Ammonia concentrations in canine whole blood, EDTA-anticoagulated whole blood, and plasma measured by use of a point-of-care ammonia meter

Adesola Odunayo DVM, MS
Karen M. Tobias DVM, MS
Chika C. Okafor DVM, PhD
Bente Flatland DVM, MS

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From the Departments of Small Animal Clinical Sciences (Odunayo, Tobias) and Biomedical and Diagnostic Sciences Sciences (Okafor, Flatland), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996.

Address correspondence to Dr. Odunayo (aodunayo@utk.edu).

OBJECTIVE
To investigate the use of canine whole blood (WB) for measurement of ammonia concentration by use of a point-of-care ammonia meter and to compare results of measuring ammonia concentrations in WB, EDTA-anticoagulated WB, and plasma.

ANIMALS
40 client-owned dogs.

PROCEDURES
A blood sample (2 mL) was obtained from each dog. One drop of WB was immediately applied to a test strip for evaluation with an ammonia meter. The remainder of the blood sample was placed in an EDTA-containing tube, and 1 drop of EDTA-anticoagulated WB was applied to a test strip. The remaining EDTA-anticoagulated WB sample was centrifuged, and the plasma was harvested and placed on ice. One drop of plasma was applied to a test strip; the remainder of the plasma sample was transported on ice and used for ammonia measurement with a reference laboratory instrument. All samples were tested within 1 hour after sample collection. Results were evaluated to detect significant differences in ammonia concentration.

RESULTS
Ammonia concentrations did not differ significantly between WB and EDTA-anticoagulated WB and between plasma samples measured with the meter and reference laboratory instrument. However, median ammonia concentration was significantly higher in plasma than in WB or EDTA-anticoagulated WB.

CONCLUSIONS AND CLINICAL RELEVANCE
Anticoagulant-free WB was a valid sample for measurement by use of the ammonia meter. Plasma samples had higher ammonia concentrations than did WB samples. Results for each sample type should be interpreted by use of specimen- and method-specific reference intervals. (*Am J Vet Res* 2017;78:1239–1244)

Ammonia is produced in the gastrointestinal tract by bacterial metabolism of nitrogenous products.[1] In dogs with severe liver disease, ammonia does not reach the hepatocytes because of portosystemic shunting or is not extracted from the blood or converted to urea as a result of hepatocellular dysfunction.[2] Increases in concentrations of ammonia and other toxins have been implicated in the development of hepatic encephalopathy, which is a neurologic disorder that can lead to seizures, coma, and death.[3–5] Measurement of ammonia concentrations is useful for the diagnosis of hepatic encephalopathy in dogs with liver dysfunction and is also used to monitor the response to treatment.[3–5] Measurement of ammonia concentrations may also be used to assess hepatic function in animals for which bile acid concentrations are not available or cannot be used because the serum bilirubin concentration is high or falsely low bile acid concentrations are suspected.[5]

ABBREVIATIONS
WB Whole blood

Although the analyte is commonly referred to as plasma ammonia in clinical practice, the dominant form of ammonia in plasma is the ammonium ion (NH_4^+), which may be converted to NH_3 during sample analysis.[6–8] To avoid difficulties in terms (ammonia vs ammonium ion), ammonia was the term we used in the study reported here to refer to the sum of ammonia gas (NH_3) and ammonium ion (NH_4^+). Plasma ammonia is extremely labile, and sample stability is an obstacle when ammonia concentrations are measured for clinical investigation. Reference laboratories measure ammonia concentrations in plasma by use of enzymatic methods based on the glutamate dehydrogenase–catalyzed reaction of ammonia with α-ketoglutarate and the reduced form of nicotinamide adenine dinucleotide or the reduced form of nicotinamide adenine dinucleotide phosphate.[6] Recommendations for sample handling include cooling

of samples (eg, via ice bath) immediately after collection, minimal exposure of a sample to air, and timely analysis (within 1 hour after collection).[6,9]

Point-of-care ammonia meters allow cage-side determination of ammonia concentrations, which enables decisions to be made rapidly for animals with suspected hepatic encephalopathy. Point-of-care meters are available to practitioners and obviate the need for sample cooling, transport, and centrifugation to ensure accurate results. As an added benefit, point-of-care meters permit analysis of ammonia concentrations in extremely small amounts of blood, which makes measurement of ammonia concentrations feasible in small patients. Point-of-care meters may potentially increase the use of blood ammonia concentration as a diagnostic tool in clinical practice.

A study[10] was conducted to validate the ammonia meter used in the study reported here; that study revealed acceptable precision and linearity and satisfactory agreement with results for a reference method. In that study,[10] the authors analyzed room-temperature EDTA-anticoagulated WB samples obtained from dogs within minutes after collection and recommended a clinical decision threshold for the ammonia meter of > 60 µmol/L (108 µg/dL) for the identification of hyperammonemic patients. In another study[11] conducted to evaluate the use of ammonia measurements for diagnosis of portosystemic shunting, blood samples were collected in EDTA-containing tubes and immediately placed on ice before analysis with the ammonia meter. Anticoagulant-free WB samples have been used for measurement of ammonia concentrations in human athletes[12]; however, the authors are not aware of any veterinary studies conducted on the use of anticoagulant-free WB as a sample type for an ammonia meter. Use of anticoagulant-free WB samples would allow for rapid determination of ammonia concentrations, and because analysis involves only a drop of blood, a smaller blood sample could be collected for sample analysis, compared with the amount required for analysis of anticoagulated samples.

The specific objective of the study reported here was to evaluate the effect of the type of sample (anticoagulant-free WB, EDTA-anticoagulated WB, and plasma obtained from EDTA-anticoagulated WB) on ammonia concentration measured with an ammonia meter. We hypothesized that there would be no difference in ammonia concentration on the basis of the sample used for analysis.

Materials and Methods

Animals

Forty client-owned dogs with a body weight ≥ 1 kg were prospectively enrolled in the study. To obtain a wide range of ammonia concentrations, subjects included dogs that were healthy as determined on the basis of the medical history and results of a physical examination, those with diseases not routinely associated with high ammonia concentrations, and those suspected of having or confirmed to have portosystemic shunts or other types of liver disease. Portosystemic shunting was suspected on the basis of breed, age, clinical signs (eg, stunted growth, abnormal neurologic signs, and signs of urinary tract conditions), and results of laboratory analyses suggestive of hepatic dysfunction (eg, high bile acid concentrations; low concentrations of urea, albumin, glucose, or cholesterol; low mean corpuscular volume; and ammonium biurate crystalluria). A diagnosis of portosystemic shunt was confirmed by use of nuclear scintigraphy or CT. Other types of liver disease (eg, hepatocellular injury or cholestatic liver disease) were diagnosed on the basis of high liver enzyme activities and an abnormal appearance of the liver during abdominal ultrasonography. Owner consent was obtained before inclusion of dogs in the study. The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Tennessee.

Sample collection and analysis

A blood sample (2 mL) was obtained by use of a 20- or 22-gauge needle from the jugular vein of each dog. According to the manufacturer,[13] the ammonia meter[a] used in the study reported here was designed for use with WB, with or without an anticoagulant (EDTA or heparin). Meter strips involved the use of microdiffusion technology and a colorimetric assay (bromcresol green method) to measure ammonia concentrations; measurement range was 10 to 400 µg/dL.[14]

One drop of WB was immediately applied to the reagent pad of a test strip for ammonia measurement as described by the manufacturer.[13] The remainder of the blood sample was promptly placed into a 2-mL EDTA-containing tube,[b] which was mixed by gently inverting the tube 10 times. The tube was then inserted into a cup of ice such that the surface of the blood sample was below the surface level of the ice. The cap of the tube was immediately removed, and 1 drop of blood (EDTA WB) was withdrawn from the sample and applied to the reagent pad of a test strip for incubation and ammonia measurement by use of the ammonia meter. The remainder of the EDTA-anticoagulated WB sample was centrifuged[c] (693 × *g* for 5 minutes). Plasma was harvested from the tube without disturbing the RBCs and transferred to an empty glass tube; tubes containing plasma were placed into a cup of ice. One drop of plasma was collected from the tube and placed on the reagent pad of a test strip for incubation and ammonia measurement by use of the ammonia meter. The remainder of the plasma sample was transported on ice to the Clinical Pathology Laboratory at the University of Tennessee for measurement of ammonia concentration by use of a reference laboratory instrument.[d] Measurement range of the laboratory instrument for ammonia concentrations was 17 to 1,192 µg/dL; samples were not diluted because ammonia concentrations of all samples were within this measurement range. Plasma color was recorded by the investigators and classified

as clear, hemolyzed, or lipemic. Times of sample collection from each dog and measurement of ammonia concentration for each sample were recorded. Sample processing time was then calculated as the difference between collection time and measurement time.

Statistical analysis

Statistical analysis was performed by use of commercial statistical software.[e] Only samples for which all 4 samples (WB, anticoagulated WB, plasma analyzed by use of the ammonia meter, and plasma analyzed by use of the reference instrument) yielded a numeric result were used in data analysis; dogs were excluded when any of the 4 samples yielded a result outside the reportable range of the ammonia meter. Data were assessed for normality by use of the D'Agostino-Pearson test. The Wilcoxon rank sum test was used to evaluate significant differences among sample types. A Bonferroni correction for multiple comparisons (4 groups) was used, and significance was set at values of $P < 0.0125$.

Results

Animals

For the 40 dogs, Yorkshire Terriers (n = 9), mixed-breed dogs (9), and Miniature Schnauzers (5) were the most prevalent. Body weight of the dogs ranged from 1 to 60 kg (median, 7.2 kg). Most dogs (29/40 [72.5%]) had a body weight ≤ 20 kg, and 22 of 40 (55%) dogs had a body weight < 10 kg. There were 10 (25%) healthy dogs, 21 (52.5%) dogs with suspected portosystemic shunting, 3 (7.5%) dogs with high liver enzyme activities (alanine aminotransaminase, alkaline phosphotase, or aspartate transaminase) not attributable to portosystemic shunting, and 6 (15%) with other health issues (brachycephalic syndrome, melanoma, hydrocephalus, or osteosarcoma).

Samples

Seven samples (2 WB, 3 EDTA WB, and 2 plasma measured with the ammonia meter) derived from 5 dogs yielded values outside the reportable range of the ammonia meter (5 low and 2 high values). The 5 samples with low values were from 3 healthy dogs (for both WB and EDTA WB of 2 dogs and for EDTA WB of 1 dog [WB ammonia concentration of that dog was 10 µg/dL]). The other sample types for these 3 dogs yielded numeric results (15, 19, and 18 µg/dL, respectively, for plasma measured with the ammonia meter and 52, 6, and 21 µg/dL, respectively, for plasma measured with the laboratory instrument). One sample with a high value (clear straw-colored plasma measured with the ammonia meter) was from a dog with an extrahepatic portosystemic shunt, whereas the other sample with a high value (clear straw-colored plasma measured with the ammonia meter) was from a healthy dog; both samples were tested by use of a second test strip, and both again yielded a high result. For both dogs, other sample types yielded numeric data (WB = 289 µg/dL, EDTA WB = 252 µg/dL, and plasma measured with the laboratory instrument = 403 µg/dL for the dog with the extrahepatic portosystemic shut; and WB = 36 µg/dL, EDTA WB = 44 µg/dL, and plasma measured with the laboratory instrument = 41 µg/dL for the healthy dog). All data for these 5 dogs were excluded from statistical analysis. Data for 1 additional dog were omitted because improper loading of the strip yielded a suspect value for the WB sample. Hence, data for 34 dogs were used for statistical analysis.

Analysis of samples

All data were nonparametric. Minimum, maximum, and median ammonia concentrations for each sample type were summarized **(Table 1)**. The ammonia concentration for the WB sample of dogs was determined. The range for healthy dogs was 10 to 36 µg/dL (median, 11.5 µg/dL), the range for dogs with suspected portosystemic shunting or high liver enzyme activities was 20 to 373 µg/dL (median, 83.5 µg/dL), and the range for dogs with nonhepatic diseases was 12 to 29 µg/dL (median, 13.5 µg/dL).

Ammonia concentrations did not differ significantly between WB and EDTA WB ($P = 0.875$) or between plasma samples measured with the ammonia meter and the laboratory instrument ($P = 0.229$; **Figure 1**). Significant differences in median ammonia concentrations were detected between WB and EDTA WB and plasma samples. There were significantly ($P < 0.001$) higher median ammonia concentrations in plasma samples (regardless of method of measurement), compared with median ammonia concentrations in WB and EDTA WB samples.

Values for sample processing time were determined **(Table 2)**. For EDTA WB and plasma samples, storage time reflected the amount of time each specimen was on ice. Processing time for plasma samples was significantly greater than for WB samples.

Color of all 34 plasma samples was evaluated. Most (28 [82.4%]) plasma samples were recorded as straw colored and clear. There were 3 (8.8%) plasma samples recorded as hemolyzed, 2 (5.9%) recorded as lipemic, and 1 (2.9%) recorded as both hemolyzed and lipemic. The 3 hemolyzed samples were from 3 Yorkshire Terriers (body

Table 1—Ammonia concentration in various sample types obtained from 34 dogs and measured by use of an ammonia meter or a reference laboratory instrument.

Sample type	Minimum (µg/dL)	Maximum (µg/dL)	Median (µg/dL)
WB	10	373	51[a]
EDTA WB	10	373	58[a]
Plasma*			
Ammonia meter	16	397	92[b]
Laboratory instrument	12	520	79[b]

*Plasma was obtained from EDTA-anticoagulated WB.

[a,b]Values with different superscript letters differ significantly ($P < 0.001$).

EDTA WB = EDTA-anticoagulated WB.

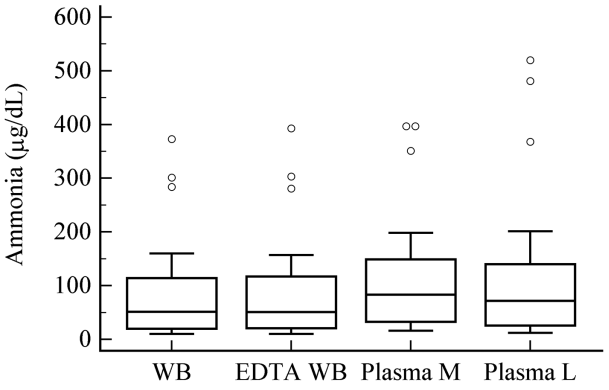

Figure 1—Box-and-whisker plot of ammonia concentrations in various sample types obtained from 34 dogs. Each box represents the values from the 25th to 75th percentiles, the horizontal line in each box represents the median, whiskers represent the minimum to maximum values (excluding outliers), and circles represent outliers. EDTA WB = EDTA-anticoagulated WB. Plasma L = Plasma obtained from EDTA-anticoagulated WB and measured with a laboratory instrument. Plasma M = Plasma obtained from EDTA-anticoagulated WB and measured with an ammonia meter.

Table 2—Sample processing time for various sample types obtained from 33 dogs and measured by use of an ammonia meter or a reference laboratory instrument.

Sample type	Minimum (minutes)	Maximum (minutes)	Median (minutes)
WB	3	4	3.0[a]
EDTA WB	3	6	4.0[b]
Plasma*			
Ammonia meter	6	15	13.0[c]
Laboratory instrument	10	57	16.5[d]

Sample processing time was calculated as the difference between time of collection of the original venous blood sample and time of measurement of the ammonia concentration; it was not recorded for 1 dog.

[a–d]Values with different superscript letters differ significantly ($P < 0.001$).

See Table 1 for remainder of key.

weight, 1.0, 3.3, and 3.8 kg, that had a patent ductus arteriosus, a portosystemic shunt, and a portosystemic shunt, respectively), and the sample recorded as hemolyzed and lipemic was from a healthy mixed-breed dog (body weight, 7.7 kg). The number of hemolyzed samples was too small to permit statistical analysis between hemolyzed and nonhemolyzed samples and interpretation of whether hemolysis caused interference with measurement of ammonia concentrations. Ammonia concentrations of the hemolyzed and lipemic sample were low for WB and EDTA WB, 15 µg/dL for plasma measured with the ammonia meter, and 52 µg/dL for plasma measured with the laboratory instrument.

For plasma samples measured with the laboratory instrument, data for ammonia concentration and the instrument hemolysis index (a unitless numeric index calculated by the analyzer that represented the degree of sample hemolysis) were not normally distributed. The Spearman rank correlation coefficient between these 2 variables was –0.406.

Discussion

Analyte stability and sample handling logistics are major factors limiting the use of ammonia measurement. Contamination of laboratory air and glassware by ammonia-containing detergents, sweat, and cigarette residues from clinical workers or laboratory personnel and hemolysis (erythrocyte ammonia concentration is higher than plasma ammonia concentration) may all affect results.[9] Clinicians submitting samples and laboratories measuring plasma ammonia concentrations must ensure appropriate sample handling (sample cooling, separation of plasma, timely analysis, and minimal exposure to air and potential contaminants). This may lead to increased costs of supplies and time associated with performing the test. Sample stability makes it challenging for practitioners to send samples to laboratories for ammonia measurement.

The study reported here revealed that the ammonia concentration in WB was not significantly ($P = 0.875$) different from the ammonia concentration in EDTA-anticoagulated WB when measured by use of the ammonia meter. From a practical perspective, this implied that 1 drop of blood could be obtained from a catheter hub during catheter placement and used to quickly determine blood ammonia concentration. This would be similar to cage-side measurement of the glucose concentration and could potentially eliminate the need to collect larger volumes of blood, especially from small-breed dogs.

Ammonia concentrations differed significantly between WB and plasma. It is possible that there was a higher ammonia concentration in the plasma samples because of the longer sample processing time. This was particularly true for plasma samples analyzed by use of the reference laboratory instrument, which had to be transferred to the clinical pathology laboratory. Ability of clinical personnel to rapidly transport samples to a laboratory and workload of personnel at a laboratory can affect sample processing times, as was evident in the present study. Delayed separation of cells and plasma affects the ammonia concentration because of the production of ammonia by erythrocytes and leukocytes.[9] This likely did not impact ammonia concentrations in the study reported here because plasma was separated from cells within 6 minutes after blood collection for all samples. Degradation of plasma proteins and amino acids in vivo can also increase plasma ammonia concentrations.[15] It is possible that such degradation may have played a role in the present study. In other studies,[9,16,17] plasma ammonia concentrations increased as storage time increased. In 1 study[9] that involved human volunteers, ammonia concentration increased by 5.6% when plasma samples were stored for 1 hour at 4°C and by 12.8% when stored for 1 hour at 22°C, and ammonia concentrations in WB samples increased by 31.7% and

39.9% when stored for 1 hour at 4° and 22°C, respectively. The authors of that study[9] recommended the use of nonhemolyzed plasma samples and immediate separation of plasma from RBCs after venipuncture to reduce effects of storage time on the ammonia concentration. Although differences in storage time may have led to differences in ammonia concentrations between WB and plasma samples in the present study, it is also possible that some intrinsic characteristic of plasma samples (matrix effect) led to higher ammonia concentrations as measured with the meter. This emphasized the need for different reference intervals for each sample type used (WB vs plasma) to best assess the results obtained.

We elected to use EDTA-anticoagulated plasma for the study because EDTA-anticoagulated plasma was required for the ammonia measurement method of the chemical analyzer[d] at our institution. It is likely we would have obtained similar results had we used heparin-anticoagulated plasma instead of EDTA-anticoagulated plasma for analysis with the ammonia meter, given that the manufacturer recommended either anticoagulant type as appropriate. However, further studies of the meter would be needed to prove that ammonia measurement by use of EDTA-anticoagulated and heparin-anticoagulated canine plasma yields equivalent results.

Hemolysis was noted in 4 samples, all of which were from small-breed dogs. It was likely hemolysis occurred because small patient and vein size predisposed to more difficulty with venipuncture and iatrogenic cell rupture. The ammonia concentration is 3-fold as high in RBCs as in plasma, and studies[15,16] have found hemolysis to be 1 cause of increased ammonia concentrations, although those studies were performed with methods that differed from the methods for the ammonia meter. Although hemolysis did not appear to interfere with the ability of the meter to generate ammonia concentrations from affected samples, further studies are needed to evaluate the effect of hemolysis on ammonia concentration as measured by use of the ammonia meter.

In the study reported here, we elected to use healthy dogs, dogs with suspected elevated ammonia concentrations, and dogs with other clinical conditions so that we could include samples with a wide range of ammonia concentrations. There were 7 samples (obtained from 5 dogs) that had ammonia concentrations outside the measurement range of the meter, but most of the samples had ammonia concentrations that could be determined with the meter. There would be no important clinical consequences for dogs with ammonia concentrations below the low end of the meter's measurement range. Concentrations at the high end of the measurement range may pose a source of limitation in situations whereby response to treatment must be monitored because it might be challenging to determine and monitor the response to treatment if the ammonia concentration remains > 400 µg/dL. The reason that a clinically normal dog had 2 high results for the plasma sample measured by use of the ammonia meter is not known. To our knowledge, there was no meter or strip malfunction or operator error. Ammonia concentrations in WB, EDTA-anticoagulated WB, and plasma determined by use of the laboratory instrument for this dog were within expected limits for a healthy animal, and the chemical analyzer hemolysis index was low (25). It is likely that the high result was erroneous; however, the exact source of the error was not determined. Similar to any result for a point-of-care instrument that is incongruent with clinical findings, evaluating instrument function, repeating the test, and (if still suspect) sending an aliquot of the sample to a reference laboratory are recommended steps.

Investigators of 1 study[10] found an agreement of 83.3% when they compared results for the same ammonia meter used in the present study with results for a reference laboratory method. They identified both constant and proportional bias between results for the ammonia meter (EDTA-anticoagulated WB was used) and the reference laboratory method (EDTA-anticoagulated plasma was used) because the meter consistently underestimated the ammonia concentration relative to the reference laboratory method throughout the range of measured values and disagreement was greater at higher ammonia concentrations.[10] Samples of EDTA-anticoagulated WB and plasma used in that study[10] were processed in parallel within minutes after sample collection, and although storage time for EDTA-anticoagulated plasma was not explicitly reported, it appears to have been less than the storage time for plasma samples evaluated by use of the reference laboratory instrument in the present study. It appears unlikely that sample storage time would explain all of the bias identified in that study[10]; however, a contribution of sample processing factors or matrix effects to the identified bias cannot be definitively excluded.

In the present study, the ammonia meter provided a means for rapidly measuring ammonia concentrations in a wide variety of patients. The use of WB allowed for rapid analysis and eliminated the need for special sample handling procedures. For patients that require frequent measurement of ammonia concentrations, it would be best to evaluate the same sample type (WB or plasma) because there appeared to be differences in ammonia concentrations for WB and plasma. Further studies are warranted to develop reference intervals for WB and plasma ammonia concentrations measured by use of the ammonia meter.

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Footnotes

a. PocketChem BA, Arkray Group, Kyoto, Japan.
b. Microtainer, Becton, Dickinson and Co, Franklin Lakes, NJ.
c. Sero-Fuge centrifuge, Becton, Dickinson and Co, Franklin Lakes, NJ.

d. Cobas c501, Roche Diagnostics, Indianapolis, Ind.
e. MedCal software, version 16.2.0-64 bit, MedCalc Software, Ostend, Belgium.

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