Brief Communication
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Comparison of Four Refractometers for the Investigation of the Passive Transfer in Beef Calves

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Background: Failure of passive transfer (FPT) in beef calves can be detected by refractometry. Nevertheless, different models of refractometers are available, and few studies compare them for the detection of FPT.

Objectives: To compare the accuracy of 4 different refractometers for measuring serum total protein concentrations in comparison with results obtained by the biuret method and, based on the serum IgG threshold of 1,600 mg/mL, to determine, for each refractometer, the optimal serum protein concentration's lowest threshold for successful passive transfer.

Animals: One hundred and eight healthy beef calves, 3–8 days of age.

Methods: Observational study. The concentrations of serum total proteins were determined with 4 different models of refractometers and compared with the biuret method by a Bland–Altman statistical method. The optimal serum protein concentration's lowest threshold for successful passive transfer was determined for each refractometer by receiver operating characteristic (ROC) analysis. In addition, the serum immunoglobulin G (IgG) concentration was compared with the serum gamma-glutamyl transferase (γ-GT) activity and with the total immunoglobulin concentration.

Results: The refractometric measurements were highly correlated with those obtained by the biuret method. Serum total protein concentration threshold values of 56, 58, 54, and 56 g/L were found respectively for the Atago, Atago ATC, Wolf ATC, and digital ATC refractometers. Immunoglobulins were highly correlated with IgG, whereas γ-GT only reflected colostrum uptake by the calf.

Conclusions and Clinical Importance: All refractometers could be used for the assessment of passive transfer using their individual serum protein concentration threshold.

Key words: Colostral immunity; Failure of passive transfer; Refractometry; Serum protein.

Despite progress in veterinary medicine, total or partial failure of passive transfer (FPT) still commonly occurs with prevalence ranging between 11 and 31% in North America[1] in beef calves, with prevalence of approximately 40% in dairy calves.[2] Calves presenting with FPT are more susceptible to infectious diseases and have higher morbidity and mortality rates. The detection of FPT, at an individual level as well as at herd level, therefore is fundamental for improving the health and management of calves. The use of refractometry for detecting FPT frequently was investigated because this method is inexpensive, quick, and easy to perform under farm conditions. The refractometric measurement of serum total protein concentration is based on the refraction index (n) of the serum and its conversion to serum total protein concentration using a known conversion factor. Depending on the manufacturer, 2 different conversion factors are applied, 1 determined by Wolf and the other by the Atago Corporation. The Wolf conversion factor is reputed to give higher serum total protein concentrations compared with the Atago conversion factor.[3] As the index of refraction is influenced by the temperature of the solute, Automatic Temperature Compensation (ATC) refractometers were commercialized to avoid the impact of potential temperature variations on the results. Recently, digital refractometers have been available on the market but, to our knowledge, only 1 study has been performed to assess FPT with this type of refractometer.[4]

The objectives of this study were to compare the accuracy of 4 different refractometers for measuring serum total protein concentration in beef calves and, based on the serum IgG concentration threshold of 1,600 mg/dL, to determine the optimal threshold of serum protein concentration above which adequate passive transfer can be concluded. In addition, the gamma-glutamyl transferase (γ-GT) activity and the total immunoglobulin (Ig) concentration in serum were compared with the serum IgG concentration for assessment of passive transfer in beef calves.

Abbreviations:

γ-GT	gamma-glutamyl transferase
ATC	automatic temperature compensation
AUC	area under the curve
ELISA	enzyme-linked immunosorbent assay
FPT	failure of passive transfer
HPLC	high-performance liquid chromatography
IgG	immunoglobulin G
Ig	immunoglobulin
ROC	receiver operating characteristic
Se	sensitivity
Sp	specificity
SRID	single radial immunodiffusion

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Materials and Methods

Calves and Samples

One hundred and eight Belgian Blue beef calves, 60 females and 48 males, originating from 3 beef farms in the Liege area and aged between 3 and 8 days were included in this study. All calves were healthy and normally hydrated at the time of sampling. Blood sample was collected from the jugular vein. Serum was collected after centrifugation (mini-centrifugal machine, 1,500 × ***g*** for 10 minutes) and stored at −20°C before analysis.

Experimental Protocol

Serum total protein concentration was determined using 4 different models of refractometers. The first 2 refractometers were ATC handheld refractometers, 1 using the Atago conversion factor,[a] and the other using the Wolf conversion factor.[b] The third refractometer was a standard laboratory refractometer without automatic temperature compensation using the Atago conversion factor,[c] whereas the last was a digital ATC handheld refractometer.[d] All these refractometers had a precision of 2 g/L.

All refractometric measurements, except those for interobserver variability, were performed at room temperature by the same person. The observer was blinded to previous results and the reading sequence was randomized for each refractometer. Before use, all refractometers were zeroed with distilled water and the prism of each refractometer was cleaned with distilled water before each reading.

The reference method used for comparison was the biuret method.[e] Serum protein electrophoresis was performed on an agarose gel[f] to calculate the Ig concentration. Serum IgG concentration was assessed by **HPLC**[g] and serum γ-GT activity was determined using a commercially available kit.[h]

Statistical Analysis

Validation of the Refractometers. Different statistical analyses were performed for the validation of the refractometers. The Pearson correlation between the individual measures (n = 108) was computed and compared using a test based on Fischer's Z-transformation. The agreement between biuret and each refractometer model was determined by Bland–Altman plots. Individual differences between biuret and refractometric measurements were tested in a mixed linear model, which included random effects for the animal, age at sampling, and refractometer effects (Proc Mixed). In addition, a conditional dependence model based on a Bayesian model of test accuracy also was performed assuming that the biuret method could not be considered as the gold standard method for the protein measurement. The biuret and each of the 4 refractometric tests (= 4 combinations of 2 assays) were applied to each sampled animal, and the resulting data were cross-classified in four 2 × 2 tables. Calves with serum IgG concentrations <1,600 mg/dL were considered to have FPT, and the thresholds for the 4 measurements of serum total protein concentration were set at 56 g/L. For each animal and each combination (i = 1 to 4), a conditional dependence model[5] was considered to take into account the dependence between the 2 test outcomes. The n_i observations and the data vector for the joint test results $y_i = (y_{11i}, y_{12i}, y_{21i}, y_{22i})$, where y_{11i} (y_{22i}) was the number of sampled animals that tested positive (negative) on the ith test and on the biuret, and y_{12i} (y_{21i}) was the number of animals that tested positive (negative) on the ith test and negative (positive) on biuret. The data vector y_i was assumed to have multinomial probabilities given by

$$p_{11i} = p\,[(SE_i\ SE_b + cov^+)] + (1-p)\,[(1-SP_i)\,(1-SP_b) + cov^-]$$
$$p_{12i} = p\,[(SE_i\,(1-SE_b) + cov^+)] + (1-p)\,[(1-SP_i)\,SP_b + cov^-]$$
$$p_{21i} = p\,[((1-SE_i)\,SE_b + cov^+)] + (1-p)\,[SP_i\,(1-SP_b) + cov^-]$$
$$p_{22i} = p\,[((1-SE_i)(1-SE_b) + cov^+)] + (1-p)\,[SP_i\,SP_b + cov^-].$$

Because prior information was not available, prevalence of AFP, the 2 sensitivities, and the 2 specificities all were assumed to have independent uniform [0, 1] prior distributions. Similarly, covariances were given a bounded domain using uniform prior distributions:

$$Cov^- \sim dunif[(SP_i-1)*(1-SP_b)],\ min[(SP_i,SP_b)-(SP_i*SP_b)]$$
$$Cov^+ \sim dunif[(SE_i-1)*(1-SE_b)],\ min[(SE_i,SE_b)-(SE_i*SE_b)]$$

We performed an initial burn-in of 500 iterations, followed by a subsequent 9,500 MCMC iterations, to generate the parameter estimates. We checked convergence by running a minimum of 3 multiple chains and verifying the results provided by the Sample Monitor Tool of Winbugs.[i]

For intraobserver and interobserver variability, serum total protein concentration was assessed using each refractometer in 15 serum samples by 5 different observers under farm conditions. Intraobserver and interobserver variability was estimated by calculating the intraclass correlation coefficient (ICC).

Determination of Serum Protein Concentration Threshold Values. In this study, calves with serum IgG concentration <1,600 mg/dL were considered to have FPT. For each refractometer, data were subjected to receiver operating characteristic (ROC) analysis to select the most appropriate serum protein concentration threshold for having adequate transfer of colostral immunity. Sensitivity (Se), specificity (Sp), area under curve (AUC), proportion of correctly classified calves, and Kappa statistic were used to assess the accuracy of each serum protein concentration threshold value. The percentage of correctly classified calves was calculated as follows: $[Se \times p] + [(Sp) \times (1-p)]$, where p represents the prevalence of FPT observed in the studied population.

Relationship between Serum IgG, γ-GT Activity, and Ig. Pearson correlation coefficients were computed to assess the relationship between the serum IgG and the γ-GT activity and between the serum IgG and Ig. All calculations were made using the program SAS.[j] Differences were considered statistically significant at the $P < .05$ level.

Results

Validation of the Refractometers

Serum total protein concentrations ranged from 48 to 83 g/L for the biuret method, from 46 to 80 g/L for the Atago and Atago ATC refractometers, from 45 to 78 g/L for the Wolf ATC refractometer, and from 45 to 79 g/L for the digital ATC refractometer. The serum total protein concentrations determined by each refractometer were highly correlated with those obtained by the biuret method. The correlation coefficients were 0.961 for the Atago ATC and digital ATC refractometers, 0.953 for the Wolf refractometer, and 0.964 for the Atago refractometer. No significant difference was found among the correlation coefficients.

Using the Bland–Altman method, the mean bias between refractometric and biuret measurements was−4.4 ± 2.0 g/L, −4.1 ± 2.0 g/L, −6.4 ± 2.3 g/L, and−5.0 ± 2.2 g/L for the Atago, Atago ATC, Wolf ATC, and digital ATC refractometers, respectively. Average individual bias, corrected for age at sampling and calf effect (mixed model), were−3.0 ± 0.3 g/L, −2.7 ± 0.3 g/L, −5.0 ± 0.3 g/L and −3.6 ± 0.3 g/L for the Atago, Atago ATC, Wolf ATC, and digital ATC refractometers, respectively. The bias obtained with the Wolf refractometer was significantly greater than those obtained with the other refractometers. Median values for sensitivity and specificity (and their corresponding 95% probability interval) for the combinations of all assays are presented in Table 1. The intraclass correlation coefficients (ICC) were 0.922 for the Atago, Atago ATC, and Wolf ATC refractometers, and 0.995 for the digital ATC refractometer.

Selection of Serum Total Protein Concentration Threshold Values

Mean serum IgG concentration for the 108 calves was 2,310 ± 750 mg/dL (range, 730–4,530 mg/dL). Based on the threshold point of 1,600 mg/dL of IgG, 18 calves (16.7% of all calves) had FPT. To have a successful passive transfer, serum total protein concentration threshold values of 56, 58, 54, and 56 g/L were found for the Atago, Atago ATC, Wolf ATC, and digital ATC refractometers, respectively. Sensitivity, specificity, AUC, proportion of correctly classified calves, and Kappa statistic for the serum total protein concentration threshold values of each refractometer are presented in Table 2.

Relationship between IgG, γ-GT, and Ig

The IgG and Ig were highly correlated with a correlation coefficient of 0.956, whereas γ-GT was only poorly correlated with IgG (0.495).

Analysis of the electrophoretic profiles shows a fusion of the beta-2 and gammaglobulin peaks on the third and fourth days of life, owing to passive transfer of colostral IgG after colostrum intake. A progressive separation of the 2 peaks begins around the end of the first week of life.

Discussion

All refractometers had the same accuracy for serum total protein concentration assessment in calves despite a significantly higher bias for the Wolf ATC. Choosing the serum IgG of 1,600 mg/dL as threshold for diagnosing FPT, the use of the specific serum protein threshold for each refractometer allows the investigation of FPT by the bovine practitioner. Ig is highly correlated with the IgG concentration, whereas γ-GT activity is poorly correlated with the IgG concentration.

The high correlation coefficient between the refractometric and biuret measurements (0.953–0.964) obtained with each refractometer indicates a nearly linear relationship. To our knowledge, few data concerning the comparison between refractometric and biuret methods in cattle are available in the literature. Quigley[6] described a correlation coefficient of 0.92 for calves aged about 3–5 days, but all measurements were performed on plasma, and not on serum. In adult cattle, McSherry and Al-Baker[7] reported a correlation coefficient of 0.982, and Caprita and Caprita[8] reported a correlation coefficient of 0.990 for plasmatic protein values, which are similar to results obtained in the present study.

In our study, correlation coefficients were not significantly different among the 4 refractometers, indicating similar accuracy for measuring serum total protein concentrations. This result is in agreement with the

Table 1. Median values for the sensitivity and specificity (and their corresponding 95% confidence interval) for the combinations of all assays.

Combinations	Sensitivity (%)	95% PI	Specificity (%)	95% PI
Biuret/Atago	89.9/83.3	80.7–96.6/73.7–91.7	83.0/90.1	62.4–96.0/74.9–97.8
Biuret/Atago ATC	90.3/82.8	82.0–96.8/73.5–91.3	83.3/90.1	62.7–96.0/74.2–98.0
Biuret/Wolf ATC	89.5/84.6	80.7–96.0/75.5–92.5	83.5/89.8	63.7–96.2/73.6–97.8
Biuret/Digital ATC	89.6/84.0	86.7–96.3/74.4–92.0	83.3/89.9	63.8–95.9/74.8–97.8

Table 2. Sensitivity, specificity, area under curve, proportion of correctly classified calves, and Kappa statistic for the serum total protein concentration threshold values of the Atago, Atago ATC, Wolf ATC, and digital ATC refractometers.

Refractometer	Atago	Atago ATC	Wolf ATC	Digital ATC
Threshold values (g/L)	56	58	54	56
Sensitivity (%)	100	100	100	100
95% CI	82–100	82–100	82–100	82–100
Specificity (%)	91.1	90.0	93.3	92.2
95% CI	83.4–95.4	82.1–94.6	86.2–96.9	84.8–96.2
AUC	0.984	0.986	0.989	0.987
Proportion of correctly classified calves (%)	92.6	91.7	94.4	93.5
Kappa statistic	0.77	0.75	0.82	0.80
95% CI	0.63–0.92	0.60–0.90	0.69–0.96	0.66–0.94

study of Calloway et al,[9] which identified a similar ability to detect FPT with 3 different refractometers, and with the study of Wallace et al,[4] which found a correlation coefficient of 0.980 between the serum total solid concentrations measured by 2 different refractometers (Atago ATC versus digital refractometer). In contrast to the findings of George,[3] no significant difference of serum total protein concentration was found between the Wolf and the Atago refractometers. This absence of significant difference could be explained by the range of measurements performed in our study. Indeed, according to George,[3] Atago refractometers give approximately 5 g/L lower protein results, but this occurs mainly in the 5–30 g/L range of measurement.

Our results show that compared with the biuret method, all refractometers underestimated the serum total protein concentrations by about 2.7–5.0 g/L. This difference in protein measurement agrees with the study of Green et al,[10] in which mean total protein values measured in cattle by the biuret method were higher by about 3 g/L compared with results obtained by refractometry. This bias could be explained by the heterogeneity of the serum proteins involved, leading to interference with the specific technique of measurement of both methods.

The bias obtained with the Wolf ATC refractometer was significantly higher than that obtained using the other refractometers. This finding is probably because of lower protein results obtained with the Wolf ATC refractometer compared with the other refractometers, although the refractometric measurements between the Wolf ATC and the other refractometers were not significantly different. Moreover, the difficulty of correctly visualizing the demarcation line with the Wolf ATC refractometer during measurement could increase the risk of erroneous results. For all combinations, median sensitivities and specificities obtained with the Bayesian model of test accuracy were relatively close, indicating that these 2 methods of protein measurements were conditionally dependent. This conditional dependence reflects the high correlation coefficients obtained between the biuret method and refractometry using the Bland–Altman method. Even if values of sensitivities and specificities obtained with noninformative prior data are elevated, they could be improved by the use of more precise prior data.

The high values of intraclass correlation coefficient (ICC) indicate excellent reliability of the serum protein measurement with each refractometer, under farm conditions. In our study, the serum IgG concentration of 1,600 mg/dL was chosen as the threshold point below which calves were considered to have FPT. This IgG concentration is much higher than the generally used concentration of 1,000 mg/dL.[9] However, the choice of an appropriate serum IgG threshold concentration for the maintenance of health in calves depends on many factors, such as the environment, the presence of infection, the breed or the prevalence of FPT in the studied population. For example, several researchers have suggested serum IgG concentrations of 500 mg/dL[11] and 800 mg/dL[12] for decreasing mortality linked to septicemia. Likewise, for decreasing preweaning morbidity and mortality, serum IgG concentrations of 800 mg/dL,[13] 1,000 mg/dL,[14] 1,600 mg/dL,[15] and even 2,400 mg/dL[16] are found in the literature.

Our choice of a higher serum IgG concentration was motivated by the good colostral immunity transfer observed in these Belgian Blue calves resulting in a low number of calves ($n = 2$) having serum IgG concentration <1,000 mg/dL. Serum total protein concentration threshold values obtained in our study are higher than the value of 52 g/L generally used,[9] but this concentration was determined for a serum IgG concentration of 1,000 mg/dL and mainly in dairy calves where FPT occurs more frequently.

The sensitivity, the specificity, the AUC, proportion of correctly classified calves, and Kappa statistic were not significantly different among refractometers using their individual serum concentration protein thresholds. However, under field conditions, it is easier to use only 1 serum protein concentration threshold, independently of the type of refractometer used. When using the same serum protein concentration threshold of 56 g/L for the 4 refractometers, sensitivity and specificity will only change for the Atago ATC (Se = 88.8%, Sp = 93.3%) and for the Wolf ATC refractometers (Se = 100%, Sp = 70%). When this serum protein concentration threshold is used for the 4 refractometers, the digital ATC (followed by the Atago) is the most accurate with the best sensitivity and specificity.

Specificities obtained with our serum protein concentration thresholds are in the same range as those obtained by Calloway et al,[9] whereas sensitivities are higher. Nevertheless, it is possible that with temperature variations at the time of measurement, sensitivity and specificity of the non-ATC Atago refractometer would change, with probably a decrease in its accuracy.

The AUC between 0.984 and 0.989 indicates very good performance of refractometry for diagnosing FPT in calves. The low prevalence of FPT observed in our study (about 17%) leads to a high proportion of calves being correctly classified. The kappa statistic values between 0.75 and 0.82 indicate good agreement among the refractometers for the diagnosis of FPT in calves.

The low correlation coefficient between IgG and γ-GT activity indicates that γ-GT activity is not a valuable test for the evaluation of the passive transfer in calves. Perino et al[13] and Wilson et al[17] obtained similar results with correlation coefficients of 0.41 and 0.438, respectively. However, Parish et al[18] and Güngör et al[19] found better correlation coefficients of 0.63 and 0.57, respectively, for dairy calves <10 days of age. One explanation for this disparity of results could be the important interindividual variations of γ-GT activity in the dams' colostrum[20] and the absence of significant correlation of γ-GT activity between calf plasma and ingested colostrum.[20] Perino et al[13] suggest that the γ-GT activity does not indicate the amount of colostrum absorbed, and that some degree of activity >200 IU/L simply reflects that the calf has absorbed some colostrum.

As described in another study,[21] the high correlation coefficient (0.956) between serum IgG and Ig obtained in our calves indicates a very good linear relationship. In fact, Ig represents the electrophoretic portion that contains the immunoglobulins transferred via the colostrum. To our knowledge, the fusion of the beta-2 and gammaglobulin peaks during the first days of life, and the separation of these 2 peaks during the first week were only described by Godeau et al.[22] Therefore, the interpretation of the serum Ig obtained by electrophoresis for the assessment of passive transfer during the first week of life must take the age of the calves into account. Additional studies are needed for a better understanding of this peak fusion and separation during the first week of life.

Footnotes

[a] RF 5612 ATC refractometer, Euromex, Arnhem, Netherlands
[b] Rhino Vet 360 ATC refractometer, Reichert Analytical Instruments, Depew, NY
[c] Atago SPR-T2 refractometer, Atago Co, Tokyo, Japan
[d] RD 5712 refractometer, Euromex, Arnhem, Netherlands
[e] Total Protein FS, Diagnostic Systems Gmbh, Holzheim, Germany
[f] SAS-MX Serum Protein, Helena Biosciences Europe, Gateshead, UK
[g] Biokema, Lausanne, Switzerland
[h] Gamma-GT SL, Elitech France, Puteaux, France
[i] WinBUGS, 1.4 Edition
[j] Statistical Analysis System, 6.03 Edition

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