Tierärztliche Hochschule Hannover

Untersuchungen zur Sauerstoffsättigungskurve des Breitmaulnashorns (*Ceratotherium simum*) sowie Evaluierung von Pulsoximetriesensoren für Unpaarhufer (Breitmaulnashorn und Pferd)

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Meiner Familie

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Einleitung

Die Afrikanischen Nashörner stecken in der Krise.

Seit 2008 bedroht die Afrikanische Wilderei-Krise (African Poaching Crisis) ihre Existenz. Der Schutz der verbliebenen Populationen in situ und ex situ ist eine große Aufgabe für multidisziplinäre Teams, zu denen auch Tierärzte gehören. Die Managementaufgaben umfassen den Transport von Tieren ebenso wie das prophylaktische Enthornen und die tiermedizinische Behandlung verletzter Tiere. Um die entsprechenden Maßnahmen durchführen zu können sind chemische Immobilisationen der Nashörner notwendig; in der Regel kommen dabei hochpotente Opioide zum Einsatz. Dabei sind sichere und zuverlässige Narkoseprotokolle und Überwachungstechniken von besonderer Bedeutung.

Das Breitmaulnashorn (*Ceratotherium simum*) stellt als Narkosepatient eine besondere Herausforderung dar. Mehr noch als das Spitzmaulnashorn neigt es unter chemischer Immobilisierung mit hochpotenten Opioiden zur Ausbildung gravierender Nebenwirkungen. Muskeltremor, Tachykardie, Hypertension, Hyperkapnie, ein niedriger arterieller Sauerstoffpartialdruck (pO_2) und Azidose treten regelmäßig auf und sind in der Literatur detailliert beschrieben [1]. Die ausreichende Oxygenierung des Gewebes ist dadurch gefährdet.

Zur Überwachung der Gewebeoxygenierung kommen im Feld zwei Techniken zum Einsatz: die Blutgasanalyse und die Pulsoximetrie. Bei der Blutgasanalyse handelt es sich um ein invasives Verfahren, es werden arterielle Blutproben untersucht, um intermittierend eine Aussage über den Oxygenierungsstatus zu erhalten (SaO_2). Im Gegensatz dazu stellt die Pulsoximetrie ein photometrisches, nicht-invasives Verfahren dar, bei dem kontinuierlich Messwerte generiert werden (SpO_2). Als Goldstandard zur Bestimmung der Sauerstoffsättigung des Hämglobins gilt die Co-Oxymetrie, welche jedoch in der klinischen Praxis keine Anwendung findet. Sie beruht als einziges der drei genannten Verfahren auf einer direkten Messung der Sauerstoffsättigung, während die anderen beiden Verfahren die Sättigung über hinterlegte Algorithmen (Blutgasanalyse) bzw. Kalibrierungsdaten (Pulsoximetrie) errechnen, die für die Physiologie des Menschen optimiert sind.

Die in der Literatur beschriebenen Werte der arteriellen Sauerstoffsättigung des Hämoglobins bei Nashörnern unter Allgemeinanästhesie sind dramatisch niedrig. Haymerle et al. berichteten von SaO_2-Werten von 39% und SpO_2-Werten von 42% bei klinisch gesunden

Tieren [2]. Im Widerspruch dazu stehen die unproblematischen Aufwachphasen ohne beschriebene Spätfolgen eines dramatischen Sauerstoffmangels unter der Immobilisation. Die Aufklärung dieses scheinbaren Widerspruchs war das Ziel der vorliegenden Dissertation. Die Grundannahme war dabei, dass es sich um technologiebedingte falsch-niedrige Messwerte handelt.

Für die Pulsoximetrie sind verschiedene potentielle Fehlerquellen identifizierbar. Zum einen ist die typischerweise kommerziell erhältliche Hardware der Sensoren für die Anwendung an derart großen Tieren ungeeignet. Es werden häufig Clip-Sensoren verwandt, für welche es am Körper des Breitmaulnashorns kaum geeignete Applikationsstellen gibt. Zum anderen basiert die Software, das heißt die eingebauten Algorithmen zur Analyse der photometrischen Messungen, auf den Absorptionskurven des humanen Oxy- und Desoxyhämoglobin. Entsprechende Photometrie-Kurven für das Breitmaulnashorn lagen zu Beginn dieses Dissertationsprojekts nicht vor.

Auch bei der Blutgasanalyse stellt die Software eine potentielle Fehlerquelle dar: die Algorithmen basieren auf der Sauerstoffbindungskurve des Menschen mit entsprechendem p50 und Bohr-Koeffizienten. Für das Breitmaulnashorn lagen nur ausgewählte Informationen zur Sauerstoffsättigungskurve vor [3], die komplette Sauerstoffbindungskurve war bisher noch nicht beschrieben worden.

Unsere Hypothese lautete, dass die vermeintlich falsch-niedrigen Messwerte durch die Anwendung von für den Gebrauch an humanen Patienten optimierten Geräten zustande kommen.

Der erste Teil des Projekts ist der Optimierung der Hardware von Pulsoximetriesensoren zur Anwendung beim Breitmaulnashorn gewidmet. In Zusammenarbeit mit dem Institut für Medizintechnik an der Technischen Universität Berlin wurde im Rahmen einer Masterarbeit systematisch ein geeignetes Sensordesign zur Anwendung an der bukkalen Schleimhaut entwickelt. Ein entsprechender Prototyp wurde anschließend im Rahmen einer experimentellen *in vivo* Studie an Pferden evaluiert. Die Ergebnisse dieser Untersuchungen sind in PUBLIKATION 1 („Development and clinical evaluation of a new sensor design for buccal pulse oximetry in horses“) veröffentlicht.

Im zweiten Teil des Projekts wurden *in vitro* die biophysikalischen Grundlagen der beiden betrachteten Messtechniken untersucht. In Zusammenarbeit mit dem Institut für molekulare Biophysik an der Universität Mainz wurden die Sauerstoffbindungskurve des Hämoglobins

sowie das Absorptionsverhalten des oxygenierten und desoxygenierten Bluts des Breitmaulnashorns beschrieben. Die Ergebnisse dieser Untersuchungen sind in PUBLIKATION 2 (“Odd haemoglobins in odd-toed ungulates: Impact of selected haemoglobin characteristics of the white rhinoceros (Ceratotherium simum) on the monitoring of the arterial oxygen saturation of haemoglobin”) veröffentlicht.

Ziel der vorliegenden Dissertation ist es,

1. Erklärungsansätze für die vermeintlich falsch-niedrigen Messwerte der Pulsoximetrie und Blutgasanalyse beim Breitmaulnashorn zu finden und
2. praktische Verbesserungsansätze zur Anwendung im Feld zu präsentieren.

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Development and clinical evaluation of a new sensor design for buccal pulse oximetry in horses

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Summary

Background: The use of pulse oximetry in horses is limited due to inadequate readings with conventional transmission sensor probes.
Objectives: The objectives of this study were to 1) develop an improved sensor design for horses to be used at an appropriate anatomical site, and 2) evaluate this design in an experimental study.
Study design: In vivo experiment.
Methods: A new sensor design for reflectance pulse oximetry at the buccal mucosa was developed. A conventional Nonin 2000SL sensor for transmission pulse oximetry was included into this design. Three different prototypes (N1, N2a, N2b) were constructed and used with the Nonin 2500A Vet pulse oximetry monitor. Thirteen anaesthetised warmblood horses were included into a desaturation protocol (100–70% SaO_2). SpO_2 and pulse frequency values were recorded, using SaO_2 calculated from blood gas analysis and invasive pulse frequency measurements as reference methods. Bias and precision were evaluated by calculations of the root mean square deviation (A_{rms}). The agreement of the methods was tested with Bland-Altman analysis.
Results: The quality of the pulse frequency readings determined the quality of the SpO_2-readings. Good pulse signal strength resulted in a SpO_2-accuracy comparable to that of the original sensor (Nonin 2000SL: A_{rms} = 3%; N1: A_{rms} = 3.60%; N2b: A_{rms} = 3.46%). Especially at heart rates ≤30 bpm, pulse rate readings that were about twice as high as the reference value occurred. Their exclusion from the dataset resulted in a pulse rate accuracy similar to that of the original sensor. Bland-Altman plots showed limits of agreement typical of pulse oximeters.
Main limitations: The pulse frequency accuracy requires further improvement. The usability in clinical cases needs to be tested.
Conclusions: The new sensor design has been shown to be suitable for buccal pulse oximetry in horses.

Keywords: horse; anaesthesia; intraoperative monitoring; reflectance pulse oximetry; biomedical engineering

Introduction

General anaesthesia in horses remains a challenging task for the equine practitioner. The need for proper clinical and technical monitoring is generally accepted. In a recent survey among equine veterinarians [1], monitoring was among the most frequently mentioned areas in which improvements were recently implemented or perceived as additionally needed by the respondents.

Pulse oximetry provides information on both the cardiovascular and respiratory function and is recommended for routine use in small animal medicine, where it has been shown to reduce the odds of anaesthetic-related death in cats [2]. The survey cited above found that 60% of veterinarians performing equine anaesthesia used pulse oximetry routinely in both elective and emergency procedures [1]. This technique enables the operator to monitor the arterial oxygen saturation of haemoglobin in a continuous, noninvasive manner and allows early detection of haemoglobin desaturation. The photometric principle of measurement can be implemented either as transmission or reflection pulse oximetry.

Even though pulse oximetry is nowadays widely used in equine medicine [3,4], the usability of commercially available devices is not always satisfactory. With the commonly used sensor clips for transmission pulse oximetry, the correct alignment of the two sides of the sensor is essential to receive a valid signal from the tissue. In horses, it can be challenging to find an appropriate anatomical site to place such sensors. Commonly used sites include the tongue, lip, nostrils, ear, vulva and prepuce. Factors impairing the applicability at these sites are the thickness of the tissue and the presence of hair and pigmentation [5]. The correct alignment of sender and receiver is more difficult in thicker tissues, as the two sides of the clip sensor are likely pressed into a larger angle rather than lying in the correct parallel position, while hair and pigmentation compromise the transmission of the tissue by the light emitted by the sender. These difficulties compromise the practicability of transmission pulse oximetry in horses.

Alternatively, reflectance pulse oximetry sensors have been shown to produce clinically usable readings on the lip, tongue and the base of the tail in foals [6,7]. In this implementation of pulse oximetry, sender and receiver of the photo signal are placed next to each other, making alignment less of an issue. Nevertheless, there are few references to reflection pulse oximetry in the adult horse literature.

Due to the described difficulties, we decided to take a systematic approach to the development of a sensor design for reflectance pulse oximetry that considers the species-specific anatomy of horses to improve the practicability of pulse oximetry in horses. The objectives of this study were to 1) develop an improved sensor design for reflectance pulse oximetry in equine patients to be used at an appropriate anatomical site, and 2) evaluate the new sensor design in an experimental study in horses under general anaesthesia.

Materials and methods

Development of the sensor design and construction of prototypes

The development of a new sensor design was undertaken at the Department of Medical Engineering, Technische Universität Berlin as preparatory work for the study presented here. The work flow was structured by the recommendations given in guideline VDI 2221 [8]. It was decided to focus on a sensor to be attached to mucosal tissue. Different appropriate anatomic sides were identified (mucosa of nose, mouth, lips, ear canal, vulva, prepuce, urethra and rectum) and evaluated regarding the following criteria: accessibility, ease of fixation of the sensor, available volume and the risk of injury [9]. Safety of both patient and handler was considered, just as biocompatibility and the capability to sterilise the materials, to meet the same high standards as for medical devices for

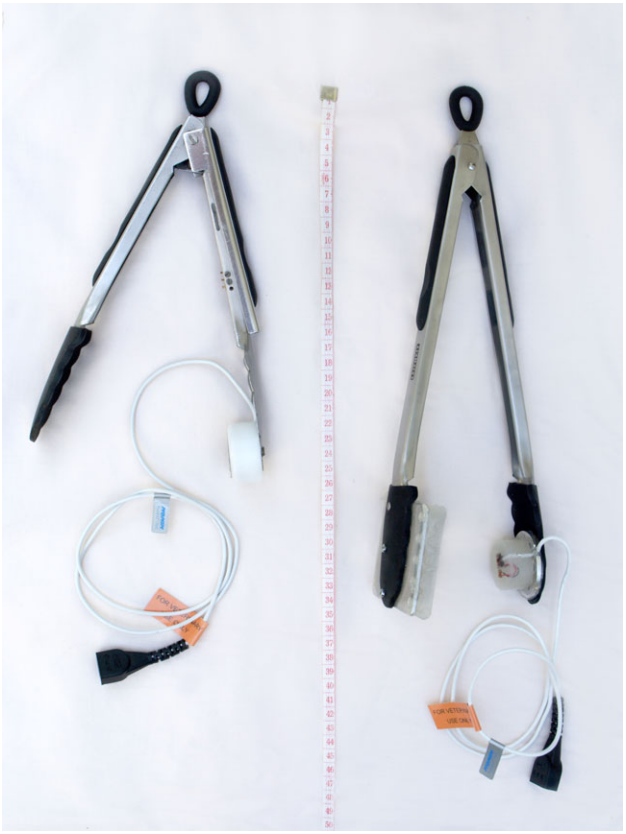

Fig 1: Comparison of the two pulse oximetry sensor prototypes N1 (left) and N2b (right) for buccal reflectance pulse oximetry in horses: general view. Please note the difference in the length of the tongs (prototype N1: 23 cm, prototype N2a and N2b: 31 cm).

human use. Different design approaches were considered. Preference was given to a tongs design for the attachment at the buccal mucosa. Risk management was conducted in accordance with DIN EN ISO 14971:2012 [9,10].

For the construction of the tongs design, commercially available (Barbecue tongs no.3041 and 3083)[a] were used. The distance of the two sides of the tongs is continuously adjustable and can be fixed in any position. A commercially available pulse oximetry sensor (Nonin 2000SL)[b] was disunited. Sender and receiver were placed next to each other at one inner side of a pair of tongs. Thereby, the mode of pulse oximetry was changed from transmission oximetry to reflectance oximetry. Sender and receiver were embedded into silicon. Three prototypes were built to be tested in controlled desaturation studies. They differ in the length of tongs used (23 cm in prototype 1, 31 cm in prototype N2a and N2b), the distance between sender and receiver (13 mm in prototypes N1 and N2a, 10 mm in prototype N2b), the shape of the silicon bedding towards the mucosa (plane in prototypes N1 and N2a, convex in prototype N2b) and presence of the surrounding plastic from the original sensor (present in prototype N1, removed in prototypes N2a and N2b). Figures 1 and 2 show prototypes N1 and N2b in comparison.

Desaturation studies and evaluation

Animals: Thirteen experimental warmblood horses (six mares, one stallion, six geldings) were included into this study (age: 8 ± 8 years (mean ± s.d.); body weight: 535 ± 40 kg). All animals were owned by the University of Veterinary Medicine, Hanover, Foundation. The animals were considered healthy based on clinical and echocardiographic examinations. All horses were part of an unrelated terminal study.

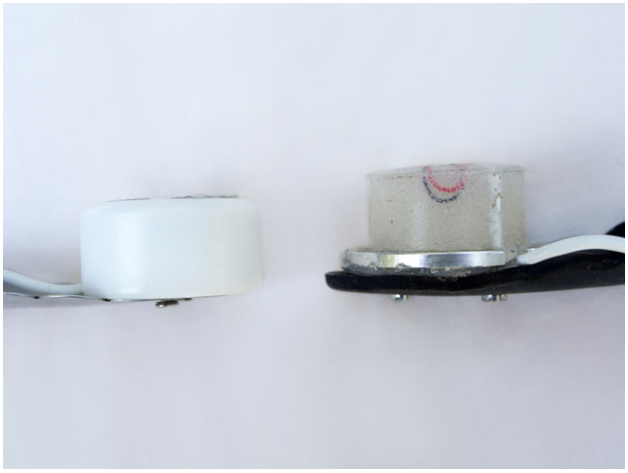

Fig 2: Comparison of the two pulse oximetry sensor prototypes N1 (left) and N2b (right) for buccal reflectance pulse oximetry in horses: close-up view of the sensor heads. Please note the different distance between sender and receiver (prototype N1 and N2b: 13 mm, prototype N2b: 10 mm), the shape of the silicon bedding towards the mucosa (plane in prototype N1 and N2b, convex in prototype N2b) and presence of the surrounding plastic from the original sensor (present in prototype N1, removed in prototype N2a and N2b).

Anaesthesia regime and instrumentation: Premedication consisted of xylazine (Xylavet® 20 mg/mL)[c] (0.8 mg per kg bodyweight intravenously (mg/kg bwt i.v.).) or dexmedetomidine (Dexdomitor®)[d] (3.5 μg/kg bwt i.v.). Anaesthesia was induced with ketamine (Narketan® 100 mg/mL)[e] (2.2 mg/kg bwt i.v.) and midazolam (Midazolam B. Braun 5 mg/mL)[f] (0.05 mg/kg bwt i.v.). All animals were intubated, positioned on an air-cushioned surgery table in dorsal recumbency and connected to a large animal anaesthesia system with a ventilator (Vet.-Tec. Model JAVC 2000)[g] for controlled ventilation with peak inspiratory pressure of 20–25 cmH_2O. Respiratory rate was constantly adjusted in order to maintain an arterial partial pressure of CO_2 of 40–45 mmHg (5.3–6 kPa). Anaesthesia was maintained with a combination of (Isofluran CP®)[c] in 100% oxygen and an infusion of xylazine (1 mg/kg bwt/h) or dexmedetomidine (7 μg/kg bwt/h). The expiratory isoflurane concentration was 1.2 Vol% initially and was adjusted during the course of anaesthesia. Ringer solution (Ringer Ecobag click)[h] was administered at a constant rate of 10 mL/kg bwt/h. The animals were connected to an anaesthesia monitor (Datex-Ohmeda Cardiocap/5)[i] to monitor the heart rate, respiratory rate, arterial blood pressure, inspiratory oxygen concentration and expiratory CO_2 and isoflurane concentration.

Controlled desaturation protocol: The protocol was designed in accordance with the procedures described in DIN EN ISO 80601-2-61 [11] as a controlled desaturation study. To date, there are no simulators available to test pulse oximetry devices, so experimental studies on human volunteers (or in our case, experimental animals) remain necessary. The subject is connected to a breathing circuit and both devices. The fraction of oxygen in inspired gases (FiO_2) is stepwise reduced to study the performance in the clinical relevant saturation ranges of 100–70% SaO_2.

In our study, the custom-made sensor was used in connection with a commercial Nonin 2500A Vet pulse oximetry monitor (Palm Sat Nonin 2500A Vet)[j], which is designated for the use with the original Nonin 2000SL sensor. The monitor has a pulse quality light-emitting diode (LED) indicator using a colour coding, where green indicates good pulse strength, amber indicates marginal pulse strength and red indicates inadequate pulse strength. The buccal mucosa was examined for pigmentation or injuries to avoid sensor placement in such areas. The sensor was introduced into the mouth and positioned at the level of the upper premolar teeth (P3, approximately). The tongs were locked and rested on the teeth which led to a stable sensor placement (Fig 3). The pressure of the tongs on the tissue was adjusted manually with the aim to reach good coupling and consequently correct transduction of the pulse signal, while the pressure should not impair the perfusion in this area. For reference measurements of the pulse rate (PR_{ref}), an arterial catheter (Venocan™ PLUS IV Catheter

Fig 3: Pulse oximetry sensor prototype N1 for buccal pulse oximetry in horses in place.

20G.33 mm)[k] was placed into the facial artery and connected via noncompressible lining to a precalibrated pressure transducer system (Gould Statham Transducer, PD 23 ID; "Cardiocap 5"-monitor)[l], which was placed at the level of the base of the heart and zeroed to ambient air. The arterial oxygen saturation (SaO_2) was used as a reference value for the arterial oxygen saturation estimated by pulse oximetry (SpO_2). It was calculated by a blood gas analyser (AVL995)[m] from arterial blood gas samples taken from the facial artery of the same side of the head. The equine p50-value [12] was included into the calculation of SaO_2 by the blood gas analyser.

The desaturation protocol was started following completion of another unrelated study, four hours after induction of anaesthesia. After a baseline measurement, nitrogen was added to the ventilation gas mixture upstream of the vaporiser to achieve a stepwise reduction of FiO_2 and gradual desaturation. The saturation level was allowed to stabilise for 15 min before an arterial blood sample was drawn anaerobically into heparinised syringes and immediately processed. At the same time, measurements of the arterial oxygen saturation of haemoglobin (SpO_2) and pulse rate (PR) were conducted with the prototypes. Prototype N1 was used in all trials, prototype N2a was used in six trials and prototype N2b was used in five trials. When two prototypes were used in the same trial, measurements were taken at the same location right after each other. SaO_2, PR_{ref}, systolic and diastolic arterial blood pressure and SpO_2 and pulse rate readings of both sensors including the results of the pulse quality indicator were recorded. Over a course of 120 min, the horses were gradually desaturated and measurements were taken every 15 min to cover the range of 100–70% haemoglobin saturation. For the assessment of the pulse rate accuracy, the values of the desaturation study were evaluated together with further values that were generated before and after the desaturation protocol at physiological ranges of SaO_2 >95%. The saturation range was not expected to influence the accuracy of pulse rate measurements.

Data analysis

In accordance with DIN EN ISO 80601-2-61, the overall accuracy of both SpO_2 and pulse rate readings was evaluated by calculating the root mean square deviation (A_{rms}) using the formula

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

The results of the A_{rms}-calculation are influenced by both precision (representing random error) and bias (representing systematic error), including both mean and local bias. Mean bias describes the average offset over the entire SpO_2 range (70–100%), while local bias specifies the variability in the different ranges [13].

The inclusion of all this statistical information into the A_{rms}-calculation makes it the most comprehensive statistic available to describe the performance of pulse oximeters and the statistic of choice for regulatory agencies [13]. DIN EN ISO 80601-2-61 demands an accuracy of $A_{rms} \leq 4\%$. The original pulse oximetry system (sensor Nonin 2000SL in combination with monitor Nonin 2500A Vet) shows an accuracy of $A_{rms} \leq 3\%$, according to the manufacturer.

In a first step, all values obtained during the trials were included into the statistic of the SpO_2 accuracy, regardless of their pulse indicator signal ("complete dataset"). Second, values with a marginal or inadequate pulse indicator signal (amber or red) were excluded from the dataset ("corrected dataset"). For the evaluation of the pulse frequency accuracy, only values with a good pulse indicator signal (green) were included ("good pulse indicator signal"). Third, outliers were defined as pulse rate readings which are about two times as high as the reference values obtained from the arterial catheter ($PR \approx 2 \times PR_{ref}$) and excluded ("outliers excluded").

Additionally, Bland-Altman-plots for repeated measurements [14] were used to compare the SpO_2 values obtained from the new pulse oximetry probe to the reference method, the SaO_2 values calculated from blood gas analysis. The plots were generated using the MedCalc Software[n].

Results

Accuracy of SpO_2 measurements

A scatter plot of the raw data can be found in Supplementary Item 1.

A_{rms}-calculations of SpO_2 measurements: The results of the A_{rms}-calculation are presented in Table 1 for all three prototypes. The protocol was aiming for desaturation down to 70% SaO_2 by stepwise reduction of FiO_2. In fact, even lower saturation levels down to 50% SaO_2 were observed during the trials. Therefore, both ranges (50–100% SaO_2 and 70–100% SaO_2) are presented. One animal was excluded from the calculations for N1 due to technical problems.

The International Organization for Standardization (ISO) guideline requires equal groups for the three ranges 70–79% SaO_2, 80–89% SaO_2 and

TABLE 1: Determination of SpO_2-accuracy of the three pulse oximetry sensor prototypes (N1, N2a and N2b) for buccal reflectance pulse oximetry in horses: results of the A_{rms}-calculations. SpO_2-accuracy of the original sensor and monitor: $A_{rms} \leq 3\%$. The complete dataset consists of all data-pairs recorded during the desaturation protocol. In the corrected dataset, values with a marginal or inadequate pulse indicator signal were excluded. Some animals reached even lower levels of arterial oxygen saturation (down to 50% SaO_2) than aimed for by the protocol (down to 70% SaO_2). Both ranges (50–100% SaO_2 and 70–100% SaO_2) are presented separately

Prototype	Complete dataset	Corrected dataset	Note
N1	70–100%: A_{rms} = 4.84% n = 90	70–100%: A_{rms} = 3.60% n = 74	One horse excluded
	50–100%: A_{rms} = 5.02% N = 105	50–100%: A_{rms} = 4.01% n = 88	
N2a	70–100%: A_{rms} = 5.11% n = 48	70–100%: A_{rms} = 4.87% n = 40	
	50–100%: A_{rms} = 7.63% n = 58	50–100%: A_{rms} = 6.73% n = 48	
N2b	70–100%: A_{rms} = 3.58% n = 46	70–100%: A_{rms} = 3.46% n = 34	
	50–100%: A_{rms} = 3.75% n = 52	50–100%: A_{rms} = 3.69% n = 40	

90–100% SaO_2; this requirement was not fulfilled in this study. To get an idea of how same sized groups would influence the results, calculations with groups of the same size were conducted for the corrected dataset of prototype N1. The sample size was adjusted for the smallest group (70–79% SaO_2, n = 14). Depending on whether the best or worst results were excluded from the other two groups, A_{rms}-results were A_{rms} = 2.48% to A_{rms} = 4.65% (mean A_{rms} = 3.57%). Due to smaller sample sizes in the range of 70–79% SaO_2, these calculations were neither conducted for N2a (n = 8) nor N2b (n = 3).

Bland-Altman plots for the SpO_2 agreement: The Bland-Altman plots for the SpO_2 agreement of prototypes N1 and N2b in the range of 70–100% SaO_2 are presented in Figures 4 and 5. While prototype N1 slightly underestimated the oxygen saturation of haemoglobin in comparison to the reference method (mean −0.3%), the two other prototypes overestimated it (N2a: mean +4%; N2b: mean +2%). The limits of agreement were widest for prototype N1 (+7% to −8%). Limits of agreement for prototype N2a and prototype N2b were +10% to −2% and +8% to −4%, respectively. The Bland-Altman plot for the SpO_2 agreement of prototype N2a can be found in Supplementary Item 2.

Accuracy of pulse rate measurements

A_{rms}-calculations of pulse rate measurements: Table 2 shows the results of the A_{rms}-calculations for all three prototypes. Measurements with a poor perfusion signal as defined above (amber or red pulse quality indicator signal) were excluded from the dataset. The accuracy of pulse rate measurements is specified as ±3 beats per minute (bpm) for the Nonin 2500A Vet by the manufacturer. When all valid results with a good (green) pulse quality indicator signal were evaluated, all three prototypes failed to stay within these limits (N1: A_{rms} = 19.45%; N2a: A_{rms} = 11.06%; N2b: A_{rms} = 4.81%. The accuracy could largely be increased by the elimination of values which can be recognised as incorrect by a clinician on-site: these outliers indicate pulse rate readings which are about two times as high as the reference values ($PR \approx 2 \times PR_{ref}$). The incidence of these outliers was higher at very low heart rates (≲30 bpm). The exclusion of these outliers resulted in an increased accuracy within the limits of ±3 bpm for the prototypes N2a (A_{rms} = 2.61%) and N2b (A_{rms} = 2.94%); only prototype N1 failed to stay within these limits (A_{rms} = 4.56%).

Bland and Altman plots for the pulse rate readings: The pulse rate reading of both N1 und N2b were 1 bpm higher than the pulse rate taken from the blood pressure trace. Prototype N2a tended to underestimate the true value (−1 bpm). The limits of agreement were (+10 bpm to −8 bpm for prototype N1, +4 bpm to −6 bpm for prototype N2a and +7 bpm to −5 bpm for prototype N2b. The Bland-Altman plots for the pulse rate readings can be found in Supplementary Items 3–5.

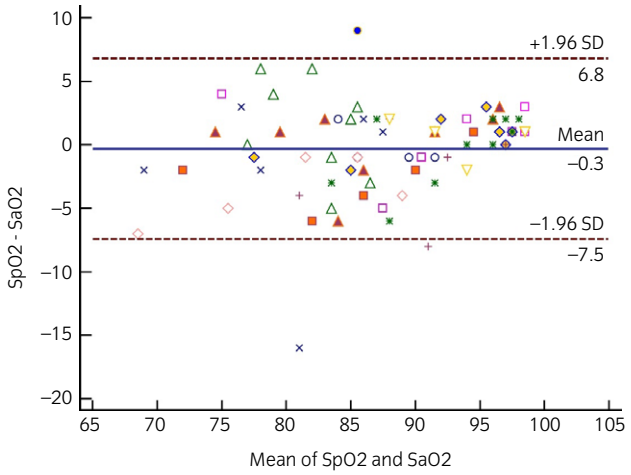

Fig 4: SpO_2 accuracy of pulse oximetry sensor prototype N1 in the range 70–100% SaO_2: Bland-Altman plot for multiple comparisons per individual with the difference between the two methods (SpO_2 measured by prototype N1 and SaO_2 calculated from the results of blood gas analysis) plotted against their mean. The solid blue line indicates the mean bias; the two dashed lines indicate the limits of agreement.

Discussion

The commercially available pulse oximetry sensor for transmission pulse oximetry was successfully integrated into the new sensor design for reflectance pulse oximetry. Analysable readings were generated at the buccal mucosa. When the pulse signal strength was good, the accuracy of SpO_2 measurements by prototype N1 and N2b met the requirements of the ISO regulations in the range of 70–100% SaO_2. After the exclusion of outliers ($PR \approx 2 \times PR_{ref}$), the accuracy of pulse rate readings of the prototypes N2a and N2b was comparable to the original sensor.

The results of the controlled desaturation trials reinforce the dependence of the quality of SpO_2 measurements on the quality of pulse rate measurements. The results from the complete datasets show that only N2b met the legal requirements ($A_{rms} \leq 4\%$). All three prototypes showed

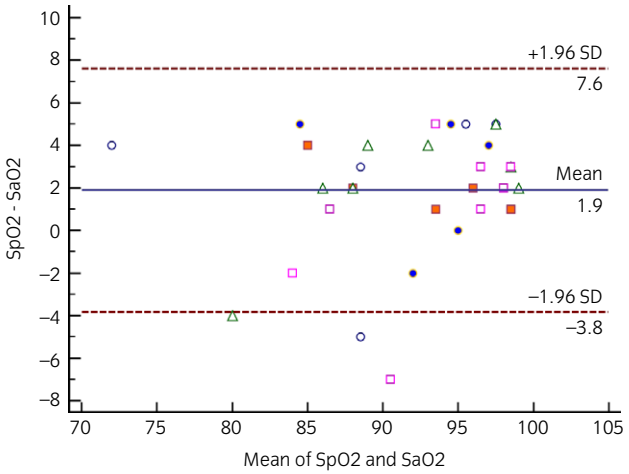

Fig 5: SpO_2 accuracy of pulse oximetry sensor prototype N2b in the range 70–100% SaO_2: Bland-Altman plot for multiple comparisons per individual with the difference between the two methods (SpO_2 measured by prototype N2b and SaO_2 calculated from the results of blood gas analysis) plotted against their mean. The solid blue line indicates the mean bias; the two dashed lines indicate the limits of agreement.

TABLE 2: Determination of the accuracy of pulse rate measurements of the three pulse oximetry sensor prototypes (N1, N2a and N2b) for buccal reflectance pulse oximetry in horses: Results of the A_{rms}-calculations. Pulse accuracy of the original sensor and monitor: ±3 bpm. Only values with a good pulse indicator signal were included ("good pulse indicator signal"), while values with a marginal or inadequate signal were excluded. Then, outliers were defined as pulse rate readings which are about two times as high as the reference values ($PR \approx 2 \times PR_{Ref}$) and excluded ("outliers excluded")

Prototype	Good pulse indicator signal	Outlier excluded	Note
N1	A_{rms} = 19.45% n = 149	A_{rms} = 4.56% n = 131	n = 35 amber n = 18 outliers
N2a	A_{rms} = 11.06% n = 64	A_{rms} = 2.61% n = 60	n = 17 amber n = 4 outliers
N2b	A_{rms} = 4.81% n = 84	A_{rms} = 2.94% n = 83	n = 30 amber n = 1 red n = 1 outlier

higher A_{rms}-results than announced by the manufacturer for the sensor NONIN 2000SL ($A_{rms} \leq 3\%$). The A_{rms}-values for the SpO_2 readings of all three prototypes improved markedly after the rejection of doubtful data-pairs. These results resemble those of other authors, who also stated that correct SpO_2 readings require correct pulse rate measurements [3,5,15]. In particular, the new sensor had a tendency to produce readings which equalled approximately double the results provided by the reference method ($PR \approx 2 \times PR_{ref}$). This phenomenon was first described in horses by Moens *et al.* in 1991 [4]. Presumably, the two prominent amplitudes of one pulse wave are considered as two pulse waves, especially at very low heart rates (≤30 bpm). These inaccurate readings can be recognised by the clinician on-site by conducting a clinical reference measurement, e.g. by palpation of the facial artery. Therefore, given that the pulse rate readings are clinically verified by the operator, the prototypes N1 and N2b can be useful tools for the clinical monitoring of SpO_2 in horses.

Buccal pulse oximetry has been used in humans [16] and primates [15] by transmission pulse oximetry. To the best of our knowledge, our study was the first systematic attempt to make the buccal mucosa accessible as a probe site for reflectance pulse oximetry in horses. The buccal mucosa was chosen under the assumption that perfusion and temperature were more constant at this anatomic side under anaesthesia than at other commonly used sides for pulse oximetry, e.g. the tongue or the ears. In addition, external light sources would not interfere with the measurements. Throughout all trials, the buccal mucosa was indeed found to be well perfused and warm even after several hours of anaesthesia. After a short familiarisation phase, the handling and application of the new sensor design were convenient for the user. Therefore, it seems conceivable to pursue further development of this design approach.

The basic principle of transmission and reflectance pulse oximetry is the same: one photoelectric unit emits a light signal (sender), while a second one receives the signal that comes back from the tissue (receiver) [17]. Typically, the sender is a LED, while the receiver can be a LED, photocell or photoresistor [18]. The same technical components are used in both kinds of sensors. Pulse oximetry can by approximation be explained by the Lambert-Beer law: the light sent into the tissue is partially absorbed, the degree of absorbance allows for conclusions on the oxygen saturation of the haemoglobin in the arterial blood. The first necessary step is the detection of the pulse, which enables the device to distinguish between the artery and the surrounding tissues and to take only the absorbance by the arterial blood into account. The degree of absorbance can be evaluated from both the transmitted and the reflected portions of light that are received back from the tissue [17]. The fact that the same basic principle and the same technical components are implemented in both kinds of sensors made it possible to integrate the commercially available pulse oximetry sensor for transmission pulse oximetry into the new sensor design for reflectance pulse oximetry.

After the first preliminary tests with prototype N1, it became obvious that the accuracy of the pulse rate measurements required further improvement. Possible sources of error were identified: the point of counter pressure of the outer tong was situated right opposite the reading point, possibly causing pressure on the tissue in this area that impaired the perfusion of the tissue. The solid edges of the sensor head were also suspected to impair the perfusion. These considerations guided to the development of the N2a prototype, characterised by the relocation of the point of counter pressure and modifications of the surface and edges of the sensor head. In addition, the distance between sender and receiver was modified in N2b. Finally, prototype N2b showed an improved accuracy concerning both SpO2 and pulse rate in comparison to prototype N1. It seems reasonable to assume causality between the adjustments in the details of the design and the improvement of the accuracy of measurement.

The data from one animal had to be excluded from the dataset of prototype N1 due to inexplicable variations in the readings. At the simultaneous measurements at the same trial, prototype N2b produced reliable results. In the performance of pulse oximeters, dynamic or changing conditions in which the readings of a pulse oximeter do not follow the oxygenation trend but provide higher values (pop-ups), lower values (drop-downs), no trend at all (frozen readings) or no signal at all (periods of no reading) have been described [13]. The changes seen in our study resemble the description of a drop-down. These conditions have not been described in animals before, but there are descriptions of temporary failures in different studies that could be interpreted as such [7,15,19]. The A_{rms}-statistic was established by ISO and other regulatory agencies to measure the accuracy of a pulse oximetry device, taking into account both bias and precision. Dynamic performance conditions are not being reflected by A_{rms}, even though they are of clinical relevance for the operator. Therefore, the idea of Batchelder and Raley [13] to develop and implement a more comprehensive assessment of pulse oximeter performance appears advisable for veterinary purposes as well.

Even though the Nonin 200A Vet is marketed exclusively for veterinary use, it was calibrated on the finger of human subjects. In the user's manual [20], the manufacturer indicates that *"although animal haemoglobin has similar optical characteristics, other types of haemoglobin or alternate sensor locations may affect the calibration."* The degree of interference and the kind of variations cannot be predicted by the operator. Various studies from both human and veterinary medicine found a crucial impact of the sensor placement side on the accuracy of pulse oximeter readings [7,21,22] and pulse oximeters from different manufacturers perform differently in different animal species [19]. Therefore, it is reasonable to expect that in addition to the unpredictable effects of the mechanical modifications, the mode of calibration of the Nonin 2500A Vet influenced the results of this study to an unknown degree. Given these impacts, both clinicians and researchers would benefit from the calibration of pulse oximeters for veterinary use based on data from the target species.

The choice of the reference method for the controlled desaturation trials deviates from the instructions given in DIN EN ISO 80601-2-61 [11]: instead of measuring SaO_2 by co-oximetry, it was calculated from the results of blood gas analysis based on the equine oxygen dissociation curve. While co-oximetry is considered the gold standard to determine the arterial oxygen saturation of haemoglobin, blood gas analysis can be considered the "clinical standard" among equine practitioners and calculated SaO_2 values have been used as references for studies on reflectance pulse oximetry in horses previously [23]. Young *et al.* found an adequate agreement of pulse oximetry with both co-oximetry and calculated SaO_2 [15]. SpO_2 cannot be expected to agree completely with neither SaO_2 from co-oximetry nor calculated SaO_2. Nevertheless, both methods should provide useful reference values.

In conclusion, the new sensor design has been shown to be suitable for buccal pulse oximetry in horses. Future developments should focus on improving the accuracy of pulse rate readings. The necessity for testing the reliability of the pulse rate readings on-site by the operator was addressed in a master's thesis that was conducted consecutively to the study presented here. The technical solution consists of an additional sensor and monitor unit providing both pulse rate readings and an adjustable plethysmogram to be compared to the pulse rate readings provided by the Nonin monitor [24]. Clinical evaluation of this device is still to be conducted. Further studies are needed to investigate the performance under the influence of different anaesthetic agents, the usability under field conditions, the performance in animals with cardiorespiratory pathologies and the practicability in other horse breeds and potentially other large animal species.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

All procedures were reviewed and approved by the Ethics Committee for Animal Experiments of Lower Saxony (Lower Saxony State Office for Consumer Protection and Food Safety, approval number 33.14-42502-04-14/1547).

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Authorship

J. Reiners contributed to the study design and execution, data collection, data analysis and preparation of the first draft of the manuscript. W. Roßdeutscher was responsible for the development of the new sensor design and construction of the prototypes and contributed to the interpretation of the data. K. Hopster was involved in the execution of the study and the data analysis. S. Kästner contributed to the study design, data analysis and interpretation of the data. All three co-authors were actively involved in the revision of the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Supplementary Item 1: Scatter plot of the raw data of all three prototypes (N1, N2a, N2b). The solid black line indicates the regression line between SaO2 calculated from the results of blood gas analysis and SpO2 measured by the prototypes. The colour coding indicates the pulse quality signal (green circle = good, amber square = marginal, red triangle = inadequate). The encircled data indicates the values measured by prototype N1 in animal no.9 that resemble the description of a "drop-down" phenomenon (see Batchelder and Raley 2007).

Supplementary Item 2: SpO_2 accuracy of pulse oximetry sensor prototype N2a in the range 70–100% SaO_2: Bland-Altman plot for multiple comparisons per individual with the difference between the two methods (SpO_2 measured by prototype N2b and SaO_2 calculated from the results of blood gas analysis) plotted against their mean. The solid blue line indicates the mean bias; the two dashed lines indicate the limits of agreement.

Supplementary Item 3: Accuracy of pulse rate measurements by pulse oximetry sensor prototype N1: Bland-Altman plot for multiple

comparisons per individual with the difference between the two methods (pulse rate measured by prototype N1 and PF_{ref} measured by the arterial catheter) plotted against their mean. The solid blue line indicates the mean bias; the two dashed lines indicate the limits of agreement.

Supplementary Item 4: Accuracy of pulse rate measurements by pulse oximetry sensor prototype N2a: Bland-Altman plot for multiple comparisons per individual with the difference between the two methods (pulse rate measured by prototype N1 and PF_{ref} measured by the arterial catheter) plotted against their mean. The solid blue line indicates the mean bias; the two dashed lines indicate the limits of agreement.

Supplementary Item 5: Accuracy of pulse rate measurements by pulse oximetry sensor prototype N2b: Bland-Altman plot for multiple comparisons per individual with the difference between the two methods (pulse rate measured by prototype N2b and PR_{ref} measured by the arterial catheter) plotted against their mean. The solid blue line indicates the mean bias; the two dashed lines indicate the limits of agreement.

PLOS ONE

RESEARCH ARTICLE

Odd haemoglobins in odd-toed ungulates: Impact of selected haemoglobin characteristics of the white rhinoceros (*Ceratotherium simum*) on the monitoring of the arterial oxygen saturation of haemoglobin

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Abstract

Background

Due to the current poaching crisis in Africa, increasing numbers of white rhinoceroses (*Ceratotherium simum*) require opioid immobilisation for medical interventions or management procedures. Alarmingly, the results of both blood gas analysis and pulse oximetry regularly indicate severe hypoxaemia. Yet, the recovery of the animals is uneventful. Thus, neither of the techniques seems to represent the real oxygenation level. We hypothesized that unusual haemoglobin characteristics of this species interfere with the techniques developed and calibrated for the use in human patients.

Methods

Haemoglobin was isolated from blood samples of four adult, white rhinoceroses. Oxygen dissociation curves at pH 7.2 and 7.4 (37˚C) were determined based on the absorbance change of haemoglobin in the Soret-region (around 420 nm). Absorbance spectra of oxy- and deoxyhaemoglobin extending into the infrared region were measured.

Results

Oxygen dissociation curves of rhinoceros haemoglobin showed the typical high oxygen affinity (p_{50} of 2.75 ± 0.07 and 2.00 ± 0.04 kPa for pH 7.2 and 7.4, respectively) under near-physiological conditions with respect to pH, temperature and DPG. The infrared absorbance spectra of oxy- and deoxyhaemoglobin showed only marginal deviations from standard human spectra, possibly due to the presence of a few percent of methaemoglobin *in vitro*.

design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Conclusions

Our data enables the development of a rhinoceros-specific blood gas analysis algorithm, which allows for species-specific calculation of SaO_2 levels in anaesthetized animals. The inconspicuous absorbance spectra do not contribute to the systematic underestimation of SpO_2 by pulse-oximetry.

Introduction

The white rhinoceros (*Ceraotherium simum*) has been the subject of numerous publications in the field of veterinary anaesthesia in the past few years. While medical training in animals under human care allows for minor procedures like blood sampling to be performed without sedation, more invasive medical interventions and management procedures (e.g. wound treatment, dehorning, relocations) generally require so-called chemical immobilisation.

Furthermore, the current rhinoceros poaching crisis in Africa requires intense management of the populations, emphasizing the need for safe and reliable anaesthetic protocols and monitoring techniques. As standard protocols include highly potent opioids, the monitoring of the arterial oxygen saturation of haemoglobin is of paramount importance. Severe side effects (e.g. muscle tremors, tachycardia, hypertension, hypercapnia, low partial pressure of oxygen (pO_2) and acidosis) occur regularly and are well described [1].

Under field conditions, blood gas analysis (providing calculated SaO_2) as well as pulse oximetry (providing SpO_2) are being used. Both monitoring techniques were developed for the use in human patients and have been implemented in veterinary anaesthesia without further adaptation of the algorithms and calibration data. In the case of certain domestic species (namely dog, cat, horse, cow and pig) the infrared spectra were shown to be indeed sufficiently close to justify this approach [2,3] for pulse oximetry. However, limited information is available for large herbivores, as the species-specific absorbance characteristics of oxyhaemoglobin and deoxyhaemoglobin in the white rhinoceros have not been studied before.

Standard blood gas analysis and pulse oximetry indicate an alarmingly low oxygenation status under opioid immobilisation that is in conflict with uneventful recoveries [1,4–6]. Haymerle et al. reported SaO_2 values as low as 39% and SpO_2 values as low as 42% in clinically healthy, opioid-immobilised animals generated by commercially available devices [7]. Baumann et al. presented the p50 and Hill coefficient determined for white rhinoceros haemoglobin (based on measurements on one blood sample from a single white rhinoceros) and showed that its oxygen binding properties are modulated by pH and CO_2 but not by DPG [8]. When Haymerle et al. modified the algorithm developed by Siggaard-Andersen et al. [9] to yield the p50 value and Hill coefficient provided by Baumann and et al., SaO_2 levels of at least 80% were calculated. Since only p50 and Hill coefficients are available from Baumann et al., we felt that the data analysis could be further improved by modifying the analysis algorithm based on more detailed experimental data, which led us to perform the corresponding experiments.

We hypothesized that deviating haemoglobin characteristics may interfere with the monitoring techniques developed and calibrated for the use in human patients. Possible sources of error include 1) deviating oxygen binding properties of haemoglobin of the white rhinoceros that interfere with the calculation of SaO_2 by blood gas analysers using human or equine algorithms and 2) deviating light absorbance characteristics of the haemoglobins interfering with pulse oximetry as described in human patients with haemoglobinopathies [10].

Our objectives were 1) to provide oxygen dissociation curves of the white rhinoceros to allow for proper species-specific calibration of blood gas analysers and 2) to investigate the haemoglobin infrared absorbance characteristics of oxy- and deoxyhaemoglobin to check for possible deviations in the extinction coefficients relevant to pulse-oximetry.

Methods

EDTA blood samples (5ml each) of four adult white rhinoceroses (one male, three females; aged seven to twenty-six years) housed in European zoological institutions were used for this study. All of these Institutions are members of the *European Association of Zoos and Aquaria* (EAZA) and participate in the *European Endangered Species program* (EEP). The samples were taken between June 2014 and December 2018. All animals were considered healthy based on the clinical assessment of the veterinary clinician in charge. All samples were collected during routine blood sampling for health monitoring and leftover specimens were secondarily donated to our study. Therefore, the obtainment of ethical approval was not required.

Equine blood samples were also examined. In exotic species, clinically relevant basic information is often rare; therefore, it is common practise to consult the literature on closely related domestic animals for approximation and comparison. As the haemoglobin characteristics of the domestic horse (*Equus caballus*) had been described before [3], we decided to include both members of the order *Perrisodactyla* into our investigations for internal comparison.

We used blood samples from two clinically healthy warmblood horses (one gelding, one mare; two and twelve years old) that were experimental horses in the possession of the equine clinic at the University of Veterinary Medicine Hanover. For the blood sampling of the horses, ethical approval was granted by the Ethics Committee for the Animal Experiments of Lower Saxony (Lower Saxony State Office for Consumer Protection and Food Safety, approval number 33.19-42502-04-18/2856). The skin over the left jugular vein was clipped and surgically prepared for catheter placement. After infiltration of the skin with mepivacaine hydrochloride (Scandicain 2%, AstraZeneca, Wedal, Germany), a 12 G catheter (EquiCath Fastflow, Braun, Tuttlingen, Germany) was placed into the left jugular vein. Blood was drawn from the catheter aseptically. The first 10 ml were discarded, then the sample (approximately 10 ml) was withdrawn and placed in EDTA tubes.

1. Determination of the oxygen dissociation curve (ODC)

1.1. Chemicals. Buffer components (TRIS, NaCl) were obtained from Roth (Roth Chemicals, Karlsruhe, Germany). All components of the Hayashi assay and Sodium Dithionite were purchased from Sigma (now Merck KGaA, Darmstadt, Germany). Buffer for oxygen binding experiments contained 0.05 M TRIS/HCl with a concentration of chloride of 0.1 M adjusted with NaCl.

1.2. Oxygen dissociation curves (ODC). Haemoglobin was isolated from secondarily donated samples from rhinoceroses housed around Europe. Therefore, pre-analytic handling of the samples (including shipping, preparation and storage) was necessary. Upon arrival at the laboratory, haemoglobin was extracted from the blood sample, using the method described by Paoli und Nagai [11]: first, the blood was centrifuged for 30 min at 100 g (4˚C). Then the supernatant was removed, the pellet carefully mixed with at least 10 times the volume of 0.9% NaCl and centrifuged again (30 min, 100 g, 4˚C). The supernatant was removed and the procedure repeated until the supernatant was clear. Then the erythrocytes were lysed by addition of 1x volume of water. After 15 min, 9% NaCl was added to obtain a final concentration of about 5% (w/v) NaCl. Next, the cellular debris was removed by centrifugation (4100 g, 30 min, 4˚C). The supernatant contained the haemoglobin. The purified haemoglobin was stored at 4˚C.

The procedure was performed on the day of arrival of the blood sample. Isolated oxygenated haemoglobin can be stored at 4˚C for at least two months [12]. Samples were used within this time period and always checked for increased met-formation beforehand. For increasing concentration or buffer exchange, centrifugal concentrators were used (Centricons Vivaspin 20 ml, 30.000 Da, Sartorius Stedim Biotech GmbH, Göttingen).

Oxygen dissociation curves were measured in a Gill-cell [13] with an optical path length of about 0.05 mm. In order to prevent formation of methaemoglobin during the measurement, the regeneration assay after Hayashi [14] was included into the experiments. ODCs were performed with a solution of the following composition: haemoglobin at a concentration of about 200 μM haemoglobin tetramer, 0.04 μM catalase (about 6 U/ml), 0.5 μM ferredoxin, 0.27 U/ml Glucose-6-phosphate-dehydrogenase, 0.15 mM NADP, 3 mM Glucose-6-phosphate, 0.15 μM ferredoxin $NADP^+$ reductase (about 0.1 U/ml). The solution was pre-incubated at 37˚ C to convert all methaemoglobin into oxyhaemoglobin, since some degree of methaemoglobin formation had already occurred during shipping of the blood sample. Prior to the measurement, the sample was shortly spun in a table centrifuge to remove air bubbles. Gas of defined mixing ratios of 20% O_2 and 100% N_2 (Linde Group, Pullach, Germany) was prepared in a self-built gas mixing system and led into the chamber next to the semipermeable membrane (Model 5794, High Sensitivity) from YSI Incorporated (Yellow Springs, Ohio, USA). The actual pO2 was measured in the gas chamber with an oxygen electrode (MicroEletrodes Inc., Bedford, New Hampshire, USA) with a self-built amplifier. Measurements were performed under continuous gas flow. Calibration was performed with gas of a known composition of N_2 and O_2 (Linde Group, Pullach, Germany). Oxygen partial pressure (pO_2) was increased gradually. A spectrum was measured after the electrode voltage and absorption values were stable. The fraction of oxygenated protein (f_{oxy}) was calculated based on a superposition of the spectra obtained for the oxygenated and the deoxygenated sample including a variable small offset:

$$S_{pO_2} = a_{oxy}\ S_{oxy} + a_{deoxy} S_{deoxy} + off$$

Here S_{oxy} and S_{deoxy} refer to the spectra of oxy- and deoxyhaemoglobin measured for the respective set of data, and a_{oxy} and a_{deoxy} are parameters adjusted by the fitting routine to obtain the best agreement between measured and calculated spectrum. In order to allow for baseline drifts, a constant offset („off“) was also included. The fraction of oxygenated haemoglobin was then calculated as

$$f_{oxy} = a_{oxy}/(a_{oxy} + a_{deoxy})$$

The pO_2 was calculated from the voltage output of the Clark-electrode, taking into account the water vapor pressure (6.27 kPa at 37˚C) as well as the actual ambient pressure.

2. Infrared absorption spectra of oxygenated and deoxygenated haemoglobin

Spectra of the undiluted haemoglobin solutions in 5% NaCl were measured employing a Lambda 465 (Perkin Elmer Inc., Waltham, Massachusetts, USA) with a cuvette of 1 cm pathlength. Deoxy-haemoglobin was prepared by adding Sodium-Dithionite to the stock solution, about 20 μl of a 10 mg/ml solution in H_20 for 1 ml haemoglobin solution. For comparison, spectra of equine haemoglobin were also recorded under the same conditions. In order to allow a comparison of the spectral shapes, they were normalized to the value measured at 940 nm (oxy-spectra) and to 660 nm (deoxy-spectra).

Results

1. Oxygen dissociation curves

Oxygen dissociation curves were measured at 37˚C, at pH 7.2 and pH 7.4, using haemoglobin from three individuals. All data points lie on a common curve (Fig 1). For comparison with available data [8], p50 and Hill coefficient n50 were obtained based on the Hill plot of part of the data (25–80% saturation), yielding values of 2.5 and 1.78 kPa, and n50 = 2.1 and 2.2 for pH 7.2 and 7.4, respectively. The Bohr coefficient calculated from the shift in p50 amounts to -0.74, which is slightly higher than the one reported by Baumann et al. (-0.62, [8]). The p50 values reported by these authors were 2.29 kPa at pH 7.2 and 1.48 kPa at pH 7.5, being in reasonable agreement with the ones reported here. The Hill coefficients were somewhat higher, ranging from 2.8 to 2.6 between pH 7.0 and 7.5. An analysis of all data by non-linear regression based on the Hill equation yielded the following parameters for pH 7.2 and 7.4: p_{50} = 2.75 ± 0.07 and 2.00 ± 0.04 kPa, n_{50} = 2.0 ± 0.2 and 2.2 ± 0.1, respectively.

2. Haemoglobin absorbance spectra

The haemoglobin absorbance spectra of oxyhaemoglobin and deoxyhaemoglobin of horse and rhinoceros are presented in Fig 2. No significant differences in the spectral features could be observed between the haemoglobins of human, horse and rhinoceros. The slightly enhanced absorbance in the lower wavelength range in case of the horse and rhinoceros spectra can be attributed to formation of methaemoglobin. While the Hayashi assay successfully removed methaemoglobin from the solution used for measuring the oxygen dissociation curves, it did

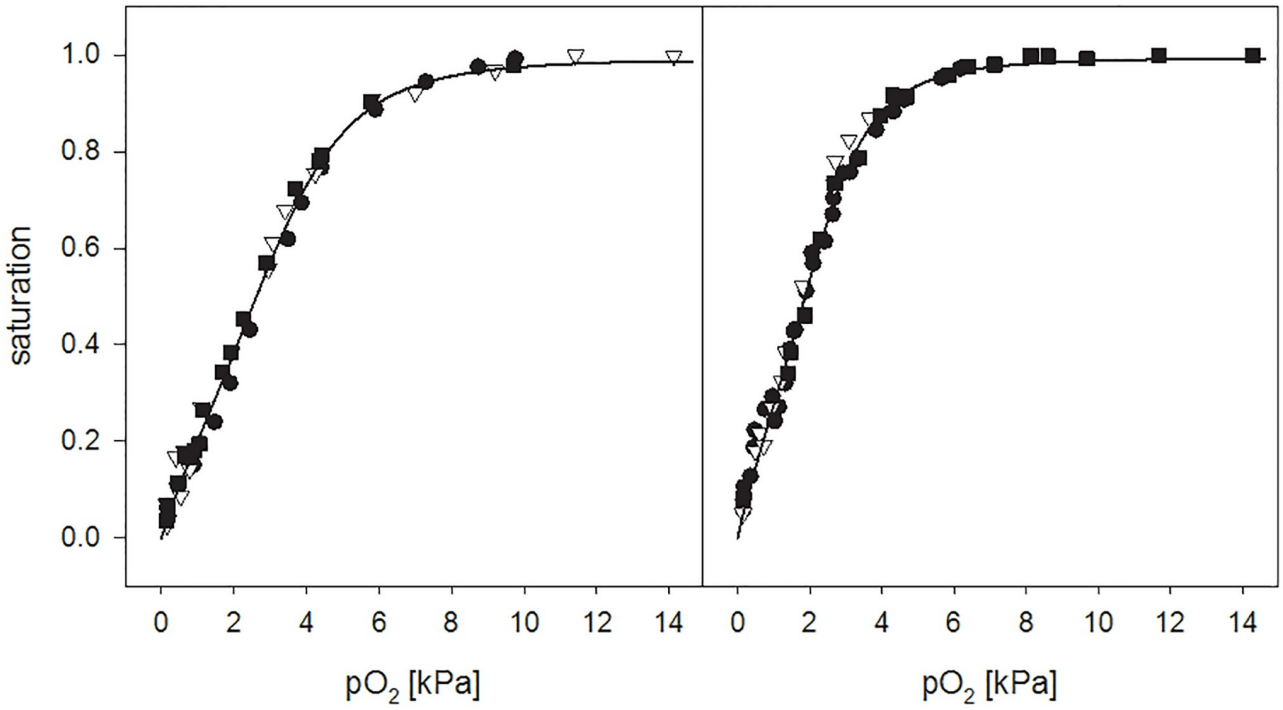

Fig 1. Oxygen dissociation curves of white rhinoceros haemoglobin at 37˚C. ODCs were measured at pH 7.2 (left panel) and pH 7.4 (right panel) in 50 mM TRIS/HCl at 0.1 M chloride. Measurements were performed with haemoglobin isolated from three different animals, indicated by the different symbols. The data does not indicate variations in oxygen affinity for different individuals. The solid lines represent the fit based on the function described in the supplemental material (eq.1), corresponding to a modified version of the one employed for human haemoglobin for the determination of SaO_2 from blood gas analysis.

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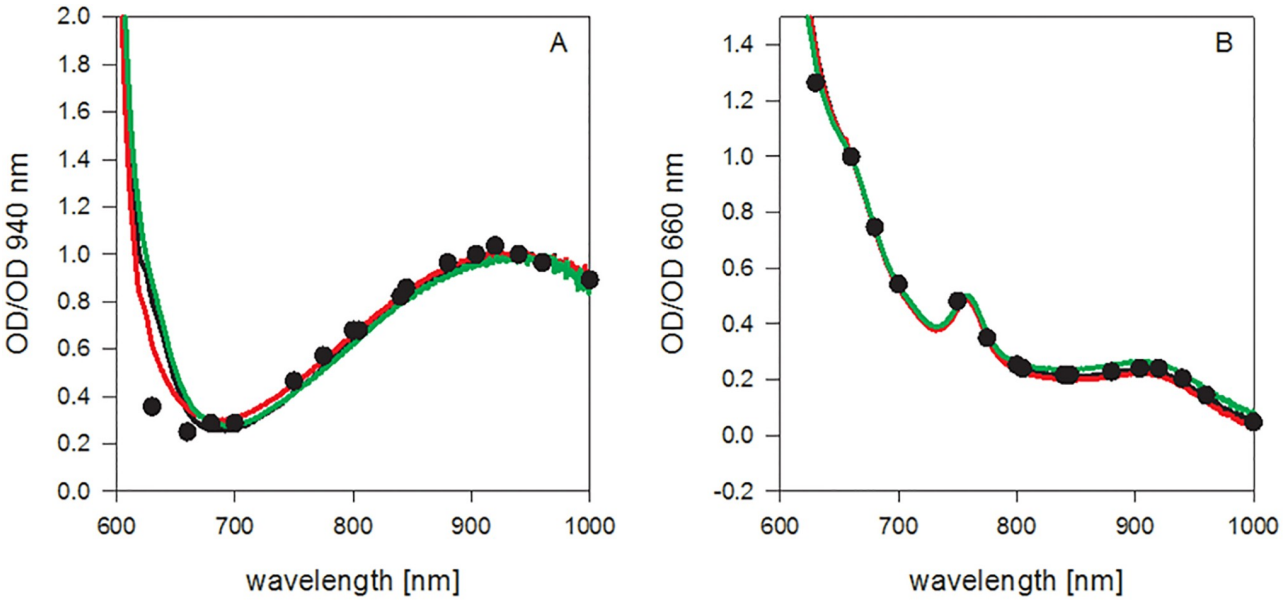

Fig 2. Absorption spectra of oxygenated (A) and deoxygenated (B) haemoglobin from rhinoceros (two individuals, black and red line) and horse (one individual, green line). For comparison, the spectrum of human haemoglobin, taken from Zijlstra et al. is also shown (circles). The Spectra are normalized to OD at 940 nm (panel A), and to 660 nm (panel B) to allow comparison of the spectral shape. The absorbance values were about 0.2 at 940 nm for oxyhaemoglobin and about 0.47 at 660 nm for deoxyhaemoglobin.

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apparently not work equally well with the high haemoglobin concentrations necessary for the measurement of the absorbance spectra.

Discussion

As expected, the ODC of the white rhinoceros showed a marked left shift at both pH = 7.4 and pH = 7.2 compared to the human ODC under near-physiological conditions. The main effector responsible is DPG, which significantly lowers the oxygen affinity of human haemoglobin, but has no effect on white rhinoceros haemoglobin [8]. However, the absorbance spectra of oxy-and deoxyhaemoglobin in both white rhinoceros and domestic horse showed only minor deviations from human absorbance patterns, most likely due to the presence of residual methaemoglobin *in vitro*.

Based on the two ODCs presented, mathematical models like the one established by Siggaard-Andersen et al. [9] and modified by Haymerle et al. [7] can now be fitted directly to measured curves, yielding rhinoceros-specific parameters (Tab A in S1 Appendix). These species-specific parameters are valid only under the specified measuring conditions (pH, temperature, salt concentration). The effect of further modulators such as CO_2 on the p50 can be incorporated as demonstrated by Haymerle et al. ([7], see S2 Fig and Tab B in S1 Appendix), Thus, SaO_2 can now be estimated based on pO_2, pCO_2 and pH determined through blood gas analysis. To demonstrate this, we used the experimental values reported by Haymerle et al. [7] and combined them with our parameters to calculate SaO_2, resulting in saturation levels above 80% in most cases (S2 Fig). Arterial haemoglobin saturation values below 90% still indicate hypoxaemia, but saturation levels above 80% seem more plausible than values of less than 40% as reported in the literature.

Acidosis, hypercapnia and a low arterial partial pressure of oxygen (PaO_2) are common in opioid-immobilised white rhinoceroses, indicating marked cardiorespiratory depression [1,4–

6]. Our findings help to explain why despite the seemingly extremely low SaO_2 values reported in these animals, recovery is usually uneventful, without obvious clinical signs of long-term damage associated with such severe hypoxaemia. As stated above, some level of hypoxaemia is still very likely to occur in immobilised white rhinoceroses, underlining the importance of adequate anaesthetic protocols and management by trained professionals.

It should be kept in mind that the model is based on ODCs measured *in vitro*; we cannot exclude that other, so far unknown factors are present under physiological conditions *in vivo*, having additional effects on SaO_2 [6]. The presented ODCs can now be used to construct a model to estimate SaO_2 more reliably, allowing for a better understanding of the true oxygen saturation status of white rhinoceroses during opioid immobilisation. Meanwhile, tabulated saturation levels at both pH 7.2 and pH 7.4 (Tab C in S1 Appendix) can aid colleagues in the field to interpret the results of blood gas analysis.

A high oxygen affinity of haemoglobin under in vivo-like conditions (characterised by a left-shifted ODC) is a typical finding in large mammals, as it facilitates oxygen uptake in the lungs and its transport via blood over longer distances. However, O_2 delivery cannot be characterized by haemoglobin's oxygen binding characteristics only; instead, physiological determinants of oxygen transport are "redundant and numerous", including blood flow (cardiac output), ventilation and acid-base-status [15]. Interestingly, Haymerle et al. found evidence that white rhinoceroses have a higher cardiac output than humans (expressed per square meter of body surface) [7], which could be interpreted as an evolutionary adaptation to ensure adequate O_2 supply.

Specific mechanisms facilitate the deoxygenation of haemoglobin and therefore the final delivery of the oxygen to the target tissues, including the Bohr effect, Haldane effect and other allosteric factors. Baumann et al. reported that in the haemoglobin of the white rhinoceros, only protons and chloride anions are major allosteric factors controlling its oxygen affinity, while DPG and CO_2 are not [8]. The Bohr and Haldane effects are closely linked and are characterised by the species-specific Bohr coefficient; values reported for the white rhinoceros (-0.62 by Baumann et al. and -0.74 in our study) are high compared to other species.

It is interesting to consider the clinical consequences of the high Bohr coefficient for the situation under immobilisation: especially under field conditions, where chasing occurs prior to darting, animals show severely elevated levels of lactate [16], indicating an anaerobic metabolic status. According to Lapennas [17], a higher Bohr coefficient is favourable under these circumstances, as it produces a right-shift of the ODC, which ensures sufficient oxygen supply of the metabolic active tissues and contributes to overcome the anaerobic situation. This information facilitates a better understanding of how white rhinoceroses are actually able to cope with the physiologically extreme situation under immobilisation with highly potent opioids.

In human patients, certain haemoglobin anomalies (Hb Bonn, Hb Cheverly, HbM Iwate, Hb Köln) are known to cause falsely low SpO_2 readings due to deviating absorbance curves, which interfere with the calculation of SpO_2 based on the standard 660nm/ 940nm absorbance ratio [10]. This led us to hypothesize that deviating absorbance characteristics of white rhinoceros oxy- and deoxyhaemoglobin might contribute to the low SpO_2 readings. However, only minor deviations in the absorbance curves measured by us for horse and rhinoceros haemoglobin from the data on human haemoglobin published by Zijlstra et al. [18] were observed.

The residual methaemoglobin, which was formed during shipping and could not be removed from the sample material at the high haemoglobin concentrations needed for the measurements, could be held accountable for this deviation. Due to methaemoglobin's extinction coefficients at the two wavelengths recorded, presence of methaemoglobin will increase the ratio measured (readings at 630 nm/readings at 940 nm), and pulse-oximetry would indeed indicate artificially decreased levels of SaO_2.

The relevance of these findings for the situation *in vivo* remains unclear. Interestingly, due to elevated free tyrosine levels in the erythrocytes of all members of the order *Perissodactyla*, order-specific processes of free radical and antioxidant metabolism have been discussed before [19]. Indicators of oxidative stress are well described in exercising horses [20]. One could argue that an opioid immobilisation including the severe side effects resembles exercise, potentially–as it is a drug-induced and uncontrolled effect—challenging the antioxidative capacity of rhinoceros erythrocytes, leading to oxidative stress and potentially the formation of methaemoglobin *in vivo*.

In addition, the absorbance of purified haemoglobin does not necessarily represent the situation *in vivo*, where components of the erythrocytes, whole blood and surrounding tissues might interfere with the photometric measurement. Other known interference factors of pulse oximetry include excessive movement, venous pulsation, poor perfusion and poor probe positioning [21], all of which might well be relevant in the case of opioid-immobilised white rhinoceroses.

A limitation of our study is the small sample size, which is a common problem in veterinary studies on rare species. In this context it is worth mentioning that the southern white rhinoceros population experienced an extreme bottleneck effect in the late 19th century with not more than 50 animals left [22], so today's population of around 20 000 animals worldwide can be expected to have a low genetic diversity.

Hypoxaemia remains a major issue in white rhinoceroses under opioid immobilisation. The results of this study provide evidence that the arterial oxygen saturation of haemoglobin is regularly underestimated by SaO_2 derived from blood gas analysis based on human data. Furthermore, our findings help to construct a model that estimates the oxygen saturation more accurately. However, no obvious deviation in absorbance spectra could be found *in vitro* to explain a potential underestimation of SpO_2 by pulse oximetry. An extraction and analysis of the crystal structure of the white rhinoceros haemoglobin is in progress in order to further investigate this question.

Supporting information

S1 Appendix. Main, combined file. Supporting Information on 1. The construction of an equation describing white rhinoceros ODCs and 2. The determination of haemoglobin saturation by blood gas analysis.
(DOCX)

S1 Fig. Employing eq.1 and eq.2, ODCs were calculated for different pH and different pCO_2. The corresponding p50 values show a decreasing impact of pCO_2 at decreasing pH-values, in agreement with the results reported by Baumann et al.
(TIF)

S2 Fig. Employing the value of blood gas analysis (pH, CO_2, pO_2, temperature) of anaesthetized rhinoceros published by Haymerle and colleagues, the saturation level of white rhinoceros haemoglobin was estimated based on eq.1+eq.3 (black circles) or in a simplified version, where temperature, Hi and HbCO is not included (eq.1 + e.q2., red circles).
(TIF)

S3 Fig. Based on the function suggested by Haymerle et al., oxygen binding curves were calculated for two different pH values and two different pCO_2 values, in the range found in the blood of anaesthetized rhinoceros. Note that an increase in pCO_2 and a decrease in pH leads to a left-shift of the ODC, in contrast to experimental data by Baumann and colleagues. In contrast, the function represented by eq.2 and eq.3 reflect the experimentally observed

shifts, since the experimental data were used to generate the equations.
(TIF)

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Diskussion

Im ersten Teil des Projekts konnte gezeigt werden, dass mithilfe des neuen Sensordesigns pulsoximetrische Messungen an der bukkalen Schleimhaut von Unpaarhufern möglich sind. Im zweiten Teil des Projekts wurde die Sauerstoffabsorptionskurve des Breitmaulnashorns beschrieben, welche deutliche Unterschiede zur humanen Kurve aufweist. Das Absorptionsverhalten des oxygenierten und desoxygenierten Hämoglobins hingegen wies keine signifikanten Abweichungen von humanem Hämoglobin auf.

Nach unserem aktuellen Kenntnisstand ist diese Arbeit zum Sensordesign bei Unpaarhufern die erste ihrer Art. Während kommerzielle Hersteller die aus der Humanmedizin bekannten Designs mit marginalen Abwandlungen für tiermedizinische Geräte verwenden, wurde unser Design im Rahmen der Masterarbeit von Bahnemann und Hebbe methodisch gemäß der VDI-Richtlinie 2221 für die Anwendung am Tier entwickelt [4]. Von der systematischen Wahl des Applikationsorts bis zur Konstruktion der in unserer Studie verwendeten Prototypen entsprach das Vorgehen aktuellen medizintechnischen Standards. Auf Grundlage unserer Publikation ist es für interessierte Kollegen mit überschaubarem Aufwand möglich, ihre vorhandenen Sensoren in das von uns entwickelte Design zu überführen.

Die Applikation von Sensoren zur Reflexionspulsoximetrie an der bukkalen Schleimhaut des Pferdes stellte sich in unserer Studie als praktikabel dar. Durch unser neu entwickeltes Design ist es möglich, den Sensor an einem gut durchbluteten Gewebe mittels Arretierung zuverlässig, aber gewebeschonend zu befestigen. Dies bietet potentiell einen Vorteil gegenüber herkömmlichen Sensorclips, deren Anwendung oft dadurch erschwert wird, dass sie nur an sehr peripheren Körperregionen angebracht werden können, wo sie sich leicht lösen oder gar nicht platziert werden können.

Pulsoximetrie bietet gegenüber der Blutgasanalyse den Vorteil, ein nichtinvasives, kontinuierliches Verfahren zu sein. Im Idealfall wird der Sensor einmal platziert und liefert anschließend ohne Unterbrechung SpO_2 und Pulsfrequenz. Über ein

akustisches Signal ist es möglich, dass der behandelnde Tierarzt die Information sogar erhält, ohne dass der Monitor in seinem Blickfeld ist. Aus diesem Grund ist die Technik auch insbesondere für den Einsatz bei Feldanästhesien interessant sowie in Situationen mit dünner Personaldecke, in denen die für die Narkoseüberwachung zuständige Person noch andere Aufgaben übernehmen muss. In solchen Situationen wird es als besonders lästig empfunden, wenn der Clip wiederholt neu platziert werden muss oder wenn die vom Pulsoximeter gelieferten Werte oder Alarme erst hinterfragt werden müssen, weil sie unplausibel erscheinen. Unser neuentwickeltes Design verspricht eine bessere, zuverlässigere Arretierung des Sensors (Verbesserung der Hardware). Die Messgenauigkeit der Pulsfrequenzmessung, welche für die Qualität der SpO_2-Messung entscheidend ist, war in unserer Studie hingegen nicht optimal. Zur Verbesserung wäre eine Anpassung der Software notwendig, welche nur durch den Hersteller erfolgen kann, da die der Messung zugrundeliegenden Algorithmen von der Industrie unter Verschluss gehalten werden.

Sowohl in situ als auch ex situ haben Nashörner einen hohen ideellen und (indirekt) auch finanziellen Wert. Wohl kaum ein Nashornhalter wäre bereit, seine Tiere für eine Entsättigungsstudie zur Verfügung zu stellen und auch die Bewilligung eines derartigen Tierversuchsantrags erscheint fraglich. Aus diesem Grund entschieden wir uns, unser Sensordesign an Versuchspferden zu evaluieren, in der Annahme, die Ergebnisse auf das derselben taxonomischen Ordnung angehörende Nashorn übertragen zu können. In diesem Zusammenhang ist es erwähnenswert, dass die Durchführung von Entsättigungsstudien an freiwilligen gesunden humanen Probanden ein standardmäßiges Vorgehen in der Entwicklung von Pulsoximetriesystemen ist, welches als unproblematisch angesehen wird. Interessante Einblicke dazu liefert die Publikation von Bickler et al. [5]: die Autoren erläutern, dass eine akute hochgradige Hypoxie (definiert als SaO_2 von 50 % - 70% für etwa 10 Minuten) von gesunden Menschen problemlos toleriert wird. Erst wenn zeitgleich zur reinen Hypoxie kardiovaskuläre Einschränkungen und damit Perfusionsstörungen wie z.B. eine Ischämie im Bereich des Gehirns auftreten, seien negative Folgen wie z.B. kognitive Langzeitschäden zu erwarten. Die von den Autoren beschriebene, unter kontrollierten

Laborbedingungen gezielt herbeigeführte reine Hypoxie ist sicherlich nicht mit der klinischen Situation immobilisierter Nashörner vergleichbar. Dennoch führt dieser Übersichtsartikel dem Leser vor Augen, wie robust und redundant die Sicherungssysteme des Körpers zur Sicherstellung der Gewebeoxygenierung grundsätzlich sind.

Zur weiterführenden Evaluierung des neuen Sensordesigns sind klinische Studien notwendig; solche stehen noch aus. Allerdings wurden bereits mehreren Zoo- und Wildtierärzten Prototypen für die klinische Anwendung und weiterführende Forschungsprojekte zur Verfügung gestellt. Dabei hat sich gezeigt, dass der Sensor im klinischen Gebrauch grundsätzlich bei einer Vielzahl von Spezies (Antilopen, Großkatzen, Neuweltkameliden) anwendbar ist (Dr. Pierre Grothmann, persönliche Kommunikation). Darüber hinaus kam der Prototyp zwischenzeitlich bereits in einer weiteren Studie zum Einsatz: Mtetwa et al. verwendeten ihn in einer experimentellen Studie zur Pulsoximetrie an Impalas [6].

Auf Grundlage unserer Arbeit zur Sauerstoffsättigungskurve des Breitmaulnashorns ist eine Anpassung von verfügbaren Algorithmen zur Bestimmung von SaO_2 speziell für diese Tierart möglich. Dabei kann an die Arbeit von Haymerle et al. angeknüpft werden: diese Arbeitsgruppe nutzte zwei mathematische Modelle zur Analyse von Messergebnissen aus Blutgasanalysen von Breitmaulnashörnern. Zum einen passten sie den Algorithmus nach Siggaard-Andersen [7,8] an (Methode 1), zum anderen nutzten sie eine von ebendiesem Autor bereitgestellte open source software, den Oxygen Status Algorithm (OSA) (Methode 2). Methode 2 stellte sich als nicht praktikabel heraus, da der p50-Wert auf der Benutzeroberfläche der Software grundsätzlich nicht geändert werden kann und somit für die Berechnungen manuell rückgerechnet werden musste. Der als Methode 1 benutzte Algorithmus stellte sich hingegen als sehr flexibel heraus. Der speziesspezifische p50-Wert und Bohr Effekt [3] sowie die Messwerte für $PaCO_2$, pH und Körpertemperatur der Tiere konnten berücksichtigt werden. Die Steigung der Kurve wurde mangels publizierter Daten für

das Breitmaulnashorn nicht verändert, jedoch wurde die Kurve etwas abgeflacht, um eine Überschätzung der Messwerte zu vermeiden.

Durch die von uns gemessenen Kurven inklusive p50 und n50 bei pH 7,4 und pH 7,2 ist es nun möglich, die Anpassung des Algorithmus um die Details der genauen Lage und Steigung der Sauerstoffsättigungskurve bei pH 7,4 und pH 7,2 zu ergänzen. Im zweiten Schritt wäre dann aufbauend auf diesem angepassten Algorithmus die Programmierung einer Software wie dem OSA zur praktischen Nutzung und klinischen Anwendung möglich. Zwischenzeitlich können die tabulierten Daten aus unserer PUBLIKATION 2 klinisch tätigen Kollegen bei der Interpretation von Blutgasanalysedaten vom Breitmaulnashorn helfen. Die Tabelle ermöglicht im Feld eine Abschätzung des tatsächlich vorliegenden SaO_2 des Patienten auf Grundlage der mittels Blutgasanalyse gemessenen Werte (pO_2 und pH). Die Idee zu dieser Tabelle entstand bereits in der Planungsphase des Projekts in Rücksprache mit im Feld tätigen Kollegen. In Hinblick auf die während der Feldarbeit zu beobachtenden Messwerte wurde deshalb auch entschieden, die Sauerstoffsättigungskurve nicht nur für den physiologischen pH von 7,4 zu bestimmen, sondern ebenfalls Messungen bei einem pH-Wert von 7,2 durchzuführen, um auch unter der häufig beobachteten respiratorischen Azidose eine Abschätzung der Oxygenierung zu ermöglichen.

Azidose, Hyperkapnie und niedrige arterielle Sauerstoffpartialdrücke (PaO_2) treten bei immobilisierten Nashörnern regelmäßig auf und sind Indikatoren für eine deutliche kardiorespiratorische Depression [9]. Unsere Ergebnisse zur Sauerstoffsättigungskurve zeigen, dass die SaO_2-Werte weniger erniedrigt sind als die bisher veröffentlichten Werte es annehmen ließen. Diese Information hilft zu verstehen, warum die meisten Tiere eine Immobilisation ohne offensichtliche klinische Anzeichen und neurologische Langzeitschäden durch eine vorangegangene massive Hypoxie tolerieren. Dennoch ist es wichtig zu betonen, dass immobilisierte Breitmaulnashörner ein hohes Risiko aufweisen, eine klinisch relevante Hypoxämie zu entwickeln. Die Verwendung geeigneter Narkoseprotokolle und die Überwachung durch erfahrene Anästhesieteams ist daher von großer Bedeutung.

SpO_2 wird über das Verhältnis der Absorption bei 660nm zur Absorption bei 940nm ermittelt. Aus der Humanmedizin ist bekannt, dass gewisse anormale Formen des Hämoglobins (Hb), namentlich Hb Bonn, Hb Cheverly, Hb Iwate, Hb Köln, aufgrund abweichender Absorptionskurven falsch-niedrige SpO_2-Werte generieren [10]. Wir vermuteten, dass dies beim Breitmaulnashorn ebenfalls der Fall sein könnte. Allerdings ergaben unsere Untersuchungen der Absorptionsspektren des Oxy- und Desoxyhämoglobins des Breitmaulnashorns lediglich minimale Abweichungen von den von Zijlstra et al. [11] publizierten humanen Messkurven. Ein möglicher Erklärungsansatz für die minimalen Abweichungen ist das technisch bedingte Vorliegen geringer Mengen an Methämoglobin *in vitro*. Das Methämoglobin bildete sich wahrscheinlich während des Probenversands durch Autooxidation mit in der Probe befindlichem Sauerstoff und konnte bei den hohen Hämoglobinkonzentrationen, welche für die Messungen notwendig waren, nicht vollständig eliminiert werden. Aufgrund der abweichenden Extinktionskoeffizienten könnte das Methämoglobin *in vitro* tatsächlich zu einem erniedrigten SaO_2-Messwert führen.

Die Bedeutung dieser Befunde für die Situation *in vivo* bleibt unklar. Es gibt Hinweise darauf, dass in der Ordnung *Perissodactyla* (Unpaarhufer) spezielle Mechanismen innerhalb der Erythozyten zum Schutz vor oxidativen Schädigungen existieren. Zur Aufklärung des Krankheitsbildes der akuten hämolytischen Anämie des Spitzmaulnashorns (*Diceros bicornis*) wurden eine Vielzahl hämatologischer Studien durchgeführt, unter anderem von Weber et al. [12]. Dieses Autorenteam berichtet von deutlich erhöhte Leveln der Aminosäure Tyrosin in den Erythrozyten verschiedener Equiden und Nashörnern, unter anderem auch beim Breitmaulnashorn. Die Werte waren gegenüber humanen Erythrozyten um das bis zu 50-fache erhöht. Die Autoren vermuten dahinter einen speziellen Stoffwechselweg zum Schutz der Zelle vor freien Radikalen, wie es in humanen Neutrophilen und Epithelzellen für Taurin beschreiben wurde [13].

Bei arbeitenden Pferden sind Indikatoren für oxidativen Stress umfassend beschrieben [14]. Man könnte argumentieren, dass die massiven Nebenwirkungen, welche mit einer Immobilisation mit hochpotenten Opioiden einhergehen, sich ähnlich auf den Körper auswirken wie harte Arbeit: es kommt zu Muskeltremor, Temperaturanstieg,

Tachykardie, Hypertension und respiratorischer Depression mit Hypoxämie, Hyperkapnie und Azidose. De Lange et al. weisen außerdem auf die Rolle der „*fight or flight*“- Reaktion bei chemisch immobilisierten Wildtiere hin, welche mit einer sympatischen Aktivitierung und Katecholaminausschüttung einher geht. In ihrer Summe könnten diese Prozesse die antioxidative Kapazität der Nashornerythrozyten überlasten, oxidativen Stress auslösen und damit zur Bildung von Methämoglobin *in vivo* führen. Dieses Methämoglobin könnte dann wiederum mit pulsoximetrischen Messungen interferieren.

Darüber hinaus ist das Absorptionsverhalten von hoch gereinigtem Hämoglobin nicht zwingend repräsentativ für die Situation *in vivo*. Bestandteile der Erythrozyten und des Vollbluts sowie des umgebenden Gewebes können die photometrische Messung beeinflussen. Weitere bekannte Störfaktoren für Pulsoximetrie umfassen Bewegungen des Patienten, venöse Pulsation, schlechte Perfusion und suboptimale Positionierung des Sensors [15]. All diese Faktoren können auch beim immobilisierten Breitmaulnashorn eine Rolle spielen.

Es ist ein erstaunliches Detail, dass ein Überwachungsgerät wie das Nonin 2500A Vet Pulsoximeter, welches explizit für den veterinärmedizinischen Gebrauch vermarktet wird, an menschlichen Probanden kalibriert wird [16]. Für humanmedizinische Geräte wird gefordert, eine Diversität der Probanden sicherzustellen (Geschlecht, Hautfarbe), um die Praxistauglichkeit der Kalibrierung zu gewährleisten. Im Gegensatz dazu wird bei Tieren noch nicht einmal die entsprechende Zielspezies zur Kalibrierung genutzt. Die Publikation von Batchelder und Raley [17] zeigt eindrücklich, was für ein komplexer Prozess eine Kalibrierung ist und wie viele Einflussfaktoren es gibt. In der Gebrauchsanweisung des Nonin 2500A Vet Pulsoximeters wird sogar darauf hingewiesen, dass Abweichungen zu erwarten sind (“Although animal haemoglobin has similar optical characteristics, other types of haemoglobin or alternate sensor locations may affect the calibration.”). Es ist für den Anwender nicht abschätzbar, ob, welche und wie starke Abweichungen es gibt. Analog dazu ermöglichen es viele moderne Blutgasanalysegeräte nicht mehr, Konfigurationen an der Software vorzunehmen. Die Anpassung des Algorithmus zur Berechnung von SaO_2 z.B. durch Eingabe des p50 Wert der Zielspezies ist somit nicht möglich. Die sich daraus

ergebenen Messungenauigkeiten sind in der praktischen tierärztlichen Tätigkeit unerwünscht und im Bereich von wissenschaftlichen Studien inakzeptabel. Vor diesem Hintergrund erscheint es wünschenswert, Pulsoximeter für den veterinärmedizinischen Gebrauch auch an den entsprechenden Zielspezies zu kalibrieren und bei Blutgasanalysegeräten eine Anpassung der SaO_2-Algorithmen an die Zielspezies zu ermöglichen.

Basierend auf unseren Ergebnissen sind weitere Projektideen entstanden und inzwischen teilweise bereits realisiert worden. Wie bereits erwähnt, führten Mtetwa et al. eine klinische Studie an Impalas durch [6]. An der Universität Mainz läuft aktuell noch eine Arbeit zur Kristallstruktur des Hämoglobins des Breitmaulnashorns, welche weiteren Aufschluss zum Sauerstoffbindungsverhalten des Proteins geben könnte. Am Alfred Wegener Institut in Bremerhaven wurde basierend auf unserem Entwurf ein Prototyp zur Anwendung bei der Weddellrobbe konstruiert.

Die gesetzten Ziele konnten erreicht werden. Die vorliegende Arbeit liefert praktische Ansätze zur Optimierung der Überwachungsmöglichkeiten der Sauerstoffsättigung des Hämoglobins beim Breitmaulnashorn und Pferd mittels Pulsoximetrie. Die neuen Erkenntnisse zur Sauerstoffsättigungskurve des Breitmaulnashorns erleichtern die Interpretation der Ergebnisse von Blutgasanalysen, erhöhen dadurch potentiell die Narkosesicherheit und stellen somit einen Beitrag zum Artenschutz dar.

Zusammenfassung

Julia Reiners

Untersuchungen zur Sauerstoffsättigungskurve des Breitmaulnashorns (*Ceratotherium simum*) sowie Evaluierung von Pulsoximetriesensoren für Unpaarhufer (Breitmaulnashorn und Pferd)

Die vorliegende Doktorarbeit befasst sich mit technischen und physiologischen Grundlagen der Überwachung der Sauerstoffsättigung des Hämoglobins bei Unpaarhufern (Breitmaulnashorn und Pferd) mit dem Ziel, die Überwachungsmöglichkeiten für das Breitmaulnashorn zu verbessern.

Im ersten Projektteil wurde in Zusammenarbeit mit Medizintechnikern ein neues Sensor-Design zur Pulsoximetrie an der bukkalen Maulschleimhaut entwickelt und im Rahmen einer experimentellen Studie am Pferd evaluiert. Es wurde ein kommerziell erhältlicher Sensor zur Transmissionspulsoximetrie (Nonin 2000SL) in das neue Sensordesign zur Reflexionspulsoximetrie integriert. Drei Prototypen (N1, N2a, N2b) wurden konstruiert und mit dem Nonin 2500A Vet Pulsoximeter benutzt. Es wurde eine Entsättigungsstudie (100 – 70% SaO_2) an dreizehn Warmblutpferden durchgeführt. SpO_2- und Pulsfrequenzmessungen wurden dokumentiert. Als Referenzmethoden dienten SaO_2 (errechnet aus den Ergebnissen einer Blutgasanalyse) sowie invasive Pulsfrequenzmessungen. Der systematische Fehler (Bias) sowie die Messgenauigkeit (Precision) wurden mittels Kalkulation der mittleren quadratischen Abweichung (A_{rms}) bestimmt. Die Übereinstimmung der beiden Methoden wurde mit der Bland-Altman Analyse untersucht. Es zeigte sich, dass die Qualität der Pulsfrequenz-Messergebnisse ausschlaggebend war für die Qualität der SpO_2-Messungen. Eine gute Pulssignalstärke resultierte in einer SpO_2-Messgenauigkeit, welche mit der des Originalsensors vergleichbar war (Nonin 2000SL: Arms = 3%; N1: Arms = 3,60%; N2b: Arms = 3,46%). Insbesondere bei Herzfrequenzen ≤30 bpm wurden Pulsfrequenzmessungen beobachtet, welche in etwa doppelt so hoch waren wie die Referenzwerte. Ihr Ausschluss aus dem Datensatz führte zu Messgenauigkeiten bezüglich der Pulsfrequenz ähnlich der des Originalsensors. Bland-Altman Plots

Im zweiten Projektteil wurden die Absorptionscharakteristika von Oxy- und Desoxyhämoglobin untersucht und die Sauerstoffsättigungskurve des Breitmaulnashorns beschrieben. Dafür wurde aus den Blutproben von vier adulten Breitmaulnashörnern aus europäischen Zoos Hämoglobin isoliert. Es wurden Sauerstoffsättigungskurven basierend auf den Absorptionsänderungen in der Soret-Region (c.a. 420 nm) bei pH 7,2 und pH 7,4 bei einer Temperatur von 37°C bestimmt. Die Absorptionsspektren wurden bis in den infraroten Bereich hinein unter physiologischen Normalbedingungen hinsichtlich pH, Temperatur und DPG gemessen. Die Sauerstoffsättigungskurve zeigte eine deutliche Linksverschiebung (p50 von 2,75 ± 0,07 und 2,00 ± 0,04 kPa bei pH 7,2 und 7,4). Die Absorptionsspektren zeigten nur marginale Abweichungen von humanen Spektren, welche möglicherweise durch Spuren von Methämoglobin in der *in vitro* Untersuchung zu erklären sind. Basierend auf unseren Daten ist die Entwicklung eines spezies-spezifischen Algorithmus zur Berechnung von SaO_2 beim Breitmaulnashorn möglich. Die systematische Unterschätzung der arteriellen Sauerstoffsättigung des Hämoglobins durch die Pulsoximetrie lässt sich durch die von uns gemessenen unauffälligen Absorptionskurven nicht erklären.

Summary

Julia Reiners

Study of the oxygen dissociation curve in the white rhinoceros (*Ceratotherium simum*) and evaluation of pulse oximetry sensors in odd toed ungulates (white rhinoceros and domestic horse)

The subject of this thesis are the technical and physiological foundations for monitoring the arterial oxygen saturation of haemoglobin in odd-toed ungulates (white rhinoceros and domestic horse). The objective was to improve the possible monitoring in the white rhinoceros.

In the first part of the project, a new sensor design for pulse oximetry at the buccal mucosa was developed in cooperation with medical engineers and evaluated in an experimental study in horses. A commercially available sensor for transmission pulse oximetry (Nonin 2000SL) was included into the new sensor design for reflectance pulse oximetry. Three different prototypes (N1, N2a, N2b) were constructed and used with the Nonin 2500AVet pulse oximetry monitor. Thirteen anaesthetised warmblood horses were included into a desaturation protocol (100–70% SaO_2). SpO_2 and pulse frequency values were recorded, using SaO_2 calculated from blood gas analysis and invasive pulse frequency measurements as reference methods. Bias and precision were evaluated by calculations of the root mean square deviation (A_{rms}). The agreement of the methods was tested with Bland Altman analysis. The quality of the pulse frequency readings determined the quality of the SpO_2-readings. Good pulse signal strength resulted in a SpO_2-accuracy comparable to that of the original sensor (Nonin 2000SL: Arms = 3%; N1: Arms = 3.60%; N2b: Arms = 3.46%). Especially at heart rates ≤30 bpm, pulse rate readings that were about twice as high as the reference value occurred. Their exclusion from the dataset resulted in a pulse rate accuracy similar to that of the original sensor. Bland-Altman plots showed limits of agreement typical of pulse oximeters. The new sensor design has been shown to be suitable for buccal pulse oximetry in horses.

In the second part of the project the absorbance characteristics of oxy- and desoxyhaemoglobin were determined and the oxygen dissociation curve of the white

nm) at 37°C and pH 7.2 and pH 7.4. The absorbance spectra were measured extending into the infrared region under physiologically normal conditions (pH, temperature, DPG). The oxygen dissociation curve showed a distinct left shift (p50 of 2.75 ± 0,07 and 2,00 ± 0.04 kPa at pH 7,2 and 7,4). The absorbance spectra showed only marginal deviation from human spectra, possibly due to the presence of traces of methaemoglobin in the *in vitro* examination. Our data allows for the development of a species-specific algorithm for the calculation of SaO_2 in the white rhinoceros. The systematic underestimation of SpO_2 by pulse-oximetry cannot be explained by our unsuspicious absorbance spectra.

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