

RESEARCH PAPER

Evaluation of the reliability of pulse oximetry, at different attachment sites, to detect hypoxaemia in immobilized impala (*Aepyceros melampus*)

Thembeke K Mtetwa^{a,b}, Gareth E Zeiler^c, Liesel Laubscher^{d,e}, Silke Pfitzer^{f,g} & Leith CR Meyer^{a,b}

^aDepartment of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

^bCentre for Veterinary Wildlife Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

^cDepartment of Companion Animal Clinical Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

^dWildlife Pharmaceuticals, Rocky Drift, White River, South Africa

^eDepartment of Animal Science, Faculty of Agriculture, University of Stellenbosch, Matieland, South Africa

^fMurdoch University, School of Veterinary and Life Sciences, Murdoch University, Perth, Western Australia, Australia

^gWildlife Vets, Ngongoni Farm, Tipperary, South Africa

Correspondence: Thembeke K Mtetwa, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, P. Bag X04, Onderstepoort, Gauteng, 0110, South Africa. E-mail: thembeke.mtetwa@up.ac.za

Abstract

Objective Evaluation of the reliability of pulse oximetry at four different attachment sites compared to haemoglobin oxygen saturation measured by a co-oximeter and calculated by a blood gas analyser in immobilized impala.

Study design Randomized crossover study.

Animals A total of 16 female impala.

Methods Impala were immobilized with etorphine or thiafentanil alone, or etorphine in combination with a novel drug. Once immobilized, arterial blood samples were collected at 5 minute intervals for 30 minutes. Then oxygen was insufflated (5 L minute⁻¹) intranasally at 40 minutes and additional samples were collected. A blood gas analyser was used to measure the arterial partial pressure of oxygen and calculate the oxygen haemoglobin saturation (cSaO₂); a co-oximeter was used to measure the oxygen haemoglobin saturation (SaO₂) in arterial blood. Pulse oximeter probes were attached: under the tail, to the pinna (ear) and buccal mucosa (cheek) and inside the rectum. Pulse oximeter readings [peripheral oxygen haemoglobin saturation (SpO₂) and pulse quality] were recorded at each site and compared with SaO₂ and cSaO₂ using Bland-Altman and accuracy of the area root mean squares (A_{rms}) methods to determine the efficacy. *P* value < 0.05 was considered significant.

Results Pulse quality was 'good' at each attachment site. SpO₂ measured under the tail was accurate and precise but only when SaO₂ values were above 90% (bias = 3,

precision = 3, A_{rms} = 4). The ear, cheek and rectal probes failed to give accurate or precise readings (ear: bias = -4, precision = 14, A_{rms} = 15; cheek: bias = 12, precision = 11, A_{rms} = 16; and rectum: bias = 5, precision = 12, A_{rms} = 13).

Conclusions and clinical relevance In order to obtain accurate and precise pulse oximetry readings in immobilized impala, probes must be placed under the tail and SaO₂ must be above 90%. Since SaO₂ values are usually low in immobilized impala, pulse oximeter readings should be interpreted with caution.

Keywords immobilization, impala, oxygen saturation, pulse oximetry.

Introduction

Chemical immobilization is an essential tool for wildlife conservation and management worldwide. It is commonly used in impala, mainly, but not exclusively, for translocation and disease surveillance purposes. To immobilize impala, potent opioids (such as etorphine or thiafentanil) are primarily used. Impala are particularly sensitive to the adverse effects of these drugs and deaths often occur when they are captured (Meyer et al. 2008; Zeiler & Meyer 2017a). These opioids impair ventilatory function by causing ventilatory depression, intrapulmonary shunting, ventilation-perfusion mismatch and probable impedance to oxygen diffusion, ultimately leading to

severe hypoxaemia (Zeiler & Meyer 2017a,b). Untreated hypoxaemia may cause tissue hypoxia, organ failure and death (Fahlman 2014). Therefore, it is important that blood oxygenation is continuously and accurately monitored during immobilization to ensure that hypoxaemia is detected and treated early to guarantee the welfare and survival of an animal.

Currently, co-oximeters and blood gas machines are frequently used for the detection of low arterial oxygen haemoglobin saturation (SaO₂) or oxygen partial pressures or both (Wong et al. 2011; Bardell et al. 2017). However, these devices do not give continuous readings, they can be costly and the arterial samples they require can be difficult to obtain in some species, including immobilized impala (Proulx 1999). Furthermore, these devices are not always practical to use in the field (Proulx 1999). Pulse oximetry (SpO₂), which gives an indirect peripheral measure of SaO₂ (Pedersen et al. 2009), has become a popular method used for the early detection of hypoxaemia in human and veterinary medicine (van de Louw et al. 2001; Young et al. 2002; Giguere et al. 2014; Haymerle et al. 2016). It is widely used in the hospital and field setting because pulse oximetry devices are cheap, portable, easy to apply and use, and give continuous noninvasive measures. Their use and effectiveness have been determined and well documented in humans (Lee et al. 2000; van de Louw et al. 2001) and domestic animals (Chaffin et al. 1996; Mathews et al. 2003; Giguere et al. 2014; Grubb & Anderson 2017; Reiners et al. 2018). However, although they are commonly used during the immobilization of wildlife (Martin-Jurado et al. 2011; Haymerle et al. 2016), their accuracy and utility have not been adequately determined. Therefore, the validity of their use for this purpose is still questionable.

Studies in human and domesticated animals show that the accuracy of pulse oximetry is dependent upon probe placement (Chaffin et al. 1996; Lee et al. 2000; van de Louw et al. 2001; Mathews et al. 2003; Giguere et al. 2014). In order to obtain a pulse oximeter reading, veterinarians working with immobilized wildlife place probes at different sites including the ear, lip, rectum, tongue, vulva and the tip of the penis (Proulx 1999). However, whether the readings from these sites are accurate and precise still needs to be elucidated. Although several studies have previously compared SpO₂ readings at different attachment sites in domestic animals (Chaffin et al. 1996; Mathews et al. 2003; Giguere et al. 2014), no such studies have been performed in immobilized wildlife.

Therefore, this study aimed to establish, in immobilized impala, whether pulse oximetry is a reliable method for the determination of SaO₂. The study also aimed to determine which attachment site of the pulse oximeter probe provided the most accurate SpO₂ measurement. We hypothesized that pulse oximetry can be used to accurately measure SaO₂ in immobilized impala under field conditions.

Materials and methods

Animals

The study was approved by the Animal Ethics Committee of the University of Pretoria (V035-17) and was conducted at Ngongoni Farm (25°31'25.2"S, 31°06'50.8"E). A total of 16 free-ranging adult female impala (*Aepyceros melampus*), temporarily housed together in a 6 × 8 m outdoor enclosure (boma), were used. The impala [body weight 34.1 ± 5.2 kg — mean ± standard deviation (SD)] were habituated to the housing conditions 14 days prior to the study. Lucerne (*Medicago sativa*) and water were supplied *ad libitum* with game pellets supplemented as needed. For exclusion, animals were checked for overt signs of cardio-respiratory and other diseases at the first immobilization.

Immobilization

Data for this study were collected during an unrelated study (see Pfitzer et al. 2019) which aimed to assess the dose-response effect of the serotonin R-enantiomer of 8-hydroxy-2-(di-n-propylamino) tetralin drug on opioid-induced respiratory depression. This 'novel drug study' used a randomized crossover study design, in which the impala were immobilized (one at a time) on six different occasions. In each trial the impalas were darted with either etorphine alone [0.09 mg kg⁻¹, Captivon, Wildlife Pharmaceuticals (Pty) Ltd., South Africa], thiafentanil alone [0.09 mg kg⁻¹, Thianil, Wildlife Pharmaceuticals (Pty) Ltd.] or etorphine (0.09 mg kg⁻¹) in combination with different doses of the novel drug, using a 1.5 mL dart (P-type Pseudarts with 19 mm long needle; DAN-INJECT, International S.A, South Africa) projected into a muscle mass of the pelvic girdle by a carbon dioxide (CO₂) gas powered rifle [230 kPa; X-Caliber dart gun; Wildlife Pharmaceuticals (Pty) Ltd.]. The dart rifle operator stood on an elevated walkway above the boma wall and darted the impala over a distance ranging from 5 to 12 m.

Experimental procedures

Following immobilization, the impala were blindfolded and carried to an under roof workstation and placed in sternal recumbency on a table. A peripheral artery (either *arteriae auricularis posterior* or *arteriae digitales palmares communes*) was aseptically cannulated (22 gauge; Jelco Smiths Medical, UK) for arterial blood sampling. A total of four pulse oximeters (Nonin PalmSat 2500 A; Kyron Laboratories (Pty) Ltd., South Africa) were connected, using four probes [Kyron Laboratories (Pty) Ltd.] at different locations on the impala, as follows (Fig. 1):

- 1) Tail—reflectance probe (2000T Transflectance probe; Kyron Laboratories (Pty) Ltd.) placed and secured gently, using adhesive tape (25 mm ElastoPlast Tape; BSN Medical (Pty) Ltd., South

- Africa), on unpigmented skin on the ventral aspect of the tail base within 20–30 mm from the root of the tail (Fig. 1a & a₁).
- 2) Rectum—reflectance probe (2000T Transflectance probe) inserted approximately 10 mm into the rectum and secured in position by taping the connecting wire of the probe to the tail base using adhesive tape (Fig. 1b & b₁).
 - 3) Ear—transmission probe (2000SL Lingual probe; Kyron Laboratories (Pty) Ltd.) clipped onto a shaved unpigmented area on the caudal margin of the pinna (Fig. 1c & c₁).
 - 4) Cheek—reflectance probe (2000T Transflectance probe) secured to a holding clip and positioned inside the mouth and placed against the buccal mucosa approximately 30 mm from the commissure of the lip (Fig. 1d & d₁).

These sites were chosen based on practical application of the probes. The Nonin PalmSAT 2500 veterinary probes were used in the study (2000T and 2000SL) because they are similar to the Nonin PalmSAT 2500 human probes (8000Q2-ear clip

sensor and 8000R-reflectance sensor), which are compatible with each other (<https://www.nonin.com/wp-content/uploads/2018/09/Sensor-Compatibility-Guide.pdf>). The device's set averaging time for SpO₂ readings is 10 seconds (<https://www.nonin.com/wp-content/uploads/Operators-Manual-2500A-Vet.pdf>).

In all the trials, 1 mL of arterial blood was collected in heparinized plastic syringes (B Braun, Germany) and stored on ice at 5, 10, 15, 20 and 30 minutes from the time the impala became recumbent. At 40 minutes of immobilization, seven impala were given etorphine alone or thiafentanil alone and were supplemented with oxygen for 10 minutes using a high-pressure oxygen cylinder [Ecomed Medical (Pty) Ltd, South Africa]. The flowmeter was set at 5 L minute⁻¹, administered intranasally via a nasal tube to increase the animal's oxygen haemoglobin saturation. Thereafter, arterial samples were also

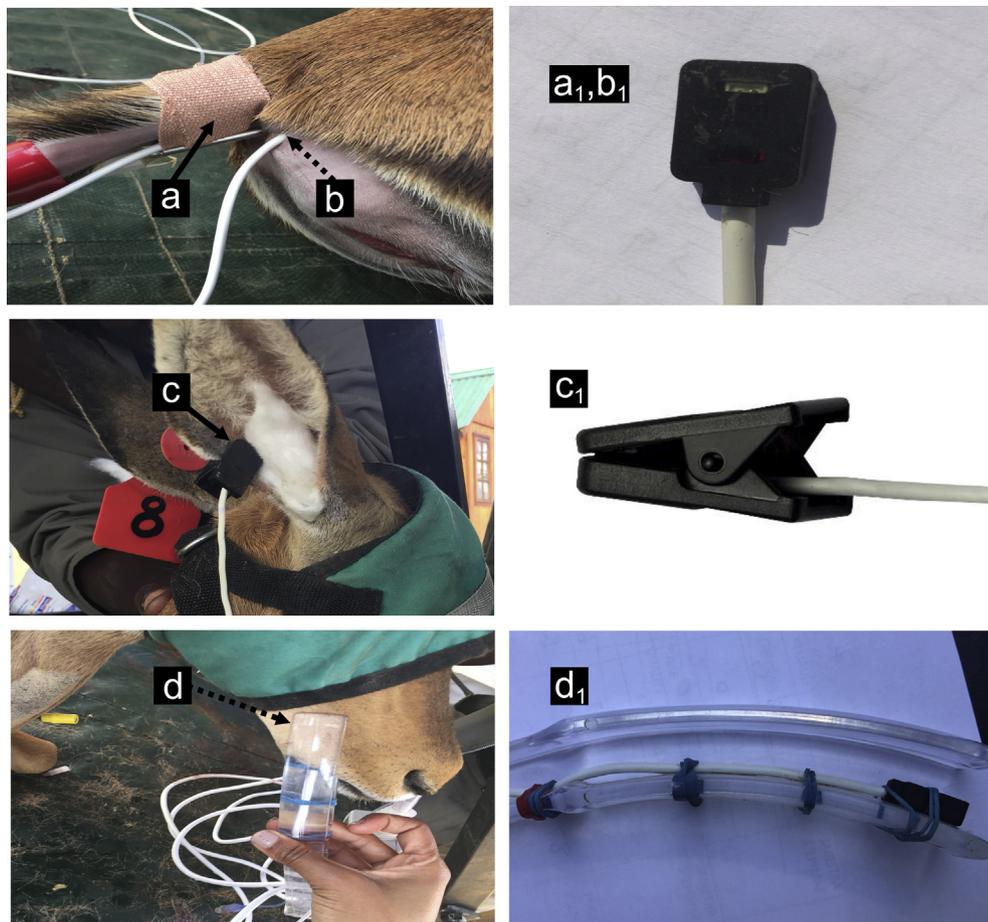


Figure 1 Placement of pulse oximeter probe at four different attachment sites. The tail (a) probe was a transreflectance probe (a₁). It was placed on unpigmented skin at the base of the tail and secured with tape. The probe inserted into the rectum (b) was a transreflectance probe (b₁). It was placed 10 mm inside the rectum facing the mucosa next to the temperature probe and indicated by the black arrow. The lingual transmission probe (c₁) was clipped to the skin of the shaved margin of the ear (c) of the impala. The probe attached to the cheek (d) was a transreflectance probe. It was attached to a plastic clip with rubber bands (d₁) and the plastic clip secured the probe adjacent to cheek (d) approximately 30 mm from the commissure of the lips. White dotted arrows represent internal probes placed on the mucosa and the white solid arrows are probes that are placed externally on the skin.

collected at random intervals during oxygen supplementation. Peripheral oxygen haemoglobin saturation (SpO_2), pulse rate (beats minute^{-1}) and pulse quality data detected by the pulse oximeter were recorded in triplicate, within 30 seconds of collecting each arterial blood sample. Pulse quality (pulse signal strength) was indicated by a flashing coloured light emitting diode built into the pulse oximeter; green indicated good, amber intermediate and red a poor quality. The pulse oximetry readings were recorded in the following order: 1) tail; 2) rectum; 3) cheek; and 4) ear.

Arterial blood analysis

After withdrawal, arterial blood samples were immediately stored on ice and analyzed within 10 minutes after collection using: 1) an EPOC blood gas analysis system and self-calibrating BGEM3 test cards (Epcal Inc., ON, Canada); and 2) a daily calibrated Avoximeter 4000 co-oximeter with cuvettes [Surgical Innovations (Pty) Ltd., South Africa]. The blood gas analyser was used to measure the arterial partial pressure of oxygen (PaO_2) to calculate the arterial oxygen haemoglobin saturation (cSaO_2) using a default equation programmed into the blood gas analyser by the manufacturer. The co-oximeter was used to measure the fractional arterial oxygen haemoglobin saturation of the arterial blood (SaO_2).

Statistical analysis

For Bland-Altman method analysis to be performed at an $\alpha = 0.05$ and $\beta = 0.90$, this study needed a minimum of 17 paired data sets (i.e. $\text{SpO}_2 - \text{SaO}_2$, $\text{cSaO}_2 - \text{SaO}_2$ and $\text{SpO}_2 - \text{cSaO}_2$) to have power when the expected mean of differences is 3%, expected SD of differences is 3% and the maximum allowed difference between methods is $\pm 10\%$.

The SaO_2 measured by the co-oximeter is accepted by the International Organisation for Standardisation (ISO) 80601-2-61:2011 guidelines as the gold-standard measurement of SaO_2 to validate the use of pulse oximetry (DIN EN, 2011). The cSaO_2 calculated by the blood gas analyser is used as a 'clinical standard' (Whitehair et al. 1990; Reiners et al. 2018). Both measures (SaO_2 and cSaO_2) were used to assess the accuracy and utility of the pulse oximeters in this study. The Bland-Altman method for multiple observations (Bland & Altman 2007) was used to determine the accuracy and precision of the measurements recorded using the three monitors. More specifically: 1) how SpO_2 measured by the pulse oximeters at each site compared with the SaO_2 ; 2) how cSaO_2 calculated by the blood gas analyser compared with the measured SaO_2 ; and 3) how SpO_2 measurements at each site compared with the cSaO_2 .

The Bland-Altman method calculates the bias [the measure of accuracy—by calculating the mean difference between the two variables (methods) that are compared, i.e. $\text{SpO}_2 - \text{SaO}_2$, $\text{cSaO}_2 - \text{SaO}_2$ and $\text{SpO}_2 - \text{cSaO}_2$], the precision (a measure of

random error—by calculating the SD of the differences), and limits of agreement (LOA) (calculated as the bias ± 1.96 SD) at 95% confidence interval for the mean difference and LOA. A frequency distribution test and column statistics were performed to determine whether the mean differences between the two variables (methods) were normally distributed.

The combined accuracy and precision of the compared variables were determined by using the accuracy of the area root mean square (A_{rms}) (Batchelder & Raley 2007; Equation (1) in Appendix A). According to ISO 80601-2-61:2011 guidelines, pulse oximetry is regarded as accurate and precise when the bias, precision and A_{rms} are $\leq \pm 3\%$, $\leq 3\%$ and $\leq \pm 4\%$, respectively (DIN EN, 2011).

Data handling

The SpO_2 data set was paired with the SaO_2 and cSaO_2 data sets to determine the total number of pairs available for analysis. Initially, all of the SpO_2 measurements (all-data) were used to calculate the mean of the triplicates for each data point and these values were used for analyses using the methods described. Then, exclusion criteria were applied to the SpO_2 data set to filter out poor pulse quality data points, thereby to obtain a good quality data set (pass-data). It was done by excluding any triplicate data sets: 1) with red oximeter pulse quality light reading; and 2) where the pulse quality light indicated green or amber, but the SpO_2 triplicate data had an SD of more than 3%.

To determine the performance of the pulse oximeters at different SaO_2 ranges, the SpO_2 data were compared at the following different SaO_2 or cSaO_2 ranges: 0 – 100%, 70 – 100% (range claimed by the manufacturer to be most accurate for the pulse oximeter), < 70% (range claimed by the manufacturer to be inaccurate for pulse oximetry), 70 – 79%, 80 – 89% and 90 – 100% for 'all-data' and the 'pass-data' sets.

Analyses were performed using commercially available software MedCalc Version 19, MedCalc Software Ltd., (Belgium) and GraphPad Prism, Version 7, (GraphPad Software, CA, USA).

Results

Quality of data collected

All immobilization procedures were successful (Pfitzer et al. 2019). Overall, there were 167 paired SaO_2 readings measured by the co-oximeter and the SaO_2 data ranged from 16.3% to 99.3%. There were 194 paired cSaO_2 readings calculated by the blood gas analyser and the data ranged from 15.5% to 99.9%. The SaO_2 readings had a good agreement (bias -1 , LOA 5 to -7) with cSaO_2 readings (Fig. 2).

All probe sites gave good pulse quality readings, i.e. percentage readings that had a good pulse quality—green light,

(Tables 1 and 2), with the highest values obtained from the tail and the lowest from the ear. The ear, the cheek and the rectum had SpO₂ readings indicating poor pulse quality (poor signal strength—red light).

Although the tail gave the best quality SpO₂ readings, there was a larger variability in the readings obtained from the tail, and likewise from the ear (triplicate readings with SD>3%, Tables 1 and 2) compared with the other two sites (rectum and cheek).

Pulse oximeter performance at each site

The mean difference between the two variables (methods) that were compared, i.e. SpO₂–SaO₂, cSaO₂–SaO₂ and SpO₂–c-SaO₂, were normally distributed.

Tail

Compared with SaO₂ (Tables 3), the pulse oximeter had an acceptable performance (i.e. it was accurate and precise) in the SaO₂ range of 90–100% for ‘all-data’. In the SaO₂ range of 80–89%, below this range (70–79% and <70%) and in the whole range of SaO₂ (0–100%), the pulse oximetry gave inaccurate and imprecise readings. For the ‘pass-data’, at the range of 90–100%, pulse oximetry was inaccurate but precise. Below 90%, however, pulse oximetry was inaccurate and imprecise.

When cSaO₂ was compared with SpO₂ at the range of 90–100%, the pulse oximeter gave accurate but imprecise

readings, however, the A_{rms} indicated that pulse oximetry was accurate for ‘all-data’ and ‘pass-data’. Below these ranges, pulse oximetry was inaccurate and imprecise. In the manufacturer’s claimed performance range (70–100%) the readings were accurate but imprecise (Table 4).

Ear

When compared with SaO₂ (Tables 3 and 4), ‘all-data’ readings were accurate but imprecise in all ranges except within the 70–79% and 90–100% ranges, where the readings were both inaccurate and imprecise. These findings were similar to the ‘pass-data’. At the range of 70–100%, the pulse oximeter gave accurate but imprecise readings for ‘all-data’ and ‘pass-data’ (Fig. 3). When compared with cSaO₂, the ear probe was inaccurate and imprecise at all ranges.

Rectum

Within the SaO₂ ranges of 80–89% and 90–100%, the pulse oximeter gave accurate but imprecise readings (Table 3). At ranges below 80%, the pulse oximeter was inaccurate and imprecise. For ‘pass-data’, the pulse oximeter gave accurate but imprecise readings at the range of 90–100%. At the range of 70–100%, the pulse oximeter gave accurate but imprecise readings for ‘all-data’ and ‘pass-data’ (Fig. 3). The comparisons of the pulse oximeter readings with cSaO₂ were similar to those of SaO₂ (Table 4).

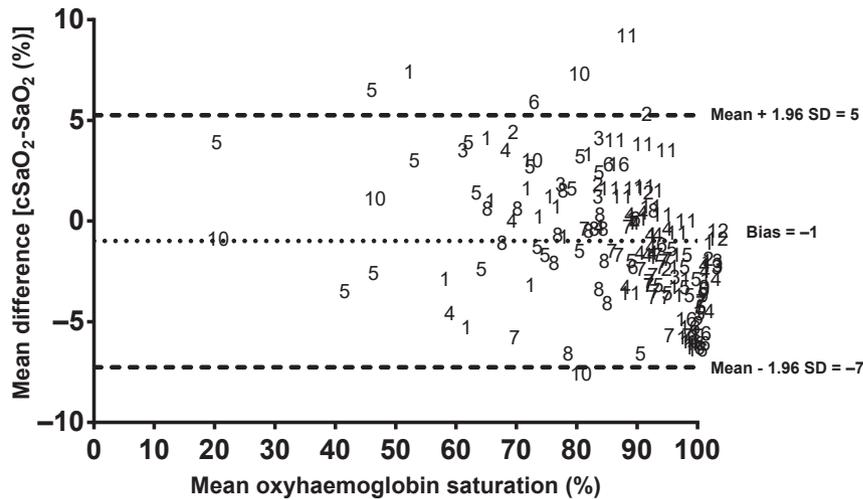


Figure 2 Bland-Altman plot showing the agreement between arterial oxygen haemoglobin saturation measured by the co-oximeter (SaO₂) and calculated by the blood gas analyser using the measured partial pressure of oxygen (cSaO₂). The mean difference between the SaO₂ and the cSaO₂ is plotted against the mean oxygen haemoglobin saturation (cSaO₂ and SaO₂) values obtained from the two devices. Each point on the plot represents one paired measurement. Each number represents the animal identification number (ID) of 16 individual impala from which the arterial samples were taken. The dashed lines represent the limits of agreement [mean ± 1.96 standard deviation (SD)] and the solid line represents the estimated bias.

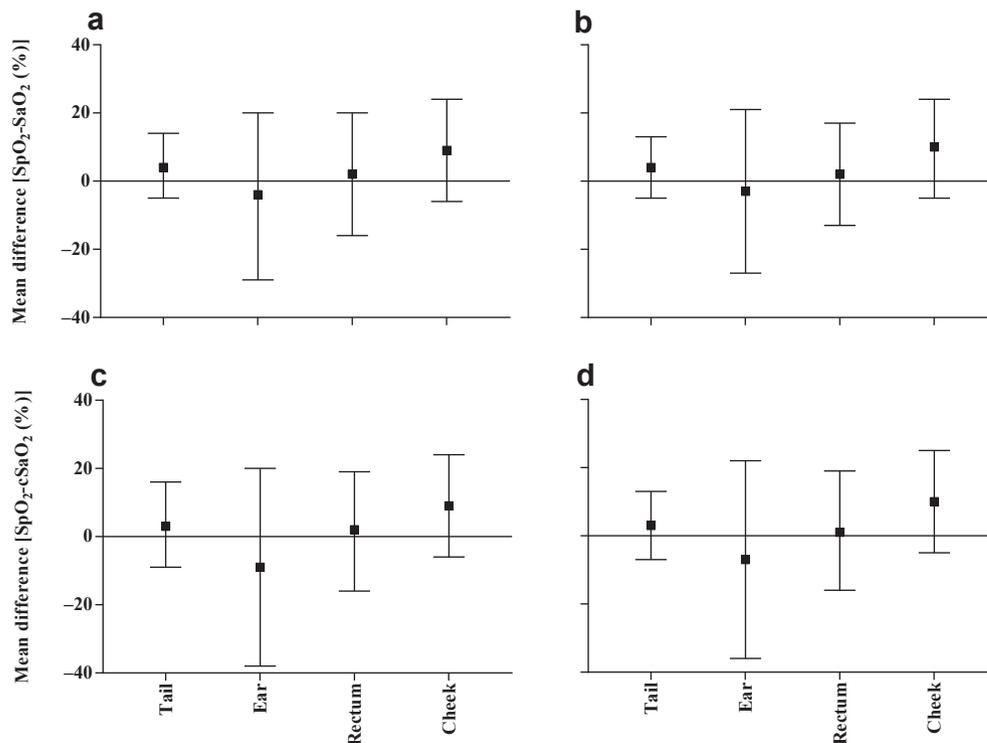


Figure 3 The performance of the peripheral oxygen haemoglobin saturation (SpO_2 ; measured by pulse oximetry) compared with arterial oxygen haemoglobin saturation (SaO_2 ; measured by the co-oximeter) (graphs a and b) and arterial oxygen haemoglobin saturation (cSaO_2 ; calculated by blood gas analyser) (graphs c and d) in the range of 70–100% (manufacturer's claimed performance range of the pulse oximeters) shown on the y axis, at four probe attachment sites in 16 impala, shown on the x axis. a and c indicate 'all-data' sets (i.e. when exclusion criteria were not applied) and b and d indicate "pass-data" sets (i.e. when exclusion criteria were applied). The data were analysed using the Bland-Altman method for multiple comparisons per individual. The mean differences (bias—black squares) ($\text{SpO}_2\text{-SaO}_2$; $\text{SpO}_2\text{-cSaO}_2$) and the limits of agreement [mean \pm 1.96 standard deviation (SD)] are plotted for the four probe attachment sites. The black line at 0 indicates a bias of 0; the closer the mean differences are to this line, the greater the accuracy of the pulse oximeter measurements and the smaller the limits of agreement, the greater the precision of these measurements.

Cheek

When compared with SaO_2 (Table 3), pulse oximetry was inaccurate and imprecise at all ranges except within the range of 90–100%, where readings were inaccurate but precise for 'all-data' and 'pass-data'.

When compared with cSaO_2 (Table 4), the cheek probe gave inaccurate but precise readings in the 90–100% range for 'pass-data'. Within all the other ranges analyzed, the cheek probe gave inaccurate and imprecise readings.

Discussion

In the immobilized impala, SpO_2 measurements using a commercial veterinary pulse oximeter device were accurate and precise indicators of oxygenation. However, it was only acceptable at high SaO_2 and was influenced by the probe's location on the impala. Pulse oximetry was most accurate and precise when the probe was placed at the base of the tail and the saturation values were greater than 90%. The tail probe

provided good pulse quality readings; however, the variability of these readings was also high. The accuracy of pulse oximetry was poor when the probes were placed on the ear, inside the rectum and against the cheek. The differences in the performance of the pulse oximeter at different attachment sites highlights the importance of appropriate probe placement when monitoring and assessing oxygen levels in free-ranging wild ungulates during immobilization.

The co-oximeter was not available on all study days, which resulted in a smaller sample size of SaO_2 measurements when compared with cSaO_2 readings. However, the number of samples was still adequate for the comparisons made, and similar findings were obtained when SpO_2 values were compared with cSaO_2 . Many studies also compare the pulse rate measured by the pulse oximeter with the heart rate (HR) of the animal to determine the quality of the data measured. If the two values are similar, this indicates that the pulse oximeter is measuring a pulse and not movement artefact (Bohnhorst et al. 2002; Young et al. 2002). However, normal

Table 1 The number of time-matched paired data obtained from the co-oximeter (SaO₂) and the pulse oximeters (SpO₂) at the different attachment sites (ear, cheek, tail and rectum). The number of pairs for all the data (all-data) and their pulse quality are presented. Number of data pairs excluded, and the criteria of exclusion and the total number of data pairs not excluded (pass-data) are also presented. SD, standard deviation. sample size (*n*).

Attachment site	All-data <i>n</i>	Pulse quality indicator light colour (%)	Pass-data <i>n</i> (%)	Exclusion criteria <i>n</i> (%)	
				Triplicate SpO ₂ with SD >3*	Red light (poor pulse quality) [†]
Tail	167	Green: 98 Amber: 2 Red: 0	124 (74)	43 (26)	0 (0)
Ear	134	Green: 75 Amber: 21 Red: 4	91 (68)	35 (26)	8 (6)
Rectum	153	Green: 83 Amber: 16 Red: 1	115 (75)	37 (24)	1 (1)
Cheek	111	Green: 88 Amber: 11 Red: 1	88 (79)	22 (20)	1 (1)

*Triplicate SpO₂ data excluded because the SD was more than 3% between the SpO₂ triplicate data, even though the pulse quality indicated a green or amber light for the readings.

[†]Triplicate SpO₂ data excluded because one or more SpO₂ readings had a poor pulse quality indicated by a red light.

variability between HR measured from an electrocardiograph and the pulse rate measured by pulse oximetry have been observed in cats, dogs and horses (Mathews et al. 2003). Therefore, using HR alone to assess data quality can have its limitations. Most studies that validated their readings using pulse rate did not take pulse quality, or its variability, into consideration, and this practice may limit the ability to make valid comparisons. We were unable to record the HR at the same time as our data collection. Therefore, the quality of our

data was based solely on the pulse quality determined by the internal light emitting diode function and the variability of the data. In some instances, more than 30% of good pulse quality data were excluded because they were highly variable, possibly indicating an error in the measurement of pulse quality, or acute changes in SaO₂ of the animal at the time of measurement or movement.

Previously, the accuracy of pulse oximetry has been assessed by either comparison of SpO₂ values with calculated SaO₂

Table 2 The number of time-matched paired data obtained from the blood gas analyser (cSaO₂) and the pulse oximeters (SpO₂) at the different attachment sites (ear, cheek, tail and rectum). The number of pairs for all the data (all-data) and their pulse quality are presented. Number of data pairs excluded, and the criteria of exclusion and the total number of data pairs not excluded (pass-data) are also presented. SD, standard deviation. sample size (*n*).

Attachment site	All-data <i>n</i>	Pulse quality indicator light colour (%)	Pass-data <i>n</i> (%)	Exclusion criteria <i>n</i> (%)	
				Triplicate SpO ₂ with SD >3*	Red light (poor pulse quality) [†]
Tail	194	Green: 97 Amber: 3 Red: 0	134 (69)	60 (31)	0 (0)
Ear	139	Green: 72 Amber: 24 Red: 4	80 (58)	50 (36)	9 (6)
Rectum	176	Green: 80 Amber: 19 Red: 1	129 (73)	46 (26)	1 (1)
Cheek	136	Green: 87 Amber: 12 Red: 1	105 (77)	30 (22)	1 (1)

*Triplicate SpO₂ data excluded because the SD was more than 3% between the SpO₂ triplicate data, even though the pulse quality indicated a green or amber light for the readings.

[†]Triplicate SpO₂ data excluded because one or more SpO₂ readings had a poor pulse quality indicated by a red light.

Table 3 The performance of the pulse oximeter readings (SpO₂) compared with the gold standard co-oximetry (SaO₂) at different attachment sites in immobilized impala (number of impala = 16). SaO₂, oxygen haemoglobin saturation measured by co-oximetry.

Attachment site	SaO ₂ Ranges	'All-data'					'Pass-data'				
		n	Bias	Precision	A _{rms}	LOA	n	Bias	Precision	A _{rms}	LOA
Tail	0–100%	167	6	7	10	21, –8	124	6	7	9	19, –7
	70–100%	144	4	5	6	14, –5	110	4	5	6	13, –5
	<70%	23	18	9	20	36, –1	14	1	9	19	35, –1
	70–79%	22	9	5	10	20, –2	9	11	5	11	20, –2
	80–89%	53	4	6	7	15, –7	42	4	6	7	15, –6
	90–100%	69	3*	3*	4*	9, –3	59	4	3*	4*	9, –2
Ear	0–100%	134	–4	14	15	24, –32	91	–2*	14	14	26, –30
	70–100%	120	–4	13	13	20, –29	82	–3*	12	12	21, –27
	<70%	14	0*	25	23	49, –49	9	8	27	26	61, –42
	70–79%	14	–4	15	15	25, –33	5	1*	9	8	20, –17
	80–89%	44	–1*	11	14	20, –23	32	–1*	11	11	21, –24
	90–100%	62	–6	13	14	19, –31	45	–5	13	14	20, –30
Rectum	0–100%	153	5	12	13	28, –18	115	5	11	12	27, –18
	70–100%	132	2*	9	9	20, –16	102	2*	8	8	17, –13
	<70%	21	23	13	26	48, –2	13	24	18	26	59, –11
	70–79%	20	8	13	15	34, –18	11	9	9	12	26, –9
	80–89%	50	3*	8	9	19, –13	34	4	6	7	16, –9
	90–100%	62	–1*	7	6	13, –14	57	–0*	7	7	13, –14
Cheek	0–100%	111	12	11	16	33, –10	88	12	11	16	33, –9
	70–100%	97	9	7	12	24, –6	78	10	7	12	24, –5
	<70%	14	30	12	33	54, –7	10	32	13	34	58, –5
	70–79%	18	17	11	20	39, –5	11	22	5	23	32, 12
	80–89%	37	10	5	11	21, –1	28	11	5	12	20, 3

*Results that are acceptable according to manufacturer guidelines and DIN EN ISO 80601-2-61 (bias $\leq \pm 3\%$, precision $\leq 3\%$ and A_{rms} $\leq 4\%$). n, sample size; A_{rms}, area root mean squares; LOA, limits of agreement (maximum and minimum).

derived from arterial blood gas data (Chaffin et al. 1996; Coghe et al. 1999; Uystepuyst et al. 2000; Wong et al. 2011) or by SaO₂ data measured by co-oximetry (Young et al. 2002; Mathews et al. 2003; Quinn et al. 2013; Dawson et al. 2014; Giguere et al. 2014; Grubb & Anderson 2017). All these studies were performed in anaesthetized domesticated animals and humans. Our study is the first study designed to formally validate the accuracy of pulse oximetry in an immobilized wildlife species using both calculated and measured SaO₂.

Although the co-oximeter and the blood gas analyser were designed and tested for human use, the paired SaO₂ and cSaO₂ values measured by both devices showed good agreement with each other. This finding suggests that impala may have a similar oxygen dissociation curve, with similar light absorption characteristics, to that of humans. This finding may be unique, as the human oxygen dissociation curve differs from that of other species such as sheep and goats (Whitehair et al. 1990; Clerbaux et al. 1993).

It has been well documented that bias, precision and A_{rms} are the best methods for properly assessing the performance of clinical devices such as pulse oximetry, when compared with linear regression analysis (Bland & Altman 1986; Uystepuyst et al. 2000; Batchelder & Raley 2007; Reiners et al. 2018). Linear

regression shows linearity and the direction of the relationship between the methods but not the agreement. Earlier studies have shown that data, which have a good correlation, do not always have good agreement (Serfontein & Jaroszewicz 1978; Oldham et al. 1979), meaning that the use of correlation is insufficient to assess the efficacy of clinical devices. Therefore, in this study we used a statistical approach to determine the agreement and hence the accuracy of pulse oximetry.

In the impala, pulse oximetry values measured at the tail base were only accurate and precise within the SaO₂ range greater than 90%. Inaccuracy and imprecision of pulse oximetry at ranges below 90% have been widely reported in several species and age categories, including foals (Chaffin et al. 1996), calves (Uystepuyst et al. 2000), cats (Mathews et al. 2003), dogs (Burns et al. 2006) and humans (DeMeulenaere 2007). Pulse oximeters are predominantly calibrated by their manufacturers using algorithms determined from healthy humans that have SaO₂ values >80% (Uystepuyst et al. 2000). This methodology may account for the inaccuracy in pulse oximeter readings at low SaO₂ levels.

Many factors should be considered when determining the utility of pulse oximetry in different species. These factors include the effects of ambient light, thickness of tissue and its

Table 4 The performance of the pulse oximeter readings compared with the blood gas analyser (cSaO₂) at different attachment sites in immobilized impala (number of impala = 16). A_{rms}, area root mean squares; LOA, limits of agreement (maximum and minimum); cSaO₂, oxygen haemoglobin saturation was calculated from arterial partial pressure of oxygen as measured by EPOC blood gas analyser.

Attachment site	cSaO ₂ Ranges (%)	'All-data'					'Pass-data'				
		n	Bias (%)	Precision (%)	A _{rms}	LOA	n	Bias (%)	Precision (%)	A _{rms}	LOA
Tail	0–100	194	6	9	11	24, –12	134	5	8	9	20, –10
	70–100	157	3*	6	7	16, –9	113	3*	5	6	13, –7
	<70	37	17	10	20	37, –3	21	16	9	18	34, –18
	70–79	27	9	7	11	23, –5	10	9	6	11	21, –2
	80–89	56	4	7	8	18, –11	36	5	5	7	14, –5
Ear	90–100	74	0*	4	4*	8, –7	67	1*	4	4*	9, –7
	0–100	139	–9	15	17	20, –38	80	–7	15	16	22, –36
	70–100	119	–9	15	17	20, –38	72	–7	15	16	22, –36
	<70	20	–10	14	17	17, –38	8	–8	18	18	27, –42
	70–79	17	8	16	18	25, –40	8	–6	18	18	30, –42
Rectum	80–89	42	–8	17	18	24, –41	22	–5	16	16	26, –36
	90–100	60	–9	13	16	16, –35	42	–8	14	15	19, –35
	0–100	176	5	13	14	31, –21	129	4	13	14	30, –21
	70–100	144	2*	9	9	19, –16	115	1*	9	9	19, –16
	<70	32	22	16	27	54, –10	14	29	16	33	60, –2
Cheek	70–79	26	9	8	13	24, –5	18	10	8	13	26, –6
	80–89	50	3*	7	7	18, –11	37	4	6	7	15, –7
	90–100	68	–3*	8	8	13, –19	60	–3*	8	9	14, –19
	0–100	136	12	11	16	34, –10	105	12	11	16	34, –9
	70–100	115	9	8	12	24, –6	90	10	8	12	25, –5
Cheek	<70	21	28	13	31	53, 3	15	28	14	31	56, 1
	70–79	24	16	10	19	36, –3	16	21	6	22	33, 9
	80–89	44	11	5	12	21, –1	30	12	5	13	21, 3
	90–100	48	4	4	5	10, –3	44	4	3*	5	11, –2

*Results that are acceptable according to manufacturer guidelines and DIN EN ISO 80601–2–61 (bias $\leq \pm 3\%$, precision $\leq 3\%$ and A_{rms} $\leq 4\%$). n, sample size; A_{rms}, area root mean squares; LOA, limits of agreement (maximum and minimum).

perfusion, skin pigmentation, artefacts created by motion and site of probe placement (Young et al. 2002).

The site of probe placement arguably plays the most important role in the accuracy and precision of pulse oximetry (Chaffin et al. 1996; Mathews et al. 2003; Grubb & Anderson 2017) and is species-dependent (Mathews et al. 2003). In the impala, the pulse oximeters only met the ISO guidelines when measurements were taken from the base of the tail, when blood oxygenation was high (SaO₂ 90–100%). Similar findings were observed using pulse oximetry measured at the base of calves' tails, where the bias was low (0.6%), precision high (3.2%) and predominantly when SaO₂ was above 90% (Uystepruyst et al. 2000). In contrast, Chaffin et al. (1996) found that pulse oximetry measured at the tail base performed poorly at all SaO₂ ranges in anaesthetized foals. Indeed, the efficacy of pulse oximetry will differ between species because anatomical sites will have species-specific factors that influence measurements. In the impala, the higher accuracy of the SpO₂ values obtained at the tail could be explained by several factors. The skin under the tail has a well perfused vascular bed with little pigment and the probe was protected from ambient light by the tape used to secure the probe to the tail.

The ear has been used widely in humans because it is known to be the least vasoactive site when compared with other sites (Grap 2002). In the impala, SpO₂ values measured at the ear were accurate, with a slight underestimation of SaO₂, but imprecise. These findings were similar to those in horses where SpO₂ measurements taken from the ear were accurate but imprecise (Chaffin et al. 1996). During immobilization, there was a large degree of ear movement, it was difficult to find areas without pigmentation and the curvature and thickness of the cartilage hindered good probe placement. These factors could account for the imprecision of these measurements.

In cynomolgus monkeys, good agreement occurred between measures of pulse oximetry from the cheek mucosa, SaO₂ measured by co-oximetry (bias = 2.7%) and cSaO₂ from a blood gas analyser (bias = 1.8%) (Young et al. 2002). From the cheek of the impala, this agreement was poor, possibly because contact was inadequate, owing to the buccal papillae on the cheek mucosa, saliva from hypersalivation (or pseudoptilism) or movement from chewing.

In critically ill dogs, the rectal mucosa is a useful and reliable site to measure SpO₂ when there is limited access to other peripheral sites (Barton et al. 1996). Unlike our rectal probe

placement, [Barton et al. \(1996\)](#) used a sensor specifically designed for the rectum and secured it using a soft silicone plug, factors that may account for their consistent results. However, a similar study performed by [Giguere et al. \(2014\)](#) also indicated larger bias (23%) at saturations below 85%, when the probe was placed inside the rectal mucosa of anaesthetized neonatal foals.

Etorphine is known to cause severe hypoxaemia in immobilized impala ([Meyer et al. 2010](#); [Zeiler et al. 2015](#)). During immobilization, overestimation of pulse oximeter measurements is arguably worse than underestimation, since it may give the impression that an animal has normal oxygen levels when it is hypoxaemic in reality. In this study, probes that were placed in the rectum and on the cheek overestimated SaO₂, therefore these sites should be avoided. This fact is particularly important especially in clinically ill animals.

Conclusion

We have shown that pulse oximetry, using a commercial veterinary device, is valid in immobilized impala, but only when oxygen haemoglobin saturations are high (>90%) and when a transreflectance probe is used at the base of the tail. Since chemical immobilization often results in severe hypoxaemia in impala, the interpretation of pulse oximetry readings to measure haemoglobin oxygen saturation should be done with caution. The authors recommend the use of additional monitoring equipment such as blood gas analysis or co-oximetry. However, given that pulse oximetry is cost-effective, noninvasive and rapidly measures trends in blood oxygenation, the development of more reliable pulse oximeters for use in wild antelope species is warranted. Using the tail base as an optimal site for pulse oximetry measurements in impala may help with this development and refinement of future clinical practices.

Funding

The study was funded by the University of Pretoria, Faculty of Veterinary Science, Department of Paraclinical Science, South African Veterinary Association (Wildlife Group) and the National Research Foundation.

Acknowledgements

The authors would like to thank [Wildlifevets.com](#) and Wildlife Pharmaceuticals for their generous support and help with data collection. Additionally, we thank Dr Etienne Bason and Penelope Miya for their help. We also thank Kyron Laboratories and Ascendis Medical for loaning the Nonin pulse oximeters and Co-oximeter devices, and the University of Pretoria, Faculty of Veterinary Science, Department of Paraclinical Science and National Research Foundation for funding.

Authors' contributions

TM: preparation of equipment, data collection, statistical analysis and preparation of manuscript. LM, GZ: idea of the study, data collection, data interpretation and preparation of manuscript. LL: data collection and edited manuscript. SP: data collection and edited manuscript.

Conflict of interest statement

SP and LL work for Wildlife Pharmaceuticals, the company that sells the drugs that were used to immobilize the animals in this study. The aim of this study was not to determine the effects of these drugs and the authors do not advocate their specific use in this manuscript. The other authors declare no conflict of interest.

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Received 18 February 2019; accepted 6 August 2019.

Available online 4 February 2020

Appendix A

$$A_{rms} = \sqrt{\sum_{i=1}^n (SpO_2 - SaO_2)^2 / n} \quad (1)$$

$$A_{rms} = \sqrt{\sum_{i=1}^n (SpO_2 - SaO_2)^2 / n}$$

$$A_{rms} = \sqrt{\left(\left(\sum_{i=1}^n (\hat{SpO}_2 - \hat{SaO}_2)^2 \right) / n \right)}$$

$$A_{rms} = \sqrt{\frac{\sum_{i=1}^n (SpO_2 - SaO_2)^2}{n}}$$