardiographic research in this species and other pinnipeds that will improve our ability to evaluate and diagnose cardiopulmonary abnormalities and diseases.

Acknowledgments

We thank Mar del Plata Aquarium and its qualified staff: Marcelo Villalba, Jose Luis González, Nicolás Cárdenas, Matías Vanoni and Javier Olguin for the sea lions training and management. We thank MV Adrián Faiella for guidance in fieldwork and data acquisition. We thank Ricardo Frid, from Fridimex (http://www.fridimex.com.ar/) for providing the transducer for the ultrasound system.

Figure legends

Figure 1. Two-dimensional (left panel) and M-mode (rigth panel) echocardiographic image of the left ventricle in a Southern sea lion *Otaria flavescens* with details of the left ventricular internal dimension at end-diastole (a and b) and end- systole (c).

Figure 2. Transthoracic examination in a Southern sea lion *Otaria flavescens* at left lateral recumbency (with slight inclination to 45°), with detail of transducer orientation and position.

Table legends

Table 1. Mean ± SD values of echocardiographic measurements obtained in trained non-sedated Sothern Sea Lions (*Otaria flavescens*) from two-Dimensional and M mode transthoracic examinations. Echocardiographic variables resulted from the average of 3 different echocardiographic measurments in each animal.

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