Application of a smartphone modulated ECG device for use in equines.

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List of Abbreviations:

App: Application (i.e. software on a cell phone or other mobile device)
Ausc: Auscultated/auscultate
AV: Atrioventricular
AVN: Atrioventricular node
BA: Bland Altman
BPM: Beats per minute
ECG: Electrocardiogram/Electrocardiography
ECG_{AKM}: *Alivecor KardiaMobile* ECG
ECG_{TV}: *Televet 100* ECG
ECG_{VET}: *Alivecor Veterinary Heart Monitor*
FDA: Food and Drug Administration
H: Bundle of HIS
HR: Heart rate
ICS: Intercostal space
LA: Left atrium
LAM: Left arm
LV: Left ventricle
MEA: Mean electrical axis
Ms: Milliseconds
RA: Right atrium
RAM: Right arm
RV: Right ventricle
SA: Sinoatrial node
T_a: Atrial t wave showing atrial repolarization
USA: United States of America

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Abstract

Application of a Smartphone Modulated ECG Device for Use in Equines.

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Introduction
Reason for performing the study: This research was performed to determine whether a novel ECG device, that uses smartphone technologies and applications, can be used to obtain electrocardiogram (ECG) readings in horses.
Objectives: The main objectives of the present study focused on determining the best method of application for the *Alivecor KardiaMobile*[a] (ECG_{AKM}) smartphone modulated electrocardiographic device in horses including body site, skin preparation as well as device orientation. The study also focused on the comparison to the *Televet 100* (ECG_{TV}) electrocardiographic device which is commonly employed for ECG recordings in horses.

Study design
A cross sectional study.

Materials and Methods
Research was completed in two parts. In the first part, 36 Nooitgedacht pony mares were used to determine the best site of application, method of skin preparation as well as ECG_{AKM} device orientation for reliable ECG tracings. The different body sites for application of the device included the fourth intercostal space of the left thorax, fourth intercostal space of the right thorax and right triceps muscle mass. The automatically calculated heart rate from the device application was compared to that acquired via auscultation.
Once the best method of ECG_{AKM} application had been established, 31 Nooitgedacht pony mares were used to compare the device with a standard ECG_{TV} device. ECG tracings were evaluated for agreement between the two devices considering the PQ, RR, QT and QRS intervals as well as the overall rhythm assessment for the ECG devices.

Results
The ECG_{AKM} device is best applied in the fourth intercostal space on the left hemithorax in a vertical orientation with reference to the ground and with the skin dampened with 70 % alcohol. Using this configuration, the ECG_{AKM} device was able to achieve acquisition of complete decipherable ECG tracings in 91.67 % of readings.
Arrhythmias were noted in 3/31 horses and were determined to conform to sinoatrial blockade/arrest in both the ECG_{AKM} and ECG_{TV} tracings. Independent t tests conducted on the mean values for RR; QT and QRS intervals yielded p-values of 0.73; 1 and 0 respectively. As such the mean values determined for RR and QT intervals were not significantly different, however it was determined that the mean values for the QRS interval were significantly different for the two devices. Independent-samples Mann Whitney U test indicated that the distribution of values of mean PQ interval were the same over the ECG_{AKM} and ECG_{TV} categories. These values for PQ, RR, QT, and QRS intervals were then also analysed using Bland-Altman plots.

Discussion and Conclusion

The left fourth ICS with a vertical device orientation yielded the best diagnostic quality ECG tracings. There was acceptable agreement between the ECG_{TV} and ECG_{AKM} devices. The ECG_{AKM} device appears to have potential to become entrenched as a basic screening tool with applicability in the field.

Chapter 1: Introduction to Research

1.1-Introduction

In the digital era, technological advancement and smart devices are becoming not only a driving force in the workplace but are now thought of as a mainstay for daily living. The technological competence of the younger generations has revolutionised modern medical science and serves to continue advancements in diagnostic evaluation and treatment of biological disease. Smart devices particularly focusing on the smartphone are at the forefront of this technological revolution.

Electrocardiographic (ECG) monitoring is however, by no means a new concept. As early as the 1960s, researchers have applied this technology to determine pathological conditions in the equine species (Rose et al., 1979). The idea of equine heart score as an index of presumptive performance, although it proved diagnostically controversial (Lightowler et al., 2004, Marlin and Nankervis, 2002), showed that the use of ECG in the horse presented interesting analytical conundrums for several decades (Rose et al., 1979).

The horse is known to have a higher incidence of cardiac dysrhythmias than any other domesticated species (Verheyen et al., 2010b) due to a high vagal tone. As such, electrocardiographic evaluation is an integral part of the cardiovascular examination (Verheyen et al., 2010b).

1.2-The cardiac cycle:

Electrical activity in the heart is initiated in the area of the atria known as the sinoatrial node (Figure 1). The electrical impulse then travels across the atria causing depolarisation and contraction of the cardiac muscle. This impulse can however not penetrate through to the ventricles at any point. It must first travel via the atrioventricular (AV) node to reach the ventricular conduction system. Once the impulse has been directed through the AV node, it can then follow the pathway created by the bundle of His and bundle branches. The impulse is then directed to the ventricular musculature via a conduction system known as the Purkinje fibre network. This then culminates in ventricular contraction.

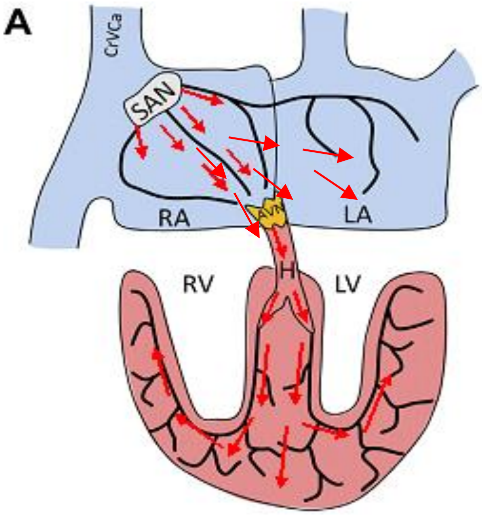


Figure 2*: Adapted from (Mitchell, 2019): Diagram of the heart with arrows showing direction of travel of electrical activity during the cardiac cycle; SAN-Sinoatrial node; AVN-Atrioventricular node; H- Bundle of HIS; RAM- right atrium; LA-left atrium; RV- right ventricle; LV- left ventricle*

1.3-The Electrocardiogram:

The basis of electrocardiography is the measurement of an electrical potential difference created by the electrical activity in cardiac musculature. This electrical potential can be measured via the skin if a sufficient amount of the cardiac musculature or “critical mass” displays electrical activity (Fish, 1988, Noble, 1979).
In order to measure the potential difference between two sites, a positive and negative electrode are placed at each of these two sites respectively. The system, thus formed, is then termed a “lead” (van Loon and Patteson, 2010, Mitchell, 2019). This lead system records changes in potential difference between two sites. The ECG device then displays the cardiac activity as an electrical tracing over time. If the summation of electrical activity is towards the positive electrode, it is seen as positive on the subsequent tracing. If it is towards the negative electrode, it is seen as a negative waveform on the tracing (Fish, 1988).

The normal ECG tracing is made up of groupings of waveforms denoted the P-QRS-T complex. Several of these complexes make up a tracing, with the total number dependent on the heart rate as well as total time of recording. When a single P-QRS-T complex is analysed, it is important to separate it into its components. The P wave denotes atrial depolarization and is rarely followed by an atrial T wave denoting repolarization (T_a) (Mitchell, 2019, Tilley, 1985). A delay is then normally seen without any deviation of the tracing as the impulse travels to the ventricles. Ventricular depolarization is denoted by the QRS complex. Finally, the repolarization that occurs in the cardiac cycle is denoted by the T wave.

A standard notation has been developed for labelling these waveforms. Following this notation, the equine ECG is generally denoted as seen in Figure 2. Standard convention suggests that the first negative waveform following the P wave is denoted the Q wave. The equine base-apex ECG rarely yields the Q wave deflection in these tracings. The first positive waveform is then denoted the R wave, and the next negative waveform the S wave. Due to this notation, the largest of the deflections in equines is regularly noted as the S wave. This differs to human and small animal cardiology where the largest deflection is regularly noted as the R segment (Noble, 1990, Mitchell, 2019).

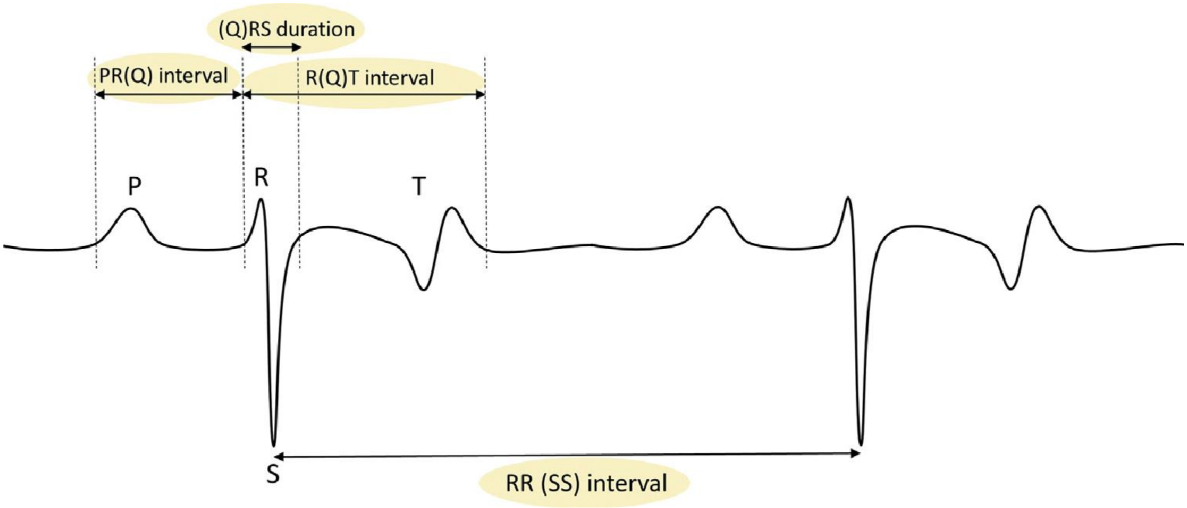


Figure 3*: (Mitchell, 2019): Tracing showing the labelling of waveforms in the equine ECG. The main intervals that are examined are the PR(Q) interval, (Q)RS Interval, R(Q)T interval and the RR(SS) interval. The labelling may require adjustment from that seen in other species due to differences in the configuration of the equine ECG.*

It is also of importance to note that several of these complexes are known to present with differing morphologies between different individuals and even between complexes in the same horse. Due to their naturally large atrial size, the equine P wave can present with a bifid or biphasic waveform in addition to the more standard single positive deflection (Verheyen et al., 2010a). A phenomenon known as “wandering pacemaker” is also prevalent in the equine species and can lead to differences in the morphology of sequential P waves. The morphology of the equine T wave is also noted to be highly variable with changes seen both due to high levels of resting parasympathetic tone as well as changes in heart rate (Broux et al., 2016).

QT interval values provide an indication of the time period consumed by the depolarization and repolarization of the functioning myocardial tissue and are highly dependent on individual heart rate (Paslawska et al., 2012). QT interval has been noted to shorten with increasing heart rate values (Pedersen et al., 2013). Breed has also been identified to contribute to the variability between different individuals (Paslawska et al., 2012). It has been reported that increased variability of the QT interval in the human heart is associated with predisposition to arrhythmic events and the propensity for sudden death (Pedersen et al., 2013). Similar findings relating to lengthening in the QT interval and pathological cardiac events have also been identified in equines (Kiryu et al., 1999), however the significance of changes in QT interval in the equine species requires further evaluation before a definitive conclusion can be made (Pedersen et al., 2013). It is also of importance to note that accurate measurement of the QT interval is fraught with difficulty (Postema and Wilde, 2014). This is due to several reasons including the decision of which lead to measure the QT interval in and knowing where the QT interval ends, which is especially true in the arrhythmic patient or when the morphology of the T wave is aberrant (Postema and Wilde, 2014). This is further complicated in equines by the fact that horses have immensely labile morphology in the T wave deflection (Mitchell, 2019). All of this must be taken into account when a clinically acceptable variation in the two methods is determined, especially considering the possibly reduced acuity provided by the diminished dipole of the ECG_{AKM} device.

The time intervals determined from ECG tracings are also dependant on the bodyweight of the horse examined and as such should be evaluated taking patient size into account (Mitchell, 2019).
Normal ECG complex and intervals durations for horses in the base apex lead configuration are expressed as follows (Table 1):

Table 1: (Verheyen et al., 2010b): Duration of common components of the equine ECG

Interval/Complex	Duration (s)
P wave	≤ 0.16
P-R	≤ 0.5
QRS	≤ 0.14
Q-T	≤ 0.6

*Duration (seconds)

1.4-Pitfalls in the electrocardiogram recording and analysis.

It is important to note that interpreting the equine ECG with regards to changes in the tracing, is different from that in man (Hanák and Jago, 1983). This is due to differences in the slope of the anatomic and electrical axis of the heart, different distribution of electrical potentials on the skin and importantly due to differences in the spread of electrical activity through the ventricular myocardium during depolarization and repolarization (Hanák and Jago, 1983). This difference is seen in ungulates and has previously been referred to as “tertiary arborisation” (Physick-Sheard, 2016). In essence this means that these species have an extension of the cardiac conduction system that penetrates the ventricle muscle and terminates almost at the level of the epicardium rather than the sub-endocardium as well as showing increased myocyte junctions (Physick-Sheard, 2016). This peculiarity in the comparative ECG between man and equids leads to much of the horse’s myocardium showing “silent depolarization” when viewed through the surface ECG (Physick-Sheard, 2016). This means that the equine ECG mainly holds value for analysis of rate and rhythm (Physick-Sheard, 2016).

Artefacts in the ECG tracing can be problematic and may hinder appropriate diagnosis of dysrhythmias. These so-called artefacts can be defined as any deviation in the tracing from the baseline value that is not a direct result of the electrical activity from the heart cycle. One of the most encountered artefacts is related to movement of either the patient or the leads. This can be seen as prominent deflections occasional resembling the QRS complex (Verheyen et al., 2010b). Muscle fasciculations (sharp, narrow and multiple deviations) as well as the motion associated with respiratory effort (large undulating deviations) often result in movement artefacts (Verheyen et al., 2010b). Sharp, narrow, and regular deflections over the entire ECG tracing are artefacts seen due to interference of electrical mains supply on the recordings. These must be carefully examined as they can be mistaken for the so-called “fibrillation (f) waves” seen in atrial fibrillation.
It is also important to be aware that changes in the P and T waves are commonly identified in horses that are exercised or stressed yet have no clinical significance (Verheyen et al., 2010b). The P wave may change in amplitude and shape and is often seen to displace toward the preceding T wave (Verheyen et al., 2010b). T wave polarity often becomes opposite to that of the QRS waveform. An elevation in the ST segment may also be seen (Verheyen et al., 2010b).

1.5-Lead Placement:

Various lead placement strategies have been suggested in the equine. These generally conform to either a base-apex (Figure 3) or modified base apex configuration (Figure 4). A general “rule of thumb” for electrode placement in the equine is that electrodes should be positioned in the same direction as the mean electrical axis. This axis is generally from cardiac apex to the base of the heart and slightly to the right cranial thorax. As such a single lead should be positioned with one electrode nearer the cardiac apex and one nearer the cardiac base (Verheyen et al., 2010b). Positioning leads all in a vertical direction will however result in loss of the cranio-caudal axis of the MEA thereby resulting in P waves with smaller amplitudes (Verheyen et al., 2010b).

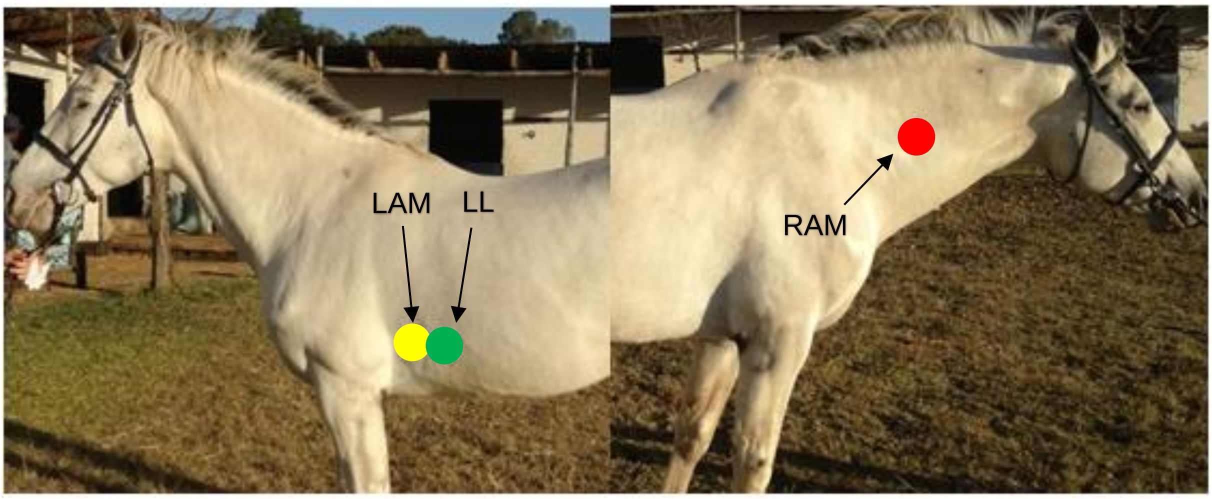


Figure 4*: Adapted from (Mitchell, 2019); Positioning of leads to obtain a base apex ECG- LAM: Left arm; LL: Left leg; RAM: Right arm. These are modified from the Einthoven lead system.*

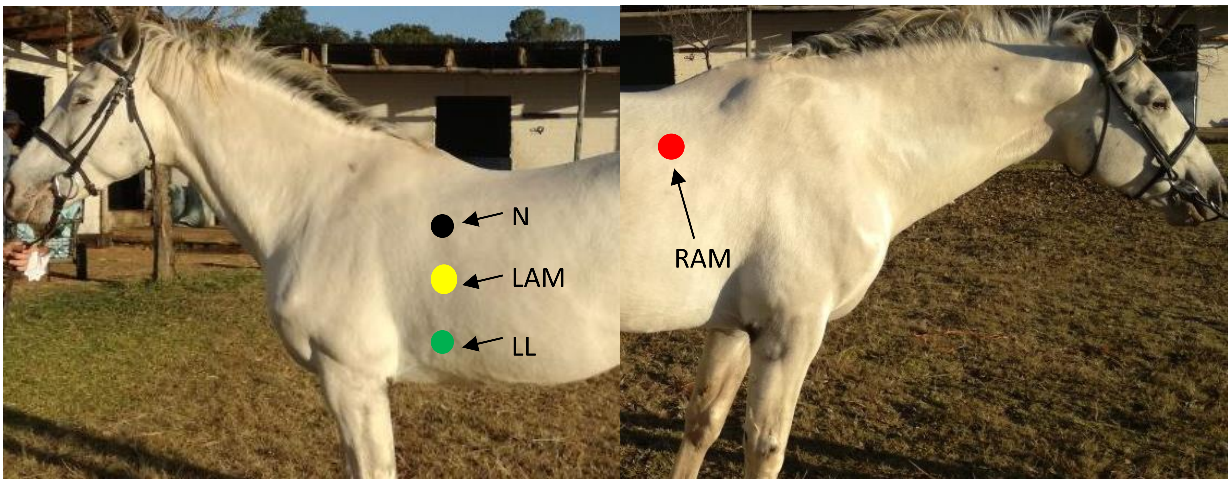


Figure 5: *Adapted from (Mitchell, 2019): Positioning of leads to obtain a modified base apex ECG- Electrodes coloured for use with Televet 100 (ECG_{TV}) recording system. LAM: Left arm; LL: Left leg; RAM: Right arm; N: Neutral.*

1.6-Additional Information

Currently, most field veterinarians are heavily reliant on simple clinical examination for the identification of abnormalities such as heart rate determination via peripheral pulse palpation and cardiac auscultation. Electrocardiographic monitors such as the *Televet 100* (ECG_{TV}) can be impractical and relatively expensive for the average ambulatory veterinarian considering the infrequency of their use. The *Alivecor KardiaMobile*[a] *(ECG_{AKM})* investigated in this study holds the possibility of providing additional diagnostic data as an inexpensive and convenient stall-side test. This information can then be applied when making decisions about the clinical health and wellbeing of equine athletes, safety for riders or to recommend further diagnostic evaluation. This improved diagnostic capability will serve to increase and evolve the concept of a "minimum database" in the diagnostic panel, thereby reducing the need for clinical extrapolation and creating a clearer "physiological picture" of the disease process. The veterinary professional is now, more than ever, expected to provide a thorough and reliable diagnostic service while retaining the affordability and alacrity of these work-ups. This novel device, if validated through scientific research, holds the potential to provide diagnostically significant information that conforms to these requirements.

Recent reports have attributed a portion of "sudden cardiac death episodes" to arrhythmogenic cardiovascular events (Navas de Solis, 2016). The sudden and often unexpected onset of these emergencies lends itself to the use of a simple, utilitarian and serviceable ECG device that couples convenience and ease of use with instantly available, diagnostically reliable data readouts.
The ECG_{AKM} offers this convenient size and user-friendliness at an affordable price. The ECG_{AKM} device utilises a non-invasive, non-painful application method; ensuring animal welfare is not compromised. For these reasons it is hypothesised that this device has the potential to be used in extended diagnostics applied in field conditions.
The company *Alivecor*[a] have subsequently launched a mobile ECG device, the "*Alivecor KardiaMobile*[a] (ECG_{AKM})", which is marketed worldwide for human use. The ECG_{AKM} device has not yet been evaluated for use in equines under clinic or field conditions.

1.7-Aim:

1. To determine the validity and diagnostic reliability of electrocardiographic measurements obtained using a smart-phone modulated electrocardiographic device i.e. the ECG_{AKM}.
2. To determine the most useful device application method.
3. To compare the device to an industry recognised standardised system namely the ECG_{TV} applied in a modified base-apex layout.

1.8- Hypothesis:

Null hypothesis:

Part 1
There is no difference in the prevalence of diagnostic quality ECG tracings obtained using the *Alivecor KardiaMobile* in different locations, orientations and with differing site preparation.

Part 2

a. Electrocardiographic measurements obtained using smartphone modulated electrocardiographic devices are a comparable and diagnostically reliable alternative to the modified base apex lead system.
b. The mean measurements for PQ, RR, QT and QRS recorded using the ECG_{AKM} are equal to those recorded using the ECG_{TV}.

Alternative hypothesis:

Part 1
There is a notable difference in the prevalence of diagnostic quality ECG tracings obtained using the *Alivecor KardiaMobile* in different locations, orientations and with differing site preparation.

Part 2

a. Electrocardiographic measurements obtained using a smartphone modulated electrocardiographic device (ECG_{AKM}) is not a comparable and diagnostically reliable alternative to the modified base apex lead system.
b. The mean measurements for PQ, RR, QT and QRS recorded using the ECG_{AKM} are different to those recorded using the ECG_{TV}.

1.9- Benefits arising from the experiment:

The main reason for this study is to establish if a non-invasive mobile human ECG device can be applied clinically for use in equines at rest. It is of vital importance, for aspects related to safety and welfare, to ensure equine athletes are physically and mentally healthy and that the equine athletic disciplines do not significantly impinge on the animal's health. This study aims to establish the usability of a device applied in the field setting to monitor equine cardiac electrical activity so that it can be applied in different areas of ambulatory veterinary practice. If successful, it will broaden the diagnostic capabilities of the field veterinarian during the cardiovascular examination. The results, as well as the method of data collection, will hopefully act as a basic framework and "stepping stone" in the institution of more extensive yet practical methods of physiological assessment in the South African equine disciplines. This will improve identification of pathological conditions as well as the safety, welfare and sustainability of equine sporting events.

We hope that we can demonstrate that the ECG_{AKM} device can fill this niche.
Other applications for this device may include exposure of possible negative impacts of endurance and other equine competitions on aspects of the cardiovascular health of the equine individual. The research, if successful, will also provide a set of repeatable guidelines for the application and use of the ECG_{AKM} device in the field setting.

1.10- Objectives:

Part 1
To determine the best location, orientation and conditions for ECG_{AKM} device application in the standing horse at rest. Specifically, where on the body the device should be placed, in what plane the device should be orientated, as well as the best method to ensure appropriate electrode-skin conductivity is achieved. The results will thus determine the most accurate and reliable method to collect data using the device in the standing horse at rest.

Part 2
To establish if ECG_{AKM} recordings are reliable for clinical use by comparing it to an established device ECG_{TV}. Initially this will be conducted under clinically controlled conditions to obtain a representative data sample with reference to the electrophysiological parameters of the cardiac cycle. It is integral to demonstrate the feasibility of this device before use in field conditions can be examined.

Chapter 2: Literature review

2.1-General ECG

Today electrocardiography is considered an integral diagnostic modality in the assessment of persons suffering from cardiac pathology (AlGhatrif and Lindsay, 2012). This diagnostic methodology has undergone several innovations since its genesis. The first human electrocardiogram was published in 1887 by the physiologist, Augustus Waller. Waller used a capillary electrometer and electrodes and identified that the process of ventricular contraction was preceded by a degree of electrical activity (Waller, 1887, AlGhatrif and Lindsay, 2012). The sensitivity of this new electrical heart assessment, coined in 1893 as an electrocardiogram, was improved in 1901 by Dr. Willem Einthoven. Einthoven achieved this by using a string galvanometer (which was noted to be an ungainly 600 pound device), as well as electrodes in the form of cylinders filled with electrolyte solution (Einthoven, 1901, AlGhatrif and Lindsay, 2012).

Since this time, further innovations have aided in making the electrocardiogram a more practical and accessible device. Advancement in modern technologies including smartphone computational power and smaller circuit boards and batteries, have propagated the development of diminutive smartphone-based ECG devices. These devices are all but unrecognisable when compared with their forefather, the string galvanometer.

There are at present a variety of different commercially available wireless systems that utilize a single lead system to obtain an electrocardiographic tracing. Several of these devices are USA FDA approved and verified to provide acceptable ECG tracings (Haverkamp et al., 2019). A review by Bansal and Joshi (2018) identified five single lead devices that have featured in scientific publications. Many of these publications centre on the ECG_{AKM}, which is marketed as a novel ECG technology. This ECG_{AKM} device is aimed at presenting the lay person with a simple and relatively inexpensive manner in which to monitor their electrocardiographic activity.

2.2-Smartphone-based ECG in humans

Several studies have been conducted in human populations with specific interest in the use of the smartphone-based ECG device for screening procedures. The concept of using this smartphone-based monitoring device is gaining traction in the research setting. Many current publications focus specifically on the use of the device for monitoring of atrial fibrillation. Due to the risks associated with this arrhythmia in man, early identification can undoubtedly reduce morbidity and mortality.

Hendrikx et al. (2014) investigated the use of intermittent short ECG recordings compared to 24-hour Holter monitoring for arrhythmia detection. They made use of a bipolar extremity lead 1 recording device (*Zenicor EKG® thumb*) (Hendrikx et al., 2014). The study concluded that the use of short intermittent recordings, both at times of presyncope as well as at predetermined intervals, showed a greater efficacy than regular 24-hour Holter monitoring in the identification of atrial fibrillation and paroxysmal supraventricular tachycardia (Hendrikx et al., 2014). This offers some evidence in support of the use of novel ECG devices in the evaluation of arrhythmic events.

In a study conducted by Haberman et al. (2015), it was concluded that the lead 1 tracings produced by a smartphone modulated *Alivecor* device were easier to conduct and obtain than the standard 12 lead ECG. They also deduced that the smartphone equipment provided tracings with accurate baseline conduction intervals as well as imparting a high degree of sensitivity (72,4%-94,4%) and specificity (>94%) in the diagnosis atrial arrhythmia in human patients. It was also of interest that in this research, patients indicated that they preferred the

smartphone-based diagnostic modality to that of the more complicated and time consuming 12 lead system. This may be mirrored by veterinary professionals, who are often focused on the convenience, cost-effectiveness and timely acquisition of a diagnosis.
The smartphone-based ECG device contributes high sensitivity and specificity to the detection of particularly atrial fibrillation and atrial flutter (Haberman et al., 2015). Several studies have indicated that the Alivecor device offers acceptable sensitivity and specificity regarding the detection of atrial fibrillation. For example, in a study by Lau et al. (2013), the sensitivity of the device for detection of atrial fibrillation was 98% and specificity 97%.

The ease of use and general convenience of the smartphone-based ECG device also lend themselves for use as a pre-participation and acute emergency diagnostic device in sport medicine. Sudden cardiac death in humans, especially athletes, is uncommon but remains a calamitous and disturbing event. The nature of this has prompted discussions among various medical and sports entities regarding screening for ECG abnormalities as a preventative measure (Gilliland et al., 2018). Although this concept retains a large degree of controversy, it has been examined in the literature. The EKG_{AKM} device has been compared to a standardised 12 lead recording in human athletes for this purpose (Gilliland et al., 2018). Although the sample size used in this investigation is undesirably small, the results did indicate that the smartphone-based ECG device presented good to very good reliability for the between rater and between device recording/measurement of heart rate, QT interval, and QRS duration (Gilliland et al., 2018). The research also highlights the fact that the use of an ECG device that provides only single lead analysis is neither an advocated nor accepted practice for any medical entity at present. This is based on the opinion that even the most rudimentary screening process requires multifarious anatomical ECG leads. Indeed, many professionals believe that pre-screening using an ECG, no matter the format, is unjustified (Gilliland et al., 2018). Their reasoning centres around the risks associated with false positive results excluding athletes from competitive events and prompting expensive work-ups. There also remains a general consensus that, at present, many inconsistencies in the interpretation of the ECG of the athletic population exist between different members of the medical profession (Gilliland et al., 2018). This results in the author’s conclusion that although the simplified, single lead ECG device may have some applicability to the suspected acute cardiac crisis, it is not at present a candidate for use in routine pre-participation screening of athletes for competitive purposes (Gilliland et al., 2018).

In human medicine, the measurement of paediatric ECGs presents a diagnostic conundrum as research indicates that children often present with arrhythmic events of a paroxysmal nature (Gropler et al., 2018). The ease of use, simplicity, and speed of configuration of the smartphone-based ECG device has the potential to fill this niche of obtaining recordings of these paroxysmal events. Comparisons between the ECG_{AKM} device and standardised 12 lead systems in paediatric patients have also indicated that the smartphone device provides accurate ECG tracings in healthy paediatric patients as well as those displaying cardiac pathology or rhythmic aberration. This led Gropler et al. (2018) to conclude that the device provides an “accurate, non-invasive and real-time approach for ambulatory monitoring in children and adolescents”.

2.3-Smartphone-based ECG in animals (excluding the horse)

With ever increasing research into the use of smartphone-based ECG devices in human cardiology, it is only natural for the veterinary cardiology sphere to emulate this trend. These devices may offer similar benefits for the veterinary diagnostician as the human diagnostician and potentially have an application in monitoring of patients by the lay person.
The small size of many of these smartphone based devices have resulted in concerns that the diminutive dipole present between the electrodes, when applied to a patient's thorax and not via the arms as in humans, will result in poor tracing resolution and subsequent difficulty in

ECG analysis (Kraus et al., 2016). It is thus of importance to analyse the accuracy of these devices in the veterinary patient.

Kraus et al. (2016) investigated the use of a bipolar, single lead smartphone ECG device and compared it to a 6-lead digital ECG device in dogs and cats. It was found that the smartphone-based ECG monitoring was a feasible and accurate method to determine the heart rate in small animal patients when compared to the 6 lead ECG (Kraus et al., 2016). This study identified the concern that recognition and evaluation of the P wave in smartphone based tracings was complicated, as the construction and simplicity of these devices lead to the creation of small waveforms when recording miniscule electrical activity (Kraus et al., 2016). This problem is particularly evident in felines (Kraus et al., 2016).

Further studies evaluated the use of a smartphone-based device marketed for veterinary purposes (*Alivecor Veterinary Heart Monitor-* ECG_{VET}) compared with a standard 6 lead system in canines (Vezzosi et al., 2016a). In this study the smartphone-based device was applied to the left precordial area simultaneously with the 6-lead device. The area was moistened with alcohol in short haired dogs while the site was first shaved in those with longer coats (Vezzosi et al., 2016a). The smartphone tracing and the tracing of lead II of the standard device were compared.

Tracings were identified as interpretable in 96.7% of cases with the non-interpretable ECG tracings all originating from small breeds in which motion artefacts were noted as to be a regular problem (Vezzosi et al., 2016a). By comparing these devices, it was deemed that the smartphone-based ECG had an excellent capacity to determine the heart rate in the animal. It was noted however, that heart rate determination by the ECG app was less accurate than manual measurement using the ECG tracing. Poor accuracy in some dogs was attributed to the app either falsely identifying P waves as QRS complexes or not identifying smaller QRS complexes at all (Vezzosi et al., 2016a). The smartphone device displayed 100% sensitivity and 97.9% specificity when used to determine the presence of arrhythmias in these canines. The device was especially accurate in identifying atrial fibrillation, which is unsurprising as the Alivecor device is equally accurate for this purpose in humans. The smartphone device was noted to underestimate the amplitude of recorded P waves. This leads to complications in the identification of some P waves and hinders the differentiation between atrial fibrillation and sinus arrhythmia (Vezzosi et al., 2016a). It may also be required to shift the orientation of the device in an attempt to increase the size and identification of waves. Early investigations in cats suggest that tracing will be of better quality if the device is applied parallel to the long axis of the heart (Vezzosi et al., 2016a).

A smartphone device was also noted to have good agreement with a standard ECG device with regards to recording the duration and polarity of the QRS complex in canines (Vezzosi et al., 2016a). Once again however, the comparison of P wave amplitude showed underestimation by the smartphone device. Considering these tendencies to underestimate amplitudes, it suggests that using a smartphone-based tracing to assess amplitudes and hence chamber distortion is not good practice (Vezzosi et al., 2016a). Comparisons of tracings also showed reliable recognition of ectopic beats as well as agreement in the examination of the PQ interval and atrio-ventricular blockade. Vezzosi et al. (2016a) concluded that the smartphone device should not be used as a substitute for 6 lead ECG systems but may be a useful tool when used in conjunction with standard ECG devices. The investigation also makes the recommendation that treatment on the basis of the smartphone ECG alone is undesirable.

The use of the smartphone-based ECG for home monitoring in canines has also been evaluated. It was observed that 89% of recordings taken by the lay person, in the home environment and emailed to a medical professional, were interpretable (Vezzosi et al., 2019). This suggests that the device is simple to use, and its app is easily grasped even by persons with no formal medical training. This also indicates that the use of this technology may present an additional tool in the home management of canines with cardiac pathology (Vezzosi et al., 2019).

The ECG_{VET} has also been evaluated for use in dairy cattle (Bonelli et al., 2019). Similar to other studies, the device was compared to a reference ECG system applied in the typical base-apex layout (routinely used in large mammals). The smartphone device was applied to alcohol moistened skin, below the level of the olecranon with a 30-degree cranial orientation (Bonelli et al., 2019). Comparisons between heart rate and waveform polarity were conducted as seen in several other studies (Vezzosi et al., 2018a, Vezzosi et al., 2016b, Kraus et al., 2016, Kraus et al., 2019). In this study, Bonelli et al. (2019) also focused on comparison of waveform interval measurements to determine agreement between the smartphone device and that of the reference method. Since all bovids in this study were healthy and showed normal sinus rhythm, no evaluation of the ability to detect abnormal rhythm was possible. Instead, analysis centred on the duration of the P wave, PR interval, QRS complex and QT interval (Bonelli et al., 2019). Smartphone tracings were identified as interpretable in 89% of instances (Bonelli et al., 2019). The heart rate determined by the app in this study showed good accuracy in comparison to manually calculated heart rates (Bonelli et al., 2019). The research also identified that the smartphone device was reliable for evaluation of ECG waves and interval measurements with differences not being of clinical significance. The study did however identify that the smartphone device was not reliable with regards to the polarity of the P wave when compared to the standard device (Bonelli et al., 2019).

The smartphone-based ECG device has not only been evaluated in domestic animals. Research extends to animals that less commonly receive cardiac evaluations. In 2018, a comparison was made between a smartphone-based ECG and a 6-lead ECG device in the Atlantic Bottlenose Dolphin (*Tursiops Truncatus*) (Yaw et al., 2018). The study pinpointed that at the time the smartphone-based ECG had not been tested in aquatic creatures or those with class B ventricular activation. Due to small sample size, a descriptive comparison was made between these two devices. The left side was chosen for application of the device, as studies have indicated it yields a higher amplitude QRS complex (Yaw et al., 2018). The study concluded that the smartphone device allowed evaluation of parameters including heart rate, heart rhythm and QRS complex polarity in this dolphin species. Similar to other studies however, there are concerns that the amplitude of waveforms recorded using this device may be smaller than seen in the standard devices, owing to the much closer proximity of the electrodes making interpretation with regards to amplitude less accurate. It may also be of interest that owing to the use of sound waves to transmit ECG from the device to the phone, the recording is sensitive to artefacts created by sounds of similar frequency. The vocalization of these dolphins appeared to be one of the sounds which caused artefactual interference with the device recordings (Yaw et al., 2018).

The ECG_{VET} has also been evaluated for its use in buffalo (Bubalis Bubalis) calves (Smith et al., 2016). In this study the Alivecor device was applied to the thorax just medial to the olecranon with the electrodes perpendicular to the ground. The site was clipped to remove hair and ultrasound gel was applied to aid with contact. The tracings from this device were then compared to tracings recorded simultaneously with a standard 6-lead ECG device (MAC-1200) (Smith et al., 2016). The devices' tracings were compared with regards to rhythm, heart rate and overall quality. The study concluded that the ECG_{VET} offered accurate diagnosis of both heart rate as well as sinus rhythm in these buffalo calves (Smith et al., 2016). It was also noted that there was no significant difference identified between quality score for the tracing produced by the two ECG devices.

The study titled "Variations in heart rate and rhythm of harbour seal pups during rehabilitation" made use of the ECG_{VET} to evaluate ECG parameters (Fonfara et al., 2015). The device was used as the primary methodology to obtain and record ECG tracings from young seals. These tracings were reliably analysed for determination of heart rate, P wave duration, PR interval duration, QT interval duration, and QRS complex duration. Tracings also allowed the identification of several arrhythmias including second degree AV block as well as supra- and ventricular premature complexes (Fonfara et al., 2015). Although the primary aim of the study was not to determine the usability of the smartphone-device in seals, the results anecdotally display that the device can be used in this species to obtain a repeatable ECG tracing that can contribute to clinical assessment of the electrical activity of the heart. Other studies assessing ECGs have made use of the ECG_{VET} for recording purposes. Participants in these studies were noted to be pigs as well as a monitor lizard (Smith et al., 2016).

2.4-Smartphone-based ECG in equines

The practicality and the inexpensive nature of these smartphone-based ECG devices also lend themselves toward their possible use in the equine species where a significant number of clinical evaluations are conducted in an ambulatory setting. Therefore, these devices have been evaluated in equines.

Research conducted by Gunther-Harrington et al. (2018) made use of ECG_{VET} but did not apply a method-comparison, preferring to rather extrapolate the validity of data collected in equines from studies in other *Mammalia* at the time. In this study, the researchers recognise that it would be of value to determine the comparability of the *Alivecor Veterinary Heart Monitor* to a standard base-apex ECG configuration and conventional ECG devices.

Kraus et al. (2019) evaluated the “utility and accuracy” of the smartphone modulated ECG_{VET}” when compared to a standard base-apex electrocardiogram. They highlight several concerns with regards to the use of this device for recording and recognition of waveforms. The small dipole created between the two electrodes of the smartphone device in particular yield some trepidation regarding the detection of waveforms that result from smaller electrical deflections as well as the resolution of other components in the ECG tracing (Kraus et al., 2019). These large differences in dipole measurements between devices applying the standard base-apex configuration and the more fixed smartphone ECG layout are hypothesised to render comparison of intervals in the ECG invalid (Kraus et al., 2019). As such the devices in this study were evaluated for heart rate and rhythm analysis only.

For application of the smartphone device, the skin was moistened with 70% alcohol on the left thorax in a location caudal to the triceps muscle mass. The device was then applied in this area over the apical beat in an oblique orientation of approximately 60 degrees. The negative electrode was located cranio-dorsally and the positive electrode caudo-ventrally to achieve this oblique orientation (Kraus et al., 2019). The reference ECG was applied in a modified base apex configuration which resulted in the negative electrode placed in the right jugular groove and positive electrode on the area of the left apical beat. The so-called ground electrode was then located in the left jugular groove (Kraus et al., 2019). ECGs were acquired simultaneously and evaluated in a blinded fashion by three cardiologists for heart rate determination, rhythm analysis and waveform polarity.

The study concluded that both intra- as well as inter-observer variability between the two devices was extremely low. The different cardiologists agreed with regards to the rhythm determinations with each other as well as between ECG pairings for the majority of recorded tracings (Kraus et al., 2019). As such the smartphone ECG was determined to confer the correct heart rhythm in 97% of instances (Kraus et al., 2019).

Once again, the study identified that the device has a tendency to result in low amplitude P waves in equine tracings similar to those described in small companion animals (Kraus et al., 2016, Kraus et al., 2019, Vezzosi et al., 2016a). Agreement between observers and methods was noted to be good but not perfect with regards to polarity of the QRS waveform. It was also noted that this imperfection did not compromise the identification of arrhythmias in this study (Kraus et al., 2019). The heart rate automatically determined by the *Alivecor Veterinary* app was shown to inaccurately reflect heart rate when compared to actual heart rate values in several individuals. The study concluded that although the smartphone device is remarkably accurate with regards to its use in rhythm diagnosis, it should not be used as the definitive method for detailed rhythm analysis as a multiple lead ECG remains preferable (Kraus et al., 2019).

Vezzosi et al. (2018a) also compared the tracings obtained from a standard base-apex lead configuration to those recorded by a smartphone-based *Alivecor* ECG device (ECG_{VET}). Unlike Kraus et al., 2019, this study examined waveform intervals in horses with sinus rhythm rather than focusing on specific arrhythmias. The study examined and contrasted heart rate determination, P wave and QRS polarity, presence of artefacts, P wave ,and QRS complex duration as well as PR and QT interval duration (Vezzosi et al., 2018a).
The standard base-apex configuration positioned the positive electrode at the cardiac apex while the negative electrode was located in the right jugular groove. The third electrode was applied in a location distant to the heart. The ECG_{VET} was applied in a dorsoventral orientation (30 degrees cranial inclination) to unclipped skin that was moistened with alcohol on the left precordium. ECGs were recorded simultaneously for a period of 30 seconds and then compared.
The study concluded that 96 % of the ECG_{VET} tracings could be considered interpretable. This device yielded reliable heart rate values when calculated in a manual fashion when compared to the standard device but poor accuracy was identified when only the heart rate determined by the *Alivecor Veterinary* app was assessed (Vezzosi et al., 2018a). The app was noted to interpret higher amplitude T waves as QRS complexes, thereby falsely increasing heart rate in many cases (Vezzosi et al., 2018a). The smartphone device was noted to be comparatively reliable with regards to the duration of ECG waves as well as tracing intervals, with variation compared to that of the standard ECG being of no clinical significance (Vezzosi et al., 2018a). QRS polarity also revealed good agreement between the two devices, although no agreement was identified in P wave polarity. In conclusion, the ECG_{VET} could be relied on to record good quality tracings in healthy horses although it could not be considered a direct substitute for base-apex electrocardiography (Vezzosi et al., 2018a).

Publications for the use of the ECG_{VET} show that it has been applied in a diverse array of species and appears to present itself as a simple yet reliable method to apply basic screening for abnormalities in the electrical activity of the heart. Yet many of the publications in animals present findings from a healthy study group. Further research is required, specifically focusing on the device's applicability in the patient with arrhythmic electrical activity, before the device can become entrenched as a screening tool for field and ambulatory ECG analysis.

Chapter 3: Materials and Methods

3.1-Study Design

This study is a cross-sectional study design with specific emphasis on method agreement analysis. Electrocardiographic tracings were collected at a single time without any previous knowledge of the cardiovascular status of the individual in question. Data was collected in two separate concurrent time periods namely from the 11/11/2018 – 22/11/2018 and the 20/05/2019 – 24/05/2019. These time periods correspond with the traditional summer and autumn seasons in the Southern hemisphere. The study was approved by the by the Faculty of Veterinary Science Research Committee and the University of Pretoria Institutional Animal Ethics Committee (protocol number V087-18).

3.2-Study population

Clinically healthy, non-pregnant Nooitgedacht pony mares from the *Onderstepoort Teaching Animal Unit* were selected for the study. Mares were considered clinically healthy based on a history of good appetite and water intake as well as a clinical examination prior to the data collection. The clinical examination was performed by a veterinarian with the main exclusion criterion being abnormal findings on general clinical examination.

Clinical evaluation was performed by the principal investigator. Heart rate was recorded by auscultation, counting the number of beats over a one-minute period, and any abnormalities in rhythm was noted.

Mares were required to be habituated to handling as well as the management facilities i.e. races and stocks to ensure minimal stress associated with the procedures of data collection. The mares remained in their natural environment consisting of open-air paddocks of varying sizes and was only confined during the procedure of data collection.

For the purposes of this study project, mature mares were used. These animals were required to be habituated to handling as well as the management facilities i.e. races and stocks to ensure minimal stress associated with the procedures of data collection.

Part 1

The 36 selected ponies in this portion had a mean age of (range 2-25 years) and mean body condition of 3/5 (range 2-4/5). Hair coats were generally short and fine in consistency with often very sparse coverage in and around the axillary region. This corresponds with the data collection for this portion taking place during summer.

Part 2

The 31 selected mares in this portion had a mean age of 14 years (range 2-25 years) and a mean body condition score of 3.5/5 (range 3-4/5). With the exception of 5 mares, selected mares also took part in the initial research portion. Hair coats were generally longer and coarser than previously due to the fact that testing was performed in late autumn.

3.3-Sample size:

Part 1
Sample size for this study was determined using the formula for comparison of two binomial proportions as reported by ***Rosner (2011)***
The formula is reported as follows:

$$n_1 = \frac{\left[\left(\sqrt{\bar{p}\bar{q}\left(1+\frac{1}{k}\right)}z_{1-\frac{\alpha}{2}}\right) + \sqrt{p_1q_1 + \frac{p_2q_2}{k}}z_{1-\beta}\right]^2}{\Delta^2}$$

$n_1 = required\ sample\ size$
$n_2 = kn_1$
$p_1, p_2 = probability\ of\ true\ success\ for\ 2\ groups$
$q_1; q_2 = 1 - p_1; 1 - p_2$
$\Delta = |p_2 - p_1|$
$\bar{p} = \frac{p_1 + kp_2}{1+k}$
$\bar{q} = 1 - \bar{p}$
$z_{1-\beta} = 0.8$

Figure 5*: (Rosner, 2011) Formula for sample size calculation*

Calculations were based on research with a similar device which achieved 96% of its tracings as diagnostic (Vezzosi et al., 2018a). The *ECG*$_{AKM}$ in this study was? expected to obtain readable diagnostic results in a minimum of 80% of individuals. This estimate was used to determine the sample size of36 individual animals.

Part 2
The same formula was also used to determine and appropriate sample size for comparison of two binomial proportions (Rosner, 2011).
The value of p1 was set at 98 %, which seemed to be a reasonable approximation for the total number of diagnostic ECG tracings that the ECG$_{TV}$ (the gold standard device for the purpose of this research) device would obtain during its application. The p2 value was set at 80 %. This seemed to be a reasonable estimate of the total percentage of diagnostic tracings a simplified patient-side ECG device can achieve whilst retaining its diagnostic value. A sample of 31 individual animals was calculated for part 2 of the study.

3.4-Smartphone-based ECG device:

This study used the *Alivecor KardiaMobile (*ECG$_{AKM}$) smartphone-based ECG device. The device was designed to monitor arrhythmias, especially atrial fibrillation in humans. A similar device was designed for use in veterinary science and is known as the *Alivecor Veterinary Heart Monitor*. This veterinary specific device is presently indicated to be compatible with smartphones developed by *Apple Inc*.[c] whereas the ECG$_{AKM}$ allows for use in android[b] modulated devices as well as those with the iOS operating system (Alivecor Inc., 2011-2016).

The ECG$_{AKM}$ device is a small and light, pentagonal device with a narrow overall profile. It is equipped with two metal electrodes on the one side and a covered battery port on the opposite side. The device is powered by a “3-volt CR-2016 coin cell battery” (Alivecor Inc., 2011-2016) that is replaceable when required.

Once the device has been attached to the phone, the user is required to download the *Kardia* app which can be found on the “Play store” for android users and the “iTunes store” for iPhone users. The device communicates with the microphone of the smartphone using ultrasound waves at a frequency of approximately 19 kHz. These are then converted into an ECG tracing. Recording is initiated by selecting the “record your EKG” button and tracings are then automatically displayed when appropriate contact has been established with the skin. A period of 10 seconds of signal and contact is required before the tracing records. This reduces artefacts created during initial placement and movement. The app allows tracings of between 30 seconds and 5 minutes to be recorded. A 50 Hz and 60 Hz mains (alternating current electrical filter) function is available or the device can be set to an automatic mains filter. The application is also equipped with an “enhance filter” that is claimed to reduce “noise” on the tracing (Alivecor Inc., 2011-2016). Tracings are recorded at a paper speed of 25mm/s and amplitude of 10mm/mV.

In addition to recording an ECG tracing, the app also determines the heart rate and analyses the tracing for evidence of “bradycardia, tachycardia and possible atrial fibrillation”. Once a tracing has been recorded, the app allows the recording to be saved and a PDF copy of the tracing to be emailed.

The device in this study was attached to an android-based *Samsung Galaxy Note 9*[h] using the device holder and double-sided tape. The side containing the battery port was orientated to the top of the smartphone. The entire phone with attached device was then applied to the selected sites on the study subjects. In order to obtain a signal between device and smartphone, sufficient contact is required between the skin and both electrodes of the device. Overall the setup is rigid and allows minimal contouring at the application site which can complicate achieving appropriate contact.

***Figure 6**: Image showing equipment needed to record smartphone ECG. From left to right- Smartphone, Alivecor KardiaMobile, Kardia Device holder*

***Figure 7**: Alivecor KardiaMobile device secured to back of smartphone using holder*

3.5-Sampling procedure:

Part 1

Horses were placed in stocks in pairs to prevent any form of anxiety caused by isolation from a herd animal. Mares were allowed 5 minutes to relax in the stocks while a full hay net was placed within easy reach of each individual animal. The restraint facilities took the form of a single linear race. One mare was placed behind the other in a position which allowed contact between the mares without causing unnecessary infringement. Padded rubber poles were used to facilitate this process and were further restrained by an individual handler during the recording process using a halter and lead rein.

In order to reduce irritation caused by flies in the environment, that could have an impact on horse movement and the ECG tracings, a commercially available fly repellent containing cypermethrin and citronella (Buzz Off™ manufactured by V-Tech[d]) was applied according to the manufacturer's instructions.

Three body contact locations were used for data collection (Figure 8):

1) The left 4th intercostal space with the lowest end of the ECG_{AKM} device 3-5 cm above the olecranon.
2) The right 4th intercostal space with the lowest end of the ECG_{AKM} device 3-5 cm above the olecranon.
3) The centre of the right triceps muscle mass.

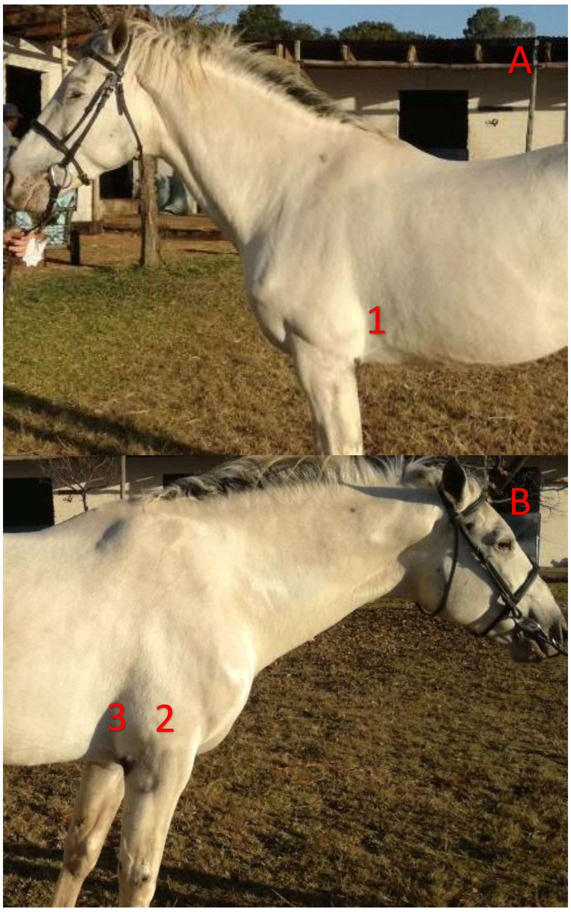


***Figure 6**: Areas that the Alivecor KardiaMobile device were applied to the thorax; **A**-device location on left thorax; **B**-device location on right thorax and triceps.*

Sites 1 and 2 were chosen as they were considered the most likely sites to measure the electrical activity of the heart and site 3 to represent a location distant to the heart and includes a large muscle mass to determine if these factors impinged on ECG acquisition.

The *ECG_{AKM}* was applied in three orientations at each site (Figure 9):

1) Vertical orientation with the device perpendicular to the ground.
2) The device horizontal to the ground (rotated clockwise 90 degrees from position 1)
3) At an angle of 45 degrees to the ground (i.e. diagonally across the chest)

***Figure 7**: Methods of Alivecor KardiaMobile device orientation for each site*

The device was tested for each of these orientations at the three predetermined sites with each of the following different skin preparations:

1) Without any skin preparation. The hair over the site was left intact and only gross contaminants such as grass or mud were removed.
2) A solution of 70% ethanol was applied to the hair and skin at the recording site. Alcohol was applied using a spray bottle and nozzle with the excess being wiped away using a gloved hand. Once recordings were completed at the site the area was dried with absorbable paper towel. The remaining alcohol then evaporated.
3) The site hair was clipped using a portable electric hair clipper. The site was clipped in a square with approximately 15cm dimensions. This allowed the device to be applied in the three methods of orientation while remaining in contact with the hair free area.

Recordings consisted of a 30-second ECG tracing recorded at a paper speed of 50 mm/sec. The device was applied to the skin using sustained, firm digital pressure from a single operator. The pressure used was sufficient to allow intimate contact of the electrodes with the skin and to prevent slippage or falling of the device.
Once all data recordings were completed the mares were returned to their paddocks and allowed to return to their normal management routines. Each mare was examined after a 24-hour period with specific emphasis on habitus and general health status as well as any potential adverse skin reactions as a result of the data capturing procedure.

Part 2
Identical procedures to those applied in part 1 were instituted for restraint and handling of the animals.
Findings from part one revealed that application of the *ECG_{AKM}* device in the left fourth intercostal space, in a vertical orientation and with the skin moistened with 70 % alcohol yielded the most repeatable yet complete ECG tracings. As such this was the method applied for part 2 of the study.
Due to the time of year during which this portion of the sample collection took place, the animal's hair coat was noted to be long and thick. This was significantly different from the short, sparse hair coats present when part 1 was conducted. As such the area of application

of the ECG_{AKM} device as well as areas of electrode attachment for the leads of the ECG_{TV} were clipped using battery operated clippers and moistened the skin with alcohol.

It was decided to use a modified base-apex configuration for application of the ECG_{TV}. This configuration is, in the author's opinion, the most versatile of the configurations as it can be used appropriately for both standing, Holter and exercising ECGs. The electrode placement is also the most similar to that of the ECG_{AKM} in a vertical orientation. This should ensure the most similarity, between the two devices, in the lead orientation relative to the heart's electrical axis.

The ECG_{TV} device was applied according to the recommendations given in the *Televet 100 Veterinary telemetric ECG*[f] system documentation supplied with the device (Figure 10): The green or "left leg" electrode was applied to the sternum, the black or "neutral" electrode applied ventral to the withers and spinal column and the red or "right arm" electrode applied 10 cm distal to the black lead. The yellow or "left arm" electrode was placed at a site corresponding to that of the red lead with the difference that it was applied to the right thorax. Electrodes were attached to the skin using ECG pads and where held in place using a surcingle.

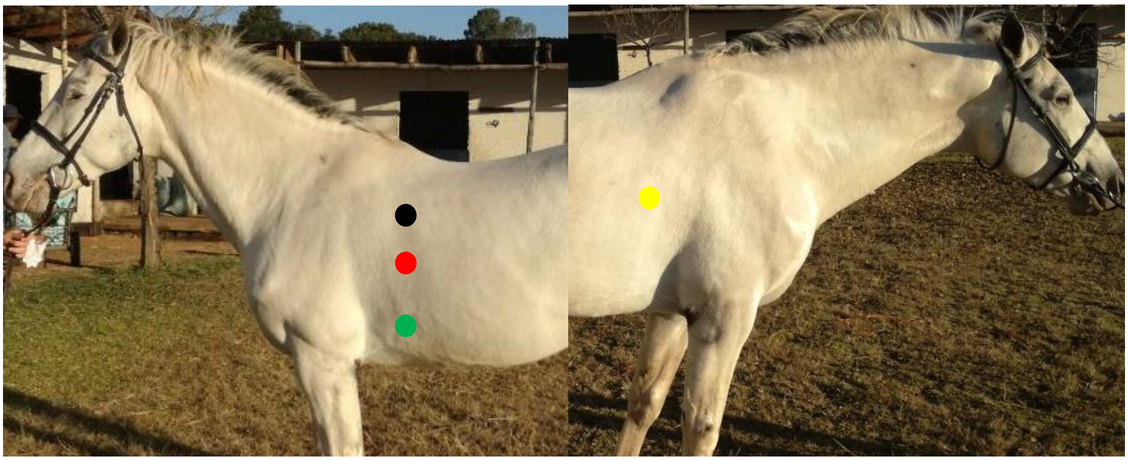


Figure 8*: Televet 100 lead placement with colours corresponding to the colours of the Televet 100 lead system*

Once the ECG_{TV} device was in place and a good quality tracing was confirmed, the ECG_{AKM} device was applied to the horse's thorax and recording of the ECG waveforms were initiated simultaneously over a period of 30 seconds.
Leads were removed and mares were returned to their respective paddocks. Each mare was examined after a 24-hour period as described for part 1 of the data capturing procedure.

3.6-Observations/analytical procedures:

Part 1
In order to obtain an ECG tracing, the ECG_{AKM} was applied at each of the sites mentioned in the experimental methods. An attempt at obtaining a tracing was regarded as the single 30 second period in which the device is set to measure ECG waveforms. The heart rate was obtained and recorded immediately after completion of recordings at each site. All recordings were analysed by the principal investigator. A random 10% of these tracings were reviewed by the study supervisors for diagnostic acceptability and agreement with the principal investigator's conclusions. A diagnostic trace was defined by the statement presented by Vezzosi et al. (2018b) requiring baseline artefacts to be absent for at least 80% of each tracing.

Part 2

The focal points for data comparison are parameters that can be measured during the ECG examination. As such, values were recorded and compared from the following dependent variables:

1. P-Q interval
2. R-R interval
3. Q-T Interval
4. QRS duration
5. Cardiovascular rhythm/ rhythmogenesis

All the intervals present in a 30 second ECG recording segment were measured for both the ECG_{AKM} and ECG_{TV} devices and recorded in a table format. These measurements were then averaged to determine a single average measurement for the specific interval category in that tracing. As such each horse had the same number of intervals measured for that interval category for both devices. The recordings were started at the same instance but due to some delays in the acquisition of an appropriate tracing in the ECG_{AKM}, it could not be ensured that the exact intervals were measured in each device. Due to variations in heart rate the number of intervals measured in different animals was not the same. Interval measurements were taken manually for each tracing. Measurements were made according to those seen in *Figure 9.*

Independent variables were maintained as constant as possible by using the same equipment each time, using a predetermined method of application of the devices and a single operator performing data collection.

3.7- Data analysis:

Data was examined and evaluated using two commercially available software programs namely *Microsoft Excel*[g] and *IBM SPSS*[e] *statistics* software. Significance was set to 5%.

Part 1

Data was summarised in the form of a frequency table with regards to the number of diagnostic quality tracings obtained in each location. ECG tracings were divided into the following categories (table 2):

Table 2: Example of categories used for evaluation of smartphone-based ECG tracings

Decipherable ECG with all major waveforms regularly present	ECG with one major waveform regularly absent	More than one major waveform regularly absent	Tracing recorded by application but no waveforms present	Non readable waveform due to artefacts	No tracing obtained at site

Major waveforms were identified as a P wave, QRS complex (or variation thereof such as rS complex where no Q wave was identified which is a normal finding in equine base-apex tracings) as well as a T wave. A non-readable tracing was defined as one that had more than 20% of the total tracing obscured by artefacts.

Heart rate data was checked for normality using the Shapiro-Wilk test. Heart rate determined by the Kardia app was then compared to heart rate determined via auscultation using a Mann Whitney test and analysed for agreement using graphical representation in the form of a Bland-Altman plot.

Part 2

Due to the nature of the ECG recordings, some parameters of interest had multiple repetitive values e.g. consecutive PR intervals. These repetitive values were then combined in the form of a mean measurement for each animal for the entire 30-second tracing. Multiple measures of this nature can serve to improve the precision of the defined data set.

Data was evaluated for normality using the Shapiro-Wilk test. If the quantitative data conformed to the expected normal distribution curve (parametric data), it was then summarized and reported in the form of mean (grouped) coupled with standard deviation. In cases where a mean was determined for individual animals (for repetitive measures), the group average value of these calculated mean values was then reported. Data that was considered not normally distributed (non-parametric) was reported using median values and interquartile range (Gunther-Harrington et al., 2018).

An independent t-test was then applied to determine if quantitative data could be considered significant, enabling rejection of the null hypothesis. The independent t-test is best applied to parametric data. The independent t-test was replaced by the Mann Whitney-U test if the data was non-parametric (Gunther-Harrington et al., 2018). The level of agreement between quantitative data pairings was determined using a Bland-Altman plot and the limits of agreement for each data set were determined (Giavarina, 2015).

Chapter 4: Results

Part 1

A total of 972 ECG recordings were attempted in part 1 of the study (Table 3). Only 11 % (107 recordings) of the total recordings were deemed decipherable and of diagnostic value. A total of 91.7% (33/36) of the tracings recorded from the left 4th intercostal space with the device in a vertical orientation and the skin moistened with 70% alcohol were decipherable with all waveforms regularly present.

Of the 107 decipherable recordings, a total of 77.6% (83/107) were obtained in the left 4th intercostal space compared to only 0.9% (1/107) over the right triceps muscle mass and 21.5% (23/107) in the right 4th intercostal space.

Of the 107 decipherable recordings, a total of 63.6% (68/107) were obtained with the skin moistened with alcohol compared to 4.7% (5/107) when there was no skin preparation applied and 31.8% (34/107) when the hair at the site was clipped.

Of the total diagnostic recordings, it was seen that 52.3% (56/107) were obtained with the device in a vertical orientation compared to only 17.8% (19/107) with the device in horizontal orientation and 30.0% (32/107) with the device in a cranioventral to caudodorsal 45 degree, oblique orientation.

Table 3: Frequency table of smartphone-based ECG categories used to divide ECG tracings into different degrees of decipherability and usability.

Site	Decipherable ECG with all major waveforms regularly present	ECG with one major waveform regularly absent	More than one major waveform regularly absent	Tracing recorded by application but no waveforms present	Non readable waveform due to artefacts (>20% is artefacts)	No tracing obtained at site	**total**
1ad	0	3	0	0	2	31	36
1bd	33	3	0	0	0	0	36
1cd	20	7	1	0	2	6	36
1ae	0	1	1	0	3	31	36
1be	7	23	2	1	2	1	36
1ce	3	14	1	0	5	13	36
1af	4	0	0	0	0	32	36
1bf	10	9	9	2	6	0	36
1cf	6	4	5	2	9	10	36
2ad	0	0	1	0	1	34	36
2bd	1	3	17	2	11	2	36
2cd	0	3	23	1	4	5	36
2ae	0	0	1	0	2	33	36
2be	0	1	7	14	11	3	36
2ce	0	0	0	12	10	14	36
2af	0	0	0	0	1	35	36
2bf	0	0	24	3	5	4	36
2cf	0	1	8	3	5	19	36
3ad	0	0	1	1	1	33	36
3bd	1	10	10	0	3	12	36
3cd	1	19	8	0	5	3	36
3ae	1	1	1	0	0	33	36
3be	8	8	11	2	4	3	36
3ce	0	7	7	2	5	15	36
3af	0	0	1	0	1	34	36
3bf	8	11	8	1	7	1	36
3cf	4	5	5	2	11	9	36
total	107	133	152	48	116	416	972

Key: Area and method of device application	
1	left 4th intercostal space
2	right triceps centre of muscle mass
3	right 4th intercostal space
a	unprepared skin and hair
b	alcohol applied
c	area shaved
d	vertical device orientation
e	horizontal device orientation
f	45-degree angle with the dorsal area closest to the triceps

Heart rate data was evaluated for normality using the Shapiro-Wilk test. Heart rate determined using the *Kardia* app conformed to a normal distribution. The data set for the auscultated heart rate was not normally distributed. Therefore, the summary statistics are presented as the median and interquartile range for both data sets to allow for comparison as seen in Table 4:

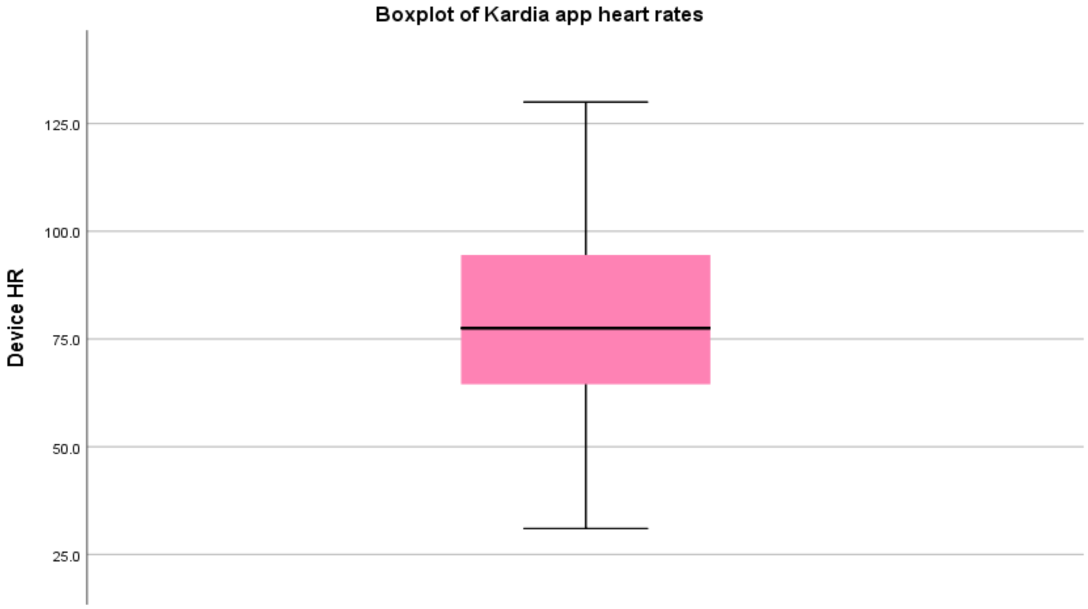
***Table 4**: Basic summary statistics for heart rate data*

method	Ausc. HR (Beats per minute (BPM))	App HR (Beats per minute (BPM))
Median	36 BPM	77.5 BPM
Interquartile Range	6 (36;42) BPM	32 (62.75;94.75) BPM

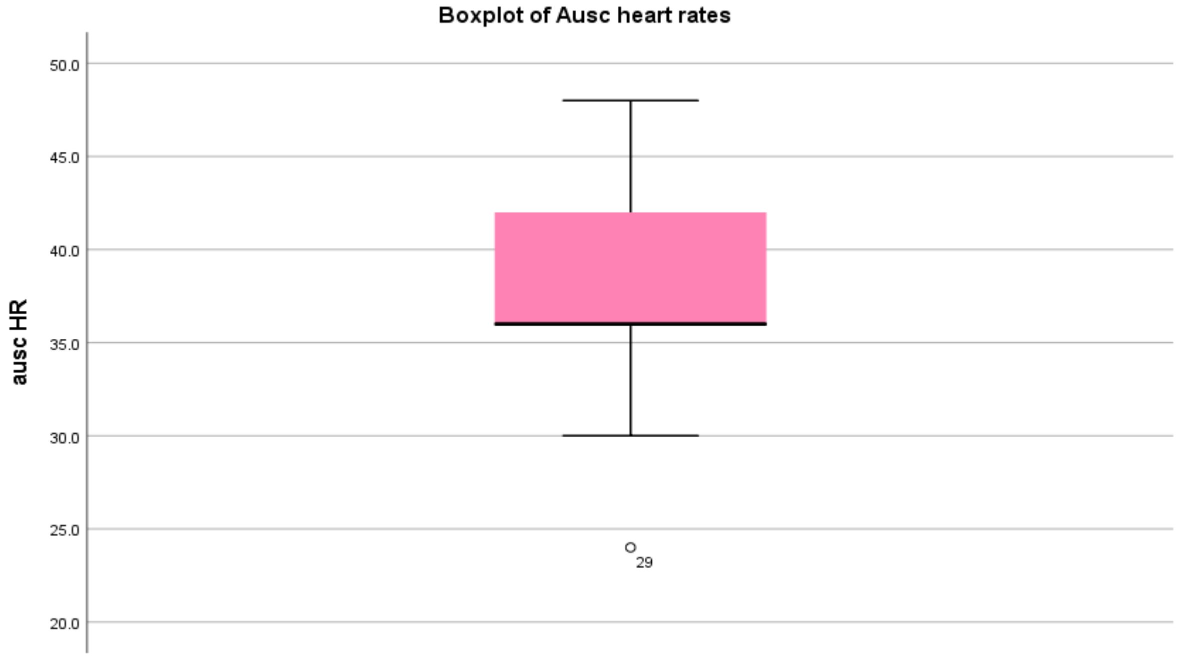
(*BPM- Beats per minute)

It can be seen when constructing boxplots of these values, that the heart rate determined by the device application has a much wider distribution of values compared to that seen when the heart rate was auscultated (Graph 1 and 2).

***Graph 1**: Boxplot of heart rate determined by the Kardia app*

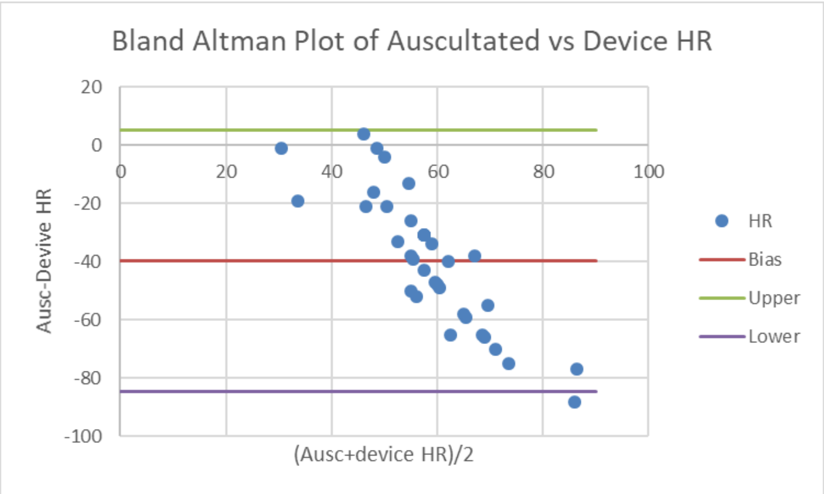


***Graph 2**: Boxplot of the heart rate determined by auscultation*



The Mann Whitney U test was used to compare the heart rates obtained using the *Kardia* app and on auscultation as the auscultated heart rate data set was not normally distributed. Due to the difference in distribution of the two data sets, the Mann Whitney U test made use of the mean rank of the data. Significance level was set at 5%. The test comparing the two data sets yielded a U value of 43 and a two-tail significance of p= 0.00. Bland Altman analysis (Graph 3) of the heart rate data sets, revealed a bias of -39.75 and upper and lower level of agreement of 5.23 and -84.73 respectively (negative values indicate overestimation by the *Kardia* app while positive values indicate underestimation by the app). As such analysis revealed that the smartphone app will, in 95% of the instances, yield a value that ranges anywhere from underestimating the heart rate by 5.23 beats per minute to overestimating the HR by 84.73 beats per minute. The relationship of the data points in the bland Altman plot also indicated that especially at increased heart rates the *Kardia* app tends to overestimate the actual value by an increasing amount.

***Graph 3:** Bland Altman plot of heart rate determined by auscultation and Kardia app*



Part 2

Mean PQ, RR, QT, and QRS interval values for both devices were analysed for normality using the Shapiro-Wilk test. Values were determined to follow normal distribution except with regards to the RR interval data set for the Alivecor device and the PQ dataset for the ECG_{TV} device. Tables 5 and 6 summarise the data sets accordingly with data presented as mean and standard deviation (if both sets are normally distributed) but as median and interquartile range if one or more of the data sets for each device is not normally distributed. These values can be seen in the table below:

***Table 5**: Summary statistics for the Televet 100 device*

	PQ (ms)	RR (s)	QT (ms)	QRS (ms)
Mean	-	-	597.31	143.292
Standard deviation	-	-	36.72	13.3
Median	265.765	2.025	-	-
Interquartile range	44	0.332	-	-

***Table 6**: Summary statistics for the Alivecor KardiaMobile device*

	PQ (ms)	RR (s)	QT (ms)	QRS (ms)
Mean	-	-	596.343	100.989
Standard deviation	-	-	38.80	10.43
Median	280	1.901	-	-
Interquartile range	54	0.564	-	-

Arrhythmias were noted in the same 3 out of 31 horses (9.67%) in both device tracings when analysed manually. These were determined to conform to sinoatrial blockade in both the ECG_{AKM} and ECG_{TV} tracings. The *Kardia* app however identified these arrhythmic tracings as unreadable in all 3 occurrences. Examples of both arrhythmic and normo-rhythmic tracings can be seen in appendix 1-4.

Significance values of p = 0.90, 0.15 and 0.85 were determined using the Shapiro-Wilk test with regards to the mean differences for the RR; QT and QRS intervals respectively. As such these were determined to follow a normal distribution curve. A significance value of 0,00 was determined for the mean difference of the PQ intervals thereby indicating these values were not normally distributed.

Independent t tests conducted on the mean values for RR; QT and QRS intervals yielded values of -0.30, 0.00 and 72.4 respectively. These values correspond with a 2-tail significance value of p = 0.73, 1,00 and 0,00 respectively. Mean values determined for RR and QT intervals were not significantly different when the two devices were compared. The mean values for the QRS interval were significantly different between the two devices.

Independent-samples Mann Whitney U test was performed for the PQ interval dataset as its mean difference was not normally distributed. The U value for the PQ dataset was calculated as 372.0 with a corresponding 2-tail significance value of p = 0.127. The Independent-samples Mann Whitney U test indicated that the distribution of values of mean PQ interval was the same over the ECG_{AKM} and ECG_{TV} categories.

Values for PQ, RR, QT, and QRS intervals were then also analysed using Bland-Altman plots (graph 4-7). Bias as well as upper and lower levels of agreement where determined from these plots and can be seen in the table 7 below:

***Table 7**: Bias and level of agreement values calculated for ECG waveform Intervals (negative values indicate overestimation of the interval by the ECG_{AKM})*

Interval	Bias (ms*)	Upper level of Agreement (ms*)	Lower level of agreement (ms*)
PQ	-9.72	27.74	-47.18
RR	27.31	237.04	-182.41
QT	0.96	35.04	-33.12
QRS	42.30	76.34	8.27

*Duration (milliseconds)

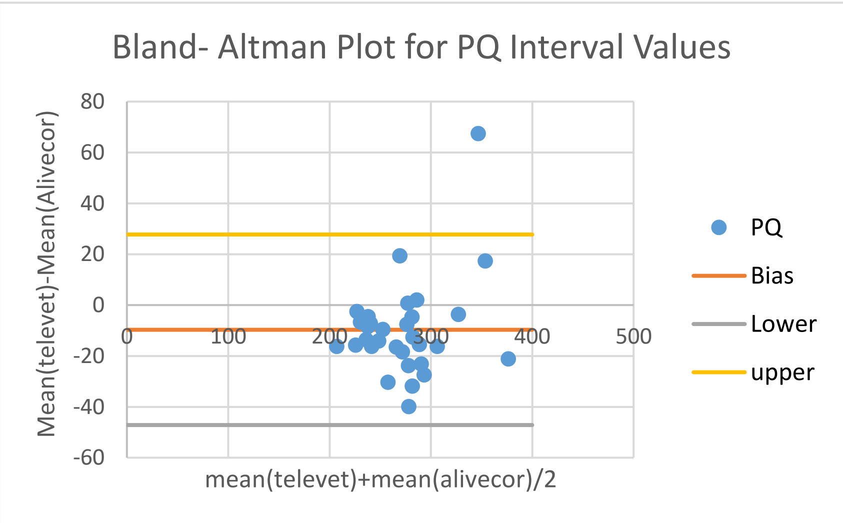
This indicated that ECG_{AKM} on average overestimates the PQ interval by 9.72 milliseconds (Graph 4). This plot also indicated that 95% of the values determined using the ECG_{AKM} device ranged from either overestimating the PQ value by 47.18 milliseconds to underestimating the value by 27.74 milliseconds.

The ECG_{AKM} device on average underestimates the RR interval by 27.31 milliseconds (Graph 5), which can be considered a slight underestimation in comparison to the total length of the equine RR interval. In 95% of cases the RR interval determined by the ECG_{AKM} device will range from overestimation by 182.41 milliseconds to underestimation by 237.04 milliseconds.

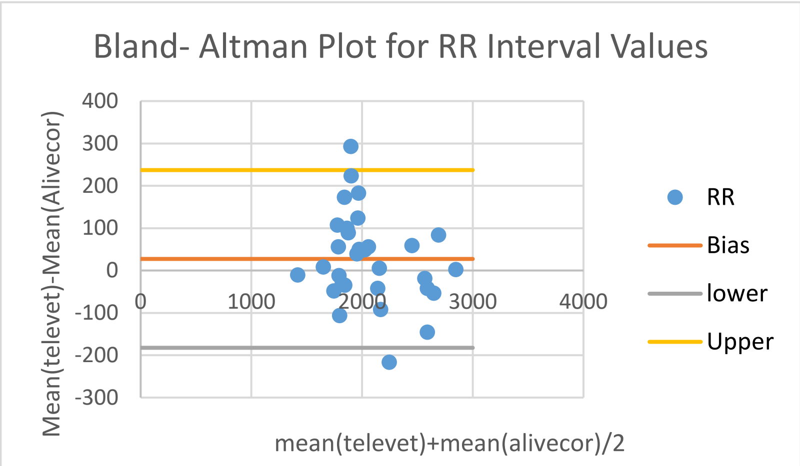
The ECG_{AKM} also underestimated the QT value by a meagre 0.96 milliseconds. This analysis also revealed that in 95% of cases the ECG_{AKM} device would yield a value that would range from underestimating the QT value by 35.04 milliseconds to overestimating the value by 33.12 milliseconds.

Bland Altman analysis revealed that the ECG_{AKM} device tended to underestimate the QRS duration by 42.30 milliseconds (Graph 6). It also revealed that in 95% of the cases the ECG_{AKM} device recorded a value that ranged from underestimating the duration by 76.34 milliseconds to underestimating the duration by 8.27 milliseconds.

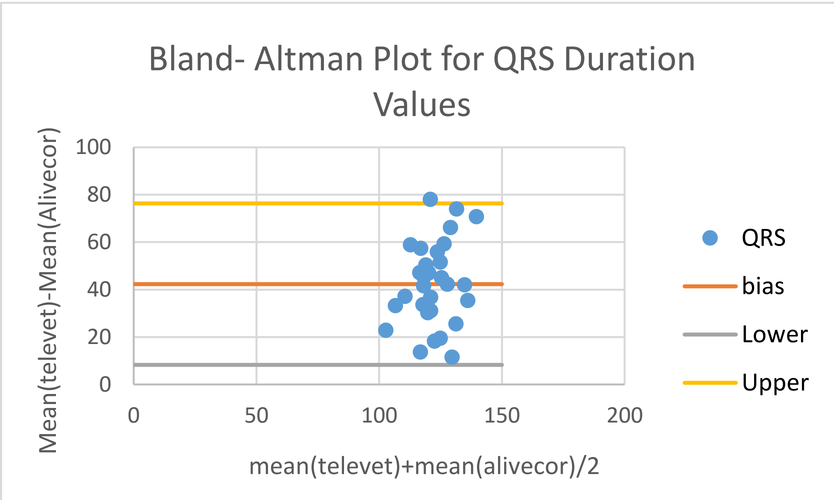
***Graph 4**: Bland Altman plot of PQ Interval Determined by ECG_{AKM} and ECG_{TV}*



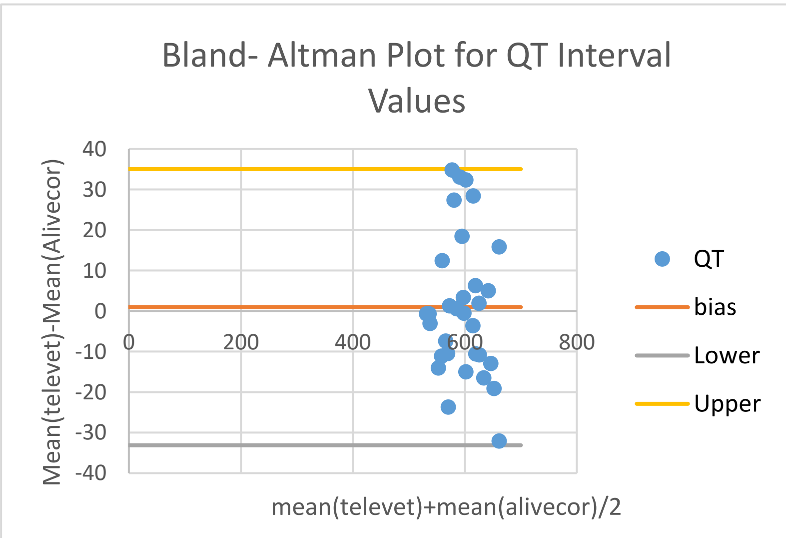
Graph 5: *Bland Altman plot of RR Interval Determined by ECG_{AKM} and ECG_{TV}*



Graph 6: *Bland Altman plot of QRS Interval Determined by ECG_{AKM} and ECG_{TV}*



Graph 7: *Bland Altman plot of QT Interval Determined by ECG_{AKM} and ECG_{TV}*



Chapter 5: Discussion

Previous studies have evaluated and concluded that the use of a veterinary specific smartphone-based ECG device (ECG_{VET}) may be applicable in equines (Vezzosi et al., 2018, Kraus et al., 2019). The present study specifically evaluates the ECG_{AKM} device, a device designed and manufactured for human use, in the equine species. The greater demand for and monetary backing applied to the human medical field means devices like the ECG_{AKM} have several advantages over veterinary specific devices. These include the fact that they are more commonly available, more regularly refined and updated and cheaper than devices specifically aimed at veterinary medicine. Therefore, supporting the idea of an inexpensive and accessible device for use by the ambulatory veterinarian, one would be amiss if a device designed for humans was not examined.

The ECG_{AKM} device and its associated smartphone application, the *Kardia* app, appear to be simple and user friendly. The use of the device and application in this study suggest that they do not require in depth technical or medical skills to apply. This ease of use however should be further evaluated in equids before definitive conclusions can be cemented.
The orientation of the device relative to the smartphone affects the polarity of complexes on the ECG recording. The polarity of the deflections can however be adjusted using a setting on the *Kardia* app.
The battery compartment is not waterproof and is prone to alcohol infiltration if large volumes are used to moisten the hair coat. In this study the device was applied with the battery side orientated to the top of the smartphone in an attempt to prevent pooling of alcohol in the battery compartment. Despite this orientation flooding of the battery compartment still occurred during the study and resulted in reduced signal between the ECG_{AKM} device and the smartphone. This necessitated the removal of the battery and drying of the battery compartment after which the device maintained its functionality.

Motion artefacts were a common occurrence in this study. Motion artefacts corresponded to obvious movement of the horses' body. Movements included foot stamping due to flies, panniculus (also often associated with irritation by flies) as well as movement associated with the breathing motion. This may have implications for the usability of the device in warmer climates and in post exercise or diseased horses where an increased respiratory rate and/or effort may occur. Climatic conditions favouring large insect populations such as flies can notably hinder the successful use of the ECG_{AKM} device. This is particularly relevant for the ambulatory setting, where fly control is more difficult than in the hospital environment.

The ECG_{AKM} has a very small dipole with regards to the orientation of the electrodes. When applied in human medicine, the ECG is recorded with a finger from each hand placed on one of the electrodes. The electrical potential is then measured across the heart making use of the arms as extensions of the lead system. This achieves some mitigation of the concerns that arise due to the small dipole. When applied in the horse however, the ECG is recorded on the thoracic wall with a small distance between the areas of electrode contact. This study supports the findings reported by Kraus et al. (2019) where the small dipole leads to a reduction in the amplitude of smaller waveforms such as the P wave and the Q deflection.

To the author's knowledge, this is the first study to not only assess the ECG_{AKM} device in equines but also to examine different methods of device application. It is also the first study to compare the ECG_{AKM} device with the modified base-apex lead system that is most commonly applied for exercising ECGs (ECG_{TV}). The electrodes in the ECG_{AKM} device are arranged in a linear pattern, similar to the linear arrangement of the modified base-apex lead system to fit underneath a surcingle. This modified base-apex configuration also enhances each of the three leads ability to emphasise different portions of the cardiac cycle (Mitchell, 2019). Comparing the two devices in these orientations hypothetically would allow for the best chance of obtaining comparable tracings. The ECG_{AKM} tracings were compared for similarity to the ECG_{TV} device and were not expected to be an exact replica.

In the first phase of the study it was determined that the most appropriate site of application for the device is the fourth intercostal space on the left thorax. This is not an unexpected finding as the site has the least amount of interfering tissue compared to the other sites investigated in this study making it a superficial area with a short distance from the cardiac musculature

Skin contact is another important aspect in the acquisition of a good quality ECG tracing. The application of 70% alcohol likely yielded the best tracings for this reason. Application of the device in a vertical orientation yielded the most diagnostically relevant and repeatable ECG tracings. This may be due to the fact that this is the easiest method to obtain appropriate contact of the electrodes with the tissue in the intercostal space, thereby reducing the amount of intervening bony tissue. Most other studies examining a smartphone-based ECG applied the device in an oblique fashion across the area of the cardiac silhouette. Taking practical, anatomic, and physiological information into account, the suggested method of application of the ECG_{AKM} device in the equine individual for acquisition of the most diagnostically appropriate ECG is as follows:

1. Apply the device in the left fourth intercostal space
2. Apply the device in a vertical orientation
3. Moisten the skin of coat with the addition of alcohol to improve the contact of the electrodes.

This leads to the rejection of the null hypothesis for Part 1 of the study.

There was a significant difference between the heart rate obtained by cardiac auscultation compared to the heart rate measured by the *Kardia* app. The *Kardia* app tends to overestimate the heart rate by 39.75 beats per minute. Considering that the average heart rate of the mature horse ranges from 28 to 44 beats per minute (Reed et al., 2017), the *Kardia* app seems to regularly double or even triple count beats. More specifically, the device appears to count the T wave in many of the ECGs as a QRS deflection. The *Kardia* app should therefore not be considered a clinically reliable alternative to auscultation when determining the average heart rate of an equine patient. This is a similar finding to that of Vezzosi et al (2018), who concluded that the smartphone application used in conjunction with the *Alivecor Veterinary Heart Monitor* was not a reliable method to determine heart rate.

Due to the extensive variability of components such as the P and T wave morphologies on a normal equine ECG, diagnosing arrhythmias is still highly depended on manual analysis of the ECG tracing (Flethoj et al., 2016). Only three (9.6%) horses in this study displayed an arrhythmia. This was identified in both the ECG_{AKM} and ECG_{TV} tracings and was determined to be sinoatrial blockade. The ECG_{AKM} device may be useful to identify rhythm discrepancies. Further studies with specific focus on the device's ability to identify even the most common arrhythmias in the equine species is needed.

Due to the limited number of abnormal rhythms present in the study, the comparative process relied on examination of the individual intervals to determine an objective indication of the similarity between tracings recorded by each device. It must be considered that both the duration, amplitude and interval measurements in the equine ECG are at present less diagnostically relevant than in other species due to differences in the pathway of ventricular depolarization (Hewetson, 2013). It was determined that the distribution of values with regards to the PQ interval conformed to the same distribution in both devices. In equines, AV blockade is common and can, in certain instances, be considered physiological (Reed et al., 2017). For this reason, the PQ interval showed a large degree of variation both between different individuals as well as between different complexes in the same ECG (Reed et al., 2017). The

PQ interval is also dependant on which lead is used for its determination (Paslawska et al., 2012). In a study conducted by Paslawska et al. (2012), it was determined that the difference between the longest and shortest PQ interval seen in resting healthy Anglo-Arabian horses was 60 ± 50 milliseconds. Taking this and clinical experience into consideration, there is good agreement between the two methods when considering the PQ interval and its associated variability. This result is similar to that concluded by Vezzosi et al. (2018b) for the use of the *Alivecor Veterinary Heart Monitor* device.

The RR interval is extremely important when analysing ECGs for signs of abnormal rhythmicity. As such it is one of the most important aspects of the comparison between the two devices in this study. Although the mean values for RR interval were determined to be the same for these two devices, further analysis using the Bland Altman plot revealed a total variability of 419.45 milliseconds for the RR interval as determined by the ECG_{AKM}. When analysing long term ECG recordings, Mitchell (2019) advocates the usage of a timed algorithm to speed up RR interval analysis. When performing this analysis, Mitchell (2019) uses a beat to beat RR interval variation of 20% as a threshold cut-off for the resting individual. This can be applied to the clinical decision-making process when determining if the above mention variability is significant or not. When taking this into account for the ECG_{TV} RR interval data set, the average RR interval variability is 413.24 milliseconds. This suggests that the variation seen in the ECG_{AKM} values when compared to the ECG_{TV} values is clinically acceptable (taking into account that the tracings were not expected to be identical replicas of one another). Considering this, the ECG_{AKM} displays good agreement with the ECG_{TV} device with regards to RR interval determination.

The QT interval was also examined for agreement between the two devices. Mean QT values were not significantly different between the two devices. Bland Altman analysis corroborated this and the variation of the QT interval estimation by the ECG_{AKM} seems to be acceptable enough for the device to be used for a screening purpose. This mirrors those conclusions of made by Vezzosi et al. (2018a) for the QT measurements made using the ECG_{VET}.

The mean values for QRS were significantly different between the two devices. Due to the small dipole created by the ECG_{AKM} device, the QRS complex often appears to be a simplified version of that seen in the ECG_{TV} tracings. Although the QRS complex is easy to identify, it often lacks the smaller components such as Q wave. This is bound to influence the duration of the complex recorded by the ECG_{AKM} device when compared to the ECG_{TV} recording system. Bland Atman analysis revealed that the ECG_{AKM} device tends to underestimate the QRS duration by 42.30 milliseconds which is the greatest bias seen over any of the waveforms recorded, even considering that the QRS duration is the smallest of the waveform durations recorded. In fact, this average underestimation corresponds to a total of 29.5% of the average value recorded for the QRS interval using the ECG_{TV} device. As such it can be expected that the ECG_{AKM} device is unlikely to yield the same interval duration as the ECG_{TV} device at any point resulting in poor agreement between the two devices. This is in contrast to a study by Vezzosi et al. (2018a). It must however be noted that the device used by Vezzosi et al. (2018b) was different than the one used in the present study.

Considering the above information, the null hypothesis for part 2 should be rejected. The ECG_{AKM} does not yield identical measurements for ECG intervals as those recorded by the ECG_{TV}. The ECG_{AKM} is also not a direct alternative to the ECG_{TV} device although still offers some practical usability as a screening tool.

Chapter 6: Study Limitations

The present study has several limitations. The evaluation of the best application method of the ECG_{AKM} device is a simplified experimental design that only serves to provide general guidelines regarding the approach of correct application of the ECG_{AKM} device. The three orientational approaches used in this study are a gross underestimation of the total number of ways in which to orientate the device on the equine thorax. Additional studies are required to provide more specific recommendations for the application of the device in the equine species.

Several important aspects of the functionality of the device were also not evaluated. For the purpose of this study the device was attached directly to the underside of the smartphone, so the range at which diagnostic recordings can be obtained was not assessed. The device battery was replaced after a maximum of 270 ECG recordings. The total number of ECGs that can be recorded using a single 3-volt CR-2016 coin cell battery was therefore not assessed in this study. It should also be mentioned that although the device was noted to overestimate the heart rate due to incorrectly identifying T waves as QRS complexes, no further evaluation was conducted to determine if different orientations of the ECG_{AKM} device had any influence on this.

Further investigation into factors that may hinder appropriate tracing acquisition is needed. These include factors such as those hindering communication between the ECG_{AKM} and smartphone as well as patient factors affecting electrode contact and ECG quality. Although anecdotal at present, there seems to be some element of reduced efficacy in an environment with a large amount of electrical equipment which requires further investigation. These factors may then limit the devices applicability for use in certain environments.

Another limitation is the relatively uniform study population. A more diverse study population may be beneficial to evaluate the applicability of the device in different breeds, hair coat and body type.

The base-apex or modified-base apex lead system is currently considered the “norm” for the standard equine ECG. Costa et. al. (2017) reported other methods for application of ECG leads which may contribute further diagnostic capabilities to the ECG work-up. This poses the question: “should the modified-base apex system be used as the gold standard in this study.” Further research into the equine ECG will likely shed light on an answer in the future.

Tracing analysis was performed by a single individual. It may be more beneficial to have several individuals with different levels of training analyse and evaluate the ECG tracings. These should range from a general equine veterinary practitioner, equine internal medicine specialist and veterinary cardiologist. This will allow evaluation of the inter- as well as intra-observer variability and provide evidence to determine the devices applicability for each of these professionals.

Chapter 7: Conclusion

In conclusion the ECG_{AKM} can record decipherable ECG tracings in horses on a repeatable basis. The device is easy to use and requires limited training to obtain diagnostic ECG recordings. The ECG_{AKM} device does not yield identical tracings to the ECG_{TV}, but the tracings recorded do appear to show sufficient agreement with the ECG_{TV} device. The ECG_{AKM} can therefore be considered as a simplified screening device in situations where the more standardised system is either unavailable or impractical. As identified by Kraus et al (2019) and Vezzosi et al (2018), the ECG_{AKM} is not currently considered to be a suitable substitute for the more standard 6-lead ECG systems and should not be considered for in-depth cardiovascular analysis. Further studies evaluating the ECG_{AKM} device's ability to identify and record arrhythmic events in the horse is warranted.

Research Funding:
Funding for this research was partially obtained from the *Agricultural Sector Education Training Authority (AgriSeta)* in conjunction with *Borbely Trading CC (Technopet)*.

Company Details

a. Alivecor KardiaMobile Reference AC-009
   Alivecor Inc.
   Mountain View, USA
   Us Patent Nos 8301232; 8509882

b. Android Inc.
   Google
   California
   USA

c. Apple Inc.
   California
   USA.

d. V-tech
   Mid-rand
   Pretoria

e. IBM SPSS Statistics 2009
   IBM
   Corporate headquarters: New York; USA

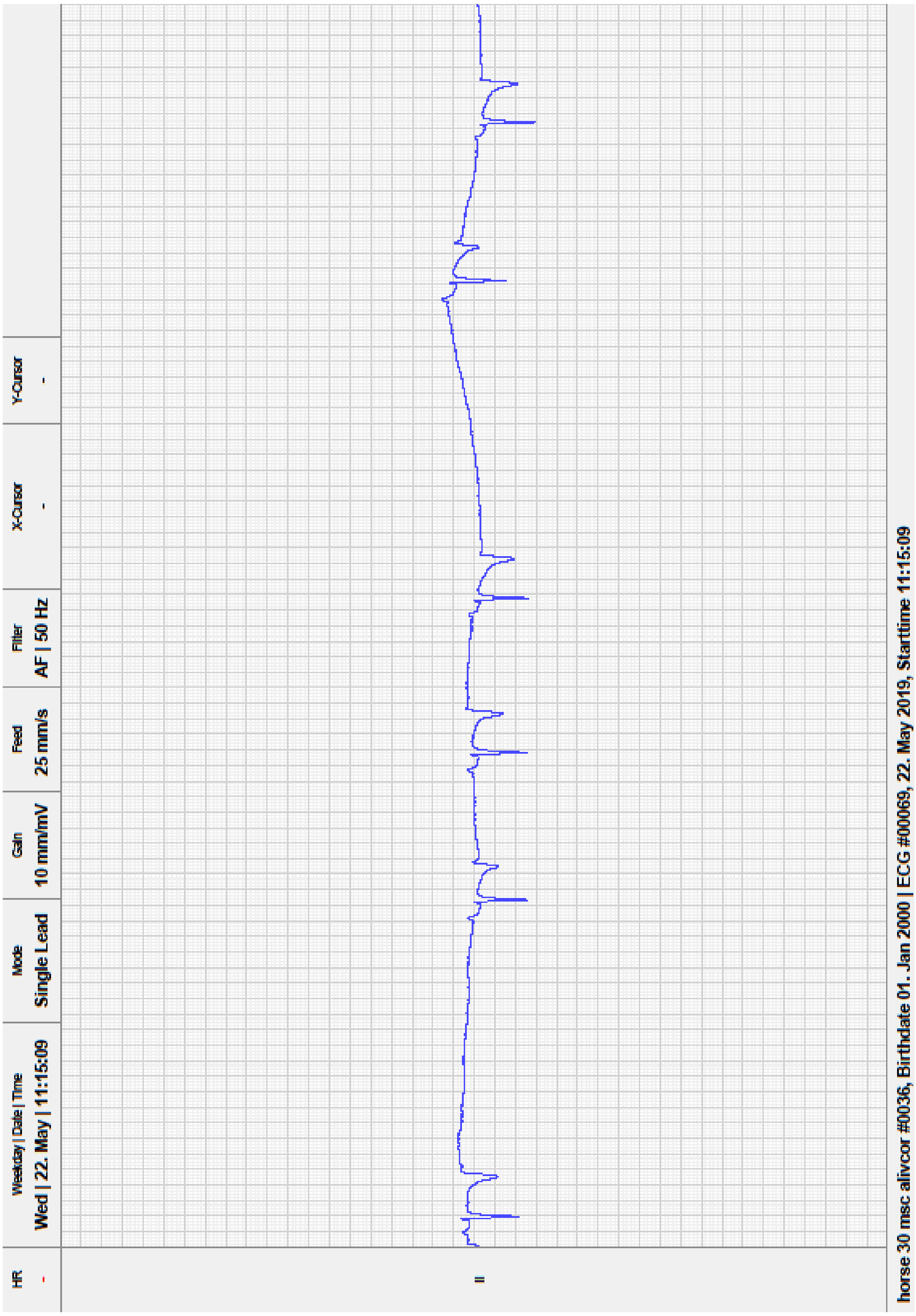
f. Kruuse Televet 100
   Rosch and Associates
   Frankfurt am Main
   Germany

g. Microsoft Excel 2013
   Microsoft Corporation
   Redmond
   USA

h. Samsung Galaxy Note 9
   Samsung
   Seoul
   South Korea

Chapter 8: Appendices

<u>**8.1-Appendix 1**: *Televet* 100 tracing of sinoatrial blockade</u>

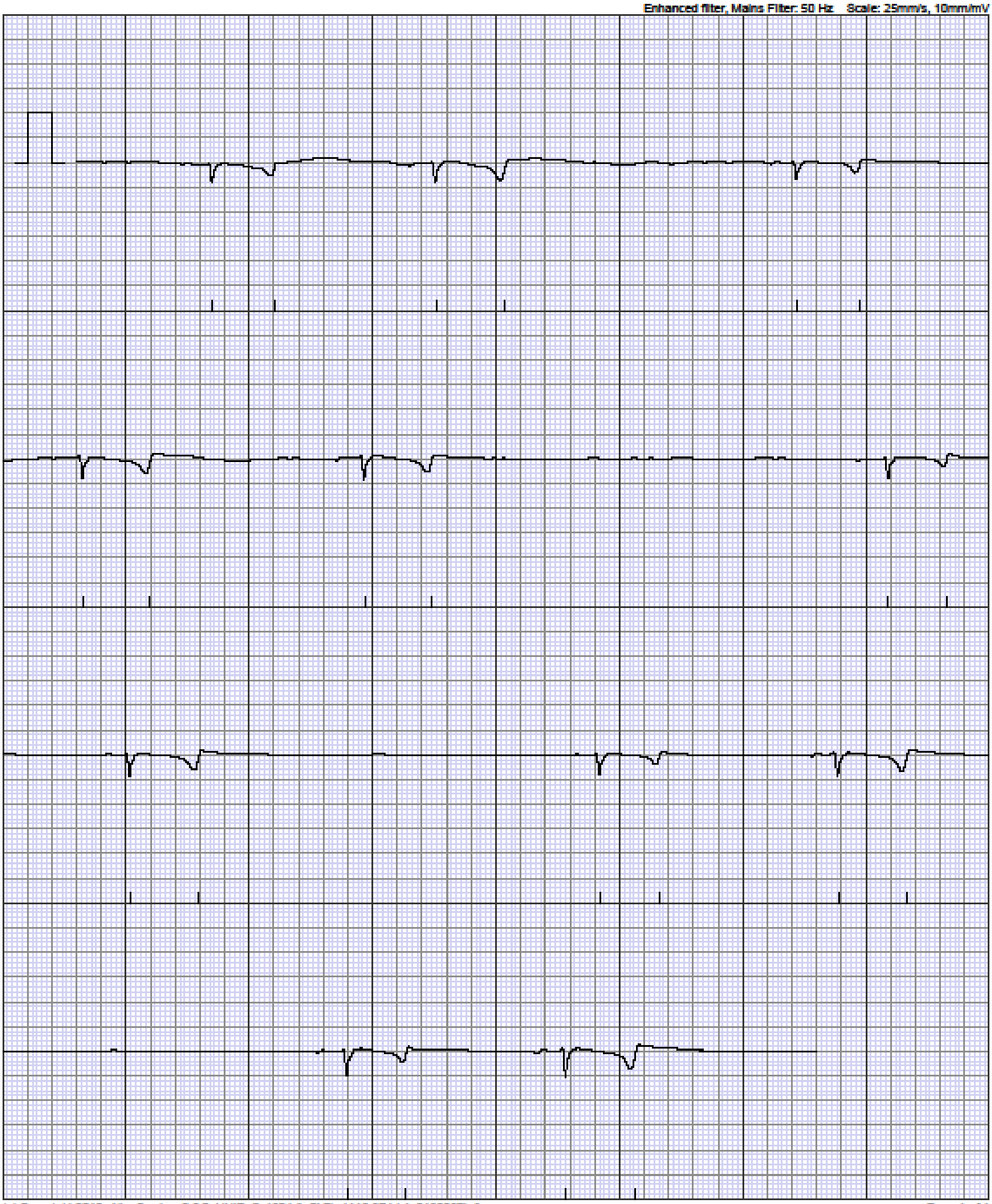


8.2-Appendix 2: *Alivecor KArdiaMobile* tracing of sinoatrial blockade

Patient:	Graeme Piketh, 1993/10/26 (25 yrs)	**Notes:**	horse 30
Recorded:	Wednesday, 22 May 2019, 10:51:24 AM		
Heart Rate:	--- bpm **Duration:** 30s	**Instant Analysis:**	Unreadable

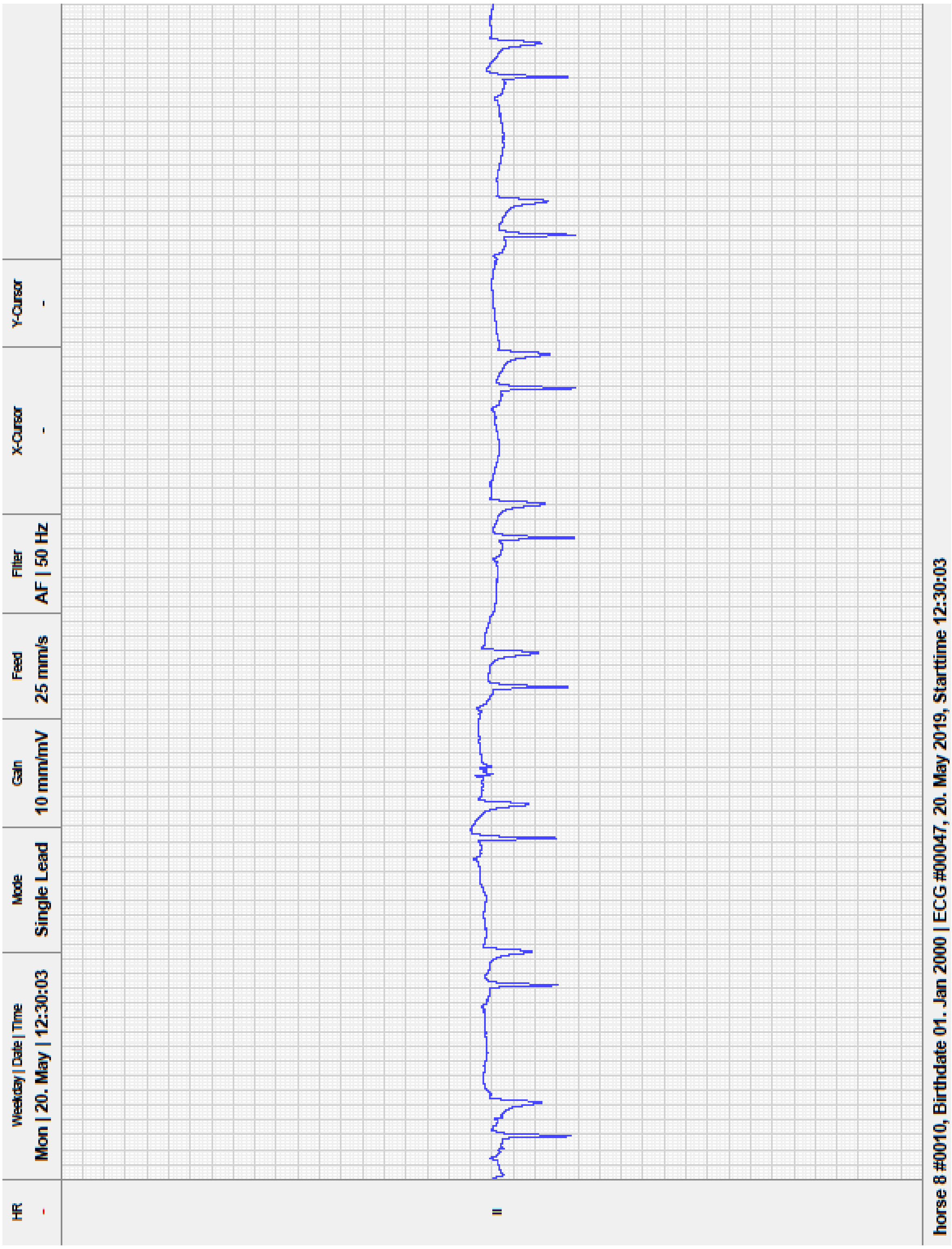
Kardia

Enhanced filter, Mains Filter: 50 Hz   Scale: 25mm/s, 10mm/mV

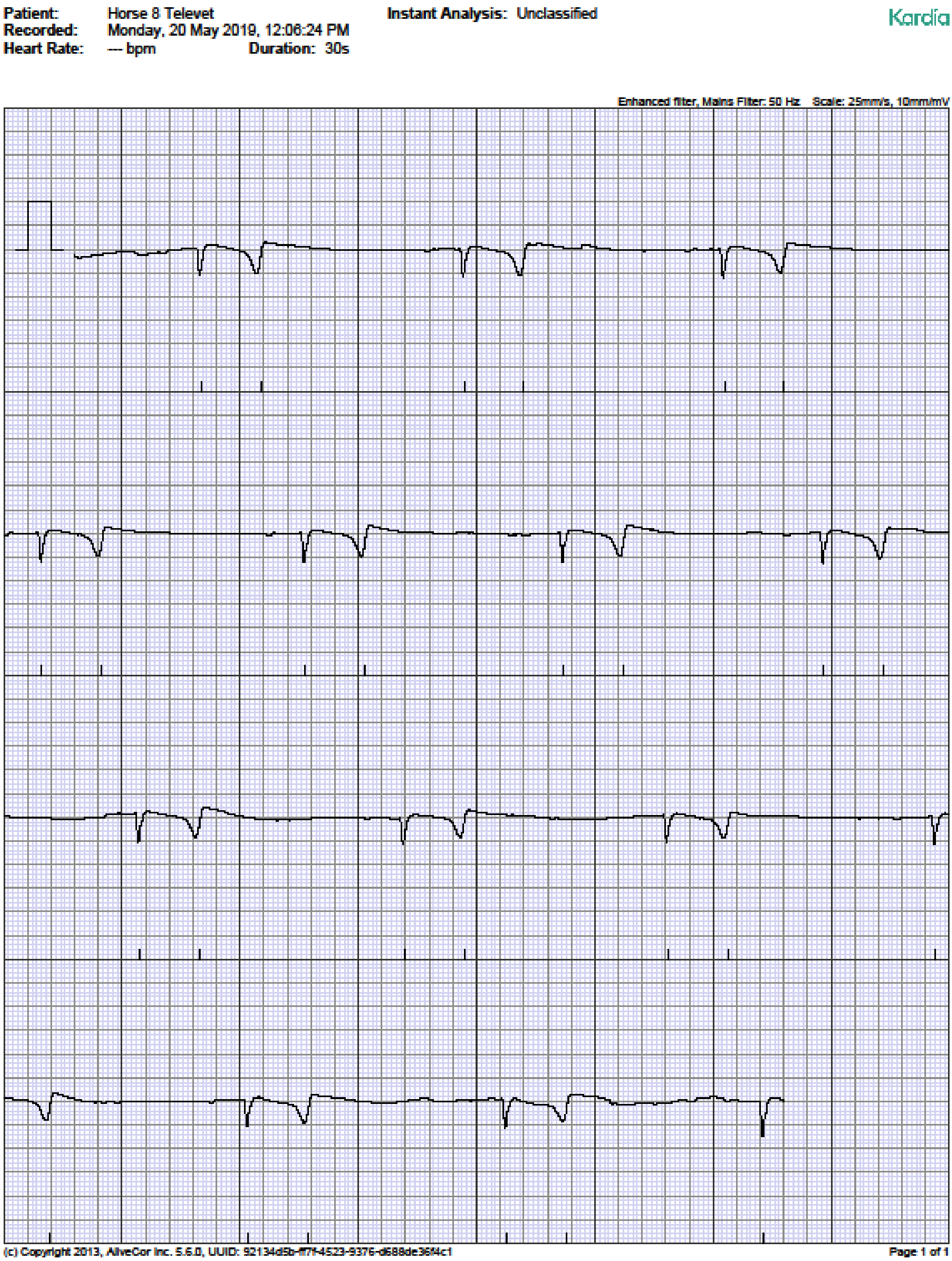


Page 1 of 1

8.3-Appendix 3: *Televet 100* tracing showing normal sinus rhythm



8.4-Appendix 4: *Alivecor KardiaMobile* tracing showing normal sinus rhythm



8.5-Appendix 5: Initial Animal Ethics Approval document

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Assessing the clinical application of a mobile ECG device for use in equine endurance athletes**
PROJECT NUMBER	**V087-18**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr. G Piketh**

STUDENT NUMBER (where applicable)	**U 12000842**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**OTAU Horses (Part 1)**	**Privately owned Horses (Part 2)**
NUMBER OF SAMPLES	**36**	**62**
Approval period to use animals for research/testing purposes		**October 2018 – October 2019**
SUPERVISOR	**Dr. A Williams**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 29 October 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

54285-15

8.6-Appendix 6: Amendment 1 of Animal Ethics Approval document

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	Assessing the clinical application of a mobile ECG device for use in equine endurance athletes
PROJECT NUMBER	V087-18 (Amendment 1)
RESEARCHER/PRINCIPAL INVESTIGATOR	Dr. G Piketh

STUDENT NUMBER (where applicable)	U 12000842
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	OTAU Horses (Part 1)	Privately owned Horses (Part 2)
NUMBER OF SAMPLES	36	62
Approval period to use animals for research/testing purposes		October 2018 – October 2019
SUPERVISOR	Dr. A Williams	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	13 October 2018
CHAIRMAN: UP Animal Ethics Committee	Signature	

Animals to stand in herringbone crush in pairs for 1 hour and 10 minutes

S4285-15

8.7-Appendix 7: Amendment 2 of Animal Ethics Approval document

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Veterinary Science

Animal Ethics Committee

26 March 201

Approval Certificate
Amendment

Ethics Reference No.: V087-18 (Amendment 2)
Title: Assessing the clinical application of a mobile ECG device for use in equine endurance athletes

Dear Dr. G Pikoth

The **Amendment** as supported by documents received in February 2019 for your research, was approved by the Animal Ethics Committee on its quorate meeting of 28 March 2018

Please note the following about your ethics approval.

- Please remember to use your protocol number (V087-18 (Amend 2)) on any documents or correspondence with the Animal Ethics Committee regarding your research. 36 OTAU animals were approved for the second phase of the study
- Please note that the Animal Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of al documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Prof V Naidoo
CHAIRMAN: UP-Animal Ethics Committee

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8.8-Appendix 8: Faculty Ethics Approval document

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Research Ethics Committee

PROJECT TITLE	Assessing the clinical application of a mobile ECG device for use in equine endurance athletes
PROJECT NUMBER	REC092-18
RESEARCHER/PRINCIPAL INVESTIGATOR	Graeme Piketh

DISSERTATION/THESIS SUBMITTED FOR	MSc

SUPERVISOR	Adele Williams

APPROVED	Date 05 November 2018
CHAIRMAN: UP Research Ethics Committee	Signature

Chapter 8: References

- ALGHATRIF, M. & LINDSAY, J. 2012. A brief review: history to understand fundamentals of electrocardiography. *J. Community Hosp. Intern. Med. Perspect.,* 2**,** 10.3402/jchimp.v2i1.14383.
- BANSAL, A. & JOSHI, R. 2018. Portable out-of-hospital electrocardiography: A review of current technologies. *J Arrhythm.,* 34**,** 129-138, 10.1002/joa3.12035.
- BONELLI, F., VEZZOSI, T., MEYLAN, M., NOCERA, I., FERRULLI, V., BURALLI, C., MEUCCI, V. & TOGNETTI, R. 2019. Comparison of smartphone-based and standard base-apex electrocardiography in healthy dairy cows, *J Vet Intern Med.,* 33**,** 981-986, 10.1111/jvim.15396
- BROUX, B., DE CLERCQ, D., DECLOEDT, A., VAN DER VEKENS, N., VERHEYEN, T., VEN, S., PARDON, B. & VAN LOON, G. 2016. Atrial Premature Depolarization-Induced Changes in QRS and T Wave Morphology on Resting Electrocardiograms in Horses. *J Vet Intern Med.,* 30**,** 1253-1259, 10.1111/jvim.13957
- EINTHOVEN W., 1901, Un nouveau galvanometer. Arch Neerl Sc Ex Nat; 6: 625-33.
- FISH, C. 1988. Electrocardiography and vector cardiography. *In:* E, B. (ed.) *Heart Disease: a Textbook of Cardiovascular Medicine.* 2nd ed. Philadelphia: WB Saunders.
- FLETHOJ, M., KANTERS, J. K., PEDERSEN, P. J., HAUGAARD, M. M., CARSTENSEN, H., OLSEN, L. H. & BUHL, R. 2016. Appropriate threshold levels of cardiac beat-to-beat variation in semi-automatic analysis of equine ECG recordings, *BMC Vet Res,* 12**,** 266, 10.1186/s12917-016-0894-2
- FONFARA, S., CASAMIAN-SORROSAL, D., SUNDERMEYER, J. & ROSENBERGER, T. 2015. Variations in heart rate and rhythm of harbor seal pups during rehabilitation. *Mar. Mamm. Sci.,* 31**,** 998-1013, 10.1111/mms.12201
- GIAVARINA, D. 2015. Understanding Bland Altman analysis. *Biochemia Medica,* 25**,** 141-151.
- GROPLER, M. R. F., DALAL, A. S., VAN HARE, G. F. & SILVA, J. N. A. 2018. Can smartphone wireless ECGs be used to accurately assess ECG intervals in pediatrics? A comparison of mobile health monitoring to standard 12-lead ECG. *PLOS ONE,* 13**,** e0204403.
- GUNTHER-HARRINGTON, C. T., ARTHUR, R., ESTELL, K., MARTINEZ LOPEZ, B., SINNOTT, A., ONTIVEROS, E., VARGA, A. & STERN, J. A. 2018. Prospective pre- and post-race evaluation of biochemical, electrophysiologic, and echocardiographic indices in 30 racing thoroughbred horses that received furosemide. *BMC Vet Res,* 14**,** 18, 10.1186/s12917-018-1336-0
- HABERMAN, Z. C., JAHN, R. T., BOSE, R., TUN, H., SHINBANE, J. S., DOSHI, R. N., CHANG, P. M. & SAXON, L. A. 2015. Wireless Smartphone ECG Enables Large-Scale Screening in Diverse Populations. *J Cardiovasc Electrophysiol,* 26**,** 520-6, 10.1111/jce.12634.
- HANÁK, J. & JAGO, P. 1983. Electrocardiographic Lead System and its Vector Verification. *Acta Vet. Brno,* 52.
- HAVERKAMP, H. T., FOSSE, S. O. & SCHUSTER, P. 2019. Accuracy and usability of single-lead ECG from smartphones - A clinical study. *Indian Pacing Electrophysiol J.,* 19**,** 145-149, 10.1016/j.ipej.2019.02.006.
- HENDRIKX, T., ROSENQVIST, M., WESTER, P., SANDSTRÖM, H. & HÖRNSTEN, R. 2014. Intermittent short ECG recording is more effective than 24-hour Holter ECG in detection of arrhythmias. *BMC Cardiovascular Disorders,* 14**,** 41, 10.1186/1471-2261-14-41.
- HEWETSON, M. Introduction to Electrocardiography in the Horse and Recognition of Common Abnormalities Proceedings of the 13th International Congress of the World Equine Veterinary Association 2013 Budapest, Hungary.

- KIRYU, K., MACHIDA, N., KASHIDA, Y., YOSHIHARA, T., AMADA, A. & YAMAMOTO, T. 1999. Pathologic and electrocardiographic findings in sudden cardiac death in racehorses. *J Vet Med Sci,* 61**,** 921-8, 10.1292/jvms.61.921
- KRAUS, M. S., GELZER, A. R. & RISHNIW, M. 2016. Detection of heart rate and rhythm with a smartphone-based electrocardiograph versus a reference standard electrocardiograph in dogs and cats. *J Am Vet Med Assoc,* 249**,** 189-94, 10.2460/javma.249.2.189.
- KRAUS, M. S., RISHNIW, M., DIVERS, T. J., REEF, V. B. & GELZER, A. R. 2019. Utility and accuracy of a smartphone-based electrocardiogram device as compared to a standard base-apex electrocardiogram in the horse. *Res. Vet. Sci.,* 125**,** 141-147, 10.1016/j.rvsc.2019.05.018.
- LIGHTOWLER, C., PICCIONE, G., GIUDICE, E., ROMEI DEL OLMO, G. & LAURA CATTANEO, M. 2004. Echocardiography and electrocardiography as means to evaluate potential performance in horses. *J. Vet. Sci.,* 5**,** 259-62.
- MARLIN, D. & NANKERVIS, K. J. 2002. *Equine exercise physiology*, Blackwell Science.
- MITCHELL, K. J. 2019. Equine Electrocardiography. *Vet Clin North Am Equine Pract,* 35**,** 65-83, 10.1016/j.cveq.2018.12.007.
- NAVAS DE SOLIS, C. 2016. Exercising arrhythmias and sudden cardiac death in horses: Review of the literature and comparative aspects. *Equine Vet. J.,* 48**,** 406-413, 10.1111/evj.12580
- NOBLE, D. 1979. *The Initiation of the Heart Beat.,* Oxford, Oxford University Press;.
- PASLAWSKA, U., ZYSKO, D., NOSZCZYK-NOWAK, A. & PASLAWSKI, R. 2012. Short-term and long-term PQ, QT and R-R intervals' variability at the resting condition and after exercise testing in healthy Anglo-Arabian horses. *Pol J Vet Sci,* 15**,** 55-9, 10.2478/v10181-011-0114-8.
- PEDERSEN, P. J., KANTERS, J. K., BUHL, R. & KLAERKE, D. A. 2013. Normal electrocardiographic QT interval in race-fit Standardbred horses at rest and its rate dependence during exercise. *J Vet Cardiol,* 15**,** 23-31, 10.1016/j.jvc.2012.08.002.
- PHYSICK-SHEARD, P. W. 2016. Equine electrocardiography revisited. *Equine Vet. Educ.,* 28**,** 65-67.
- POSTEMA, P. G. & WILDE, A. A. 2014. The measurement of the QT interval. *Curr Cardiol Rev,* 10**,** 287-94, 10.2174/1573403X10666140514103612.
- REED, S., BAYLY, W. & SELLON, D. C. 2017. *Equine Internal Medicine: Fourth Edition*. Elsevier, St. Louis, Missouri
- RJ. NOBLE, J. S. H., D.A. ROTHBAUM . 1990. Electrocardiography. *In:* HK. WALKER , W. H., JW. HURST (ed.) *Clinical Methods: The History, Physical, and Laboratory Examinations.* Boston: Butterworths.
- ROSE, R. J., ILKIW, J. E. & HODGSON, D. 1979. Electrocardiography, Heart Score and Haematology of Horses Competing in an Endurance Ride. *Aust Vet J,* 55**,** 247-250, 10.1111/j.1751-0813.1979.tb00389.x.
- ROSNER, B., 2011, Fundamentals of biostatistics. Boston, Brooks/Cole, Cengage Learning.
- SMITH, J., WARD, J., URBANO, T. & MUELLER, M. 2016. Use of AliveCor heart monitor for heart rate and rhythm evaluation in domestic water buffalo calves. *JSci. J. Anim. Sci.,* 94**,** 146-146, 10.2527/msasas2016-312
- TILLEY, L. P. 1985. General principles of electrocardiography. *In:* TILLEY, L. P. (ed.) *Canine and Feline Electrophysiology.* Philadelphia: Lea & Febiger.
- VAN LOON, G. & PATTESON, M. 2010. Chapter 6 - Electrophysiology and arrhythmogenesis. *In:* MARR, C. M. & BOWEN, I. M. (eds.) *Cardiology of the Horse (Second Edition).* Edinburgh, W.B. Saunders.

- VERHEYEN, T., DECLOEDT, A., CLERCQ, D., DEPREZ, P., SYS, S. U. & VAN LOON, G. 2010a. Electrocardiography in horses - Part 1: How to make a good recording. *Flem. Vet. J.,* 79**,** 331-336.
- VERHEYEN, T., DECLOEDT, A., DE CLERCQ, D., DEPREZ, P., SYS, S. U. & VAN LOON, G. 2010b. Electrocardiography in horses - part 2: how to read the equine ECG. *Flem. Vet. J.,* 79**,** 337-344.
- VEZZOSI, T., BURALLI, C., MARCHESOTTI, F., PORPORATO, F., TOGNETTI, R., ZINI, E. & DOMENECH, O. 2016a. Diagnostic accuracy of a smartphone electrocardiograph in dogs: Comparison with standard 6-lead electrocardiography. *Vet J,* 216**,** 33-7, 10.1016/j.tvjl.2016.06.013.
- VEZZOSI, T., BURALLI, C., MARCHESOTTI, F., PORPORATO, F., TOGNETTI, R., ZINI, E. & DOMENECH, O. 2016b. Diagnostic accuracy of a smartphone electrocardiograph in dogs: Comparison with standard 6-lead electrocardiography. Vet J. 216:33-7 10.1016/j.tvjl.2016.06.013.
- VEZZOSI, T., SGORBINI, M., BONELLI, F., BURALLI, C., PILLOTTI, M., MEUCCI, V. & TOGNETTI, R. 2018b. Evaluation of a Smartphone Electrocardiograph in Healthy Horses: Comparison With Standard Base-apex Electrocardiography, *J. Equine Vet. Sci.,* 67**,** 61-65, 10.1016/j.jevs.2018.03.006.
- VEZZOSI, T., TOGNETTI, R., BURALLI, C., MARCHESOTTI, F., PATATA, V., ZINI, E. & DOMENECH, O. 2019. Home monitoring of heart rate and heart rhythm with a smartphone-based ECG in dogs. *Vet Rec,* 184**,** 96, 10.1136/vr.104917.
- WALLER AD., 1887, A demonstration on man of electromotive changes accompanying the heart's beat. J Physiol; 8(5): 229.
- YAW, T. J., KRAUS, M. S., GINSBURG, A., CLAYTON, L. A., HADFIELD, C. A. & GELZER, A. R. 2018. Comparison of a Smartphone-based Electrocardiogram Device with a Standard Six-lead Electrocardiogram in the Atlantic Bottlenose Dolphin (Tursiops Truncatus). *J Zoo Wildl Med,* 49**,** 689-695, 10.1638/2017-0140.1.