Cardiac Output Measurements with UDCO and ECHO under general anesthesia during normotension, hypotension and hypertension in adult alpacas

by

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Abstract

Cardiac output (CO) is defined as the quantity of blood pumped into the aorta each minute by the heart. Measurement of CO is considered an important tool to determine oxygen deliver to the tissues. Currently, there are several technics available to measure CO, some being considered more invasive than others. The accepted gold standard for CO measurement is pulmonary thermodilution with the use of a pulmonary arterial catheter (Swan Ganz technic). Ultrasound dilution (UDCO) and transthoracic echocardiography (ECHO) are minimally invasive methods of CO measurement and were investigated in alpacas.

Six alpacas (3 female, 3 male, 62.6 to 88.7 kgs) were anesthetized with propofol and maintained in right lateral recumbency with isoflurane and mechanically ventilated. An external arterial-venous loop with ultrasound probes for UDCO measurement was placed. A 30 ml saline bolus was injected into the venous end of the loop and CO was calculated as area under the curve from transient blood protein dilution. An ultrasound probe was placed against the right chest wall and CO was estimated by stroke volume (SV) measured via three different methods and multiplied by heart rate: ECHO M-mode left ventricular internal diameter (LVID), aortic valve flow (Ao Linear), and mitral valve flow (Min flow). Measurements were taken during normotension (NORM, MAP 60 to 80 mm Hg), hypotension induced with isoflurane (HYPO, MAP < 60 mm Hg) and hypertension induced with phenylephrine (HYPER, MAP > 80 mm Hg). Bland-Altman plots were used to compare UDCO with ECHO. Data are reported as median (range). A Friedman test with a post hoc Dunn´s test for multiple comparisons between groups when significant was used for analysis. A p value < 0.05 was used for significant.

The UDCO and ECHO M-mode had the best agreement (Bias: NORM -0.44, HYPO 1.04, and HYPER -1.14 L mim^{-1}). Mean bias between UDCO and ECHO-Ao Linear or ECHO-min flow were considered clinically unacceptable. For UDCO, CO was 4.3 (3.03, 5.74), 4.46 (2.14, 4.63), and 6.62 (4.22, 8.9) L min^{-1} for NORM, HYPO and HYPER respectively.

For CO measurement in alpacas, only ECHO M-mode appears to have an acceptable level of agreement with UDCO. UDCO may prove useful results for CO measurement in alpacas.

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List of Abbreviations

π	Radian
%	Per cent
2D	Two-dimensional
AA	Aortic Annulus
Amax	Peak left ventricular late filling velocity
ACVI	Active Circulation Volume Index
Ao	Aorta
AoD	Aortic root Dimension
AoDN	Normalized Aortic Root Dimension
AoET	Aortic Ejection Time
AV	Arterio venous
A-V	Atrio-ventricular
$CaCO_2$	Arterial Carbon Dioxide Content
CBV	Central blood Volume
CFM	Color-Flow Mapping
CI	Cardiac Index
CO	Cardiac Output
C_aO_2	Oxygen Arterial Content
C_vO_2	Oxygen Content in Venous blood
CSA	Cross Sectional Area
CW	Continuous-Wave
D	Diameter

D_aO_2	Oxygen Delivery
D_{Ao-AA}	Diameter of the Aorta at the level of th Aortic Annulus
DCM	Dilated Cardiomyopathy
E: A	Ratio between Emax and Amax
Emax	Peak left ventricular early filling velocity
EDP	End-diastolic pressure
EDV	End-diastolic volume
ECG	Electrocardiogram
ECHO	Echocardiography
ECHO-Ao Linear	CO measured with ECHO at Aortic Valve
ECHO-Minflow	CO measured with ECHO at Mitral Valve
$etCO_2$	End Tidal Carbon Dioxide
FS	Fractional Shortening
GDT	Goal Direct Therapy
HBH	Hypobaric Hypoxia
HCL	Hydrochloride
HCM	Hypertrophic Cardiomyopathy
HR	Heart Rate
ICU	Intensive Care Unit
IPPV	Intermittent Positive Pressure Ventilation
IV	Intravenous
IVSd	Thickness Interventricular Septum end-diastole
LAD	Left Atrium Diameter

LAD:Ao	Ratio between Left Atrium diameter and Aortic Root dimension
LADN	Normalized Left Atrium Diameter
LiDCO	Lithium Dilution Cardiac Output
LV	Left Ventricle
LVID	Left Ventricular Internal Dimension
LVIDd	Left Ventricular Internal Dimension at end-diastole
LVIDs	Left Ventricular Internal Dimension at end-systole
LVIDdN	Normalized Left Ventricular Internal Dimension end-diastole
LVIDsN	Normalized Left Ventricular Internal Dimension end-systole
LVET	Left Ventricular Ejection Time
LVFWd	Left Ventricular free wall thickness at end-diastole
LVOT	Left Ventricular Outflow Tract
LVVd	Left Ventricular Volume at end-diastole
LVVs	Left Ventricular Volume at end-systole
MAC	Minimal Alveolar Concentration
MICOM	Minimally Invasive Cardiac Output Monitoring
M-mode	Motion based-mode
MAP	Mean Arterial Pressure
PAC	Pulmonary Artery Catheter
PEEP	Positive End Expiratory Pressure
PiCCO	Pulse Contour Cardiac Output
PIVA	Partial Intravenous Anesthesia
P_aO_2	Partial pressure arterial Oxygen

P_aCO_2	Partial pressure arterial Carbon Dioxide
PRAM	Pressure Recording Analytic Method
PW	Pulsed - Wave
SV	Stroke Volume
SVI	Stroke Volume Index
SVV	Stroke Volume Variation
SVR	Systemic Vascular Resistance
SVRI	Systemic Vascular Resistance Index
TD	Thermodilution
TEB	Thoracic Electrical Bioimpedance
TED	Transesophageal Doppler
TEDV	Total End Diastolic Volume
TEDVI	Total End Diastolic Volume Index
TEF	Total Ejection Fraction
TIVA	Total Intravenous Anesthesia
TTE	Transthoracic Echocardiography
TOT SV	Total Stroke Volume
TOT SV_{ao}	Aortic derived Total Stroke Volume
TOT SV_{MI}	Total Stroke Volume Mitral Inflow
TOT SV:BW	Total Stroke Volume indexed to body weight
UDCO	Ultrasound Cardiac Output
UV	Ultrasound Velocity
UV_a	Change in the arterial blood UV measured by the arterial sensor

UV_{blood}	Ultrasound Velocity of blood
UV_{saline}	Ultrasound Velocity saline
VCO_2	Carbon Dioxide Consumption
VOL_{inj}	Volume of injected isotonic saline
VO_2	Oxygen Consumption
V_T	Tidal Volume
VTI	Velocity Time Integral
VTI_{Ao-AA}	VTI of the Aortic Flow at the level of the Aortic Annulus
VTI_{MI}	Velocity Time Integral Mitral Inflow
VTI_{MV}	Velocity Time Integral of the Mitral Valve

1. Introduction

Cardiac output (CO) is a fundamental component of oxygen delivery to end organs. It is defined as the volume of blood that is transferred from the left ventricle to systemic circulation over time. This is measured as ml/kg/min in veterinary medicine (Marshall K et al, 2016). Because of various sized animals in veterinary medicine, CO can be difficult to compare between different animals. Therefore, another way to consider CO in species of variable size is to divide CO by the animal´s body surface area. This is known as cardiac index and is measured in l/min/m^2. Normal CO in anesthetized alpacas is 4.5 l/min (Garcia-Pereira FL et al, 2007) or 95.2 ml/kg/min at sea level (Sillau, AH et al, 1976).

There are different methodologies for directly monitoring CO that have been adopted in veterinary medicine. These methods are broadly classified as invasive, minimally invasive and non-invasive. For several decades, the main method for the determination of the CO has been intermittent thermodilution involving the insertion of a catheter in the pulmonary artery (PAC) (García X. et al, 2011). However, its use is associated with potential complications such as pneumothorax, arrhythmias, pulmonary artery rupture, valve injury, and embolism (Domino KB, et al. 2004) and its applicability in veterinary medicine is limited to research settings. Due to the associated risks, it is preferred to use minimally or non-invasive methods to measure CO, as is considered as a helpful variable for the adequate management of critically ill patients (Joosten A. et al, 2017). Moreover, the measurement of CO is an important component to achieve goal directed therapy,

facilitating the administration of fluids and vasopressors, and potentially improving patient outcome (Metha Y and Dheeraj A. 2014). The determination of CO and understanding the associated variables involved can be useful in identifying animals in shock that required fluid therapy. Previous research in human medicine has shown that only approximately 50% of critically ill patients are fluid responsive to changes in blood pressure (Marik EP, et al., 2009). Knowing that an unstable patient is no longer fluid responsive might also help the clinician to seek different therapies to improve organ perfusion with the use of vasopressors or inotropes, or even with the direct administration of blood products.

An ideal CO monitor should be minimally or non-invasive, continuous, cost effective, reproducible, reliable during various physiological states and have fast response times (De Waal EE, et al., 2009). Unfortunately, there is not a universally accepted way to measure CO in veterinary medicine (Marshall K, et al,. 2016). Currently, research has shown that dilution techniques of CO monitoring (PiCCO and LiDCO) are the most accurate in veterinary patients; however, their invasiveness has limited their use (Marshall K et al, 2016).

The lack of data available regarding cardiovascular parameters in anesthetized alpacas, was one of the main impetus for this study, as this species is becoming more popular as companion animals. Thus, the demand for high quality, advanced veterinary care is also increasing (Margiocco M, et al. 2009). Furthermore, alpacas are commonly domesticated for various uses including as pack and fiber animals. Alpacas (Lama pacos Linnaeus, 1758; reclassified Vicugna paco, Kadwell et al. 2001) are one of the four species of South American camelids.

In their native South American high-altitude location, the Andean altiplano, alpacas develop, reside and reproduce in chronic low oxygen environments. At these altitudes (3000 to 5000 meters above sea level) there is a decrease in barometric pressure and a reduction in the partial pressure of oxygen available for respiration. The physiologic state that occurs when living in areas where the partial pressure of oxygen is reduced is termed hypobaric hypoxia (HBH) (Muthuraju S, et al. 2014). Because of this environmental pressure, physiologic evolutionary adaptations have occurred in South American camelids (Moraga et al. 2018). Of great clinical importance are the cardiovascular adaptations necessary to compensate for high altitude living such as increased hemoglobin concentrations and oxygen affinity within the blood, decreased musculature within the pulmonary arteries, and increases in myoglobin concentration and lactate dehydrogenase activity (Margiocco ML, et al. 2009). While these adaptations are critical for survival in their native environment, they may also create challenges for their anesthetic management. It can be said that the cardiovascular response of alpacas is less predictable during general anesthesia when compared to other common domestic species. To date, specific investigation of the cardiovascular response of alpacas under common anesthetic protocols has not been reported.

COstatus®, is a newer, minimal invasive system based on ultrasound dilution monitoring technology. This technique was first used to measure hemodynamic parameters during hemodyalisis in human patients. Later on, the technique was adapted for cardiac output measurement and tested in vitro (Krivitski N, et al. 2008) and in vivo in animals (Melchior R, et al. 2005). It was also introduced to measure CO in children (Crittendon I 3rd, et al. 2012). This system follows the theory of ultrasound dilution, which is based on

changes in blood ultrasound velocity (Crittendon I 3rd, et al. 2012). Only a standard arterial and a central venous catheter are necessary. An extracorporeal arterial-venous (AV) loop primed with saline connect the artery catheter with the central venous catheter through a roller pump. This pump is used to circulate blood from the artery to the vein for the duration of measurements. In the arterio venous loop there are two ultrasound flow-dilution sensors, one for the venous side and for the arterial side. There is a difference between the velocity of ultrasound in blood versus the velocity of ultrasound in isotonic saline. Thus, the injection of isotonic saline into the bloodstream will lead to a decrease in ultrasound velocity and this can be used to obtain a dilution curve (de Boode, W.P et al, 2010). The sensor on the venous side measures the amount of the injected volume and checks the quality of the injection. The sensor on the arterial side measures the change in dilution and develops the curve. A small bolus of isotonic saline (0.5 to 1 ml/kg) is injected in the venous circulation, which will create a transient hemodilution of the blood proteins (Shih, A., et al. 2011). This temporary hemodilution is detected by the arterial sensor as a decrease in ultrasound velocity that is due to mixing of blood in the system. CO is then calculated from the ultrasonic speed dilution curve based on a derivation of the Stewart-Hamilton principle (Shih, A., et al. 2011). Among the non-invasive cardiac output measurement methods, transthoracic echocardiography (TTE) has been reported as a hemodynamic assessment tool (Azcarate AJM, et al. 2012). Echocardiography satisfies some of the requirements of the ideal CO monitor. TTE can be used to estimate CO in several ways. The most frequently recommended method involves measuring the blood flow velocity (using a Doppler technique) at the left ventricular outflow tract (LVOT) and thus obtaining the stroke volume (SV) (Mercado P, et al., 2017). Another approach

for measuring CO with TTE is following the M-Mode (motion-based), which is the oldest and simplest ultrasonic method to measure cardiac output (Wallerson D.C et al., 1990). Whether echocardiography can replace thermodilution (TD) methods in CO measurement remains controversial (Zhang Y, et al., 2019). Some studies suggested that echocardiography is not interchangeable with TD for measuring CO (Wetterslev M, et al., 2016). However, in human medicine, use of echocardiography to evaluate CO is clinically acceptable utilizing either the M-mode or the Doppler method. (Uehara, Y., et al.1995).

For the reasons discussed above, the main goal of this study was to evaluate the measurement of CO with two different methods, one considered minimally invasive and the other non-invasive, in adult alpacas during general anesthesia.

2. Literature Review

2.1 Cardiac and hemodynamic physiology

The cardiac cycle is a complex and organized process that occurs from the beginning of one heartbeat to the beginning of the next one. Each cardiac cycle is initiated through the spontaneous generation of an action potential in the sinus node. This sinus node, also called the sinoatrial node, is integrated with specialized cardiac muscles. These specialized muscle fibers have the capability of self-excitation, a process that can cause automatic rhythmical discharge and contraction. For this reason, the sinus node controls the rate of contraction of the entire heart. Thus, the sinus node is recognized as the pacemaker of the heart. Fibers of this node connect directly to the atrial muscle fibers, so any action potential that starts in the sinus node spreads into the atrial wall and then through the atrioventricular bundle into the ventricles. The atria work as primer pumps for the ventricles, and the ventricles provide the major source of power for moving blood through the system.

The cardiac cycle is divided in two periods, a period of relaxation called diastole followed by a period of contraction called systole. About 80 percent of the blood flows directly into the ventricles through the atria, even before the atria contract. Then, atrial contraction causes an additional 20 percent filling of the ventricles. But under normal conditions, the heart can pump 300 to 400 percent more blood than is required by the resting body. Even when the atrial fail to function, the difference is unlikely to be of any physiological consequences at rest. (Guyton AC, Hall JE. In: Guyton AC, Hall JE, editors. Textbook of medical physiology. 13th edition. Philadelphia). During diastole, a

period of rapid filling of the ventricles occurs. Once systole has ended and ventricular pressure decreases to its lowest values, and pressure increases in the atria, the atrioventricular valves open allowing blood to rapidly fill the ventricles. This period lasts for about the first third of the diastole. During the middle third of diastole, only a small amount of blood normally flows into the ventricles. This is blood that continues leaving the atria from the veins and passes directly into the ventricles. The last third of the diastole, atria contraction happens sending the additional 20 percent of the blood into the ventricles.

During systole, there is a period of isovolumic contraction, a period of ejection and a period of isovolumic relaxation. The period of isovolumic or isometric contraction is characterized by increasing tension in the walls of the ventricles, but no ejection occurs due to the muscle fibers from the myocardium not shortening. The period of ejection is initiated when left ventricular pressure rises above aortic semilunar valve closing pressure. At this point semilunar valves open allowing blood ejected from the heart to the rest of the body.

Following this, a period of isovolumic or isometric relaxation occurs. That happens immediately after the systole, when pressure of the wall of the ventricles decreases, but pressure increase in the wall of the arteries, (pulmonary and aorta), which induce the closing of the semilunar valves to avoid the flow back into the ventricles. The ventricular muscle continues to relax, even though the ventricular volume does not change. When the intraventricular pressures rapidly decrease back to their low diastolic levels, A-V valves open to begin a new cycle (Guyton AC, Hall JE. In: Guyton AC, Hall JE, editors. Textbook of medical physiology. 13th edition. Philadelphia).

The total volume during diastole is called end diastolic volume. From this volume, approximately 60% is pumped from the heart during a single contraction. That volume is known as stroke volume. There is a remaining volume in the heart, around 40%, which is called end-systolic volume. The fraction between the stroke volume and the end-diastolic volume that is ejected is called the ejection fraction. The amount of these volumes can be measured and provides information about the left ventricular systolic function.

The primary goal of the cardiovascular system is to supply adequate amounts of oxygen rich blood to tissues to meet the metabolic demands of the body. Systemic oxygen delivery (D_aO_2) and oxygen consumption (VO_2) are parameters which can be calculated with the formulas:

$D_aO_2 = CO \times C_aO_2$

where CO is cardiac output and C_aO_2 is the oxygen content

$VO_2 = CO \times (C_aO_2 - C_vO_2)$

where C_aO_2 is the oxygen content in arterial blood and C_vO_2 is the oxygen content in the venous blood.

A common factor from both formulas is CO (cardiac output), this represents the clinical manifestation of cardiac function. Cardiac function results from the interaction of four interdependent factors: heart rate, preload, contractility and afterload. (Tibby SM, Murdoch IA, 2002).

2.2 Cardiac output measurement

Cardiac output is defined as the amount of blood the heart pumps per unit of time, conventionally 1 minute. It is logically equal to the product of the stroke volume and the

number of beats per minute (heart rate). The heart rate is one of the simplest determinants of cardiac output to visualize and to measure (Jean-Louise Vincent, 2008). Cardiac output can be adversely affected by extreme sinus tachycardia, bradycardia or any arrhythmia producing loss of atrioventricular synchrony (Tibby SM, Murdoch IA, 2002). Stroke volume is defined as the volume of blood ejected by the ventricles in one beat. This variable is dependent on three interdependent factors, preload, contractility and afterload. Contractility directly affects CO. It is defined as the inherent capacity of the cardiac muscles to shorten in the absent of load (Spinale, GF, 2015). A clinically adequate bedside measurement technique of contractility does not exist. (Tibby SM, Murdoch IA, 2002). It is influenced by preload augmentation and afterload impedance (Kurt A. Grimm et. al; Veterinary Anesthesia and Analgesia, The 5th edition of Lumb and Jones). Preload is the hemodynamic load or stretch on the myocardial wall at the end of the diastole just before the contraction begins (Kurt A. Grimm et. al; Veterinary Anesthesia and Analgesia, The 5th edition of Lumb and Jones). There are several techniques described for direct and indirect measurement of preload: end-diastolic pressure (EDP), end-diastolic volume (EDV), wall stress at end-diastole, end diastolic sarcomere length, central venous pressure (right heart) and pulmonary artery occlusion pressure (left heart) (Tibby SM, Murdoch IA, 2002). The concept of preload can be explained with the Frank-Staling relationship or heterometric autoregulation. This mechanism means that the greater the heart muscle is stretched during the filling phase (diastole), the greater is the force of contraction and the greater the quantity of blood pumped into the aorta. Finally, the afterload of the ventricle is the pressure from the aorta pushing back toward the ventricle, or in other words, the left ventricular wall stress at the end of the systole. It has an inverse

relationship with the stroke volume and a direct relation with myocardial oxygen consumption. The most commonly used clinical measure of afterload is systemic vascular resistance.

Cardiac output is a primary determinant of global oxygen transport from the heart to the body, for that reason it appears reasonable to measure cardiac output in critically ill patients. Additionally, CO can act as an indicator of cardiovascular health, and it is important to monitor for assessment of cardiovascular function. Clinically in veterinary medicine, it is uncommon to monitor CO. Moreover, in pediatric medicine, the decision to measure CO represents a balance between the risks involved with the measurement process, and the potential benefits gained from the additional hemodynamic information (Tibby SM, Murdoch IA, 2002). Maintenance of an adequate perfusion pressure is vital to organs and from the formula it can be seen that blood pressure is affected by CO and SVR:

Mean blood pressure ≈ CO x SVR

Thus, a low blood pressure may be secondary to a low CO, low SVR, or both. Conversely, a normal blood pressure can exist in the face of decreased CO if SVR is high. A low CO may occur for many reasons, for example inadequate vascular volume, excessive afterload, poor contractility, myocardial restriction (pericardial effusion), diastolic dysfunction, vascular stenosis/insufficiency, or an arrhythmia. Additionally, delivery of sedative drugs, analgesic and anesthetic agents often results in significant impact on CO in most animal species.

In very sick patients, the measurement of CO is an important component of goal directed therapy, or in other words, in the optimization of stroke volume and cardiac output

(Duke-Novakovski T. et all., BSVA Manual of Canine and Feline Anaesthesia and Analgesia, 3rd Edition). Measurement of CO can determine if an animal in shock or showing evidence of hypoperfusion requires fluid therapy (Marshall K, et al. 2016). Previous studies in human medicine have shown that approximately 50% of critically ill patients are fluid responsive (increase cardiac output in response to IV fluid administration) (Marik PE. et al. 2009). So, the measurement of CO is beneficial since it provides information about cardiac function, guide titration of therapies, and facilitates the identification of low CO states that might compromise vital organs (Crittendon I 3rd et al. 2012).

2.3 Different Technics to measure CO

Eight desirable characteristics have been identified for any CO monitoring technic: accuracy, reproducibility, rapid response time, operator independence, easy of application, no morbidity, continues use, and cost effectiveness (Shephard JN, et al. 1994). Unfortunately, a device with all of these characteristics does not exist, As a result, the choice of method may depend on availability, cost, the patient and clinical situation. Invasive monitoring with a pulmonary artery catheter (PAC) was the previous gold standard, however less invasive alternatives devices have been developed. Studies have demonstrated that minimally invasive CO monitoring (MICOM) combined with goal direct therapy (GDT) protocols improve perioperative outcomes in high-risk surgical patients (Kobe J, et al. 2019). Methods of CO monitoring are broadly classified as: Invasive methods, minimally invasive and non-invasive. The first technic for the calculation of cardiac output in humans was described by Adolph Fick in 1870. He

postulated that the total uptake or release of oxygen by the lungs is the product of blood flow through the lungs and the arteriovenous oxygen content differences. From this hypothesis CO can be calculated:

$CO = VO_2 / ([CaO_2 – CvO_2] \times 100)$

where VO_2 is the oxygen consumption, CaO_2 oxygen content in arterial blood and CvO_2 mixed venous oxygen content (Kobe J, et al. 2019).

The Fick technic was the standard for the measurement of cardiac output until the development of dye dilution techniques. Dye dilution techniques of cardiac output determination are based on the Stewart-Hamilton principle. These techniques were repeatedly shown to be as reliable as the Fick technique. Thermodilution, first described by Fegler in 1954, uses heat as an indicator instead of a dye, and relies on the same principle as dye dilution. The accuracy of thermodilution-based cardiac output has been assessed in hundreds of studies (Kurt A. Grimm et. al; Veterinary Anesthesia and Analgesia, The 5th edition of Lumb and Jones).

As previously mentioned, technics to measure cardiac out can be classified depending on their invasiveness.

2.3.1 Invasive methods:

Fick´s Method (The Original Gold Standard) (Marshall K, 2016)

This technic is the oldest and original gold standard of CO monitoring. The Fick´s method assumes that the rate of oxygen consumption is determined by the rate of blood flow and the rate of oxygen uptake by the tissues. The tissues take up oxygen presented to them by the arteries, with any excess oxygen not used by tissues being found in the

veins. The arterial oxygen comes from inspired oxygen, and the unused oxygen in the venous circulation is related to the oxygen exhaled. To perform CO calculation using the Fick´s method, the patient should be intubated and oxygen consumption is determined by comparing the oxygen concentration in the air that they inhale vs. the oxygen concentration that the patient exhales. An arterial blood sample can be compared with a mixed arteriovenous sample (usually from the pulmonary artery). Following the formula:

$CO = VO_2 / (CaO_2 – C_v O_2)$

CO can be calculated (Marshall K et al. 2016). Determining cardiac output using this method requires catheterization of both a peripheral artery and the main pulmonary artery for arterial and mixed venous blood sampling respectively (Laszlo G, 2004). The measurement of oxygen consumption also requires accurate collection and analysis of exhaled gases.

This method not only requires intubation of the patient to obtain the analysis of the gases, but it also requires that the patient be hemodynamically stable because results are less accurate if shunting of blood is occurring, which happens frequently in unstable patients.

Indicator Dilution methods (The New Gold Standard)

There are two types of indicator dilution methods, pulmonary artery thermodilution and transpulmonary dilution, both follow the Stewart-Hamilton principle. With transpulmonary dilution, the patient receives an injection of a predetermined amount of an indicator (dye, lithium, or cold saline) into the bloodstream (usually jugular vein). Blood is sample (usually from the femoral artery), and the concentration of the indicator is measured in the sample. The amount of indicator found in the downstream blood is

used to generate a time-dilution curve that is used to derive CO. However, in pulmonary artery thermodilution, just a pulmonary artery catheter is placed (Swan-Ganz catheter) and a predetermined amount of indicator (cold saline) is injected into the catheter and is detected by a thermistor located in the pulmonary artery to generate a temperature curve. Indicator dilution methods have replaced Fick´s method as the current gold standard of CO monitoring because of their high level of accuracy. However, the use of the pulmonary artery catheter is associated with significant complications. The most common are catheter-related infections (5%) and arrhythmias (2%), (Garcia X, et al. 2011). Other potential complications are heart block, rupture of the right heart or pulmonary artery, thromboembolism, pulmonary infarction, valvular damage, and endocarditis (Kurt A. Grimm et. al; Veterinary Anesthesia and Analgesia, The 5th edition of Lumb and Jones).

2.3.2 Minimally Invasive methods

Pulse Contour Analysis

This method is based on the principle that the area under the systolic portion of an arterial pressure waveform is proportional to the SV (Hofer CK, et al. 2009). It was first described by Erlanger and Hooker in 1904 and suggested that CO was proportional to arterial pulse pressure (Funk DJ, et al. 2009). Using current technology, an arterial wave form obtained from a patient is analyzed by software to derive CO from the area under the curve of the arterial wave form. This method is usually combined with an indicator dilution method. Lithium dilution cardiac output monitoring (LiDCO) and pulse contour cardiac output monitoring (PiCCO) are the most common methods. The LiDCO system combines pulse contour analysis with lithium indicator dilution for continuous

monitoring of SV and stoke volume variation (SVV) (Mehta Y, et al. 2014). This technic requires a venous (central or peripheral) and an arterial catheter. A bolus of lithium chloride is injected into venous line and arterial concentration is measured by withdrawing blood across a disposable lithium sensitive sensor. The lithium concentration in the blood is determined by lithium selective electrode connected to the peripheral arterial catheter. This device has been compared to PAC and has good correlation (Linton RA, et al. 1993). The main disadvantage of lithium dilution is the blood loose associated with withdrawal of arterial blood for the determination of lithium concentration. The PiCCO systems requires both a central venous (femoral or jugular) catheter and arterial cannulation. Indicator solution (cold saline) is injected via the central venous cannula and blood temperature changes are detected by an arterial thermistor-tipped catheter placed in the peripheral artery (von Spiegel T, et al. 1996). PiCCO is a relatively invasive method as it requires both arterial and central venous cannulation. This method also correlates well with PAC (Rocca G, et al. 2003).

Pulse Pressure Analysis

In an effort to find less invasive ways to measure CO, pulse pressure analysis was developed. Cardiac output devices based on the arterial waveform analysis include FloTrac, Vigileo and PRAM (Pressure recording analytic method). All of these devices measure CO based on pulse pressure analysis. The main difference with LiDCO and PiCCO is that it only requires an arterial cannulation. It is based on the principle that there is a linear relationship between the pulse pressure and SV (Mehta Y, et al. 2014). Stroke volume is calculated from the variations in pulsatility of the arterial waveform and

essentially is the area under the arterial waveform. The resulting SV is then multiplied by heart rate to calculate CO continuously (Marshall K, et al. 2016). This methodology is less invasive; however, it does have significant limitations. It correlates poorly with thermodilution methods. Also, these technics require a good arterial signal and waveform to calculate CO. Additionally, the site of the arterial cannulation also impacts accuracy. The FloTrac device has been investigated in veterinary medicine and been found to be unsuitable for use in dogs, overestimating CO with a high rate of errors (Valverde A, et al. 2011).

Ultrasound dilution velocity (UDCO)

The COstatus device estimates CO by using ultrasound dilution velocity technology. It measures changes in blood ultrasound velocity following an injection of a small saline bolus. Two reusable sensors are used to measure change in blood ultrasound velocity. The venous sensor detects the injection of saline while the arterial sensor measures the changes in the concentration of saline in the blood as a dilution and records the indicator travel time through the cardiopulmonary system. This technic is considered minimally invasive as it only requires a central venous and a peripheral arterial cannulation. This method will be described more in detail below.

Transesophageal Doppler (TED)

The transesophageal Doppler technique is based on measurement of blood flow velocity in the descending aorta by means of a Doppler transducer at the tip of a flexible probe. The probe is introduced orally in anesthetized patients, then advanced until the tip of the probe is located at the mid thoracic level. It is then rotated so that the transducer faces the aorta and a characteristic aortic velocity signal is obtained (Berton and Cholley, 2002).

An estimation of CO can be calculated by first obtaining SV. SV is the result of the product of the cross sectional area (CSA) of the aorta and the blood flow velocity detected by Doppler.

SV = CSA x VTI

The aortic diameter is obtained assuming the annulus is circular. Once the diameter is known, CSA can be calculated following the formula:

$CSA = 0.785 \times d^2$

The velocity time integral (VTI) is calculated from the area under the velocity-time curve and used as the stroke distance. The area can be calculated by nomogram or direct measurement (Mehta Y, et al. 2014). Thus, SV is calculated first and then CO is then calculated (Funk DJ, et al. 2009). This technic presents several limitations. First, it measures the flow from descending aorta which is 70% of total flow. A correction factor is needed to compensate for aortic arch flow (Mehta Y, et al. 2014). Second, the probe position is crucial to obtain an accurate measurement of aortic blood flow, so this device is operator dependent, and studies have shown that up to10-12 insertions are needed to obtain accurate measurements (Lefrant JY, et al.1998). Moreover, aortic cross-sectional area is not constant but rather dynamic in any individual patient, and this might be a problem in veterinary medicine given the wide variety of patient sizes encountered (Marshall K, et al. 2016).

2.3.3 Non Invasive methods

Partial gas rebreathing or NICO system

This method uses the indirect Fick's principle to calculate CO. Fick's principle can be applied to any gas and this method analyzes CO_2 instead of oxygen. The NICO monitor estimate cardiac output noninvasively, using intermittent partial rebreathing through a specific disposable rebreathing loop. This methodology follows the idea that at steady state, the amount of CO_2 entering the lungs via the pulmonary artery is proportional to the CO and equals the amount exiting the lungs via expiration and pulmonary veins (Mehta Y, et al. 2014). Therefore, the Fick equation applied to carbon dioxide would be (Berton and Cholley, 2002):

$CO = VCO_2 / CvCO_2 – CaCO_2$

The NICO monitor consists of a carbon dioxide sensor (infrared light absorption), a disposable airflow sensor and a pulse oximeter. VCO_2 (CO_2 consumption) is calculated from minute ventilation and its carbon dioxide content, whereas the arterial carbon dioxide content ($CaCO_2$) is estimated from end tidal carbon dioxide ($etCO_2$), with adjustments for the slope of carbon dioxide dissociation curve and the degree of dead space ventilation. (Berton and Cholley, 2002). Changes in VCO_2 and $etCO_2$ only reflect the blood flow that participates in gas exchange, and intrapulmonary shunt could affect the estimation of CO using the NICO monitor. This method has been found to work well in dogs and foals (Gunkel CI, et al. 2004 and Giguère S, et al 2005). Limitations include the need for an intubated and preferably ventilated patient and arterial blood samples (required to enter arterial oxygen tension values for shunt estimation) which somewhat tempers the noninvasive nature of the technic (Berton and Cholley, 2002).

Thoracic electrical bioimpedance and bioreactance analysis

Thoracic electrical bioimpedance (TEB) is based on the assumption that the electrical resistance of the thorax is related to intrathoracic blood volume (Thiele, RH. et al, 2014). With TEB, it is also assumed that changes in thoracic impedance are exclusively a function of changes in intrathoracic blood volume (Thiele, RH. et al, 2014). This method applies a high frequency electric current of known amplitude and frequency across the thorax. The impedance is defined as the amount of resistance a current encounter as it travels through a circuit, in this case the circuit is the thorax (Marshall K, at al. 2016). With this methodology, CO is continuously derived from electrical signals received by using skin electrodes (Kobe, et al. 2019). The impedance of the current varies in relation to the amount of fluid within the thorax and specifically owing changes in blood flow with the aorta. TEB has had variable correlation compared to thermodilution methods in patients with heart failure, and it has shown to be unreliable to predict CO (Kamath SA, et al. 2009). Another potential limitation in veterinary medicine is its decreased accuracy in awake, moving patients and loud environment, reducing its use in ICU setting on awake patient (Marshall K, at al. 2016).

Bioreactance is a modification of the thoracic bioimpedance technic, which refers to the overall sum of electrical resistance, capacitive and inductive properties of blood and biological tissues. This technique was developed to decreased erroneous readings secondary to electrode positioning, body size, temperature, and humidity that area typically encountered with bioimpedance method (Thiele, RH. et al, 2014). The Bioreactance technique analyzes the frequency spectra variations of the delivered

oscillating current. This approach is supposed to result in a higher signal-to-noise ratio and thus in an improved performance of the device (Alhashemi JA, et al. 2011). The only currently available device designed to measure CO using the bioreactance technique is the NICOM. (Thiele, RH. et al, 2014). Bioreactance has been shown to correlate well with thermodilution methods. It has been also evaluated with experimental dogs and had an excellent correlation with thermodilution (Heerdt PM, el at. 2011).

The following two CO measurement techniques (UDCO and TTE) are discussed here as they are the focus of the research project.

2.4 Ultrasound dilution technics:

Ultrasound dilution technology was introduced in 1995 and has been used to measure several hemodynamic parameters in human patients during hemodialysis (Krivitski, NM.1995). This method follows the theory of ultrasound dilution based on changes in blood ultrasound velocity. The velocity of ultrasound in blood varies between 1560 and 1585 m/s depending on total blood protein, temperature, and ion concentration in plasma. In contrast, the ultrasound velocity of saline is only 1533m/s (Krivitski, NM.1995). The injection of body temperature isotonic saline transiently reduces blood ultrasound velocity and can be used to obtain a dilution curve (Crittendon I 3rd et all, 2012). Two ultrasound flow-dilution sensors are needed, one of the venous side and one on the arterial side of the circulation. This is set up with an extracorporeal circuit, constructed by connecting a disposable AV loop between a peripheral arterial and a central venous catheter. These AV loops are available for different patient sizes. Reusable sensors, which detect transit time blood flow and ultrasound velocity, are clamped on the arterial

and venous limbs of the AV loop (de Boode, W.P et al, 2010). The sensor on the venous side measures the amount of injected volume, the quality of injection and detects the ultrasound velocity of the used saline (Krivitski N, et al. 2008). The sensor on the arterial side measures the change in ultrasound velocity and sends information to a computer to build an ultrasound dilution curve. Following the Steward-Hamilton principle, cardiac output can be calculated with the equation:

$CO = [(UV_{blood} - UV_{saline}) \times (VOL_{inj})] / UV_{a(t)dt}$

CO = Cardiac output (ml/min)

$UV_{blood} - UV_{saline}$ = difference between ultrasound velocity of blood and saline

VOL_{inj} = volume of injected isotonic saline (mL)

UV_a = change in the arterial blood ultrasound velocity measured by the arterial sensor.

Between the two sensors, there is a peristaltic pump which is used to circulate the blood through the AV loop at speed of 12 ml/min for a period of 5 to 6 minutes during cardiac output measurements. This pump prevents periodically unstable blood flow (De Boode WP, et al. 2010). A measurement session begins by entering the patient's weight, length, arterial blood pressure, central venous pressure, and heart rate into the device computer. When the peristaltic pump starts, a small amount of body-temperature physiologic isotonic saline (0.5 to 1.0 ml/kg) is injected at the outflow segment of the loop on the venous side before the venous ultrasound sensor. The saline is warmed to 37℃ (similar to body temperature) by a fluid warmer. Volume and time of the injected saline is determined by the venous ultrasound sensor. The saline is completely mixed as the blood passes through the cardiopulmonary circulation and gives rise to a homogenous blood dilution on the arterial side. The final blood dilution that occurs in the systemic arterial

circulation is detected by the arterial ultrasound sensor at the arterial in-flow segment of the loop, and an ultrasound velocity curve is generated (Sigurdsson, ST, et all, 2019). This technology can be also used to calculate total end-diastolic volume, central blood volume, and active circulating blood volume and to determine and to detect cardiac shuts.

2.5 Transthoracic Echocardiography (TTE) technics

Among the noninvasive cardiac output measurement methods, transthoracic echocardiography (TTE) has been reported as a hemodynamic assessment tool (Azcarate AMJ, et al. 2012). TTE provides valuable information on diastolic function, cardiac structures, regional motility and valve function (Oh JK, et al. The Echo Manual, 3rd Edition. 2004). There are three basic "modes" used to image the heart**:** two-dimensional (2D) imaging, M-mode imaging (motion based) and Doppler imaging. For cardiac ultrasound, Doppler is used in three ways, continuous-wave (CW) Doppler, pulsed-wave (PW) Doppler and color-flow mapping (CFM) (Ashley EA, Niebauer J. 2004, Cardiology Explained).

Continuous-wave Doppler: measures velocity along the entire length of the ultrasound beam and not at specific depth. It is used to estimate the severity of the valve stenosis or regurgitation by assessing the shape or the density of the output.

Pulse-wave Doppler: measures the blood-flow velocity within a small area at a specified tissue depth. It is used to assess ventricular in flow patterns, intracardiac shunts, and to make precise measurements of blood flow at valve orifices.

Color flow mapping: uses measurements of the velocity and direction of blood flow to superimpose a color pattern onto a section of a 2D imagine (Ashley EA, Niebauer J. Cardiology Explain, 2004).

When **pulse-wave Doppler** is used, flow velocities can be recorded within discrete regions of the heart and great vessels (Quiñones AM, et al. 2002). The most common sites are the left ventricular outflow tract (LVOT), mitral annulus and left ventricular inflow (at the tips of the mitral valve leaflets), pulmonic valve annulus and PA, tricuspid valve inflow, hepatic veins, and pulmonary veins (Quiñones AM, et al. 2002). The flow volume passing through these sites can be calculated as the product of the velocity-time integral and the cross-sectional area (CSA) of the respective site, and thus obtaining stroke volume (SV= CSA x VTI) (Quiñones, AM, et al. 2002). In a heart with normal function valves (absent of regurgitation) the volume entering the left ventricle across the mitral valve (inflow) will be equal to the volume exiting the left ventricle across the aortic valve (outflow). Meaning the SV across the aortic valve equals the SV across the mitral valve. The preferred sites for determining SV and cardiac output are as follows:

1. The LVOT or aortic annulus
2. Mitral annulus
3. Pulmonic annulus

The LVOT is the most widely use site (Lewis JF, et al. 1984).

M-mode echocardiography is the oldest and simplest ultrasonic method to measure cardiac output (Wallerson DC, et al.1990). The primary measurements utilized are LV internal dimensions measured between the papillary muscle and mitral leaflet tips (Wallerson DC, et al.1990). LV end-diastolic diameter is measured at the onset of a QRS

complex and the end-systolic dimension is measured at the nadir of posterior septal motion by the leading edge to leading edge technique (Wallerson DC, et all.1990). Investigators have recognized that these linear dimensions could be cubed to provide reasonable estimates of the volume of normally-shape left ventricles based on the fact that such ventricles resemble a prolate ellipsoid with a long axis twice the length of the short axes (Pombo JF, et al. 1971). M-mode echocardiography measures stroke volume with moderate accuracy in patients with symmetric LV contraction, allowing detection of relatively small differences between large groups of patients (Wallerson DC, et al.1990). When 2-D echocardiography is performed, variations of chamber shapes can be appreciated with more complex and appropriate geometric models of the left ventricle, this results in a more accurate volume measurement (Wallerson DC et al,1990). This method allows structures to be viewed moving in real time in a cross-section of the heart (two dimensions). The most common cross-sectional views are the parasternal short axis and the apical view (Ashley EA, Niebauer J. Cardiology Explain, 2004). However, an in vitro study demonstrated that directly measured LV cavity area was systematically underestimated by 2-D echocardiography (Helak JW and Reich N, 1981). In addition, there has been little use of this technique to assess stroke volume and cardiac output (Wallerson DC et al, 1990)

In summary, echocardiography provides three different modalities for the non-invasive measurement of stroke volume and cardiac output. M-mode methods have been most widely used. This method is reasonably accurate in patients with symmetric ventricles. Doppler methodology achieves echocardiographic measurements of blood flow across different anatomical parts of the heart. 2-dimensional echocardiography methodology is

theoretically attractive but suffer the limitation of having little data available validating their performance under clinical circumstances.

2.6 Alpacas Physiology:

Alpacas, *Lama pacos linnaeud*, 1758; reclassified *Vicugna paco*, (Kadwell et al. 2001), are one of the fours species of South American camelids. Alpacas are commonly domesticated for various uses including as pack and fiber animals. Alpacas are also acquired as pets and have become more popular as companion animals. In their native South American high-altitude location, the Andean altiplano, alpacas develop, reside, and reproduce in chronic hypobaric hypoxia secondary to high altitude (Margiocco LM, et al. 2009). At these altitudes (3000 to 5000 meters above sea level) there is a decrease in barometric pressure and reduction in the partial pressure of ambient oxygen available for respiration. Some mammalian species have adapted and can tolerate prolonged periods of oxygen shortage. Some examples include seals and other diving mammals, or the South American *Camelidae*, which includes the domesticated llama (*Lama glama*) and alpaca (*Lama pacos*) and the wild species, vicuña (*Vicugna vicugna*) and guanaco (*Lama guanicoe*) (Llanos AJ, et al. 2003).

The physiologic state that occurs when living in areas where the partial pressure of oxygen is reduced is termed hypobaric hypoxia (Muthuraju S and Pati S, 2014). Because of this environmental pressure, physiologic evolutionary adaptations have occurred in South American camelids (Moraga MD, et al. 2018). Of great clinical importance are the cardiovascular adaptations necessary to compensate for high altitude living, such as increased hemoglobin concentrations and oxygen affinity within the blood; decreased

musculature within the pulmonary arteries and increases in myoglobin concentration and lactate dehydrogenase activity (Margiocco LM, et al. 2009). Man, and cattle develop pulmonary hypertension when living at high altitude (Peñaloza D, et al. 1962) due to breathing of hypoxic gas mixtures that cause an increase in the pulmonary arterial pressure. In alpacas and llamas, the pulmonary arterial pressure is significantly higher than those animals which have been born and bred at low altitude (Harris P, et al. 1982). But anatomical and histological evidence shows that such an increase in pressure is too slight to have measurable effect on the heart or the muscular pulmonary arteries in alpacas (Harris P, et al. 1982). It seems, therefore, that the pulmonary vasoconstriction response to alveolar hypoxia is greatly reduced in the camelids (Harris P, et al. 1982). Studies in fetal camelids have demonstrated that during acute hypoxemia (low blood oxygen content) blood flow to fetal heart and adrenal increases, while it decreases in the kidney, spleen and carcass, and is maintained in the brain (Llanos AJ, et al. 1995). Additionally, in the fetus there is a mild or no increase in cerebral blood flow and the peripheral vasoconstriction is much more intense than in lowlands fetus species (Reyes RV, et al. 2020). A study of anesthesia in five llamas provided data on the following cardiovascular parameters: cardiac out 8.2 ± 0.8 L/min; stroke volume 146 ± 20ml/beat; mean systemic arterial pressure 137 ± 8mmHg; mean pulmonary arterial pressure 14 ± 0.8 mmHg; mean right atrial pressure 2.6 ± 0.7mmHg; total peripheral resistance 1,470 ± 183 dyne. seconds/cm^3; left ventricular work 15.7 ± 0.9kgm/min; cardiac output per 73kg ± 9ml/minute/kg; P_aO_2 127 ± 8.9 mmHg, $PaCO_2$ 34 ± 1.1 mmHg; base excess 2.3 ± 1.1 mEq/L; and bicarbonate 21.3 ± 1.1 mEq/L (Gavier, D. 1988).

The blood volume in llamas and alpacas approximates 63.5 ml/kg body weight, or 6.35% (6.35% to 8.65% of body weight). This is lower than in the other mammals (Fowler ME, 1998).

Regarding hematology, hemoglobin concentration for South American Camelids is higher than hemoglobin concentration in cattle. Camelid erythrocytes are small and ellipsoid (Hajduk P, 1992). The small size and shape result in a lower packed cell volume. Camelid erythrocytes are oriented with the long axis in the direction of the blood flow, this makes it possible to traverse small capillaries (Smith JE, et al. 1979). Alpaca blood has a large oxygen-carrying capacity and a low viscosity, which ideally suits the alpaca to living in an environment with low oxygen tension (Yamaguchi K, et al. 1987). For all those physiological changes mentioned above, alpacas are an object of study under general anesthesia, as they might present an unusual or exaggerated cardiovascular responses to commonly used anesthetic and analgesic drugs that are not noted in other domestic companion animal species.

2.7 Alpacas Anesthesia Currently recommended protocols for anesthetizing alpacas are generally similar to those used in domestic ruminants (Dugdale A, 2001) and do not account for the important potential differences in the cardiovascular response of these animals to anesthetic drugs.

For elective surgeries, llama and alpacas should be fasted for twenty-four to forty-eight hours (Fowler ME, 1998). Both, passive and active reflexive regurgitation are potential sequelae to general anesthesia (Fowler ME, 1998). Manipulation can be difficult due to unruly nature of these animals. Standing to the side of the animal helps to reduce the

incidence of spitting, , biting and striking at the handler (Garcia Pereira FL, et al. 2005). Intramuscular injections can be given at sites with good blood supply, such as the quadriceps, triceps or lumbar epaxial muscles (Garcia Pereira FL, et al. 2005). Catheter placement should be performed for induction of general anesthesia using injectable agents. Two major sites are suitable for jugular venipuncture: low on the neck near the thoracic inlet and high near the ramus of the mandible. The jugular vein is preferred for venous access (Garcia Pereira FL, et al. 2005). It is necessary to have an understanding of the anatomy of the vessels of the cervical region. In long neck animals, valves in the jugular veins prevent backflow of blood to the head when it is lowered for feeding and drinking. These valves may be bicuspid or tricuspid (Fowler ME, 1998). When a low venipuncture is performed, the operator should palpate for the ventral projection of the transverse process of the sixth cervical vertebra and occlude the vessel at this site by wrapping the fingers around the projection (Fowler ME, 1998).

There are many possible drug combinations which can be administered to camelids in order to provide sedation and analgesia prior to anesthesia and surgery. Alpha-2 agonists and opioids are the most common drugs used in camelids as premedication (Garcia Pereira FL, et al. 2005). Xylazine is the alpha 2-agonist most commonly described for use in alpacas and llamas (Riebold et al. 1989). Alpacas seem to be less sensitive to xylazine than llamas, therefore higher doses may be necessary to provide desirable sedation (Riebold et al.1989). Opioids are the drugs most effective in providing analgesia. Full μ-agonist as well as κ-agonist can be used in camelids (Garcia Pereira FL, et al. 2005). Morphine (0.1-0.5 mg/kg IM) has been used successfully in camelids, producing good analgesia during surgical procedures (Garcia Pereira, unpublished clinical observations).

Llamas and alpacas generally recover well from general anesthesia; therefore premedication is not mandatory (Riebold et al. 1989), however may be needed with untrained or unhandled animals. Chemical restraint techniques used in camelids can provide a range of response from mild sedation of standing patients to semi anesthetized recumbency. A popular technique among farm animal clinicians is a combination protocol called ketamine stun. This is the addition of a small dose of ketamine to a chemical restraint combination. Xylazine, Butorphanol and Ketamine are typically used (Abrahmsen JE, 2008).

Induction of anesthesia can be accomplish using injectable drugs, such as Propofol, Alfaxolone, Ketamine or inhalants (Garcia Pereira FL, et al. 2005). Propofol (2mg/kg IV) or Ketamine (2.5-5mg/kg IV) can be used alone or in combination with xylazine or guaifenesin (Riebold et al. 1989, Duke et al. 1997). Also, midazolam and diazepam (0.2-1mg/kg) may be used in combination with ketamine for intravenous induction of general anesthesia (Garcia Pereira FL, et al. 2005).

Camelids have a relatively long narrow oral cavity, which makes orotracheal intubation difficult. The animal should be kept in sternal recumbence with the neck fully extended to facilitate visualization of the larynx and intubation. For proper insertion of an endotracheal tube, the use of a long laryngoscope blade and placement of a stylet through the arytenoids a few centimeters into the trachea as a guide the endotracheal tube will be helpful. The use of lidocaine on each arytenoid prior to intubation will help reduce the potential for laryngo-spasm. Intubation is necessary to prevent aspiration of salivary secretions, even when inhalant anesthetics are not used. Moreover, the cuff should be inflated immediately (Garcia Pereira FL et al. 2005). Insufficient depth of anesthesia may

stimulate regurgitation once the larynx is stimulated by the laryngoscope. Adult alpacas typically require a 9-to 10-mm internal diameter endotracheal tube and the largest llamas rarely require more than 14-mm endotracheal tube.

Maintenance of anesthesia can be performed with inhalant anesthesia or partial intravenous anesthesia (PIVA) or total intravenous anesthesia (TIVA). There are multiple drug combinations for TIVA described in camelids and can be practice in a "field" or out of hospital setting. Ruminant "triple drip" is likely the most commonly used TIVA protocol used to maintain a stable plane of anesthesia in alpacas without inhalant anesthetics. It is created by adding ketamine (1mg/ml) and xylazine (0.1mg/ml) to 5% guaifenesin (Lin HC, Tyler JW, Welles EG, et al. 1993). Maintenance of anesthesia with a constant rate infusion of propofol has been reported in llamas. The rate of 0.4mg/kg/min was sufficient for non-invasive procedures with minimal cardiovascular depression, mild hypercarbia and respiratory acidosis (Duke T et al. 1997). Isoflurane MAC in llamas is approximately 1.1% which is slightly lower than in most species (Khursheed RM et al. 1997). The MAC of sevoflurane in spontaneously breathing llamas and alpacas is approximately 2.3% (Grubb et al., 2003). The depression in myocardial contractility caused by inhalants is less profound with isoflurane and sevoflurane when compared to halothane anesthesia in llamas and alpacas (Garcia Pereira FL, et al. 2005). Vital signs monitoring during anesthesia is important for obtaining information about patient homeostasis, increasing safety and helping to more readily recognize abnormalities. Heart rate, respiratory rate and simple reflexes are easy to assess and do not require expensive equipment. Heart rates range from 40-100 per minute depending on the species, size and age of the animal (Garcia Pereira FL, et al. 2005). Arterial blood

pressure in camelids is similar to other ruminant species and is usually higher than in other mammals, where mean arteria blood pressure varies from 70-100 mmHg (Riebold et al.1989). Invasive arterial blood pressure measurement can be achieved through placement of a catheter in the auricular, dorsal pedal or cranial branch of medial saphenous artery. Alpacas and llamas generally ventilate adequately under light planes of inhalant anesthesia without assistance (10-20 breaths per minute) (Riebold et al. 1989). However, mechanical ventilation has been shown to better maintain normal PaO_2 and P_aCO2 values (Gavier D et al.1988). Ocular reflexes are commonly used to assess depth of anesthesia in camelids and palpebral reflex remains fairly brisk and spontaneous blinking occurs at surgical planes of anesthesia (Abrahmsen EJ. 2008). Hypotension and bradycardia are the most common cardiovascular problem encountered during inhalation anesthesia of camelid patients. Dobutamine and norepinephrine appear to be appropriate choices to improve cardiac index (CI) and mean arterial pressure (MAP) and thus overall oxygen delivery (D_aO_2) in alpacas with isoflurane-induced hypotension. Moreover, dobutamine reduces relative oxygen consumption while improving D_aO2 and tissue oxygenation (Vincent CJ, et al. 2009). If bradycardia is noticed, atropine (0.01-0.02 mg/kg IV) is generally effective in treating bradycardia (Abrahmsen EJ. 2008). Alpacas should be recovered in sternal recumbency as this position assists venting of any fermentation gas trapped in the first compartment during anesthesia. It also allows saliva and any regurgitation to drain from the oral cavity. Nasal or laryngeal edema can develop in alpaca, particularly if the head is lower than the heart and/or anesthesia time was prolonged. Camelid patients should be extubated in sternal recumbency with the endotracheal tube cuff partially inflated to facilitate removal of any debris in the trachea

(Abrahmsen EJ. 2008). Furthermore, camelids should not be extubated until vigorous chewing activity is observed. Camelids typically do not attempt to stand until they are awake and have full function of their limbs. Pre-emptive analgesic support should be used in painful procedures. Local anesthetics provide inexpensive analgesia with minimal side effects and epidural anesthesia is simple and effective. In llamas and alpacas, the epidural space at the sacrococcygeal level is larger and shallower than in cattle and horses, which allows the use of shorter needle (18-20 ga x 1 inch) for epidural injections (Garcia Pereira FL et al. 2005) Preservative free lidocaine is often used at 0.22mg/kg, but other local anesthetics may also be used. Epidural injection of 1-5 ml of Lidocaine HCL will provide anesthesia of the hind limb and pelvis. Higher doses may cause incoordination, recumbency and paralysis of the hind limbs (Fowler ME, 1998).

3. Objectives:

The objectives of this study were: 1) to report findings of UDCO measurements in adult alpacas under general anesthesia during three different treatments: normotension, hypotension and hypertension, 2) to report the findings of ECHO cardiac output measurements in the same alpacas during normotension, hypotension and hypertension, and 3) to determine the level of agreement between UDCO and ECHO during normotension, hypotension and hypertension.

4. Hypothesis:

The hypothesis tested was that ultrasound velocity dilution cardiac output (UDCO) measurements for CO in anesthetized adult alpacas during normotension, hypertension and hypotension, will have a clinically acceptable level of agreement with CO measurement with transthoracic echocardiographic (ECHO) measurements during similar cardiovascular conditions.

5. Materials and Methods

5.1 Animals used:

To accomplish this study, 6 adult alpacas, 3 females and 3 males were used. The alpacas were part of the university teaching hospital herd and were acclimated to being handled. Age ranged from 9 to 17 years old and body weight ranged from 62.6 to 88.7 kg. Alpacas were determined to be healthy based on normal findings on clinical examination and complete blood count and serum chemistry panel prior to the study.

5.2 General Anesthesia and Instrumentation:

Food but not water was withheld for 12 hours prior to administration of any medications. The day before general anesthesia, alpacas were acclimated in pairs for 24 hours in a stall in a dedicated food animal medical barn to minimize stress. On the day of the study, a 14-gauge 5.5 inch polyurethane catheter (Terumo Surflo I.V catheter orange-14 Gauge) was aseptically placed in the left jugular vein under minimal physical restraint. The insertion point for the catheter was just cranial to the sixth cervical vertebra so the tip of the catheter was within the thoracic cavity. No premedication was administered to avoid any interaction with the variables measured. Anesthesia was induced with propofol 6mg/kg (Propoflo; Zoetis Laboratories, Italy) IV to effect and animals were positioned in sternal recumbency to allow for orotracheal intubation. Using a long blade laryngoscope, a polypropylene stylet was passed through the arytenoids and into the trachea. The laryngoscope was withdrawn, and a 12 mm internal diameter endotracheal tube was passed over the stylet and into the trachea. The stylet was withdrawn, and endotracheal tube place was confirmed by noting movement of air from the tube during exhalations.

The endotracheal tube cuff was inflated to secure the airway and to avoid any leaks in the breathing system. The tube was secured in place with medical tape around the mandible. Animals were positioned in right lateral recumbency on a padded table with a space between pads just caudal to the dependent elbow to allow for echocardiographic examination. Maintenance of anesthesia was performed with isoflurane (Isoflurane, Akorn, Inc., Lake Forest, IL, USA) in 100% oxygen delivered from a large animal anesthesia machine (Tafonius Jr., Hallowell EMC, MA, USA). Alpacas were mechanically ventilated with a Tidal Volume (V_T) of 15 ml/kg and a rate of 8 breaths per minute to maintain an end tidal CO_2 between 34 and 45 mm Hg. Alpacas were instrumented with electrocardiogram, pulse oximetry, and end-tidal gas analysis, from a dedicated patient monitor (Mindray Passport 12, Mindray North America, NJ, USA). A 20-gauge, 2-inch catheter (Surflash 20G x 1", Terumo, NJ, USA) was placed in a caudal branch of the right femoral artery at the midpoint of the medial aspect of the thigh just caudal to the femur. Three way stop-cocks were placed on the arterial and jugular catheters and pressure transducers were attached to one limb of each stop-cock with non-compliant, saline filled tubing. Transducers were connected to the patient monitor and zeroed to atmospheric pressure to measure arterial blood pressure and central venous pressure. Vital parameters were recorded at five minutes intervals during general anesthesia. The end tidal concentration of the isoflurane was recorded at 5 minute intervals. Mechanical ventilation was kept constant throughout the data collection period. A minimum of 15 minutes was allowed to elapse for patient anesthetic equilibration prior to initiating measurement of cardiac variables.

5.3 CO measurements via UDCO:

Ultrasound dilution cardiac output measurement was performed with the COstatus System (Transonic Systems, Ithaca, NY). During anesthesia, for each alpaca, an extracorporeal arteriovenous (AV) loop from the arterial catheter to the jugular catheter was created using sterile tubing designed to be used specifically with the UDCO monitor. The AV loop was connected to a peristaltic pump that kept blood flow constant through the loop during measurements. The AV tubing was placed in channels passing through two ultrasound velocity sensors, one was placed upstream from the venous catheter and the other was placed downstream from the arterial catheter For CO measurement, values for HR, systolic and diastolic blood pressure and animal length (from tip of the nose to the base of the tail) were entered into the COstatus monitor. The pump was started and maintained a constant flow of 14 ml/min through the AV loop. Saline (30 ml), warmed to body temperature, was then injected into a port on the venous side of the AV loop. The injection was performed manually over approximately 5 seconds. Measurements were performed by the COstatus as previously described. Measurements were performed in triplicate and then averaged to obtain a single value. Variables measured included cardiac output (CO), cardiac index (CI), stroke volume index (SVI), total ejection fraction (TEF), systemic vascular resistance index (SVRI), total end diastolic volume index (TEDVI), central blood volume (CBV), and active circulation volume index (ACVI).

5.4 CO measurements via Echocardiography:

Echocardiography measurements were obtained immediately following UDCO measurements. Echocardiographic examinations were performed with a GE Vivid E9

ultrasound machine with a phase array transducer (GE healthcare, Fairfield, CT) and simultaneous ECG monitoring. A transthoracic approach via a right thoracic window was used. The specific imaging plane used for measurements was based on what the examiner regarded as the most accurate and representative view of the LV. Cardiac measurements were done in triplicate and averaged to obtain a single value. Normalized left ventricular internal dimensions were obtained via measurement between the papillary muscle and below the mitral leaflet tips at end-diastole (LVIDdN) and at end-systole (LVIDsN). In addition, the interventricular septal (IVSd) and left ventricle (LV) posterior wall thickness were measured from M-mode obtained from a right short axis view during systole and diastole. Fractional shortening (FS), expressed as percentage, was another variable measured obtained from M-mode for each blood pressure group.

Left atrium diameter (LAD), aortic root dimension (AoD) and their ratio (LA:Ao) were measured from a 2D right short axis view. Normalized values for LAD (LADN) and AoD (AoDN) were calculated. Peak early (Emax) and late (Amax) mitral inflow velocity were measured at the tips of mitral valve leaflets from a left apical view and their ratio (E:A) was calculated. The diameter of the mitral value annulus was measured from a right parasternal long axis view. The velocity time integral of the mitral valve (VTI_{MV}) was measured using pulsed wave Doppler. The diameter of the aorta was measured at the level of the aortic annulus ($D_{Ao\text{-}AA}$) using the 2D image of the aorta obtained from the right parasternal long axis view. The velocity time integral (VTI) of aortic flow at the level of the aortic annulus ($VTI_{Ao\text{-}AA}$). The aortic ejection time (AoET) was measured from the pulsed wave Doppler tracing.

Left ventricular volume at end diastole (LVVd) and end-systole (LVVs) were calculated using the formula for the volume of a prolate ellipse with a length that is twice as long as the diameter. LVVd was calculated as $LVID^3$ and LVVs was calculated as $LVIDs^{3.}$ Both volume measurements were indexed to body weight. The total stroke volume (TOT SV m-mode) was calculated as LVVd-LVVs from M-mode derived measurements. The product of the mitral inflow velocity time integral (VTI_{MI}) and the diameter of the mitral annulus measured at the tips of the mitral valve leaflets in early diastole, was used to derive a value for total stroke volume ($TOT\ SV_{MI}$) based on the mitral inflow technique $CSA= \pi \times (D\ mitral\ annulus/2)^2$. The aortic derived stroke volume (TOT SVao) was calculated from the velocity time interval measured of the aortic valve and the diameter at the aortic annulus: $CSA= \pi \times (D\ aortic\ annulus/2)^2$.

Cardiac output was calculated by three different methods using the stroke volume derived from the M-mode, mitral and aortic valves. The cardiac output equals stroke volume × heart rate for each of the methods analyzed. Regurgitant flow was calculated by SV aorta – SV mitral/SV aorta for each time point to quantify the degree of valvular regurgitation.

5.5 Experimental design:

Alpacas had cardiac measurements under anesthesia during normotension (mean arterial blood pressure of 65 to 75 mmHg), hypotension (mean arterial pressure 45 to 55 mmHg), and hypertension (mean arterial pressure > 90 mmHg). After induction of anesthesia and instrumentation of each alpaca, at least fifteen minutes were allowed to elapse for anesthetic equilibration and stabilization of blood pressure. A similar minimum time was allowed between each treatment. The isoflurane anesthetic vaporizer was adjusted to maintain normotension. Cardiac measurements utilizing UDCO and ECHO as previously

described were performed. Following the normotensive phase (base line values), the hypotensive stage was initiated. Hypotension was achieved via increased anesthetic concentration with isoflurane. After a minimum of 15 minutes of hypotension, cardiac measurements were repeated. The final hemodynamic stage was hypertension. First, isoflurane concentration was decreased to allow mean arterial blood pressure to return to normotension. Then, phenylephrine was administered (0.5-2 mcg/kg/min) and adjusted to maintain a mean arterial blood pressure (MAP) greater than 85 mmHg. Again, a minimum of 15 minute was allowed and then cardiac measurements were repeated.

Heart rate, systolic arterial blood pressure, MAP, diastolic arterial blood pressure, oxygen saturation (measured by pulse oximetry) and end tidal concentrations of carbon dioxide and isoflurane were monitored every 5 minutes throughout all general anesthesia. A mean of each variable recorded was obtained at the time of each CO measurements.

Treatment order for changes in blood pressure was not randomized to limit the effects of hypotension and hypertension on normal cardiac function values.

After all measurements were obtained, alpacas were recovered from general anesthesia. No complications were recorded.

5.6 Statistical Analysis:

A sample size calculation was performed to detect a difference of 1L/min in cardiac output between blood pressure states with a sigma of 0.58, an alpha of 0.05 and a power of 0.8 indicating the need of 6 animals. Descriptive statistics for each measured parameter was calculated and reported as median (range). Due to a small sample size, all data were analyzed as non-normally distributed. Variables were analyzed with a Friedman test and a post hoc Dunn's test when significant. A p value < 0.05 was used for

significance. Cardiac output via UDCO and each ECHO method were assed for agreement using Bland and Altman plots (Bland & Altman 1986). Lower and upper limits of agreement were calculated.

6. Results:

All 6 alpacas completed the study successfully. No complications during general anesthesia or after recovery were recorded. There was no difference in heart rate between groups.

6.1 Cardiac Output Results for UDCO and ECHO

Results from CO measurements with UDCO and ECHO are summarized in tables 1 and 10. When measured via UDCO, ECHO-M mode, and ECHO-Ao Linear, CO during hypertension was significantly higher than CO during hypotension ($p = 0.0055$, $p = 0.0055$, $p = 0.0017$ respectively). There was no significant difference between hypertension compared to normotension or normotension compared to hypotension.

6.2 Level of Agreement with Bland-Altman plots

Bland-Altman plots for each blood pressure group comparing UDCO with each ECHO modality can be found in Figures 1, 2, and 3. ECHO-M mode had clinically acceptable agreement with UDCO for all blood pressure groups. ECHO-Ao Linear and ECHO-Minflow had clinically unacceptable agreement with UDCO for all blood pressure groups.

6.3 Other results obtained with UDCO

Findings for CI, SVI, SVR, SVRI, TEF, CBV, TEDV, and TEDVI measured with UDCO are summarized in tables 2 to 9. CI during hypertension was significantly higher than

during hypotension (*p* value = 0.0055). SVI during hypertension was significantly higher than during hypotension and normotension (*p* value = 0.0081). SVR and SVRI during hypertension were significantly higher than during hypotension (*p* value = 0.0017, *p* value = 0.005 respectively). TEF during hypertension was significantly higher than during normotension or hypotension (*p* value = 0.0087). CBV during hypertension was significantly higher than during normotension or hypotension (*p* value = 0.0081). TEDV and TEDVI were not different among groups.

6.4 Other results obtained with ECHO

Findings for IVSd, LVIDd, LVIDs, LVFWd, FS, Emax, Amax, E:A, $VTI_{Ao\text{-}AA}$, $D_{Ao\text{-}AA}$, VTI_{MV}, LVET, LAD, AoD, LA:Ao, and TOT SV: BW are summarized in tables 10 to 26. IVSd was not different between groups. LVIDd during hypertension was significantly higher than during hypotension (*p* value = 0.005).No significant differences were found in IVSd, LVIDs, LVFWd or FS among groups. LAD during hypertension was significantly larger than during normotension or hypotension (*p* value = 0.0081). AoD during hypertension was significantly larger than during hypotension (*p* value = 0.0017). LAD:Ao was not different among groups. Emax and Amax during hypertension were significantly higher than during hypotension (*p* value = 0.0081 and 0.0017 respectively). E:A during hypotension was significantly larger than during hypertension (*p* value = 0.0289). $D_{Ao\text{-}AA}$ during hypertension was significantly larger than during hypotension (p value = 0.0289). LVET during hypertension was significantly higher than during normotension (*p* value = 0.0289). $VTI_{Ao\text{-}AA}$ and VTI_{MV} were not different among groups.

TOT SV: BW during hypertension was significantly larger than during hypotension (p value = 0.0017).

7. Discussion

This study evaluated CO values measured via ECHO and UDCO in adult alpacas under general anesthesia during normotension, hypotension and hypertension. Additionally, we reported the level of agreement between the methods UDCO and ECHO in adult alpacas under these three blood pressure phases.

Values from UDCO and ECHO provided information about hemodynamic variables as well as cardiac function in alpacas under general anesthesia. In the present study CO measured via UDCO, ECHO-M mode and ECHO-Ao Linear, increased during hypertension. These results were unexpected with the use of phenylephrine. As expected, SVR and SVRI increased significantly during hypertension.

Regarding important results obtained with ECHO, LVIDd during hypertension measured with M-mode ECHO was higher than during hypotension, as well as TOT SV:BW.

Bland-Altman plots comparing UDCO and ECHO techniques for each blood pressure group showed an acceptable level of agreement only between UDCO and M-mode ECHO. Thus, we rejected the hypothesis that UDCO and ECHO would have good levels of agreement as only one ECHO CO technique agreed with UDCO measurement.

To our knowledge, this is the first report of measured CO and hemodynamic variables in anesthetized alpacas using ECHO and UDCO during normotension, hypotension via isoflurane and hypertension with phenylephrine.

There are few studies reporting cardiac output or cardiac index in alpacas and other camelids (llamas). In a study by Vincent J.C et al., 2009, they report CI (ml/kg/min) and other hemodynamic parameters measured with PAC, under general anesthesia in alpacas

describing the pharmacologic effects of dobutamine and norepinephrine but not phenylephrine. Another study (Garcia-Pereira L F, et al. 2007) reported CO values via PAC in alpacas under general anesthesia before and after the administration of butorphanol. Finally, in the study by Duke et al.1997, CI is reported in ml/kg/min in llamas under GA with halothane. Overall, there is a lack of controlled studies on CO measurements in alpacas during general anesthesia, particularly during changing hemodynamic status (normotension, hypotension, or hypertension).

In the study by Garcia-Pereira et al. 2007, CO data, measured via PAC, was collected from anesthetized alpacas during normotension (4.5 L/min). In a comparison with those results, the values we obtained with UDCO during normotension were highly similar suggesting that, at least during normotension, UDCO performs accurately in this species. This was expected as UDCO has been validated to be accurate in many species.

CO increased during hypertension with the used of phenylephrine. This finding was unexpected. TEDV, TEDVI and FS also increased during hypertension, but wasn't statistically significant. This may have been due to the small number of animals in the study. Phenylephrine was selected to induce hypertension because it was believed that it would not have a direct effect on the heart as phenylephrine lacks effect on beta-adrenergic receptors. It is considered a potent alpha-1-adrenergic receptor and is utilized as a vasopressor. Alpha adrenergic receptors are located within pre and postsynaptic regions of sympathetic nerve endings on smooth muscle cells and are minimally present on myocardial cells (Bohm M, et al., 1988). In the heart, the main adrenergic receptor is beta-1, which compromises roughly 90% of the total cardiac adrenergic receptors with alph-1, accounting for approximately 10% (O´Connell DT, et al., 2014). In general,

activation of cardiac beta-1 adrenergic receptors induces positive inotropic and chronotropic responses (Bristow RM, 2000). Less is known about the cardiac alpha-1 adrenergic receptors, but studies from human medicine indicate that long-term activation of cardiac alpha-1 adrenergic receptors activate beneficial trophic signaling (O´Connell DT et al. 2014). Stimulation of the alpha-receptors in the vascular muscle causes vasoconstriction and increases blood pressure, where myocardial alpha-receptors stimulation may have a slower onset and lead to a prolonged increased in the inotropic stated of the heart (Govier CW, 1968). Among some species, including mouse, guinea pig, rabbit, pig and cow, a greater number of alpha-1 adrenergic receptors are present in the heart and thus play a larger role in cardiac performance (O´Connell DT, et al. 2014). The results of the present study suggest the presence of these receptors in the heart of alpacas in higher concentration and their activation resulted in an increased cardiac output when activated by phenylephrine. Moreover, in a study in newborn llamas, phenylephrine caused an intense femoral vasoconstriction compared to minimal change in newborn sheep suggesting that camelids have an enhanced peripheral sensitivity to alpha adrenergic stimulation. Additionally, identification of a different population of alpha-1 adrenergic receptors subtypes was expressed in the vascular beds suggesting that not only was response increased but that a different type of alpha receptor was responsible for it (Moraga, A. F. et al., 2011). It was thought that this response may have resulted from physiologic adaptation to life at native high altitudes.

Other reasons for an increase CO during hypertension may be due to phenylephrine induced vasoconstriction centralizing blood volume from peripheral to central veins thereby raising preload, stroke volume (SV) and thus cardiac output (Krogh A, 1912).

However, this mechanism would be based on the assumption that a significant portion of venous blood is stored in the splanchnic organs and that phenylephrine induces an unloading of this reservoir (Gelman S, 2008).

Bland-Altham plots showed acceptable agreement only between UDCO and M-mode ECHO. This finding could be explained with how the two Doppler pulse wave methods were performed. In ideal conditions, the alpacas should had been placed in left lateral recumbency after measurements in right lateral recumbency, for additional measurements with a left-caudal 4-chamber view and a left caudal long axis view (Thomas WP, et al., 1993). In addition, with would allow for Doppler pulse wave measurement at a better angle (completely parallel with the estimated direction of the blood flow) (Kirberger M. R et al., 1992). This positioning is very important because Doppler ultrasonography is extremely dependent on alignment with the flow and catching the signal at the proper angle (≤20°) is fundamental to obtaining an accurate measurement (Mercado P et al., 2017). In our study, the alpacas remained in right lateral recumbency during all echocardiographic measurements. Changing animal position was not feasible in this study due to the instrumentation necessary for UDCO use and because anatomic changes in heart position may have affected blood pressure and cardiac output.

There are several limitations of the study. First, the small sample size may have affected to the results of non-cardiac output measurements as the sample size calculation was made only for CO results. Secondly, the alpacas were mature adults, as they were within the range between 9-17 years old. Alpacas' life span is reported to be between 15-20 years old (Brandford A, 2017). During ECHO several alpacas had regurgitant blood flow due to mitral valve insufficiency. Age-related degeneration of the mitral and tricuspid

valves is an uncommon finding in llamas and alpacas (Cebra C, et.al., 2014). Dilated cardiomyopathy (DCM) as well as hypertrophic cardiomyopathy (HCM) has been reported in Old and New World camelids, but neither one is a commonly encountered condition (Gentile JM, Abbott JA, 2010) and was not identified in any of the alpaca in this study. The most commonly reported cardiac conditions are congenital heart diseases (septal defects, valve dysplasia, and vascular anomalies) and acquired cardiac diseases (nutritional myopathy, cardiotoxicity, and bacterial endocarditis and myocarditis), which were also not identified in any of the alpacas in this study (Margiocco M, et al. 2009). In human patients, when CO is measured via echocardiography, aortic valve stroke volume correlates well with the stroke volume derived from mitral valve echograms in the patients without mitral regurgitation but it does not correlate well in the patients with mitral regurgitation (Corya CB et al. 1981). This finding may explain why only M-mode ECHO had acceptable agreement with UDCO. Finally, the used of intermittent positive pressure ventilation (IPPV) could had affected CO measurements. During mechanical ventilation, wide variation in CO measured with thermodilution may reflect cyclic variation in right ventricular output related to changing mechanical forces of ventilation (Snyder JV and Powner DJ, 1982). During IPPV, elevated intrathoracic pressure is transmitted against the right atrial wall, reducing venous return. This reduction in venous return is worse with high mean airway pressure and positive end expiratory pressures (PEEP). The reduction in right-sided preload, causes a reduction in right-sided cardiac output (Guyton AC, 1967), which in turn, reduces left sided preload, stroke volume and mean arterial blood pressure. Alpacas in this study were not subjected to PEEP during mechanical ventilation and the peak inspiratory pressure used during the procedure was

minimized by using tidal volume (TV) calculated to 15ml/kg. This likely resulted in minimal impact on CO as there were no appreciable variations in arterial pulse waves but could not be ruled out completely.

UDCO has been validated against PAC thermodilution in multiple in vitro studies (Krivitsi NM, et al. 2008), as well as in vivo studies with adult, pediatric and neonatal humans (Tsutsui M, et al. 2009) (Gleed R, et al., 2006), piglets (de Boode W, et al., 20010) and in a neonatal model with lambs (Vrancken LS et al. 2014). UDCO has been used in more than 100 peer-reviewed publications (Krivitski NM, 2003) and it is the second most widely used dilution technology after thermodilution to measure clinical hemodynamic parameters (Tsutsui M, et al., 2009). Further studies are needed to validate UCHO against the gold standard, PAC, in alpacas as well as with ECHO, as this method has only been validated in pregnant women (Cornette J, et al. 2017) and dogs (Lopes PC, et al. 2010). To further support the use of UDCO in alpacas is that the CO findings using PAC in normotensive, healthy, anesthetized alpacas in the study by Garcia-Pereira L F, et al., 2007, are nearly identical to the CO findings in our normotensive group measured with UDCO.

Future studies in alpacas should include investigation of the mechanism of phenylephrine on CO for a better understanding of the alpha-1 adrenergic receptors in alpacas. Additionally, comparison of the pharmacodynamic effects of other inotropic and vasopressor drugs in anesthetized alpacas should be performed for improvement in decision making during hypotension in alpacas.

In conclusion, CO measurements via ECHO and M-mode ECHO resulted in acceptable level of agreement in anesthetized alpacas. Additionally, CO can be increased with phenylephrine in alpacas under general anesthesia.

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Appendix 1: Tables

Variables measured with UDCO method:

Table 1 – CO (L/min)

Group	Median	Min	Max
Normotensive	4.30	3.03	5.74
Hypotensive	4.46	2.14	4.63
Hypertensive	6.62*	4.22	8.90

P value = 0.0055
(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 2 – CI (L/min/m^2) measured with UDCO.

Group	Median	Min	Max
Normotensive	3.83	2.84	6.68
Hypotensive	3.80	2.01	5.42
Hypertensive	6.58*	3.52	10.04

P value = 0.0055
(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 3 – Stroke Volume Index (SVI mL/m^2)

Group	Median	Min	Max
Normotensive	49.5	33.67	91.67
Hypotensive	53.5	35.33	70.67
Hypertensive	71.83*	59.67	121.00

P value = 0.0081
(*) Significantly different (Normo vs Hyper $p < 0.05$ and Hypo vs Hyper $p < 0.05$)

Table 4 – Systemic Vascular Resistance Index (SVRI dyn*s*$^{cm-5}$*m^2)

Group	Median	Min	Max
Normotensive	1370.00	689.67	1983.33
Hypotensive	808.33	563.33	1446.67
Hypertensive	1303.33*	903.33	3120.00

P value = 0.0017

(*) Significantly different (Hypo vs Hyper $p < 0.01$)

Table 5 – Systemic Vascular Resistance SVR (dyn*s*cm^{-5})

Group	Median	Min	Max
Normotensive	1211.70	996.67	1853.30
Hypotensive	796.67	590.00	1356.70
Hypertensive	1268.30*	923.33	2193.33

P value = 0.0055

(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 6 – Total Ejection Fraction (TEF %)

Group	Median	Min	Max
Normotensive	43.67	36.67	48.00
Hypotensive	40.17	34.00	45.33
Hypertensive	52.67*	42.00	53.33

P value = 0.087

(*) Significantly different (Normo vs Hyper $p < 0.05$ and Hypo vs Hyper $p < 0.05$)

Table 7 – Central Blood Volume (CBV ml)

Group	Median	Min	Max
Normotensive	1363.30	1030.00	1493.33
Hypotensive	1240.00	1080.00	1580.00
Hypertensive	1570.00*	1406.70	1973.30

P value = 0.0081

(*) Significantly different (Normo vs Hyper $p < 0.05$ and Hypo vs Hyper $p < 0.05$)

Table 8 – Total End Diastolic Volume Index (TEDVI ml/kg)

Group	Median	Min	Max
Normotensive	10.43	6.20	24.00
Hypotensive	11.67	6.10	23.50
Hypertensive	14.67	6.73	22.67

P value = 0.1840

Table 9 – Total End Diastolic Volume (TEDV ml)

Group	Median	Min	Max
Normotensive	560.00	400.67	652.67
Hypotensive	563.00	444.67	642.67
Hypertensive	623.17	587.67	779.00

P value = 0.1840

Variables measured with ECHO method

Table 10 – CO (L/min):

Table 10.a – CO M-mode (L/min)

Group	Median	Min	Max
Normotensive	5.01	2.85	7.25
Hypotensive	2.66	2.21	4.23
Hypertensive	5.79*	4.48	15.89

P value = 0.0055
(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 10.b – CO Ao Linear (L/min)

Group	Median	Min	Max
Normotensive	5.27	3.29	8.75
Hypotensive	4.45	4.04	7.64
Hypertensive	9.84*	6.28	11.82

P value = 0.0017
(*) Significantly different (Hypo vs Hyper $p < 0.01$)

Table 10.c – CO min flow (L/min)

Group	Median	Min	Max
Normotensive	8.40	4.41	11.01
Hypotensive	7.86	3.89	11.28
Hypertensive	11.00	10.10	14.73

P value = 0.1840

Table 11– Thickness of Interventricular Septum at end-diastole (IVSd) (cm)

Group	Median	Min	Max
Normotensive	1.26	1.13	1.50
Hypotensive	1.30	1.16	1.49
Hypertensive	1.21	1.09	1.41

P value = 0.1840

Table 12 – Left ventricular internal diameter at end-diastole (LVIDd) (cm)

Group	Median	Min	Max
Normotensive	4.72	4.24	5.08
Hypotensive	4.06	3.91	4.41
Hypertensive	4.94*	4.69	5.43

P value = 0.0055

(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 13 – Left ventricular internal diameter at end-systole (LVIDs) (cm)

Group	Median	Min	Max
Normotensive	4.72	4.24	5.08
Hypotensive	4.06	3.91	4.41
Hypertensive	4.94	4.69	5.43

P value = 0.1416

Table 14 – Left Ventricular free wall thickness at end diastole (LVFWd) (cm)

Group	Median	Min	Max
Normotensive	1.20	1.01	1.38
Hypotensive	1.16	0.99	1.71
Hypertensive	1.06	0.95	1.17

P value = 0.1416

Table 15 – Fractional shortening (FS %)

Group	Median	Min	Max
Normotensive	25.92	15.58	30.96
Hypotensive	24.90	19.42	36.80
Hypertensive	33.08	26.09	40.39

P = 0.0521

Table 16 – Left atrium diameter (LAD) (cm)

Group	Median	Min	Max
Normotensive	4.66	3.41	5.47
Hypotensive	4.31*	4.00	5.57
Hypertensive	5.42	4.50	5.75

P value = 0.0081
(*) Significantly different (Normo vs Hypo $p < 0.05$ and Hypo vs Hyper 0.05)

Table 17 – Diameter of the Aorta (AoD) (cm)

Group	Median	Min	Max
Normotensive	3.52	3.05	3.77
Hypotensive	3.31*	2.75	3.55
Hypertensive	3.63	3.12	3.96

P value = 0.0017
(*) Significantly different (Hypo vs Hyper $p < 0.01$)

Table 18 – Left atrium to aortic root diameter ratio (LA:Ao)

Group	Median	Min	Max
Normotensive	1.34	1.02	1.56
Hypotensive	1.35	1.20	1.60
Hypertensive	1.45	1.35	1.66

P value = 0.1416

Table 19 – Peak left ventricular early filling velocity (Emax) (m/s)

Group	Median	Min	Max
Normotensive	0.45	0.27	0.58
Hypotensive	0.44	0.38	0.67
Hypertensive	0.63*	0.54	0.72

P value = 0.0081
(*) Significantly different (Normo vs Hypo $p < 0.05$ and Hypo vs Hyper $p < 0.05$)

Table 20 – Peak left ventricular late filling velocity (Amax) (m/s)

Group	Median	Min	Max
Normotensive	0.36	0.22	0.56
Hypotensive	0.30	0.25	0.37
Hypertensive	0.64*	0.52	1.03

P value = 0.0017

(*) Significantly different (Hypo vs Hyper $p < 0.01$)

Table 21 – Ratio of peak early to late ventricular filling velocity (E:A)

Group	Median	Min	Max
Normotensive	1.40	0.48	2.05
Hypotensive	1.57*	1.04	1.89
Hypertensive	1.03	0.53	1.21

P value = 0.0289

(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 22 – Velocity time integral of aortic flow signal at level of Aortic Annulus (VTI_{Ao-AA}) (cm)

Group	Median	Min	Max
Normotensive	12.40	9.12	15.35
Hypotensive	11.71	10.21	13.00
Hypertensive	15.89	8.96	21.15

P value = 0.1416

Table 23 – Diameter of the aorta at the level of Aortic Annulus (D_{Ao} – AA) (cm)

Group	Median	Min	Max
Normotensive	2.58	2.50	3.26
Hypotensive	2.66	2.50	3.04
Hypertensive	2.84*	2.60	3.26

P value = 0.0289

(*) Significantly different (Hypo vs Hyper $p < 0.05$)

Table 24 – Velocity time integral of mitral valve flow signal (VTI_{MV}) (cm)

Group	Median	Min	Max
Normotensive	8.02	6.62	17.52
Hypotensive	9.26	5.07	17.86
Hypertensive	10.89	6.79	16.09

P value = 0.7402

Table 25 – Left Ventricular ejection time (LVET) (sec)

Group	Median	Min	Max
Normotensive	0.29	0.25	0.34
Hypotensive	0.29	0.24	0.38
Hypertensive	0.39*	0.37	0.41

P value = 0.0289

(*) Significantly different (Normo vs Hypo $p < 0.05$)

Table 26 – Total Stroke volume indexed to body weight (TOT SV:BW) (mLs/kg)

Group	Median	Min	Max
Normotensive	0.79	0.46	1.16
Hypotensive	0.50	0.40	0.62
Hypertensive	1.28	0.71	1.39

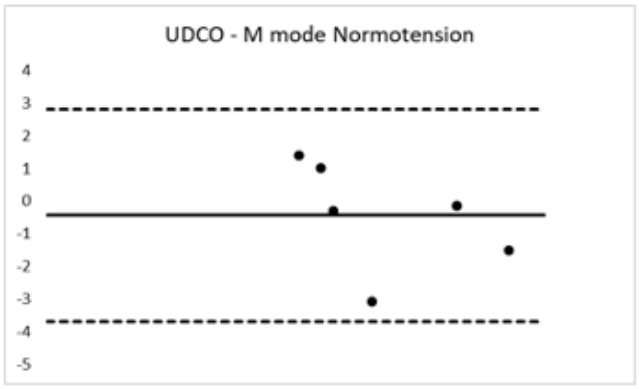
P value = 0.0017

(*) Significantly different (Hypo vs Hyper $p < 0.01$)

Appendix 2: Figures

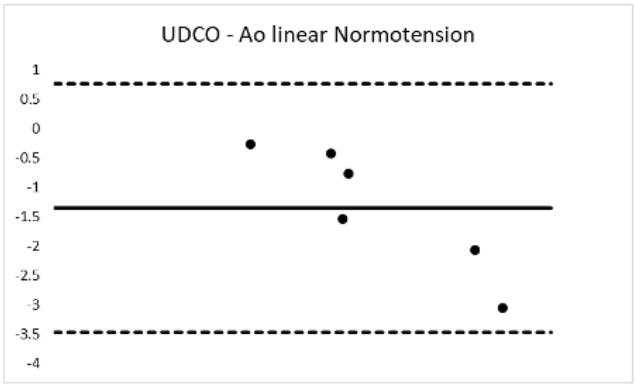
UDCO versus M Mode

Bias	-0.43921435
Stan Dev	1.657628306
Lower LOA	-3.688165829
Upper LOA	2.809737129



UDCO versus Ao linear

Bias	-1.350774142
Stan Dev	1.080681329
Lower LOA	-3.468909547
Upper LOA	0.767361263



UDCO versus Minflow

Bias	-3.757736885
Stan Dev	1.769048569
Lower LOA	-7.225072079
Upper LOA	-0.29040169

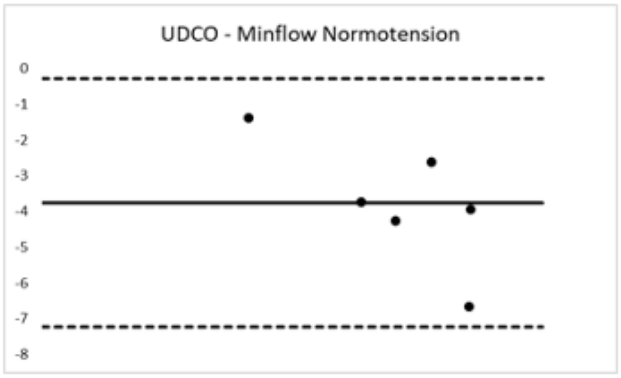
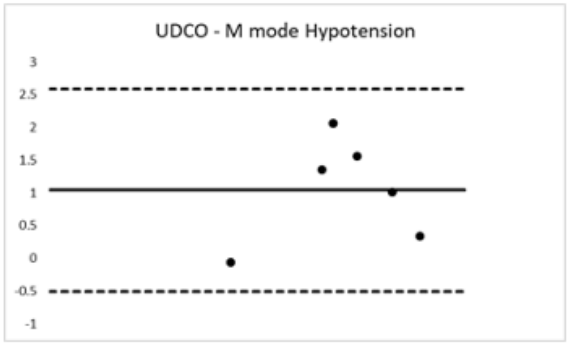


Figure 1: Bland-Altman plots for the comparison of UDCO and ECHO during normotension

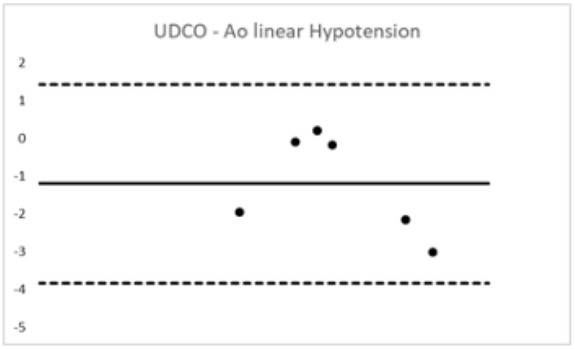
UDCO versus M Mode

Bias	1.04326738
Stan Dev	0.789349108
Lower LOA	-0.503856872
Upper LOA	2.590391632



UDCO versus Ao linear

Bias	-1.196749691
Stan Dev	1.344607728
Lower LOA	-3.832180839
Upper LOA	1.438681457



UDCO versus Minflow

Bias	-3.953885105
Stan Dev	3.011785547
Lower LOA	-9.856984776
Upper LOA	1.949214567

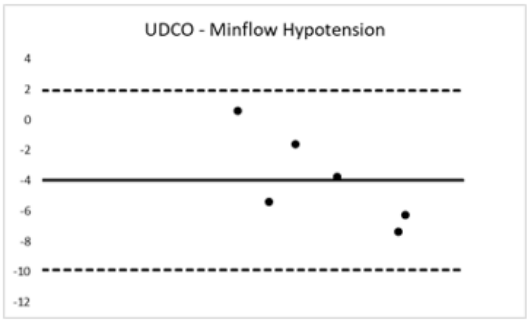
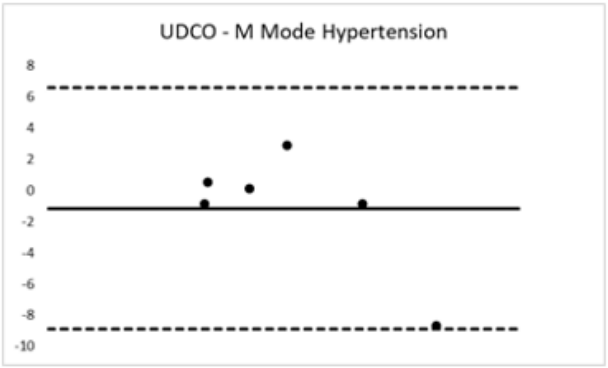


Figure 2: Bland-Altman plots for the comparison of UDCO and ECHO during hypotension

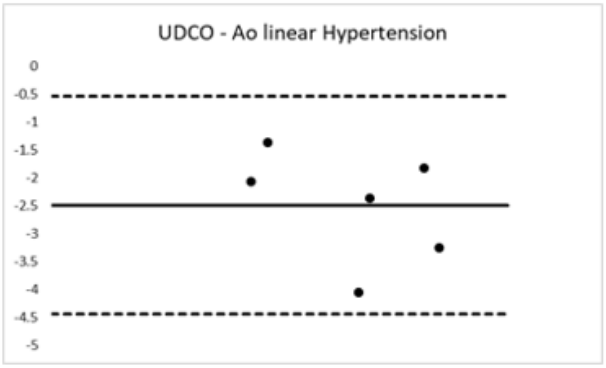
UDCO versus M Mode

Bias	-1.141572617
Stan Dev	3.953494496
Lower LOA	-8.890421828
Upper LOA	6.607276594



UDCO versus Ao linear

Bias	-2.489141122
Stan Dev	0.994054046
Lower LOA	-4.437487052
Upper LOA	-0.540795192



UDCO versus Minflow

Bias	-5.274377813
Stan Dev	2.682778046
Lower LOA	-10.53262278
Upper LOA	-0.016132844

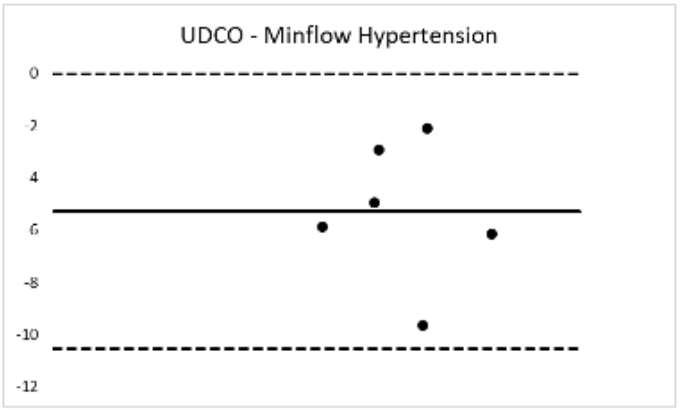


Figure 3: Bland-Altman plots for the comparison of UDCO and ECHO during hypertension