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Ultrasonographic examination of the heart in sheep

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ABSTRACT

In sheep, echocardiography (ultrasonographic examination of the heart) includes two-dimensional examination, with which right or left parasternal images are taken, employed to image the various anatomical structures of the heart, M-mode examination, employed to image one-dimensional views of the heart visualised over time, and conventional Doppler examination, employed to record normal blood flow velocities through the cardiac valves and great vessels and to detect abnormalities of blood flow through these structures and through the interatrial or interventricular septum. Several studies have proposed reference values for the various echocardiographic parametres in healthy sheep; however, differences occur between authors, which may arise from lack of consistency regarding animal age, sex, weight, status of consciousness, breed, as well as technique employed. There is limited scope in clinical use of the methodology, due to the small incidence risk and clinical significance of cardiac disorders in sheep; there are a few case reports of specific heart diseases in sheep investigated by means of echocardiography; these include congenital heart defects, calcinosis, myocardial disease (myocarditis and cardiomyopathy), endocarditis, pericarditis and cardiac tumour. The main application of echocardiography in sheep refers to using the animals as models in human cardiovascular research, where it can be applied in models for development of functional indices, for evaluating systolic and diastolic left ventricular performance, for studying valvular disorders and for investigating effects of intracoronary microembolisation; results of echocardiography applied in sheep have indicated that the model is suitable for extrapolating results in humans.

Keywords: blood flow, calcinosis, cardiomyopathy, Doppler, echocardiography, endocarditis, heart, myocarditis, pericarditis, reference values, sheep, ultrasonography, valve

1. Introduction

Ultrasonographic examination of the heart (‘echocardiography’) is a widely-applied technique, which is of particular usefulness in the diagnosis of heart diseases. By using two-dimensional (2D) echocardiography, M-mode echocardiography or conventional Doppler echocardiography, a variety of heart diseases can be accurately diagnosed, e.g., pericardial effusions, vascular stenosis, tumours, intracardiac blood clots, valvular disorders. In most cases, the technique is performed transthoracically; the transducer is placed on the chest of the animal under examination, often as part of an ultrasonographic examination of the lungs (Scott, 2017). In recent years, three- or four-dimensional echocardiographic examination has also been developed, whilst findings of the technique can be improved by using contrast media.

There is some work referring to echocardiographic examination of sheep. In most cases, the relevant publications refer to use of sheep as animal models in cardiovascular research in humans. Nevertheless, there are also references related to the study of heart diseases of sheep. Objective of this review is to present the echocardiographic examination in sheep and to discuss its applications within the frame of sheep health management.

2. Methodology of ultrasonographic examination

2.1. Equipment

Echocardiography in sheep is challenging. The heart occupies a medial position in the chest; it is cranially located and covered by the olecranon and the caudal brachial muscles (Olsson et al., 2001). Further, configuration of the chest in this species, which is keel-shaped with narrow intercostal spaces, makes difficult to position the ultrasound transducer and limits the acoustic window (Olsson et al., 2001; Donadio Abduch et al., 2013). Moreover, presence of gas in the reticulo-rumen interferes with ultrasound beams, making difficult the acquisition of subcostal and apical views (Olsson et al., 2001; Leroux et al., 2012; Donadio Abduch et al., 2013).

Choice of transducer is important, in order to acquire good quality images. A sector transducer is more useful for echocardiography than a linear one (Long, 1995). The ultrasound beam of a sector transducer diverges from a relatively small footprint, which can be easily positioned between the ribs (Long, 1995). Further, linear transducers can produce a limited number of image planes, as they must be placed vertically between the ribs to maintain good contact with the skin (Long, 1995).

Transducer frequency is another factor to be considered, as it affects depth of penetration and resolution of the image (Long, 1995; Boon, 2011). Low-frequency transducers (2.0-3.5 MHz) allow sound waves to travel deeper into the tissues before weakening, which supports their use in animals with a larger surface area (Boon, 2011; Donadio Abduch et al., 2013); this is at the expense of resolution quality (Boon, 2011). In contrast, high-frequency transducers (4.0-8.0 MHz) offer better resolution, but do not penetrate to a sufficient depth and would be more suitable for small or young animals (Long, 1995; Boon, 2011; Donadio Abduch et al., 2013). In general, for sheep in the range of 13.5 to 23 kg bodyweight, transducers with a frequency of 5.0 to 7.5 MHz can be used, whilst in heavier animals transducers with frequencies up to 5.0 MHz would be more useful (Boon, 2011). Boon (2011) has suggested that when performing two-dimensional (2D) or M-mode echocardiography, it would be better to start scanning using a higher frequency transducer before changing to one with a smaller frequency, to allow for evaluation of its applicability. This would allow acquisition of images of improved quality for diagnostic purposes. However, in conventional Doppler echocardiography it is advised to change to a smaller frequency transducer, even if all cardiac views can be obtained with only one transducer (Long, 1995; Boon, 2011). Smaller frequency transducers are able to record higher velocities of blood flow and can provide increased signal strength at greater depths (Long, 1995; Boon, 2011).

2.2. Animal preparation and positioning

Before starting the examination, hair on the area on the right and left hemithorax of the animal, from the 3rd to the 5th intercostal space, just behind the elbows, should be clipped, starting 3 to 5 cm below the olecranon and finishing 5 to 10 cm above it (Hallowell et al., 2012; Leroux et al., 2012). The skin of the area is then cleaned with water or 70% alcohol solution and coupling gel is applied onto the transducer and the skin.

Sheep can be examined in the standing position or in lateral recumbency, scanned from underneath. When animals are scanned in lateral recumbency for long periods of time, care must be taken to ensure that they would not develop bloat (Long, 1995). When sheep are examined in the standing position, they should be placed into a restraint crate (Moses and Ross, 1987; Boon, 2011). The echocardiographic examination starts from the right side of the animal for all standard long and short axis views and is then completed on the left side. In most cases, in order to acquire good quality standard images, the right front leg of the animal can be pulled forward and slightly abducted. The left front leg should also pulled forward to facilitate echocardiographic examination from the left side (Boon, 2011).

2.3. Two-Dimensional (2D) echocardiographic examination

When scanning from the right hemithorax, the transducer should be held with the thumb of the left hand on the reference mark. Conversely, when scanning from the right hemithorax, the transducer should be held with the thumb of the right hand on the reference mark (Long, 1995). The reference mark is used to orientate the image on the screen and defines the plane in which the sound leaves the transducer. Further, a symbol is displayed on the screen of every ultrasound machine, which indicates the direction of the reference mark in the body; in the case of standard echocardiographic examination, it is displayed on the right side of the image (Boon, 2011).

Descriptions herebelow regarding probe orientation during echocardiographic examination in sheep are based on previous descriptions in horses (Bonagura et al., 1985; Reef., 1990; Long et al., 1992; Long, 1995; Boon, 2011) and include adaptations from Hallowell et al. (2012).

2.3.1. Right parasternal images

In order to get the right parasternal long axis view of the left ventricle (LV) (four-chamber view), the transducer is placed on the 4th or 5th right intercostal space. The reference mark of the transducer is at the 12 o’clock position (0 ° rotation) and the transducer is oriented caudally (the cable can be pulled into the leg of the animal). The right ventricle (RV) and the tricuspid valve (TV) can be seen at the top of the image and the left ventricle (LV) and the mitral valve (MV) at its bottom. In sheep, a portion of the right atrium (RA) may be seen on the top right of the image and a portion of the left atrium (LA) on the bottom right. From this view, by slightly moving the transducer in a more dorsal location with the axial beam angled more dorsally and slightly cranially, a right parasternal long axis apical view is obtained and a larger portion of the right atrium may be visible (Long, 1995).

From the right parasternal long axis view of the left ventricle, if the transducer is held perpendicular to the thoracic wall and rotated clockwise to bring the reference mark at the 1 o’ clock position (0 to 30 º rotation), the right parasternal caudal long axis view of the left ventricular outflow tract (LVOT) is obtained. In this view, the right ventricle is at the top of the image and the tricuspid valve, which is at the top right, is visible in 75% to 80% of sheep (Hallowell et al., 2012). Below the right ventricle and the tricuspid valve, are the left ventricular outflow tract on the left and the aorta (Ao) on the right of the image. The aortic valve (AV) cusps are clearly seen as curved semilunar lines concave to the aorta. In sheep, this is considered to be the best view to detect abnormalities of tricuspid valve, aortic root and aortic valve.

In sheep, the right parasternal cranial long axis view of the right inflow and outflow tracts is obtained when the transducer is placed on the 4th intercostal space and is held with the reference mark pointed at the 12 o'clock position (0 º rotation). The resultant image includes the right ventricular outflow tract (RVOT), the pulmonary valve (PV) and the tricuspid valve.

Further, in order to obtain the short axis views of the mitral and aortic valves, the probe needs to be moved ventrally, down the chest wall and angled dorsally (Hallowell et al., 2012). For all right parasternal short axis views, the transducer is placed on the 4th right intercostal space, 2 cm above the point of the olecranon. The right parasternal caudal short axis view of the left ventricle can be obtained when the transducer is rotated 90 º from the 12 o'clock position. The resultant view includes a crescent-shaped right ventricle at the top of the image, a circular-shaped left ventricle below the interventricular septum (IVS) and symmetrically shaped papillary muscles located in the left ventricle. The shape of the left ventricle in this view is described as that of a mushroom (Boon, 2011). From this view, by pointing the transducer towards the base of the heart (i.e., dorso-cranially), the mitral valve comes into view. The leaflets of the mitral valve appear as oval-shaped in the left ventricle chamber, when the valve is open (diastole) and as touching lines when the valve is closed (systole). The right ventricle is seen as crescent-shaped at the top of the image. Pointing the transducer further dorsally a right parasternal short axis view of the aortic valve can be obtained. In sheep, this view also includes the mitral valve, the right ventricle and the tricuspid valve (Hallowell et al., 2012). By pointing the transducer slightly more dorsally and cranially, towards 1 o'clock, and by funning the crystals right and left (cranial and caudal), the base of the heart with the pulmonary artery may come into the view (Boon, 2011). The pulmonary valve is located at the 2 to 4 o'clock position and the left atrium is visible between the right atrium and the right pulmonary artery branch at the 8 to 9 o'clock positions. The bifurcation of the pulmonary artery is usually seen in-between the 5 and 6 o'clock positions, with the right main pulmonary artery extending from right to left under the descending aorta (Boon, 2011).

2.3.2. Left parasternal images

Images are obtained from the left hemithorax, usually when better visualisation of the mitral, aortic and pulmonary valves is desired (Reef, 1990). In sheep, by placing the probe on the left 4th or 3rd intercostal space with the reference mark at 12 o'clock position (0 º rotation) and the transducer pointing cranio-dorsally, a left parasternal cranial long axis view of the right ventricular outflow tract is obtained. Structures visible in this view include the aorta, the pulmonary artery, the pulmonary valve, the right ventricular outflow tract and the tricuspid valve (Hallowell et al., 2012).

When the probe is placed on the left 4th intercostal space, cranio-dorsally oriented with 0 º to 20 º rotation, the left parasternal long axis view of the left ventricular outflow tract is acquired. This view in sheep includes also the aorta, aortic valve, caudal vena cava (CdVC) and the left ventricular outflow tract (Hallowell et al., 2012). The left atrium, the mitral valve, the left ventricle and the caudal vena cava can be imaged in a left parasternal caudal long axis view, when the transducer is held on the left 5th or 4th intercostal space, 2 to 3 cm above the olecranon and is oriented caudally with 0 º rotation (Hallowell et al., 2012).

Left parasternal apical four- or five-chamber views are difficult to obtain in sheep. Kirberger and Van Den Berg (1993) succeeded to get these views by scanning from below the sheep, which laid on the side, and placing the transducer on the 4th or 5th intercostal space, just dorsal to the sternum and aiming dorsally and to the right. These authors have suggested that the heart moved closer to the thoracic wall in left lateral recumbency, which facilitated acquisition of better quality images. The apical four-chamber view included the left atrium, the mitral valve, the left ventricle and a small portion of the right ventricle and the right atrium (Long, 1995), whilst the five-chamber view included the above structures and the aorta.

In order to obtain the short axis structures of the above areas, the probe is held perpendicular to the long axis plane, scanning from apex to base, as described for the right parasternal short axis views.

2.4. M-mode echocardiographic examination

The M-mode (motion mode) echocardiographic examination is a one dimensional view of the heart, visualised over time (Reef, 1990; Boon, 2011). It is often used to measure left and right ventricular internal diametres (LVID, RVID), interventicular septum and left ventricle wall (LVW) thickness. It is also used to record subtle changes in wall and valve motion. M-mode images are obtained from the real time 2D echocardiographic examination, by placing the cursor over the structures of interest. On every M-mode image there is a Y axis, which represents the depth through the heart, and an X axis, which represents time. Standard M-mode echocardiographic images obtained from a 2D examination include the left ventricle, mitral valve and aortic valve views.

The M-mode of the left ventricle view can be obtained by scanning the animal either from the right hemithorax or from the left hemithorax. In the first case, images of the left ventricle are acquired from the right parasternal long axis view of the left ventricle (four-chamber) and the right parasternal caudal short axis view of the left ventricle, by placing the cursor perpendicularly to the septum through the left ventricle. The resultant image presents the right ventricle on the top, followed by the interventricular septum, the left ventricle and the left ventricular wall at the bottom. Measurements obtained from this view include the interventricular septum and left ventricular wall thickness and the left ventricular internal diametre with the right ventricular internal diametre. In case the animal is scanned from the left hemithorax, an M-mode image of the left ventricle can be obtained from the left parasternal caudal long axis view.

According to recommendations of the American Society of Echocardiography, all M-mode measurements should be taken by using the ‘leading edge’ method. This means that measurements are taken from the leading edge of the first endocardial surface to the leading edge of the second endocardial surface (Lang et al., 2015). End diastolic measurements are taken at the onset of the QRS complex on the ECG trace and end systolic measurements are taken at the maximal excursion of the interventricular septum, unless septal movement is abnormal (Long, 1995; Boon, 2011). An estimation of the contractility of the left ventricle can be made from its view, by measuring the fractional shortening using the below formula.

FS = 100 × (LVIDd - LVIDs) / LVIDd, where FS: fractional shortening, LVIDd: left ventricular internal diametre diastole and LVIDs: left ventricular internal diametre systole.

Modern ultrasound machines calculate automatically this parametre from the measurements obtained.

The M-mode imaging of the mitral valve is acquired from the right parasternal long axis view of the left ventricle and the right parasternal short axis view at the level of the mitral valve, by placing the cursor perpendicularly over the tips of the mitral valve’s leaflets. During diastole, an M-shaped line is visible in the M-mode image, with the first peak representing early diastolic filling (E point) and the second peak representing atrial contraction at the end of diastole (A point). The E point normally should almost touch the interventricular septum and the distance between the E point and the interventricular septum (EPSS) is an index of left ventricle function.

The M-mode images of the aortic valve can be obtained from the right parasternal caudal long axis view of the left ventricular outflow tract and the right parasternal caudal short axis view at the level of the aorta, by placing the cursor through the aortic valve. In M-mode, the aortic valve is presented as a line in the centre of the aorta during diastole and as a box during systole. Measurements obtained by M-mode imaging include the aortic diametre and the ejection time (ET), which is the time from opening of the aortic valve leaflets at the onset of systole to their closure at the end of systole.

2.5. Conventional Doppler echocardiography

2.5.1. Basics of conventional Doppler echocardiography

Conventional Doppler echocardiography (hereafter: ‘Doppler echocardiography’) is used to record normal blood flow velocities through the cardiac valves and great vessels and to detect abnormalities of blood flow through these structures and through the interatrial or interventricular septum. Doppler examination is usually combined with 2D real time echocardiography and views utilised to place the sample volume, are those described for the routine 2D echocardiographic examination (Reef, 1990). The most accurate blood flow recordings are obtained when the ultrasound beam is placed as parallel as possible with the direction of blood flow. For this reason, imaging planes used are those offering the best possible alignment with the Doppler beam. Several planes can be available to record blood flow and each of them should be tested to assure the most accurate readings (Boon, 2011).

The decision regarding which technique of Doppler echocardiography to use depends mainly on the reason of flow interrogation (Long, 1995; Boon, 2011).

Pulsed wave (PW) Doppler can be used to record flow information from a specific location, in which case the PW gate is placed on the region of interest. The property of PW Doppler to record blood flow in specific areas of the heart is particularly useful, especially in assessing diastolic flow. However, with this technique there is a limit for the maximum velocity that can be recorded. This limit ('Nyquist limit') is equal to the half of the pulse repetition frequency (sampling rate). Velocities exceeding the Nyquist limit, often associated with turbulent flow, cannot be recorded accurately and aliasing of the signal would occur.

Continuous wave (CW) Doppler is able to record very high velocities accurately. However, with this technique, recordings are obtained across the entire axis of the ultrasound beam and accurate localisation of the high velocity flow is not possible.

Colour flow (CF) Doppler is a type of PW Doppler, which provides a colour map of the size, shape and direction of blood flow. It has the same limitations as the PW Doppler regarding high flow velocities exceeding the Nyquist limit. The main advantage of the technique is that it enables simultaneous recordings of velocities from numerous sampling points within a sector. Further, CF Doppler makes easier identification of small regurgitant jets and allows proper alignment with all valvular flows (Long, 1995; Boon, 2011). CF Doppler provides semi quantitative information of the severity of a regurgitant jet. Further, colour and pattern of the flow can also give an idea of the velocity of the blood. Turbulent flows can be presented with a mosaic pattern, which either encodes the varying velocities of the flow with different colors (red, blue, yellow and cyan) or encodes the areas of disturbed flow by mixing green with other colors usually red or blue (Boon, 2011).

By convention, the direction of blood flow is considered positive (flow moves towards the transducer) when the flow is recorded above the baseline or has red colour in CF Doppler or negative (flow moves away from the transducer) when the flow is below the baseline or has blue colour in CF Doppler.

2.5.2. Normal Doppler flow patterns

The pattern of the aortic flow is negative (below the baseline), as blood in the aorta moves away from the transducer. The optimal plane to obtain the aortic flow is the left parasternal apical five-chamber view. In sheep, Kirberger and Van Den Berg (1993) recorded normal aortic flow from the apical five-chamber view with the sampling cursor in the ascending aorta at the dorsal end of the sinus Valsava. The flow had a rapid acceleration phase (downstroke) with the peak velocity being reached within the first third of systole and a deceleration phase (upstroke). The decelerating velocities were often poorly visible.

The pulmonary artery (PA) flow profile, similarly to the aortic flow, is displayed as negative (below the baseline). It has a symmetrical profile with peak velocity about midway during ejection of blood (Boon, 2011). In sheep, it has been recorded from the right parasternal short axis with the pulmonary valve and the pulmonary artery in view (Kirberger and Van Den Berg, 1993).

In sheep, similarly to other animals, the mitral valve flow is best recorded from the left parasternal apical four-chamber view (Kirberger and Van Den Berg, 1993). The flow is positive and laminar during diastole with two main peaks, which create an M-shaped pattern similar to the M-mode images of the mitral valve motion. The initial peak velocity (E wave) corresponds to the rapid filling of the left ventricle in early diastole. The second peak velocity (A wave) corresponds to atrial contraction in the end of diastole. Distance between the two peaks depends on the heart rate (HR). The faster the heart rate, the shorter the diastolic period, the closer the two peaks move together. In sheep, up to a heart rate of approximately 95 bpm, the two peaks were separated; at more increased heart rates, the two peaks begun to fuse and eventually became indistinguishable (Kirberger and Van Den Berg, 1993).

Further, tricuspid valve flow has been recorded from the left parasternal apical four-chamber view with the sampling cursor placed on the right ventricle, just ventral to the atrioventricular annulus (Kirberger and Van Den Berg, 1993). The profile of the tricuspid valve flow, similar to that of the mitral valve flow, is M-shaped with E and A waves.

3. Echocardiographic reference values in healthy sheep

Several studies have proposed reference values for echocardiographic parametres in healthy sheep. Moses and Ross (1987) have reported normal M-mode echocardiographic values in adult non-sedated sheep. In that study, 21 sheep (of which 20 females), aged 2 to 5 years, of various breeds, including purebred Hamphshire, Dorset and Suffolk animals, were studied. Bodyweight was significantly correlated with left ventricular internal dimensions in systole and diastole, septal thickness in systole and diastole, aortic root and left atrial dimensions. Finally, significant correlations were found, when heart rate was compared with bodyweight, ejection time, velocity of circumferential fibre shortening, mean velocity of mitral valve mid-diastolic closure and left atrial dimension.

A study conducted in adult non-sedated sheep by Hallowell et al. (2012) has aimed to report reliability of the echocardiographic technique and a full set of normal internal cardiac dimensions and time indices. In total, 51 Suffolk-cross sheep (of which 40 were female), aged 2 to 4 years, were included into the study. The technique and measurement repeatability and reproducibility were evaluated as excellent for all cardiac dimensions and most of the time indices. Further, results of the study for several echocardiographic parametres were compared with results of previous studies. Particularly in sheep, significant differences were found between Hallowell et al. (2012) and Moses and Ross (1987) in the right ventricular internal diametre in diastole and the left ventricular internal diametre in both diastole and systole. These differences were attributed to the variation of the level, at which measurements had been obtained and to the improved ultrasonographic technology available in the most recent study.

Yadegari (2014) aimed to establish normal reference echocardiographic values for Lori-Bakhtiary sheep. In total, 21 non-sedated Lori-Bakhtiary sheep (of which 10 female), aged one year, were included into the study. Significant differences were found between males and females in the right ventricular size at end systole, the left ventricular size at end systole, the thickness of the left ventricular free wall at end systole and the left atrial size at end diastole. A summary of reference echocardiographic values based on previous studies is in Table 1.

In a study conducted by Acorda and Pajas (2015), reference echocardiographic values in non-sedated sheep were reported, according to sex, age and reproductive status of the animals. Significant differences were evident between male and female animals. In both sexes, there was a significant correlation between bodyweight and most echocardiographic values. Several echocardiographic parametres changed significantly with age of animals. Pregnancy and lactation appeared to be of importance in cardiac function and various echocardiographic parametres. In that study, no standard technique was employed for visualisation of the heart; some animals were scanned in the standing position and some others in right lateral recumbency (Acorda and Pajas, 2015). The echocardiographic values for male or female sheep, proposed by the authors are summarised in Table 2.

Kirberger and Van Den Berg (1993) have determined transvalvular blood flow variables and described transvalvular blood flow patterns in apparently healthy, non-sedated sheep by using pulse-wave Doppler echocardiographic examination. In total, 20 Merino ewes, aged 1.5 to 5 years, at the first stage of pregnancy were included into the study. Animals were examined in the standing position or in lateral recumbency. It was concluded that, in sheep, mean ratio of early peak velocity (E wave) to late pick velocity (A wave), termed E:A ratio for the mitral valve, was smaller than 1. This parametre is used to assess ventricular diastolic function (Appleton et al., 1988); its reference value is considered to be always >1 in humans and dogs (Feigenbaum, 1986; Kirberger and Van Den Berg., 1993). In contrast, E:A ratio for the tricuspid valve was similar to that found in the dog (Kirberger and Van Den Berg., 1993), i.e. >1. Doppler derived echocardiographic values for all four cardiac valves are in Table 3.

All above studies have published reference echocardiographic values for apparently healthy, non-sedated sheep. Sheep are considered to be suitable animal models for human cardiovascular research (Borenstein et al., 2006; Locatelli et al., 2011; Zaragosa et al., 2011; Poser et al., 2013). Further, the echocardiographic examination is a non-invasive, sensitive and accurate method allowing evaluation of cardiac morphology and function, which has driven some researchers to establish baseline echocardiographic values in sedated sheep. Locatelli et al. (2011) have studied 69 lightly sedated adult male Corriedale sheep by means of echocardiographic examination. Absolute and body surface area normalised left ventricular dimensions in sheep were compared to standardised values in normal human adults. Indices of cardiac systolic and diastolic function in sheep were also compared with corresponding indices in humans. Most normalised left ventricular dimensions as well as indices of cardiac systolic and diastolic function in sheep were within the reference ranges applicable in humans. It was concluded that left ventricular echocardiographic parametres in young adult sheep were similar to corresponding parametres in humans; hence, sheep would be a reliable model for studying human heart diseases (Locatelli et al., 2011).

Poser et al. (2013) aimed to establish two-dimensional, M-mode and Doppler reference echocardiographic values in sedated, growing female sheep. In total, 8 lambs, aged 80 to 150 days, were studied over a 12-month period. The animals underwent echocardiographic examinations 90, 180, 270 and 360 days after inclusion into the study. All the two-dimensional and M-mode echocardiographic parametres, except for Left Ventricular internal diametre in systole and diastole (LVIDs, LVIDd) and fractional shortening (FS), were positively correlated with age and bodyweight. In contrast, no significant correlations were found between Doppler-derived parametres and age. With regard to use of sedation, although effects of the drugs used were considered to be minimal, the possibility of some of functional echocardiographic parametres to have been influenced could not be ruled out.

Ultrasonographic studies of the heart of sheep under *in vitro* conditions have been described by Karimi et al. (2008) and Aissi (2011). In the first study, 60 hearts of healthy sheep were collected following slaughter and imaged under water immersion using B-mode echocardiography. Thickness of left ventricle wall, right ventricle wall and interventricular septum was measured ultrasonographically and by a biometrical method (ruler). In the second study, 27 hearts of healthy sheep were collected and were examined after immersion into water, from a 2 cm distance. Thickness of left ventricular wall, right ventricular wall and interventricular septum was measured and compared to those obtained by biometrical measurements. Results of both studies indicated that biometrical methods and ultrasonography produced comparable measurements, confirming the accuracy of cardiac ultrasonographic examination as a method.

The above studies lacked consistency regarding age, sex, weight, status of consciousness, breed and echocardiographic technique employed. In some studies, techniques more commonly indicated for small animals (Moses and Ross, 1987; Locatelli et al., 2011; Poser et al., 2013) were employed, whilst in others echocardiographic techniques for horses (Hallowell et al., 2012; Yadegari, 2014) were used. Further, some studies were conducted in sedated animals, whilst others in non-sedated animals. However, all studies have contributed to the assessment of normal heart function in sheep.

4. Ultrasonographic examination for diagnosis of cardiovascular diseases in sheep

In domestic animals, cardiac congenital malformations account for 9 to 12% of all congenital defects (Dennis and Leipold, 1968; Saperstein et al., 1975; Dennis, 1993; Haist et al., 2009). In sheep, incidence risk of such disorders is estimated to be 0.1% (Dennis and Leipold, 1968; Dennis, 1993).

The most common congenital heart abnormality in sheep is ventricular septal defect (Dennis and Leipold, 1968; Hughes et al., 1972; Dennis, 1993), with the majority of these defects located in the membranous portion of the septum immediately below the tricuspid valve (Dennis and Leipold, 1968). The size of ventricular septal defects is variable, but in sheep they are usually 5 mm in diametre (Dennis and Leipold, 1968). Echocardiographic reports of ventricular septal defects are lacking in sheep. These defects can be visualised from a 2D right parasternal long axis view of the aorta and a short axis view immediately bellow the aortic valve level (Long, 1995; Boon, 2011). The significance (size) of these defects can be estimated by measuring the maximum flow velocity through the defect and calculating the pressure gradient between right and left ventricle (Long, 1995; Boon, 2011). Reports of other congenital heart abnormalities in sheep are very few and most of them identified during post-mortem examination. These include tetralogy of Fallot (Dennis and Leipold, 1968), cardiomegaly (Dennis and Leipold, 1968; Hughes et al., 1972), tricuspid valve atresia (van der Linde-Sipman and van den Ingh, 1979; Bisaillon et al., 1982), persistent *truncus arteriosus* (Haist et al., 2009), patent *ductus arteriosus* (Shivaprakash and Rao, 1997), hypoplastic left ventricle with double outlet right ventricle (van der Linde-Sipman, 1978), as well as other defects diagnosed less frequently (Hartley and Kater, 1962; Dennis and Leipold, 1968; Hughes et al., 1972). Many of these abnormalities can be detected by 2D echocardiography or, in the case of defects which are difficult to visualise, e.g., patent *ductus arteriosus*, by colour flow Doppler (Long, 1995). There is only one case report in the literature that involves echocardiography and describes congenital malformations in sheep. Sameluck et al. (2003) have presented a case report of a 12-week old lamb with patent *ductus arteriosus* and atrial septal defect; echocardiographically, there was a massive dilation of both ventricles, with the atrial septum bulging out into the right atrium and having a centrally located defect; pathological examination identified a wide patent *ductus arteriosus*, which had not been imaged echocardiographically.

Calcinosis is a chronic disease caused after ingestion of *Trisetum flavescens* (Gufler et al., 2005; Franz et al., 2007). Histological findings include cardiac dilatation, calcification of the aorta and other arteries, nephrocalcinosis, calcification and emphysema of the lungs, ascites, hydrothorax and hydropericardium (Braun et al., 2000; Gufler et al., 2005). Gufler et al. (2005) have found, by using echocardiographic examination, aortic stenosis and calcification of aortic and pulmonic valves in sheep after ingestion of *T. flavescens*. However, in a study conducted by Franz et al. (2007), ultrasonographic diagnosis of calcified areas in the heart valves of sheep was difficult and calcific lesions were identified mainly by histological examination.

Myocardial disease (myocarditis or cardiomyopathy) is infrequently diagnosed in sheep. Myocarditis may be the consequence of microbial infection. Cardiomyopathy is defined as a chronic disease of the heart muscle with no underlying anatomical valvular disease, congenital defect of the heart or vessels or pulmonary disease (Reef and McGuirk, 2015). In large animals, dilated cardiomyopathy is most common (Guglielmini, 2003; Buczinski et al., 2010; Reef and McGuirk, 2015). Cardiomyopathy can be inherited or acquired. In sheep, acquired cardiomyopathy has been associated with monensin and selenium toxicosis (Newsholme et al., 1983; Smyth et al., 1990); gross- and histo-pathological findings include damage of the conductive and contractile myofibres in the heart (Smyth et al., 1990) and myocardial lesions with predominantly epicardial distribution (Newsholme et al., 1983). Selenium deficiency is also associated with cardiomyopathy in sheep (Robson, 2007; Gunes et al., 2010). Microscopic examination of the heart reveals hyaline degeneration and necrosis in the sub-endocardial and sub-epicardial regions of the myocardium.

In cases of myocarditis, echocardiographic findings may be normal (Reef and McGuirk, 2015), whilst in animals with sustained ventricular tachycardia and decreased cardiac output, findings may include small ventricular internal diametre, thickening of the left ventricular free wall and interventricular septum and decreased shortening fraction (Reef and McGuirk, 2015). Typical echocardiographic findings in cases of dilated cardiomyopathy include decreased thickness of interventricular septum and left ventricular free wall, reduced myocardial contractility (reduced shortening fraction and ejection fraction), increased atrial size and increased end-systolic and end-diastolic ventricular dimensions (Buczinski et al., 2010; Boon, 2011; Reef and McGuirk, 2015). The dilatation of the left and/or right ventricles may cause mitral and/or tricuspid regurgitation, respectively (Buczinski et al., 2010; Boon, 2011).

In sheep, there are very few clinical reports of endocarditis (Scott, 2015), possibly because the disease is presented with no audible murmur (Scott, 2015). Incidence risk of vegetative endocarditis in sheep is increased in ewes 2 to 4 months after lambing, which would indicate the uterus is a potential source of infection (Scott, 2015). Vegetative lesions of the heart valves are difficult to image with conventional equipment and colour flow Doppler is necessary for definitive diagnosis (Scott and Sargison, 2010).

Pericarditis is the inflammation of the pericardium, which results in accumulation of fluid between the visceral and parietal pericardium (Reef and McGuirk, 2015). In sheep, diseases which may result in development of pericardial effusion, e.g., *Mannheimia haemolytica* infections, often cause sudden death (Scott and Sargison, 2010), which does not allow time for detailed diagnostic investigations. For this reason, echocardiographic examination is rarely performed (Scott and Sargison, 2010) and there are only a few reports of traumatic pericarditis diagnosed post-mortem in sheep. Abo-shehadeh et al. (1991) have presented a case of traumatic pericarditis in a 4 month-old Awassi lamb; post-mortem examination revealed a long wire penetrating the pericardium and the left ventricle; the pericardium was thickened and fused with the epicardium. There was fibrinous pericarditis, epicarditis and focal necrotic endocarditis of both ventricles. Torki et al. (2011) have reported four cases of traumatic reticulopericarditis in sheep. Gross pathological findings included presence of metallic needles or wires penetrating the reticular wall, pericardium, regional artery and myocardium. In one animal, acute cardiac tamponade and haemothorax were evident, whilst in the other three, the main findings were pyothorax, enlargement and thickening of the pericardium and peritonitis. *Arcanobacterium pyogenes* was isolated from fibrinopurulent exudates and disseminated abscesses of the affected organs. Microscopic analysis revealed fibrosis and inflammation with neutrophilic and lymphocytic infiltrate in the pericardium, epicardium and myocardium. In cattle, species in which traumatic reticulopericarditis is the most common cause of pericarditis (Streeter and Step, 2007; Bexiga et al., 2008; Buczinski et al., 2010), the main echocardiographic findings of pericarditis are pericardial effusion, which results to separation of the pericardium from the epicardium. When the pericardial effusion is haemodynamically significant, right ventricular diastolic collapse and right atrial collapse are common (Reef and McGuirk, 2015). Decreased left ventricular chamber dimension and paradoxical motion of the interventricular septum may be apparent (Reef and McGuirk, 2015).

Heart tumours may involve the pericardium, myocardium or endocardium or may be intracavitary in the left or right atrium or ventricle (Braun et al., 1995). There is only one report describing echocardiographic diagnosis of a cardiac tumour in sheep: Braun et al. (1995) have reported a case of a 6 year-old sheep with fibrosarcoma in the right atrium; echocardiographically, an echogenic round mass was identified in the right atrium immediately above the tricuspid valve, which moved freely, was not attached to the endocardium and had smooth surface. There was also dilation of the right atrium and ventricle and mild pericardial effusion.

5. Ultrasonographic examination of the heart in sheep health management

The diagnostic potential of echocardiographic examination is undeniable. It is a first-line technique for diagnosis of disorders of the cardiovascular system in companion animals and horses (Streeter and Step, 2007; Hallowell et al., 2012). It is a non-invasive, accurate and inexpensive diagnostic method, which allows early diagnosis in highly valuable animals and prevents the cost of ineffective treatment in cases with poor prognosis.

In contrast to cattle, animal species in which the method has been used for diagnosis of various cardiac disorders (Braun and Schweizer, 2001; Braun et al., 2001; Buczinski et al., 2010), in sheep, echocardiography is not widely employed and cardiovascular abnormalities in that species are of small significance and infrequently diagnosed in clinical practice. Most cardiac disorders are diagnosed post-mortem, by use of gross and/or histo-pathological examinations. As part of health management in sheep flocks, the technique might be applied in the examination of ram-lambs for selection as replacement animals, in order to exclude presence of congenital heart diseases. In adult sheep, it can be performed further to the examination of the lungs, mainly to diagnose cardiac problems, which would arise as part of pneumonic problems, e.g., to identify potential fibrinous pericarditis consequently to long-standing bacterial pneumonia (Scott, 2017). Nevertheless, correct application of the technique needs particularly proficient operators, as it presents particular challenges and requires increased experience.

The main application of echocardiography in sheep refers to using the animals as models in human cardiovascular research. The technique can be usefully applied in (among others) models for development of functional indices, for evaluating systolic and diastolic left ventricular performance, for studying valvular disorders and for investigating effects of intracoronary microembolisation, usually applied for investigating prosthetic valves, as model of heart failure and for studying myocardial infarction and atherosclerosis. The results of echocardiography applied in sheep during various studies have indicated that the model is suitable for extrapolating results in humans (Schmitto et al., 2008; Locatelli et al., 2011; Poser et al., 2013; Barka et al., 2015).

6. Concluding remarks

In sheep, echocardiography is used mostly as part of examination of animals during studies into human cardiovascular research, where it can provide useful findings. Although the technique has advantages in its use and can contribute to accurate diagnosis of heart diseases, there is limited scope in its clinical use, due to the small incidence risk and clinical significance of cardiac disorders in that species.

Conflict of interest statement

The authors have nothing to disclose.

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Table 1. Reference echocardiographic values in sheep proposed by three different authors.

	Authors		
Parametre	Moses and Ross, 1978	Hallowell et al., 2012	Yadegari, 2014
Age (years)	2-5	2-4	1
Weight (kg)	73.5±11.3	74±13	40.6± 4.36
RVIDd (cm)	2.03±0.56	1.42±0.36	1.44±0.05
RVIDs (cm)	1.36±0.53	0.84±0.14	0.38±0.05
RVWd (cm)	0.51±0.11	NR	NR
RVWs (cm)	0.94±0.22	NR	NR
LVIDd (cm)	5.17±0.74	4.42±0.54	2.94±0.18
LVIDs (cm)	3.23±0.46	2.62±0.35	1.28±0.11
LVWd (cm)	0.89±0.20	0.99±0.14	0.93±0.01
LVWs (cm)	1.53±0.33	1.50±0.28	1.69±0.10
IVSd (cm)	0.94±0.17	1.19 ±0.15	0.92±0.11
IVSs (cm)	1.41±0.22	1.55±0.18	1.45±0.16
Aos (cm)	NR	3.20±0.31	2.03±0.09
Ao (cm)	3.29±0.33	2.74±0.25	NR
LA (cm)	3.02±0.35	4.59±0.84	1.58±0.36
LA/Ao	0.92±0.10	NR	NR
FS (%)	37.2±5.7	40.2±4.8	56.3±2.7
LVVd (cm^3)	NR	NR	43.94±3.44
LVVs (cm^3)	NR	NR	16.7±1.1
SV (cm^3)	NR	NR	27.24±3.28
EF (%)	NR	76.9±4.9	61.80±3.3
HR (bpm)	96.1±21.6	84.3±5.4	87.9±3.8
EPSS (cm)	NR	0.42±0.09	0.68±0.06
LV output (cm^3 min^{-1})	NR	NR	2394±305
PA (cm)	NR	2.38±0.21	NR
PA/Ao	NR	0.89±0.06	NR
Thickening of LVW (%)	75.1±28.5	NR	NR
Thickening of IVS (%)	52.4±27.7	NR	NR
IVSd/LVWd	1.07±0.18	NR	NR
ET (s)	0.252±0.035	0.19±0.04	NR
PEP (ms)	NR	0.03±0.01	NR
Vcf (circumferences s^{-1})	1.52±0.28	NR	NR
DE slope (mm s^{-1})	347±55	NR	NR
EF slope (mm s^{-1})	205±49	NR	NR

Values are mean ± standard deviation.

Abbreviations. RVIDd: right ventricular internal diametre diastole, RVIDs: right ventricular internal diametre systole, RVWd: right ventricular wall thickness diastole, RVWs: right ventricular wall thickness systole, LVIDd: left ventricular internal diametre diastole, LVIDs: left ventricular internal diametre systole, LVWd: left ventricular wall thickness diastole, LVWs: left ventricular wall thickness systole, IVSd: interventricular septum thickness diastole, IVSs: interventricular septum thickness systole, Aos: aortic sinus, Ao: aorta, LA: left atrium, LA/Ao: left atrium to aorta ratio, FS: fractional shortening, LVVd: left ventricular volume diastole, LVVs: left ventricular volume systole, SV: stroke volume, EF: ejection fraction, HR: heart rate, EPSS: e-point to septal separation, LV output: left ventricular output, PA: pulmonary artery, PA/Ao: pulmonary artery to aorta ratio, LVW: left ventricular wall, IVS: interventricular septum, IVSd/LVWd: interventricular septum diastole to left ventricular wall diastole ratio, ET: ejection time, PEP: pre-ejection period, Vcf: velocity of circumferential fibre shortening, DE slope: mean velocity of early diastolic left atrioventricular valve opening, EF slope: mean velocity of mid-diastolic left atrioventricular valve closure.

NR: not reported.

Table 2. Reference echocardiographic values in male or female sheep proposed by two different authors.

	Reference			
	Male sheep			
Parametre	Yadegari, 2014	Acorda and Pajas, 2015		
Age (years)	1	<1	1-2	>2
Weight (kg)		13.7±2.4	18.6±1.4	26.4±3.0
RVIDd (cm)	1.441±0.022	0.88±0.17	1.14±0.09	1.29±0.30
RVIDs (cm)	0.423±0.013	0.53±0.09	0.64±0.05	0.66±0.23
RVWd (cm)	NR	0.17±0.04	0.22±0.03	0.23±0.05
RVWs (cm)	NR	0.26±0.06	0.33±0.05	0.35±0.06
LVIDd (cm)	2.96±0.06	1.78±0.07	0.20±0.14	2.27±±0.23
LVIDs (cm)	1.324±0.02	0.91±0.133	0.99±0.069	1.29±0.174
LVWd (cm)	0.99±0.03	0.23±0.04	0.30±0.05	0.36±0.05
LVWs (cm)	1.72±0.03	0.43±0.08	0.57±0.03	0.65±0.07
IVSd (cm)	0.97±0.03	0.66±0.05	0.67±0.05	0.69±0.08
IVSs (cm)	1.50±0.04	0.94±0.06	1.00±0.04	1.06±0.12
Aos (cm)	2.04±0.03	NR	NR	NR
Ao (cm)	NR	0.15±0.17	0.18±0.10	0.19±0.18
LA (cm)	1.74±0.09	0.14±0.17	0.16±0.09	0.17±0.11
RA (cm)	NR	0.14±0.16	0.17±0.10	0.18±0.01
LA/Ao	NR	0.90±0.02	0.90±0.06	0.90±0.05
FS (%)	55.26±0.64	48.90±7.56	50.20±2.74	43.20±4.25
EF (%)	63.7±0.9	76.0±7.7	77.6±2.7	69.7±5.0
HR (bpm)	88.9±1.3	NR	NR	NR
EPSS (cm)	0.70±0.02	NR	NR	NR
LV output (cm^3 min^{-1})	2598±73	NR	NR	NR
	Female sheep (non-pregnant, non-lactating)			
Parametre	Yadegari, 2014		Acorda and Pajas, 2015	
Age (years)	12		22.7±20.63	
Weight (kg)	NR		14.2±3.7	
RVIDd (cm)	1.441±0.022		0.830±0.263	
RVIDs (cm)	0.35±0.01		0.52±0.20	
RVWd (cm)	NR		0.17±0.06	
RVWs (cm)	NR		0.26±0.11	
LVIDd (cm)	2.93±0.06		0.20±0.39	
LVIDs (cm)	1.25±0.02		0.12±0.24	
LVWd (cm)	0.89±0.033		0.36±0.07	
LVWs (cm)	1.66±0.03		0.61±0.16	
IVSd (cm)	0.88±0.03		0.66±0.09	
IVSs (cm)	1.40±0.04		0.94±0.12	
Aos (cm)	2.02±0.03		NR	
Ao (cm)	NR		0.15±0.20	
LA (cm)	1.43±0.09		0.14±0.18	
RA (cm)	NR		0.14±0.19	
LA/Ao	NR		0.90±0.03	
FS (%)	57.32±0.64		42.10±5.62	
EF (%)	59.90±0.85		68.50±6.51	
HR (bpm)	86.89±1.29		NR	
EPSS (cm)	0.67±0.02		NR	
LV output (cm^3 min^{-1})	2190±73		NR	

Values are mean ± standard deviation.

Abbreviations. RVIDd: right ventricular internal diametre diastole, RVIDs: right ventricular internal diametre systole, RVWd: right ventricular wall thickness diastole, RVWs: right ventricular wall thickness systole, LVIDd: left ventricular internal diametre diastole, LVIDs: left ventricular internal diametre systole, LVWd: left ventricular wall thickness diastole, LVWs: left ventricular wall thickness systole, IVSd: interventricular septum thickness diastole, IVSs: interventricular septum thickness systole, Aos: aortic sinus, Ao: aorta, LA: left atrium, RA: right atrium, LA/Ao: left atrium to aorta ratio, FS: fractional shortening, EF: ejection fraction, HR: heart rate, EPSS: e-point to septal separation, LV output: left ventricular output.

NR: not reported.

Table 3. Reference echocardiographic values for the four cardiac valves in sheep proposed by Kirberger and Van Den Berg (1993)

	Valves			
Parametre	Mitral	Tricuspid	Aortic	Pulmonary
E peak maximum (cm s^{-1})	63.8±9.2	51.9±12.7	NR	NR
A peak maximum (cm s^{-1})	68.4±12.7	44.8±14.6	NR	NR
Fused peaks (cm s^{-1})	95±7	55	NR	NR
E/A	0.96±0.19	1.21±0.32	NR	NR
Mean diastolic velocity	50.7±6.3	36.7±11.3	NR	NR
Decelaration time (ms)	190±40	140±70	NR	NR
Peak systolic velocity (cm s^{-1})	NR	NR	99.1±16.2	92.7±13.9
Mean systolic velocity (cm s^{-1})	NR	NR	63.6±8.9	63.1±10.1
Acceleration time (ms)	NR	NR	26.4±16.8	10.1±3.7
TTP (ms)	NR	NR	50±20	100±20
ET (ms)	NR	NR	230±20	240±30
TTP/ET	NR	NR	0.23±0.07	0.42±0.1
PEP	NR	NR	60±10	60±10
PEP/TTP	NR	NR	1.34±0.66	0.67±0.16
PEP/ET	NR	NR	0.27±0.05	0.27±0.07
HR (bpm)	112.3±20.8	105.6±19.6	107.0±19.2	109.5±16.8

Values are mean ± standard deviation.

Abbreviations. E/A: Ratio E peak maximum to A peak maximum, TTP: Time to peak, ET: ejection time, TTP/ET: time to peak to ejection time ratio, PEP: pre-ejection period, PEP/TTP: pre-ejection period to time to peak ratio, PEP/ET: pre-ejection period to ejection time ratio, HR: heart rate.

NR: not reported.