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# Hematologic Parameters and Blood Cultures from the Gingival Vein Compared with the Cranial Vena Cava in Guinea Pigs

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**Blood collection methods in guinea pigs are limited due to the animals' compact neck, short limbs, and lack of a tail. Gingival venipuncture is a recently described blood sampling technique that is minimally traumatic with no significant alterations in hematologic parameters when multiple blood samples were collected weekly for 6 wk. The purpose of this study was to determine whether the gingival vein can be used as an alternative blood collection site in guinea pigs, such that: (1) hematologic parameters would be consistent with samples collected from the cranial vena cava; and (2) no contaminants from the oral cavity would be introduced into the sample. Blood samples were obtained from both the gingival vein and cranial vena cava of anesthetized Dunkin Hartley guinea pigs for CBC ($n$ = 9) and aerobic blood cultures ($n$ = 10). Only MCV was significantly different between sampling sites. Bland–Altman analyses calculated a small mean bias for all hematologic parameters, indicating clinical interpretation is unlikely to be affected by the sampling site. Bacterial growth occurred in all 5 gingival vein blood samples prepared by using saline and 2 of the 5 prepared with dilute chlorhexidine. Bacteria did not grow from any cranial vena caval blood samples prepared with dilute chlorhexidine. No clinical signs of hemorrhage or trauma were detected at either site. These results provide evidence that gingival venipuncture can be used as an alternative blood collection method for guinea pigs for hematologic analysis but should not be used for blood culture.**

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Guinea pigs share many biologic similarities to humans, including their dietary requirement for vitamin C and immune responses to various infectious diseases.[10,18] These similarities, combined with a docile nature and small size, have made the guinea pig a particularly useful animal model of human diseases for more than 200 y.[10,18,26,27] Venipuncture is an essential procedure in research studies using animal models but is often challenging to perform in guinea pigs. Compared with other rodents, safe and easily accessible blood collection techniques in guinea pigs are limited due to their compact neck, short limbs, and lack of a tail.

A myriad of venipuncture techniques with variable blood sample volumes has been described in guinea pigs. Common venipuncture sites include the lateral saphenous vein,[4,8,11,19,29] cephalic vein,[8,19,29] auricular vein,[4,29] cranial vena cava,[8,19,29] and jugular vein.[4,8,19,29] The lateral saphenous, cephalic, and auricular veins are easily accessible, allow for repeated sampling, and do not require anesthesia.[4] However, these vessels are small, and minimal blood (less than 200 µL) can be collected from each vein.[8,19,27] Therefore, blood may need to be collected from multiple peripheral veins to acquire a sufficient sample volume.[19]

The jugular vein and cranial vena cava are the most commonly used sites to collect large blood samples (1 to 2 mL).[19,27] However, due to guinea pigs' short and thick neck, the jugular vein is difficult to palpate and visualize. Manual restraint can be extremely stressful to guinea pigs, and sedation or anesthesia is often necessary. Although the cranial vena cava is frequently used, its proximity to the heart and major vessels within the thoracic cavity poses a significant risk of death due to traumatic intrathoracic or pericardial hemorrhage. Therefore, cranial vena caval venipuncture requires anesthesia to ensure precise sampling.[8,19]

The gingival vein (*labialis mandibularis* vein) is located within the gingiva just below the pair of mandibular incisors. The gingival vein was first reported as a simple and reliable intravascular injection and blood collection site in rats and mice. As much as 800 µL of blood in rats and 100 µL in mice were successfully acquired with minimal signs of pain and distress after collection.[7] A recent study[20] determined that a maximum of 500 µL of blood could be collected from guinea pigs by using this route. Samples were collected weekly from each guinea pig for 6 wk, and no significant alterations in hematologic parameters were noted.[20] In addition, histologic analyses confirmed that multiple blood collections over time were minimally traumatic to the surrounding tissue.[20] Although sedation or anesthesia is required for using the gingival vein, its safety, feasibility, and potential for large sample volume make it a promising new venipuncture site in guinea pigs.

Hematologic and biochemistry parameters in mice and rats can vary when blood is obtained from different sampling sites.[1-3,6,9,14-17,21-23] One study compared hematologic parameters in blood collected from the sublingual vein and vena cava in rats, and equivalence was only established for 5 of 14 hematologic parameters.[22] Another study in rats compared 3 peripheral blood collection sites (retroorbital plexus, dorsal anastomotic orbital vein, and sublingual vein) to a central site (abdominal aorta). Hematologic parameters, particularly the WBC count, were significantly different between peripheral sites when

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compared with the central site.[3] A study in mice compared hematologic parameters between blood sampled from a tail clip, the retroorbital plexus, and the heart. In general, the tail sample had significantly higher values for all cell types when compared with the heart.[16]

The purpose of our current study was to determine whether gingival venipuncture could be used as an alternative blood collection route in guinea pigs, such that hematologic parameters would be consistent with those of samples collected from a central venipuncture site, that is, the cranial vena cava. In addition, samples were assessed for potential contamination by oral cavity bacteria to assess the gingival vein's utility as a blood culture collection site.

## Materials and Methods

**Animals.** A total of 24 (16 male, 8 female) Dunkin Hartley guinea pigs (age, 5 to 8 mo) from Charles River Laboratories (Wilmington, MA) were used in this study. Guinea pigs were singly housed in 30.80 cm × 59.37 cm × 22.86 cm isolator cages (Maxi-Miser Interchangable IVC Caging, Thoren, Hazleton, PA) with 0.125-in. corncob bedding (Harlan, Madison, WI). Red huts (BioServe, French Town, NJ) and daily hay cubes (PMI Nutrition International, Brentwood, MO) were provided as enrichment. Caging was changed 3 times weekly. Animal rooms were maintained on a 12:12-h light:dark cycle, at 20 to 26 °C, and at 30% to 70% humidity. Teklad Global Guinea Pig Diet 2040 (Envigo, Madison, WI) and filter-sterilized water were provided without restriction. Guinea pigs were free of Sendai virus, lymphocytic choriomeningitis virus, pneumonia virus of mice, guinea pig adenovirus, guinea pig reovirus, *Helicobacter* spp., *Mycoplasma pulmonis*, and ectoparasites. All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals*[12] and approved by the Colorado State University IACUC.

**Blood collection for CBC analysis.** Each guinea pig was placed in an induction box and received 5% vaporized isoflurane with an oxygen flow rate of 0.75 to 1.0 L/min. Once the animal had lost its righting reflex, it was removed from the induction box, placed in dorsal recumbency, and maintained on 3% isoflurane and oxygen through a nose cone. Sterile ophthalmic lubricant was applied to both eyes.

An assistant retracted the mandibular lip and applied light pressure in the area of the mandibular symphysis to occlude the gingival vein. A 28-gauge, 1/2-in. needle attached to a 1-mL insulin syringe was inserted caudally 3 to 5 mm into the gingiva below the middle of the mandibular incisors at an angle of 30 to 60° (Figure 1). The needle was advanced with slight negative pressure applied to the plunger until blood appeared in the hub of the needle. At that point, advancement of the needle was stopped, and slow negative pressure was applied until blood flow ceased.

A minimal blood sample of 250 µL was required for CBC analysis. When an adequate blood sample was collected from the gingival vein, blood was immediately collected from the cranial vena cava of the same guinea pig. The area of the sternum and manubrium was prepared by using 70% isopropyl alcohol. A 25-gauge, 5/8-in. needle with a 1-mL syringe was inserted into the right clavicular notch and slowly advanced caudally into the cranial vena cava. Slight negative pressure was applied until blood was visible in the hub of the needle. Negative pressure on the plunger was continued until at least 500 µL of blood was collected in the syringe.

After each collection, the needle was removed prior to transfer of the blood sample into a 1.3-mL microtube containing EDTA (Sarstedt, Nümbrecht, Germany). Filled blood collection tubes were stored for a maximum of 3 h on ice prior to processing. Blood collection tubes were submitted to the Colorado State University Clinical Pathology Laboratory for processing. Hematologic analyses were performed by using an automated system (Advia 120 Hematology System, Siemans, Munich, Germany) and a manual differential cell count.

**Blood collection for culture.** In a separate procedure, each animal was placed under anesthesia in the same way as described earlier. Once the guinea pig was anesthetized, the gingival vein and cranial vena caval sites were prepared prior to blood collection. In the first 5 guinea pigs, the gingiva caudal to the mandibular incisors was flushed with sterile saline (Hospira, Lake Forest, IL) by using an 18-gauge, 1-in. needle with a 35-mL syringe for 3 repetitions or until all gross debris was removed. In light of the results of the first 5 blood cultures, the protocol was adjusted to prepare the gingiva by using 3 scrubs of 0.05% chlorhexidine solution (2% chlorhexidine gluconate, Vetoquinol, Ft Worth, TX) for the remaining 5 guinea pigs. To prepare the cranial vena caval site, the fur was clipped in the area of the sternum and manubrium. The area was prepared by using 3 scrubs of 0.05% chlorhexidine solution for all 10 guinea pigs.

A minimum of 100 µL was needed for blood culture analysis. When a sufficient blood sample was obtained from the gingival vein, blood was immediately collected from the cranial vena cava of the same guinea pig. Blood collections from the gingival vein and cranial vena cava were performed by using the same techniques as described previously.

Once each sample was collected, the puncture site of the blood culture vial (BD BacTec Peds Plus medium, Becton Dickenson, Franklin Lakes, NJ) was disinfected by using 70% isopropyl alcohol. For the gingival vein blood sample, the needle was fixed to the insulin syringe and was unable to be replaced. For the cranial vena caval blood sample, a new 22-gauge, 1-in. needle was placed on the syringe prior to injection of the sample into the vial. On transfer of the blood sample, the vial was gently inverted until blood was well mixed with the culture media. The blood culture vials were submitted to the Colorado State University Veterinary Diagnostic Laboratories for processing.

**Statistical Analyses.** All data were analyzed by using Prism version 8.0.1 for Windows (GraphPad Software, San Diego, CA). Normality was determined by using the D'Agostino–Pearson normality test. Normally distributed data were compared by using a paired *t* test. Nonnormally distributed data were compared by using a Wilcoxon matched-pairs signed rank test. The following hematologic parameters were compared: Hgb, Hct, RBC distribution width, MCV, MCHC, cell hemoglobin concentration mean, MPV, and total RBC, WBC, band cell, heterophil, lymphocyte, monocyte, eosinophil, basophil, and platelet counts. A $P$ value of less than 0.05 was considered statistically significant. Bland–Altman analyses[5] were performed to determine agreement between methods. The mean bias was calculated as the average of the difference between methods. The 95% limits of agreement were calculated as the mean bias plus or minus 1.96 times its standard deviation.

## Results

**CBC analyses.** The minimal blood volume of 250 µL from each site was successfully collected and processed from 9 of 24 guinea pigs. D'Agostino–Pearson tests indicated normal distributions for Hgb, Hct, RBC distribution width, MCV, MCHC, cell hemoglobin concentration mean, MPV, and platelet, heterophil, lymphocyte, monocyte, eosinophil, and basophil counts. Nonnormally distributed data included RBC and WBC counts. Band

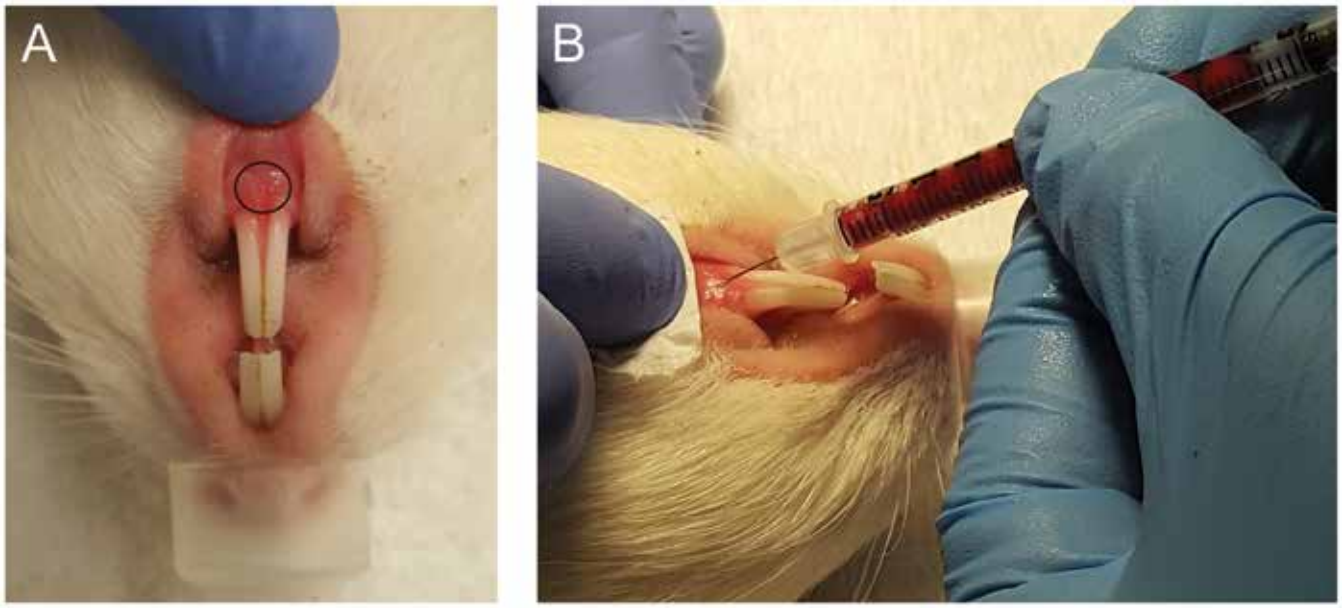


**Figure 1.** Blood collection from the gingival vein in a guinea pig. (A) The area of needle insertion for gingival vein blood collection is encircled. (B) An assistant occludes the gingival vein in the area of the mandibular symphysis. For blood collection, a 28-gauge insulin needle and syringe is held at a 30° to 60° angle and inserted into the gingiva below the middle of the mandibular incisors.

cells were not detected in any blood sample. The mean ± 1 SD, *P* value, mean bias, and 95% limits of agreement are shown in Table 1. MCV was the only hematologic parameter that differed significantly between venipuncture sites ($P = 0.0028$, $t = 4.243$, $df = 8$). Corresponding Bland–Altman plots of Hgb, Hct, MCV, and RBC, WBC, heterophil, lymphocyte, monocyte, and platelet counts are displayed in Figure 2. Comparison between the 2 sampling sites showed acceptable clinical agreement for all hematologic parameters.

**Blood culture analyses.** The minimal blood volume of 100 µL was successfully collected from both veins in 10 of 12 guinea pigs. All 5 blood samples from the gingival vein site prepared by using saline yielded bacterial growth. When the gingival vein site was prepared by using dilute chlorhexidine, bacterial growth was present in 2 of 5 blood samples. None of the 10 blood samples collected from the cranial vena cava yielded any bacterial growth (Table 2).

## Discussion

The objective of this study was to investigate the gingival vein as an alternative site for blood collection in guinea pigs. This new route would benefit both scientists and clinicians by providing a safe and high-volume blood collection site in guinea pigs, a species with limited venipuncture options. For this purpose, blood samples were collected from both the gingival vein and cranial vena cava, a central phlebotomy site in guinea pigs, and hematologic values and blood culture results were compared.

Results from the CBC data showed there was a significant difference in MCV between blood collected from the gingival vein and the cranial vena cava. The Bland–Altman analysis of MCV returned a mean bias of 1 fL, indicating the cranial vena caval samples tended to be 1 unit higher than the gingival vein samples. Given that a minimum of 250 µL was used for CBC analyses, potential underfilling of EDTA blood collection tubes might have contributed to decreased MCV values for gingival vein samples. Although a bias is present, it is quite small and unlikely to affect clinical outcomes. Furthermore, the MCV values from gingival vein samples were consistent with previously reported values for similarly aged guinea pigs.[28,29] In addition, note that MPV and lymphocyte count neared statistical significance, and the collection site may influence these parameters. However, the biases for these parameters likewise were small and unlikely to affect clinical interpretations. In addition to these parameters, the mean biases for all other hematologic parameters were not considered clinically significant when evaluated using Bland–Altman analysis. Therefore, our results provide evidence that the peripherally located gingival vein can be used a suitable alternative to the centrally located cranial vena cava for CBC analyses. These results vary from other studies that have observed differences in blood values between sample sites in other species.[1-3,6,9,14-16,17,22,23] For example, hematologic parameters were significantly different between peripheral sites when compared with a central site in rats.[3] Likely many factors account for the observed differences in those previous studies, such as the sampling sites, collection methods, and anesthetic protocols. Therefore, it remains imperative for researchers to select the most appropriate blood collection method and to maintain it consistently throughout their studies.

In addition to CBC analyses, blood collected from each site was cultured and analyzed for bacterial growth. Gingival vein samples—but not blood from the cranial vena cava—yielded many gram-positive and gram-negative organisms. We used dilute chlorhexidine, a common antiseptic for skin preparations, to prepare the cranial vena caval venipuncture site of all guinea pigs. However, due to the potential for mucosal irritation and ingestion with subsequent adverse gastrointestinal side effects, we initially prepared the gingival vein sites by using sterile saline. After saline treatment, all gingival vein blood samples yielded bacterial growth. Consequently, we decided to prepare the remaining gingival vein sites by using 0.05% chlorhexidine solution. With this preparation, only 2 of 5 blood samples yielded bacterial growth, and no signs of mucosal irritation or systemic adverse effects were appreciated. Therefore, dilute chlorhexidine reduced the bacterial load on the gingiva, yet appeared safe for the animals.

Other than from the site preparation, the bacterial growth from the gingival vein blood samples was also likely due to

**Table 1.** Hematologic parameters of blood samples collected from the gingival vein and cranial vena cava of 9 guinea pigs.

| Parameter | Gingival vein | Cranial vena cava | Paired-test *P* | Mean bias | 95% limits of agreement |
|---|---|---|---|---|---|
| Hgb (g/dL) | 15.43 ± 0.67 | 15.46 ± 0.55 | 0.8813 | 0.02 | −0.83 to +0.87 |
| Hct (%) | 45.33 ± 2.24 | 45.44 ± 2.46 | 0.8602 | 0.11 | −3.48 to +3.70 |
| RBC ($10^6$/µL) | 5.72 ± 0.28 | 5.70 ± 0.32 | 0.3711 | −0.02 | −0.42 to +0.38 |
| RBC distristribution width (%) | 12.77 ± 0.70 | 12.79 ± 0.74 | 0.6224 | 0.02 | −0.23 to +0.28 |
| MCV (fL) | 79.22 ± 4.09 | 80.22 ± 3.99 | 0.0028[a] | 1 | −0.39 to +2.39 |
| MCHC (g/dL) | 34.22 ± 1.40 | 34.00 ± 1.32 | 0.3466 | −0.22 | −1.53 to +1.08 |
| CHCM (g/dL) | 34.44 ± 1.67 | 34.11 ± 1.83 | 0.0805 | −0.33 | −1.31 to +0.65 |
| Platelet count ($10^3$/µL) | 547.11 ± 115.79 | 522.33 ± 69.22 | 0.4847 | −24.78 | −223.60 to +174.10 |
| MPV (fL) | 8.09 ± 0.33 | 8.36 ± 0.31 | 0.0535 | 0.27 | −0.43 to +0.96 |
| WBC count ($10^3$/µL) | 7.02 ± 1.65 | 6.59 ± 1.89 | 0.1172 | −0.43 | −1.69 to +0.83 |
| Band cell count ($10^3$/µL) | 0.00 ± 0.00 | 0.00 ± 0.00 | — | 0.00 | 0.00 to +0.00 |
| Heterophil count ($10^3$/µL) | 3.03 ± 1.08 | 3.16 ± 1.54 | 0.5922 | 0.12 | −1.17 to +1.41 |
| Lymphocyte count ($10^3$/µL) | 3.62 ± 0.92 | 3.18 ± 0.71 | 0.0536 | −0.44 | −1.60 to +0.71 |
| Monocyte count ($10^3$/µL) | 0.24 ± 0.16 | 0.12 ± 0.14 | 0.0836 | −0.12 | −0.49 to +0.24 |
| Eosinophil count ($10^3$/µL) | 0.11 ± 0.08 | 0.13 ± 0.09 | 0.1690 | 0.02 | −0.06 to 0.11 |
| Basophil count ($10^3$/µL) | 0.03 ± 0.05 | 0.00 ± 0.00 | 0.0805 | −0.03 | −0.13 to +0.06 |

CHCM, cell hemoglobin concentration mean
[a]Significantly ($P < 0.05$) different between sample sites.

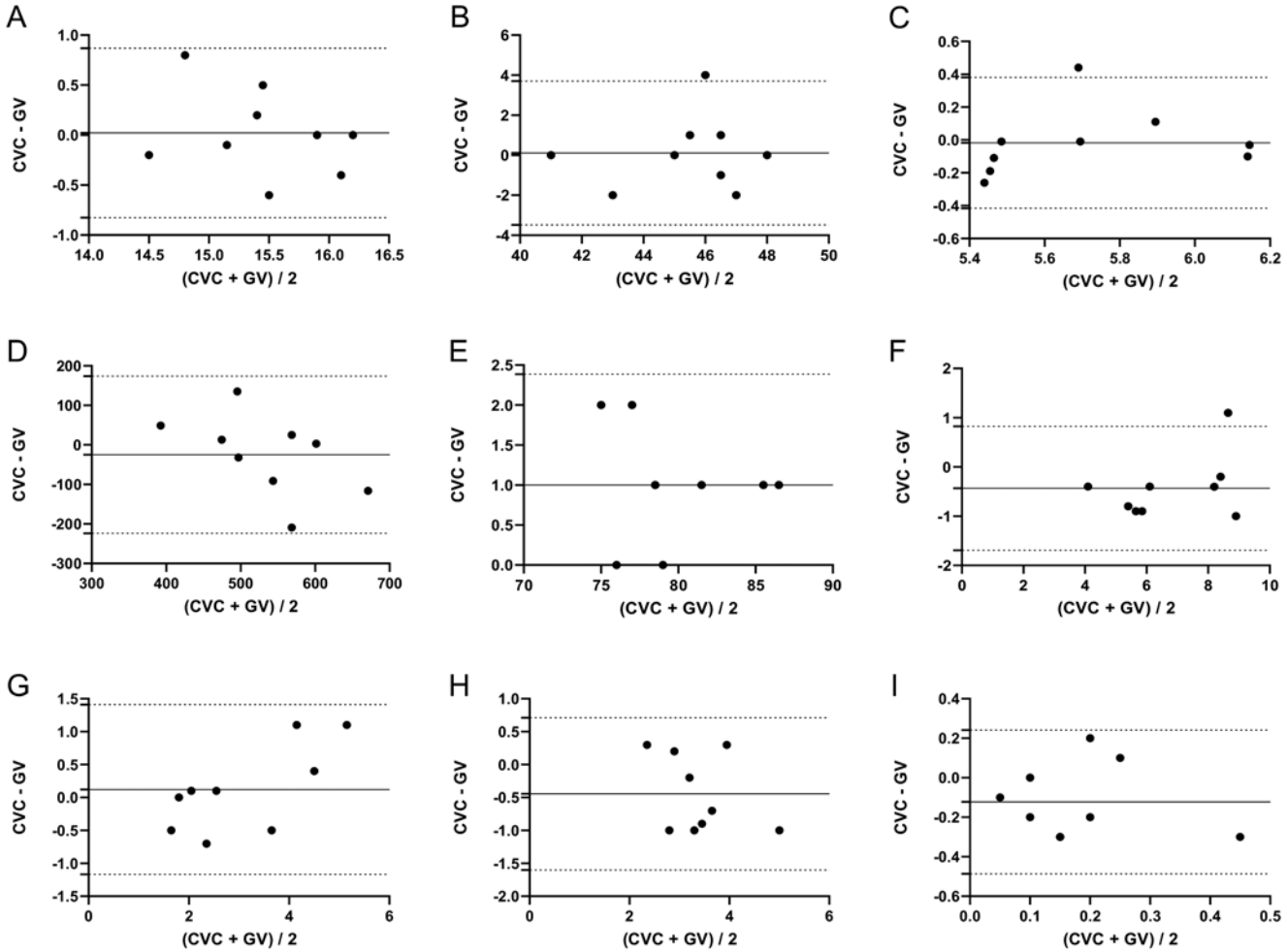


**Figure 2.** Bland–Altman plot of (A) Hgb, (B) Hct, (C) RBC count, (D) platelet count, (E) MCV, (F) WBC count, (G) heterophil count, (H) lymphocyte count, and (I) monocyte count comparing gingival vein and cranial vena cava blood sampling sites. The difference between methods (CVC – GV) is plotted on the *y*-axis, and the average of the methods ([CVC + GV] / 2) is plotted on the *x*-axis. The outer dotted lines designate the 95% limits of agreement, and the solid central line indicates the mean bias.

**Table 2.** Blood culture results from blood samples collected from the gingival vein of 10 guinea pigs.

| Preparation | Animal | Bacteria |
|---|---|---|
| Saline | | |
| | 1 | *Klebsiella oxytoca* |
| | 2 | *Streptococcus* spp., α hemolytic; *Staphylococcus* spp., coagulase negative |
| | 3 | *Streptococcus* spp., α hemolytic; *Neisseria* spp. |
| | 4 | *Staphylococcus*, coagulase negative; *Streptococcus* spp., α hemolytic; *Corynebacterium* spp. |
| | 5 | *Corynebacterium* spp. |
| 0.05% chlorhexidine | | |
| | 6 | *Streptococcus* spp., α hemolytic |
| | 7 | *Actinomyces* spp. |
| | 8 | No growth |
| | 9 | No growth |
| | 10 | No growth |

For all animals, the cranial vena cava was prepared by using 0.05% chlorhexidine; none of the sites yielded any bacterial growth.

needle contamination from the oral cavity. Immediately after blood collection from the gingival vein, the sample was injected into the blood culture vial by using the original insulin needle and syringe. Insulin syringes are produced with permanently attached needles, and there was not an efficient method for changing the needle, as was performed with the cranial vena caval samples. Although the puncture site of the blood culture vial was disinfected with 70% isopropyl alcohol, the needle may have retained bacterial flora from the oral cavity, which then was introduced into the vial. Because it is common practice in clinical veterinary medicine to attach a new needle prior to injecting blood into culture vials, we placed a new needle on the syringes with cranial vena caval blood samples, thus preventing any needle-associated contamination in the culture vials. This variation in experimental procedures may account for the differences in the blood culture results between the 2 sites.

For adequate CBC sample submission, gingival venipuncture had a success rate of 38%. It should be noted that adequate blood sample volumes (up to 800 µL) were able to be obtained from the gingival vein. However, many of these samples tended to rapidly coagulate, making them inappropriate for CBC analyses. The main contributing factor of rapid coagulation in the gingival vein samples was likely the use of 28-gauge insulin needles. Venipuncture through a small gauge needle can result in blood cell shearing, which causes activation of platelets and coagulation factors.[24] Another contributing factor may have been longer collection times for the gingival vein samples. Although the exact time required to collect each blood sample was not recorded, the gingival vein sample collections were perceived to be slower to prevent the collapse of the small vein.

Based on these results, it may be beneficial to heparinize the syringe prior to blood collection for clinical samples. However, it has been shown in other species that using preheparinized syringes results in differences in blood values.[13] Therefore, preheparinizing syringes prior to gingival venipuncture in guinea pigs requires further investigation.

Past studies have shown that phlebotomist experience has a significant effect on outcomes, and technical expertise decreased trauma,[25] corticosterone concentrations, behavioral responses, and collection times.[2] As previously noted, the gingival vein is more difficult to access in guinea pigs compared with other species.[20] Gingival venipuncture requires training, and the phlebotomist must gain confidence and precision to be successful. In our current study, gingival venipuncture involved 2 persons—the phlebotomist and an assistant to retract the lip and occlude the vein. This technique might be performed successfully by a single person, although practice and dexterity are necessary. In contrast, cranial vena caval venipuncture can easily be performed by the phlebotomist alone, given that manual occlusion of the vein is unnecessary.

No adverse effects were observed after blood collection from either site. Some mild bruising occurred in the area of the gingival vein puncture site but did not appear to have clinical effects on any of the animals. There were no signs of stress, lethargy, or pain after recovery from anesthesia, and animals were able to ambulate, eat, and drink normally. These results are consistent with the findings in a previous study.[20] One potential limitation of this study is the effect of saliva on hematologic parameters in blood collected from the gingival vein. However, a study evaluating sublingual and retrobulbar blood collection in rats revealed no significant differences in amylase, a salivary enzyme, between methods.[14] In addition, glucose levels were significantly increased in blood sampled from the sublingual vein, indicating salivary enzymes did not have an effect on this parameter.[14] Because no parameters demonstrated clinically significant differences between blood collection methods in the current study, it is rather unlikely that saliva altered hematologic parameters in gingival vein blood samples. Another potential limitation is the effect of anesthesia on hematology profiles, which has previously been demonstrated in guinea pigs.[26] Despite the potential for anesthesia to affect blood parameters in our study, both blood collections were performed during the same anesthetic event in each guinea pig. Anesthesia is a common procedure used to collect blood in this species, and neither cranial vena caval nor gingival venipuncture is recommended to be performed in awake patients.

In conclusion, gingival venipuncture can be used as an alternative blood collection method in guinea pigs for analyses such as CBC counts. However, the method is unsuitable for blood culture testing in clinical patients, particularly in the absence of 0.05% chlorhexidine. Gingival venipuncture has been successful in other rodent species, including mice, rats,[7] and hamsters,[20] and researchers could consider this route in other laboratory or free-ranging rodents. Compared with the cranial vena cava, gingival venipuncture has the advantage of minimal risk to the animal yet still yields a potentially large sample volume.

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