ORIGINAL RESEARCH

Validation of Noninvasive Methemoglobin and Carboxyhemoglobin Measurements Using Pulse Co-Oximeter in Healthy Dogs

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Purpose: To assess the agreement between pulse co-oximeter and blood co-oximeter in measuring methemoglobin (MetHb) and carboxyhemoglobin (COHb) in healthy, awake dogs.

Patients and Methods: Forty-five healthy dogs were enrolled in the study. Pulse co-oximetry was performed using the Masimo Radical 7 Pulse Co-Oximeter with a Rainbow® adhesive sensor. Simultaneously, venous blood samples were collected, and MetHb and COHb were immediately measured using a Stat Profile Prime Plus VET Critical Care Analyzer. Paired measurements of MetHb and COHb were evaluated via Spearman correlation, intra-class correlation (ICC), and Bland-Altman plots to evaluate the degree of agreement between the pulse co-oximeter and the blood co-oximeter.

Results: A total of 45 paired MetHb and COHb measurements were collected. There was a weak correlation between the pulse co-oximeter and the blood co-oximeter readings. The correlation coefficients for MetHb and COHb were 0.0 (95% CI, −0.3 to 0.3) and 0.03 (95% CI, −0.27 to 0.32), respectively. The ICC indicated poor agreement between the pulse and blood co-oximeter for MetHb (ICC = 0.00, 95% CI: −0.12 to 0.15) and COHb (ICC = 0.03, 95% CI: −0.27 to 0.33). Bland-Altman plots revealed low mean bias but wide limits of agreement, indicating that the pulse co-oximeter overestimated MetHb by on average of 0.7% (P < 0.0001) (95% LoA: −0.5 to 2.0) and COHb by on average 0.2% (P = 0.59) (95% LoA: −4.6 to 5.0).

Conclusion: Obtaining MetHb and COHb measurements with the Masimo Radical 7 Pulse Co-Oximeter is straightforward in healthy, awake dogs. However, the device does not provide accurate measurements compared to the blood co-oximeter, specifically in the range of MetHb and COHb in healthy dogs, based on the wide LoA.

Keywords: co-oximetry, methemoglobin, carboxyhemoglobin, dog

Introduction

Hemoglobins are divided into two categories based on their oxygen-binding capabilities.[1] Functional hemoglobins, including oxyhemoglobins and deoxyhemoglobins, can bind, carry, and unload oxygen. In contrast, non-functional hemoglobins, or dyshemoglobins, such as methemoglobin (MetHb), carboxyhemoglobin (COHb), and sulfhemoglobin, are incapable of binding oxygen. The dyshemoglobins present in a very small amount in healthy dogs, but in the disease process, their presence indicates clinical significance.[1–3]

Co-oximeters are specialized benchtop analyzers that employ spectrophotometry to distinguish between various hemoglobin species in blood.[1] Due to its accuracy and reliability in quantifying different forms of hemoglobin, this technique is the gold standard for measuring dyshemoglobins.[4] Using multiple wavelengths of light, co-oximeters can accurately identify and quantify these hemoglobin species, providing critical information for diagnosing and treating conditions related to abnormal dyshemoglobin levels.[1,5,6] However, obtaining blood samples in critically ill dogs, particularly small, anemic, or those with coagulopathy, poses potential complications.

Pulse co-oximeter uses multiple wavelength spectrophotometry to measure dyshemoglobins, including MetHb and COHb, through a tissue bed with pulsating blood.[7] The Masimo Radical 7 Pulse Co-Oximeter (Masimo Radical 7 Pulse Co-Oximeter, Masimo Corporation, Irvine, CA, USA) employs eight wavelengths of light compared to standard pulse oximeters with two wavelengths.[5,6] This allows for instantaneous detection of MetHb and COHb. The Masimo Radical 7 Pulse CO-Oximeter is approved for use in clinical practice in people as a bedside tool for triage, diagnosis, and monitoring.[8] The utility of pulse co-oximeter is of particular interest in dogs, as it provides a noninvasive, continuous measurement of MetHb and COHb without requiring repeated blood sampling.

To date, no studies have been published evaluating the agreement between pulse co-oximetry and blood co-oximetry in measuring MetHb and COHb levels in dogs. This study aims to compare MetHb and COHb values measured by the Masimo Radical 7 Pulse Co-Oximeter to those measured by the Stat Profile Prime Plus VET Critical Care Analyzer (Stat Profile Prime Plus VET Critical Care Analyzer, Nova Biomedical, Waltham, MA, USA) in healthy dogs. The authors hypothesized that there would be a moderate agreement between the measurements from the Masimo Radical 7 Pulse Co-Oximeter and those obtained with the Stat Profile Prime Plus VET Critical Care Analyzer.

Materials and Methods
Case Selection
This study was approved by the Institutional Animal Care and Use Committee at the Ohio State University (protocol number 2022A00000072). Only dogs that were determined to be healthy based on (1) lack of abnormal clinical signs, (2) physical examinations by the principal investigator (JH), and (3) laboratory findings, including hematology and biochemistry, within three months were included. A total of 47 dogs were enrolled, which included 35 dogs that were participants of the blood donation program at the Ohio State University Animal Blood Bank. The remaining twelve dogs were owned by staff members of the Ohio State University Veterinary Medical Center. Routine demographics, such as age, gender, breed, and weight, were collected from each patient.

Sample Measurement
Pulse co-oximetry was conducted on each dog using the Masimo Radical 7 Pulse Co-Oximeter. The principal investigator collecting the data (JH) and a veterinary technician who uses pulse oximetry daily were trained to use the device. Dogs were gently restrained, and the disposable sensor (Rainbow® adhesive sensor, R1 20L Infant or R1 25L Adult, Masimo Corporation, Irvine, CA, USA) was applied to dogs' toes, primarily the 4th or 5th digit of their hind limbs. If a strong signal (indicated by bright light in the display) was not obtained at the initial location, alternative sites such as the tail, penis, or vulva were attempted. The measurements were taken in a room with minimal lighting. Once stable measurements are achieved, MetHb, COHb, and pulse rate displayed were recorded. The principal investigator (JH) verified the readings by matching the displayed pulse rate to patients' heart rates, plethysmographic waveform, and strong signal strength in the display.

At the same time as the pulse co-oximetry readings were obtained, venous blood gas samples were collected and analyzed. Using a vacutainer, each blood sample was collected via direct venipuncture to jugular veins under anaerobic conditions. The sample were then transferred anaerobically into pre-heparinized syringes (RAPIDLyte; Siemens Healthcare Limited, Washington, D.C. USA) and immediately analyzed using the Stat Profile Prime Plus Blood Gas Analyzer. The investigator (JH) who operated the analyzer had received prior training and followed the manufacturer's guidelines (Nova Biomedical User Manual, 2020). The blood co-oximeter was calibrated daily with an automated calibration process per the manufacturer's instructions and maintained by clinical laboratory personnel.

Statistical Analysis
All data were entered into a spreadsheet (Microsoft Excel for Mac v.2011; Microsoft, Redmond, WA) and analyzed using statistical software (R V4.3.1; R Core Team 2023, SAS V9.4; SAS Institute Inc, Cary, NC). They were evaluated for normality via inspection of QQ- and PP-plots, histograms, and skewness. Normally distributed data were presented as mean and standard deviation (SD), and non-normally data were presented as median and interquartile range (IQR).

MetHb blood measurements were normally distributed but were presented as median (IQR) for comparison to pulse co-oximeter values. A two-way random effects model with single rater/measurement and absolute agreement was used to calculate intra-class correlation coefficients (ICCs) for agreement between paired MetHb and COHb values. The interpretation of ICC was based on the guidelines proposed by Koo,[9] where 95% confidence intervals (CI) were used to determine the level of agreement. ICC values >0.9 were considered excellent, 0.75–0.9 were good, 0.50–0.75 were moderate, and <0.5 were in poor agreement. Bland-Altman plots were employed as a graphical representation to assess the mean bias (compared to 0 using paired t-tests) and the agreement based on the 95% limits of agreement (LoA). Spearman correlation coefficients were also calculated to assess the correlation between MetHb and COHb values. A *P* value <0.05 was considered statistically significant.

Results

A total of forty-seven dogs participated in the study, and samples were collected from 45 of 47 dogs. Two dogs were excluded from the study due to their uncooperation to blood sample collection. None of the 45 dogs required sedation for sample collections. The mean age of dogs included was 5.2 years (SD = 2.6) and the mean weight was 30.4 kg (SD = 8.9). Sixteen (36%) were neutered males, six (13%) were intact males, eighteen (40%) were spayed females, and two (4%) were intact females. Breeds represented in this group included Greyhounds (n = 19), mixed breed (n = 4), Borzoi (n = 3), Labrador Retriever (n = 3), American Staffordshire (n = 2), Belgian Malinois (n = 2), Border Collie (n = 2), Golden Setter (n = 2), Beagle (n = 1), Bernese Mountain (n = 1), Chesapeake Bay Retriever (n = 1), French Bulldog (n = 1), Goldendoodle (n = 1), Golden Retriever (n = 1), Irish Wolfhound (n = 1), and Poochon (n = 1).

Forty-five paired MetHb measurements from pulse and blood co-oximeter were analyzed. (Figure 1) The median (IQR) MetHb values from the pulse and blood co-oximeter were 1.2% (0.6–1.6) and 0.4% (0.35–0.5), respectively. The Spearman correlation coefficient (rho) was 0.00, with a 95% CI ranging from −0.3 to 0.3. This indicates that there is at most a weak positive correlation between the pulse and blood co-oximeter measurements. The ICC was 0.00 (95% CI, −0.12–0.15) which indicates that there is poor agreement of MetHb measurements from the pulse and blood co-oximeter. When MetHb from the pulse co-oximeter was compared with MetHb from the blood co-oximeter using Bland-Altman plots, the mean (SD) bias was 0.7 (0.6) (*P* < 0.0001), meaning the pulse co-oximeter overestimated MetHb by on average 0.7% with 95% LoA −0.5% to 2.0%. (Figure 2) That is, the pulse co-oximeter provides a reading between −0.5% and 2% of the true MetHb value for 95% of all measurements, so that if the pulse co-oximeter reads 5%, the blood MetHb will be between 4.5% and 7%.

Forty-five paired COHb measurements from pulse and blood co-oximeter were analyzed. (Figure 3) The median (IQR) COHb values from the pulse and blood co-oximeter were 0% (0–2) and 0.6% (0.4–1.70), respectively. The
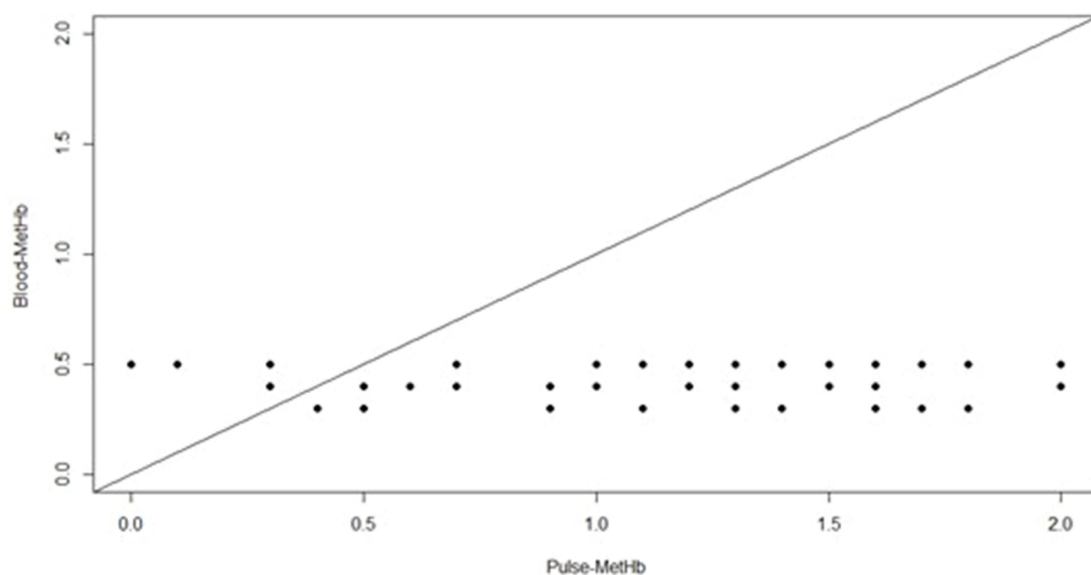

Figure 1 The scatterplot of MetHb (%) measured by pulse co-oximeter (Pulse-MetHb) and blood co-oximeter (Blood-MetHb).
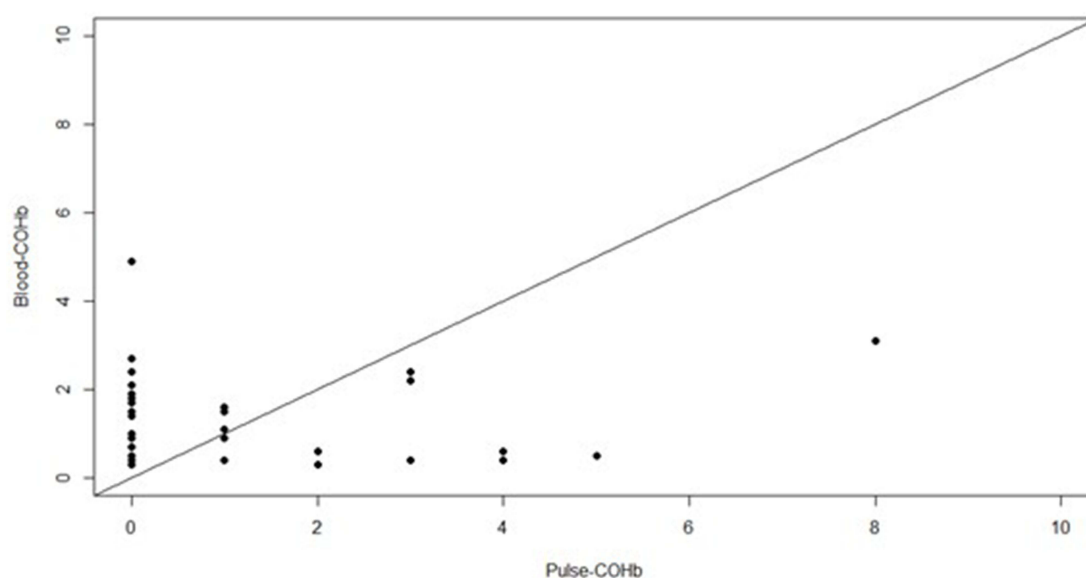
Figure 2 Bland-Altman plot for comparison of methemoglobin (MetHb) measurements by pulse co-oximeter and blood co-oximeter in 45 dogs. The middle horizontal line represents the mean bias (mean difference) and the outer lines (dashed) represent the 95% limits of agreement (LoA). The mean bias (SD) was 0.7% (SD = 0.6) with 95% LoA of −0.5 to 2.0%.
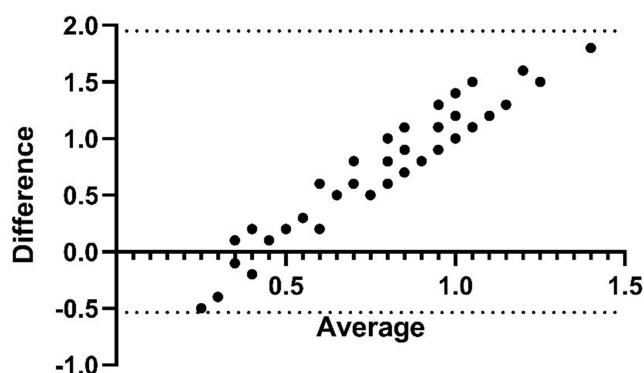

Figure 3 The scatterplot of COHb (%) measured by pulse co-oximeter (Pulse-COHb) and blood co-oximeter (Blood-COHb).

Spearman correlation coefficient (rho) was 0.03 with a 95% CI ranging from −0.27 to 0.32. This indicates that there is at most a weak positive correlation. The ICC was 0.03 (95% CI, −0.27–0.33) which shows that COHb measured from pulse co-oximeter and blood co-oximeter are in poor agreement with each other. When COHb measured with the pulse co-oximeter was compared to COHb measured with the blood co-oximeter using Bland-Altman plots, the mean (SD) bias was 0.2 (2.5) ($P = 0.59$), meaning the pulse co-oximeter overestimated COHb by on average 0.2% with 95% LoA −4.6% to 5.0%. (Figure 4) That is, the pulse co-oximeter provides a reading between −4.6% and 5.0% of the true COHb value for 95% of all measurements, so that if the pulse co-oximeter reads 5%, the blood COHb will be between 0.4% and 10%.

Discussion

This study compared the measurements of MetHb and COHb obtained from the Masimo Radical 7 Pulse Co-Oximeter to those obtained from the Stat Profile Prime Plus VET Critical Care Analyzer, the latter serving as the gold standard, in healthy awake dogs. To our knowledge, this is the first study to evaluate the agreement between pulse co-oximeter and blood co-oximeter in measuring MetHb and COHb in dogs. The Masimo Radical 7 Pulse Co-Oximeter was easy to use and capable of measuring MetHb and COHb in healthy dogs, suggesting its potential clinical application for dogs. The
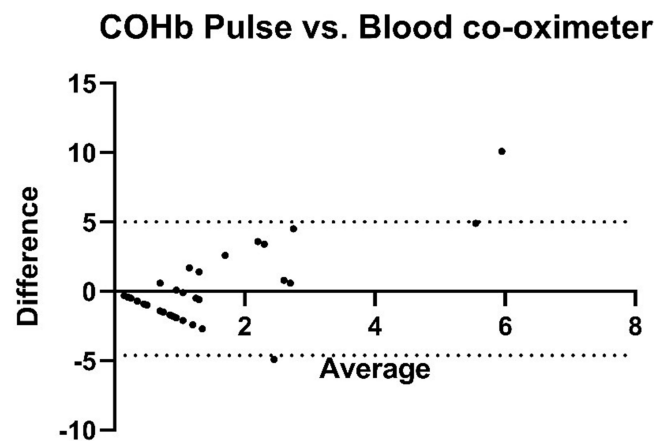
COHb Pulse vs. Blood co-oximeter

Figure 4 Bland-Altman plot for comparison of carboxyhemoglobin (COHb) measurements by pulse co-oximeter and blood co-oximeter in 45 dogs. The middle horizontal line represents mean bias (mean difference) and the outer lines (dashed) represent the 95% limits of agreement (LoA). The mean bias (SD) of the differences was 0.2 (SD = 2.5) with 95% LoA of −4.6 to 5.0.

potential for noninvasively and continuously measuring dyshemoglobins using a pulse co-oximeter is significant, especially for dogs with anemia, small blood volumes, or coagulopathy, and for clinics without access to blood co-oximeter. In these circumstances, a pulse co-oximeter could offer immediate and clinically relevant information, aiding clinicians in monitoring trends and making clinical decisions. Despite human studies describing the utility of pulse co-oximeter to detect MetHb or COHb in clinical practice,[8,10,11] only one case report exists on the clinical use of pulse co-oximeter in a dog.[12] In the case report, the pulse co-oximeter was used to diagnose methemoglobinemia, measuring MetHb levels between 15% and 17% in the dog, compared to a venous MetHb level of 21.7%. Of note, the current study assessed the agreement in healthy dogs with minimal levels of MetHb and COHb, but did not investigate the agreement in dogs with elevated MetHb and COHb, which remains an area for future research.

In the present study, the ICC showed poor agreement between the pulse and blood co-oximetry. However, the agreement assessed via the ICC could have been underestimated due to the narrow range of values measured from healthy dogs in the study.[13] While the ICC in the current study suggests poor agreement, the result should be interpreted with caution, and future studies are warranted to investigate the agreement between the pulse and blood co-oximeter in a wide range of MetHb and COHb to accurately assess the agreement.

The present study is the first study to report the normal range of MetHb and COHb measured via pulse co-oximetry in healthy dogs. The reference intervals of dogs' MetHb and COHb levels measured via blood co-oximetry have been established in the previous studies.[1,4] One study from 27 healthy adult dogs suggested reference intervals for MetHb and COHb to be 0.1–0.45% (with a median of 0.2%) and 1.3–2.7% (with a median of 2.3%), respectively.[1] Another study comparing blood co-oximetry measurements from 57 Greyhound dogs with those from 30 non-Greyhound dogs found no significant difference in reference intervals, with Greyhounds showing MetHb levels of 0.0–2.2% and COHb levels of 0.9–3.9%, versus 0.1–2.8% for MetHb and 0.4–4.5% for COHb in non-Greyhounds.[4] The reported reference intervals of blood co-oximetry are similar to the findings from our study, which reports a median of 0.4% (IQR 0.35–0.5) for MetHb and 0.6% (IQR 0.4–1.75) for COHb. Despite the Masimo Radical 7 Pulse Co-Oximeter's tendency to overestimate MetHb and COHb, with the reported range of MetHb at a median of 1.2%, (IQR 0.6–1.6) and COHb at a median of 0% (IQR 0–2), these ranges could serve as a basis for future research investigating the agreement of pulse and blood co-oximeter in elevated MetHb and COHb levels or diagnosing patients with abnormal COHb or MetHb levels using pulse co-oximeter in the clinical setting.

Recent veterinary literature has highlighted the diagnostic and prognostic utility of COHb in dogs.[2,3] COHb serves as a diagnostic marker for hemolysis, exhibiting excellent ability to distinguish hemolytic anemia from other types of anemia in dogs. In the retrospective study, COHb outperformed traditional markers like serum bilirubin concentration or the mean corpuscular volume of erythrocytes for predicting hemolytic anemia. Specifically, dogs with hemolytic anemia showed significantly higher COHb levels (median 7.7%, IQR 2.5) compared to those with non-hemolytic anemia

(median 3.6%, IQR 1.05).[2] Another retrospective study of dogs hospitalized in the ICU of a tertiary academic referral hospital investigated the prognostic utility of COHb.[3] Similar to human literature, dogs with respiratory diseases exhibited higher COHb levels (median 2.7%, range 0.3–5.0%) than those without (median 2.5%, range 0.1–5.6%), suggesting a positive association between COHb and respiratory disease in dogs. In the present study, the Bland-Altman plot of COHb showed a wide range of 95% LoA (−4.6 to 5.0). For example, if the pulse co-oximeter COHb measurement is 5%, the corresponding blood co-oximeter COHb could range from 0.4% to 10% 95% of the time. Based on the wide LoA reported herein, the role of pulse co-oximeter in investigating the diagnostic and prognostic role of COHb appears to be limited, with blood co-oximetry remaining the gold standard.

One of the strengths of the present study is that the pulse co-oximeter was performed under healthy awake dogs. Despite the lack of agreement between the blood co-oximeter and the pulse co-oximeter, the Masimo Radical 7 Pulse Co-Oximeter carries a number of advantages that support its continued clinical development. First, it was easy to use in dogs. Although not evaluated in the study, the pulse co-oximeter can also measure perfusion index, plethysmography variability index, and oxygen saturation. On the other hand, a limitation of this study is the limited number of dogs included. Future investigations are needed to assess the diagnostic and monitoring utility of the Masimo Radical-7 Pulse Co-Oximeter in a larger number of dogs.

Conclusions

This study is the first to evaluate the agreement between pulse and blood co-oximeter in measuring MetHb and COHb in dogs. MetHb and COHb levels were easily measured in healthy, awake dogs using the Masimo Radical 7 Pulse Co-Oximeter. In healthy dogs with low levels of MetHb and COHb, the agreement between the pulse co-oximeter and blood co-oximeter was poor. The numerical differences between pulse and blood co-oximeters reported in this study should be considered when using pulse co-oximeters in dogs. Future research is needed to evaluate the agreement in dogs with elevated levels of MetHb and COHb.

Ethical Approval

The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ("best practice") of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed Consent

Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

Funding

Disclosure

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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