

Comparison of refractometers and test endpoints in the measurement of serum protein concentration to assess passive transfer status in calves

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Objective—To evaluate 3 refractometers for detection of failure of passive transfer (FPT) of immunity in calves, and assess the effect of refractometric test endpoints on sensitivity, specificity, and proportion of calves classified correctly with regard to passive transfer status.

Design—Prospective study.

Animals—90 calves.

Procedure—Blood samples were obtained from calves that were < 10 days old. Serum IgG concentration was determined by use of a radial immunodiffusion assay. Accuracy of 3 refractometers in the prediction of serum IgG concentration was determined by use of standard epidemiologic methods and a linear regression model.

Results—At a serum protein concentration test endpoint of 5.2 g/dL, sensitivity of each refractometer was 0.89 or 0.93, and specificity ranged from 0.80 to 0.91. For all refractometers, serum protein concentration test endpoints of 5.0 or 5.2 g/dL resulted in sensitivity > 0.80, specificity > 0.80, and proportion of calves classified correctly > 0.85. Serum protein concentrations equivalent to 1,000 mg of IgG/dL of serum were 4.9, 4.8, and 5.1 g/dL for the 3 refractometers.

Conclusions and Clinical Relevance—The refractometers, including a nontemperature-compensating instrument, performed similarly in detection of FPT. Serum protein concentration test endpoints of 5.0 and 5.2 g/dL yielded accurate results in the assessment of adequacy of passive transfer; lower or higher test endpoints misclassified larger numbers of calves. (*J Am Vet Med Assoc* 2002;221:1605–1608)

Calves have low serum immunoglobulin concentrations at birth.¹ Initially, a calf's requirement for immunoglobulins is met by the ingestion and absorption of colostral immunoglobulins; this process is referred to as passive transfer of maternal immunity. Calves that assimilate less than optimal concentrations of maternal colostrum-derived immunoglobulins are at high risk for illness and death.²⁻⁶ Additionally, the production potential of these calves is compromised as measured by growth rate and subsequent milk production.⁷⁻⁹

Numerous assays are available to assess passive immunoglobulin transfer status in neonatal calves;

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these include serum protein determination by use of refractometry,^{2,10} zinc sulfate¹¹ and sodium sulfite turbidimetry,¹⁰ and immunoassays¹²; measurement of g-glutamyl transferase activity^{13,14}; and glutaraldehyde coagulation tests.¹⁵ Assay results are also used to assess the effectiveness of farm practices in achieving adequate passive transfer of immunoglobulins in calves.

Refractometry is a commonly used tool in veterinary practice and provides an indirect measurement of serum protein concentration. Because immunoglobulins constitute a large proportion of the protein in neonatal calf serum and nonimmunoglobulin protein concentration of calf serum is relatively constant, refractometry provides a close representation of the serum immunoglobulin concentration. Previous studies^{2,10,16} that assessed refractometry as a tool for evaluation of passive transfer status in neonatal calves used temperature-compensating refractometers. Recommended test endpoints for serum protein concentration have varied from 5.0 to 6.0 g/dL.^{2,10,16a} To the authors' knowledge, comparison of different models of refractometer in the assessment of passive transfer status has not been investigated.

The purpose of this study was to compare the performance of 3 models of refractometer in detection of failure of passive transfer (FPT) in calves. Two temperature-compensating refractometers and 1 nontemperature-compensating refractometer were used to measure protein concentrations in serum samples obtained from blood of neonatal calves for evaluation of passive transfer status. Furthermore, the effect of test endpoint on the performance of each refractometer in the assessment of passive transfer status in calves was determined.

Materials and Methods

Calves—Ninety beef and dairy calves < 10 days of age were used in the study; clinically ill and overtly healthy calves were included. Calves were examined by clinical services at the University of Missouri College of Veterinary Medicine.

Experimental protocol—Blood samples were collected by jugular venipuncture; collection tubes contained no anticoagulant. Serum was obtained from clotted blood samples < 18 hours after collection and frozen into aliquots.

Serum IgG concentration was determined via radial immunodiffusion.¹⁷ Assay plates used to measure serum IgG concentration were prepared by dissolving 1% agarose^b in sodium barbital buffer^c containing 0.1% sodium azide.^d Rabbit anti-bovine IgG^e (1%) was added to the agarose solution; this solution was transferred to 10-cm Petri dishes (11 mL/dish). After the agarose solidified, 3-mm diameter wells were cut in the agar. Serum samples were diluted 1:100 with sodium barbital buffer; 5- μ L aliquots of diluted serum samples were added to wells in

the assay plates. Plates were incubated at 23°C for 72 hours. The diameter of each observed zone of precipitation was measured. Serum IgG concentrations of study samples were determined by comparing the diameters of zones of precipitation with a standard curve ($r^2 = 0.96$) generated by assay of serial dilutions of a commercially available standard solution of bovine IgG.¹

Serum protein concentration was determined by use of 2 temperature-compensating refractometers^{a,b} (A and B) and 1 nontemperature-compensating refractometer^c (C).

Data analyses—Sensitivity and specificity of each of the 3 refractometers were calculated by use of standard epidemiologic methods.¹⁸ In this calculation, calf passive transfer status was assessed on the basis of serum IgG concentration. Failure of passive transfer was defined as serum IgG concentration < 1,000 mg/dL. Calves with serum IgG concentrations \geq 1,000 mg/dL were considered to have had adequate passive transfer.

Calves with serum protein concentrations less than the defined serum protein concentration endpoint were classified as test positive, and calves with serum protein concentrations greater than or equal to the endpoint were classified as test negative. Sensitivity was defined as the proportion of calves with serum IgG concentration < 1,000 mg/dL that were test positive. Specificity was defined as the proportion of calves with serum IgG concentration \geq 1,000 mg/dL that were test negative. These calculations used serum protein concentration endpoints that spanned the range of recommended test endpoints; selected endpoints included 4.8, 5.0, 5.2, 5.5, and 6.0 g/dL. The proportion of calves in the study population that were correctly classified by use of each refractometer and test endpoint was calculated for values of prevalence from 0.00 to 1.00 by use of the following formula:

$$\text{Proportion calves correctly classified} = (\text{sensitivity}) \times (\text{prevalence}) + (\text{specificity}) \times (1 - \text{prevalence})$$

Simple linear regression models that predicted calf serum IgG concentration as a function of serum protein concentration were also constructed.¹⁹ A separate model was constructed for each refractometer. The regression coefficient, *P* value, and correlation coefficient were reported for each regression model. The serum protein concentration equivalent to 1,000 mg of IgG/dL was calculated. For all analyses, values of *P* < 0.05 were considered significant.

Results

Of the 90 calves in this study, 45 had serum IgG concentration < 1,000 mg/dL. Mean \pm SD serum IgG concentration was 1,182 \pm 1,128 mg/dL (range, 0 to 3,041 mg/dL). At a test endpoint of 5.2 g of protein/dL of serum (Table 1), sensitivities of the refractometers

were 0.89 (A [temperature-compensating] and C [nontemperature-compensating]) and 0.93 (B [temperature-compensating]). At the same test endpoint, specificities of the refractometers ranged from 0.80 (B) to 0.84 (C). Test endpoints of 5.0 and 5.2 g/dL consistently resulted in adequate sensitivity (> 0.80), specificity (> 0.80), and proportion of calves classified correctly (> 0.85). For all refractometers, the proportion

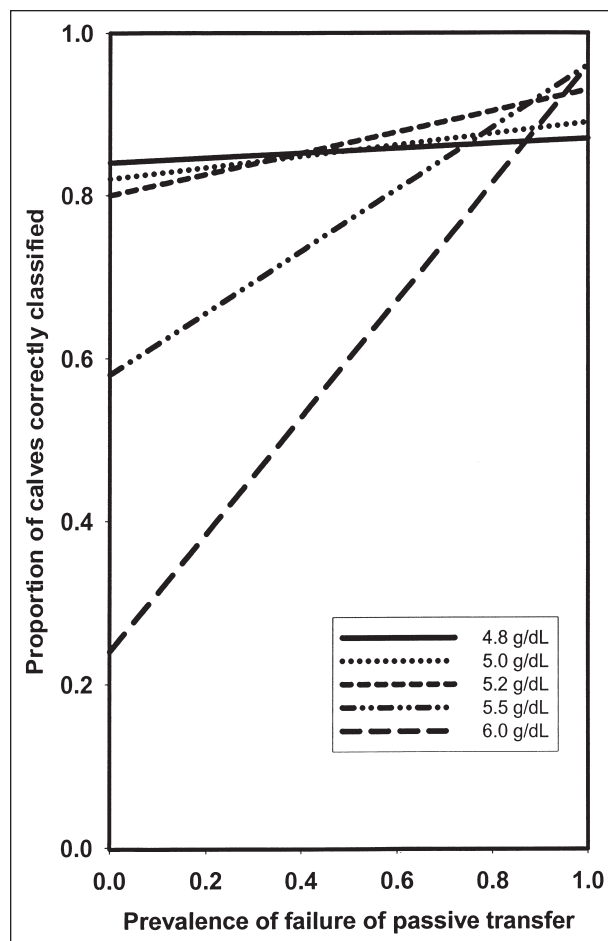


Figure 1—Proportions of affected calves correctly identified by use of refractometry with various test endpoints and prevalences of failure of passive transfer (serum IgG < 1,000 mg/dL).

Table 1—Performance of 3 refractometers at various serum protein concentration test endpoints in the assessment of adequacy of passive transfer of immunity in 90 calves

Serum protein (g/dL)	Refractometer A			Refractometer B			Refractometer C		
	Se	Sp	Proportion of calves correctly classified	Se	Sp	Proportion of calves correctly classified	Se	Sp	Proportion of calves correctly classified
4.8	0.78	0.91	0.85	0.87	0.84	0.86	0.62	0.96	0.79
5.0	0.89	0.84	0.87	0.89	0.82	0.86	0.80	0.91	0.86
5.2	0.89	0.82	0.86	0.93	0.80	0.87	0.89	0.84	0.86
5.5	0.93	0.56	0.75	0.96	0.58	0.77	0.91	0.78	0.85
6.0	0.96	0.22	0.59	0.96	0.24	0.60	0.96	0.44	0.70

Sensitivity and specificity were determined by comparing test results with results obtained by use of radial immunodiffusion assay. Calves with serum IgG concentration, determined with the radial immunodiffusion assay, \geq 1,000 mg/dL were considered to have adequate passive transfer of immunity, and calves with serum IgG concentration < 1,000 mg/dL were considered to have failure of passive transfer. For calculation of sensitivity and specificity, a positive test result was considered to be indicative of failure of passive transfer and a negative test result was considered to be indicative of adequate passive transfer of immunity. Se = Sensitivity. Sp = Specificity.

Table 2—Results of linear regression models that predict serum IgG concentration as a function of serum protein concentration measured by use of 3 refractometers

Refractometer	Y-intercept (mg of IgG/dL of serum)	Regression coefficient (mg of IgG/dL of serum/ g of protein/dL of serum)	r ²	P value
A	-3645	943	0.587	< 0.001
B	-3495	929	0.596	< 0.001
C	-2990	778	0.546	< 0.001

of calves correctly classified using the 5.2 g/dL test endpoint was greater than the proportion of calves correctly classified using a 5.5 g/dL test endpoint for FPT prevalences < 0.87. For each refractometer evaluated in this study, the proportion of calves correctly classified using a 5.2 g/dL test endpoint exceeded the proportion of calves correctly classified using a 6.0 g/dL test endpoint for all FPT prevalences < 0.90. For illustrative purposes, the proportion of calves correctly classified by use of refractometer B at test endpoints of 4.8, 5.0, 5.2, 5.5, and 6.0 g/dL are shown (Fig 1).

All 3 of the refractometers examined had similar y-intercepts, regression coefficients, and correlation coefficients (Table 2). Serum protein concentrations equivalent to 1,000 mg of IgG/dL were 4.9, 4.8, and 5.1 g/dL for refractometers A, B, and C, respectively.

Discussion

A serum IgG concentration of 1,000 mg/dL was selected as the threshold value below which FPT was considered to have occurred on the basis of a previous study.¹⁰ Tyler et al² reported results of a study of 3,479 dairy replacement heifers that indicate that the relationship between serum protein concentration and death behaves as a threshold phenomenon. All serum protein concentration strata > 5.5 g/dL had equivalent risk for death.² Furthermore, calves with serum protein concentrations ≥ 5.0 g/dL but < 5.5 g/dL had marginally increased relative risk (1.3) for death. In a previous report¹⁰ from the same group of investigators, a regression model to enable calculation of serum IgG concentrations on the basis of serum protein concentrations was presented. From application of this formula, serum protein concentrations of 5.0 and 5.5 g/dL are estimated to be equivalent to serum IgG concentrations of 890 and 1,340 mg/dL, respectively. Consequently, the serum IgG concentration that provides the optimal threshold for determining adequacy of passive transfer in calves is > 890 mg/dL but < 1,340 mg/dL. Similarly, from results of a study to evaluate the risk for respiratory disease in 410 heifer calves, Virtala et al³ estimated that the optimal serum IgG concentration threshold for determining adequacy of passive transfer is between 800 and 1,300 mg/dL. On the basis of these findings, alternative test endpoints for serum IgG concentration as low as 890 mg/dL or as high as 1,300 mg/dL could have been justified in our study, whereas test endpoints > 1,300 mg/dL could not. The selection of serum IgG concentration of 1000 mg/dL as a cut-off value is consistent with earlier observational studies.^{5,6}

Several assay systems are available to veterinary practitioners for measurement of passive transfer status in calves. Admittedly, economic considerations may

limit application of certain assays in veterinary practice; even personnel working in a typical mixed-animal veterinary clinic may find it impractical to invest in a temperature-compensating refractometer. On the basis of our results, the 3 refractometers performed similarly. The refractometers had similar sensitivities and specificities. Each sensitivity and specificity calculated in this study was superior to that previously reported for refractometry.¹⁰ Adequate sensitivities and specificities can be expected with temperature-compensating and nontemperature-compensating refractometers.

Nontemperature-compensating refractometers have limitations; the manufacturer of the instrument tested in this study recommends an interval of 30 seconds between placement of the sample on the refractometer's prism and determination of serum protein concentration to permit temperature equilibration. Nontemperature-compensating refractometers are designed to be used at room temperature (approx 22°C [72°F]). For the model used in this study, the manufacturer advises that operating temperatures between 60 and 100°F (15.5 and 37.7°C) yield the most accurate measurement.

It should be noted that the proportion of calves correctly classified will vary with the prevalence of FPT. Prevalence of FPT in the study population was 50%. Selection of a lower serum protein concentration test endpoint, such as 4.8 g/dL, classifies correctly a larger proportion of calves with adequate passive transfer as the prevalence of FPT decreases. Conversely, selection of a higher test endpoint, such as either 5.5 or 6.0 g/dL, results in a decrease in the proportion of calves correctly classified because specificity of the 3 refractometers decreases at test endpoints > 5.2 g/dL.

Serum protein concentration as great as 6.0 g/dL has been recommended previously⁴ as an endpoint for determination of passive transfer status in calves. Tyler et al¹⁰ recommended a test endpoint of 5.2 g/dL. On the basis of our findings, serum protein concentration test endpoints between 5.0 and 5.2 g/dL, as determined by refractometry, should be considered indicative of adequate passive transfer. Sensitivities of the refractometers for the detection of FPT were decreased when test endpoints lower than 5.0 g/dL were selected. Refractometer specificity for the detection of FPT was decreased when test endpoints > 5.2 g/dL were selected. The proportion of calves correctly identified was greatest when either a 5.0 or a 5.2 g/dL test endpoint was used. Higher test endpoints would correctly identify larger proportions of calves in populations with very high prevalences of FPT (> 95%; Fig 1). For example, the proportion of calves identified correctly by use of refractometer B was less at a serum protein concentration test endpoint of 6.0 g/dL than that cal-

culated at an endpoint of 5.2 g/dL until the prevalence of FPT exceeded 0.95. A prevalence of FPT of this magnitude is unlikely. Results of previous studies^{2,16,20} have indicated that prevalence of FPT does not exceed 90%, even in high-risk populations such as naturally suckled dairy calves, mixed-source Holstein heifer calves, and clinically ill calves. Consequently, the selection of serum protein concentration test endpoints > 5.2 g/dL (ie, 5.5 or 6.0 g/dL) should be discouraged because these higher endpoints increase the percentage of calves classified incorrectly.

The use of proportion of calves correctly identified as a measure of test performance has clear limitations. A change of prevalence of FPT in the study population alters the proportion of calves correctly identified. Furthermore, this calculation assumes that failure to correctly identify a calf with FPT has the same negative impact as the failure to correctly identify a calf with adequate passive transfer. The argument can be made that sensitivity in the assessment of passive transfer status will increase by selection of 5.5 g/dL as a test endpoint, rather than 5.2 g/dL. At the higher endpoint, an increase in sensitivity of 0.02 or 0.04 was observed depending on which refractometer was used. As a result, an additional 2 to 4% of calves with FPT would be identified correctly as having FPT. To monitor passive transfer in a population of extremely valuable calves, a practitioner might choose to apply a more stringent serum protein concentration test endpoint (5.5 g/dL) to facilitate correction of less-than-optimal passive transfer. However, it should be noted that an increase in test endpoint from 5.2 g/dL to 5.5 g/dL would decrease specificity by 0.06 to 0.26. As a result, an additional 6 to 26% of calves with adequate passive transfer would be incorrectly identified as having FPT and perhaps targeted for intervention. For farm personnel who use refractometry to assess management practices, accuracy of diagnostic testing may have more value than correct classification of an individual calf's passive transfer status. Furthermore, actual FPT rates may have less value than temporal changes in the measured FPT, regardless of the test endpoint used.

^aNavarre CB. Diagnosis of failure of passive transfer in calves (abstr), in *Proceedings*. North Am Vet Conf 2001;15:44.

^bAgarose, Sigma Chemical Co, St Louis, Mo.

^cBarbital buffer, Sigma Chemical Co, St Louis, Mo.

^dSodium azide, Sigma Chemical Co, St Louis, Mo.

^eRabbit IgG fraction to bovine IgG, Organon Technika Corp, West Chester, Pa.

^fBovine IgG, Sigma Chemical Co, St Louis, Mo.

^gReichert Medical Instruments, Buffalo, NY.

^hTS Meter, Leica, Buffalo, NY.

ⁱRHC-200 Refractometer for Clinical Use, Westover Scientific, Woodinville, Wash.

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