Comparison of four methods to assess colostral IgG concentration in dairy cows

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Objective—To determine sensitivity and specificity of 4 methods to assess colostral IgG concentration in dairy cows and determine the optimal cutpoint for each method.

Design—Cross-sectional study.

Animals—160 Holstein dairy cows.

Procedures—171 composite colostrum samples collected within 2 hours after parturition were used in the study. Test methods used to estimate colostral IgG concentration consisted of weight of the first milking, 2 hydrometers, and an electronic refractometer. Results of the test methods were compared with colostral IgG concentration determined by means of radial immunodiffusion. For each method, sensitivity and specificity for detecting colostral IgG concentration < 50 g/L were calculated across a range of potential cutpoints, and the optimal cutpoint for each test was selected to maximize sensitivity and specificity.

Results—At the optimal cutpoint for each method, sensitivity for weight of the first milking (0.42) was significantly lower than sensitivity for each of the other 3 methods (hydrometer 1, 0.75; hydrometer 2, 0.76; refractometer, 0.75), but no significant differences were identified among the other 3 methods with regard to sensitivity. Specificities at the optimal cutpoint were similar for all 4 methods.

Conclusions and Clinical Relevance—Results suggested that use of either hydrometer or the electronic refractometer was an acceptable method of screening colostrum for low IgG concentration; however, the manufacturer-defined scale for both hydrometers overestimated colostral IgG concentration. Use of weight of the first milking as a screening test to identify bovine colostrum with inadequate IgG concentration could not be justified because of the low sensitivity. (*J Am Vet Med Assoc* 2008;233:761–766)

Because adequate passive transfer of colostral immunoglobulins is so important to calf survival and health, numerous methods for assessing IgG concentration in bovine colostrum have been developed. Currently, the most accurate method of measuring colostral IgG concentration is radial immunodiffusion. However, this method is impractical for field situations because test results are not available for 48 to 72 hours. Thus, there is a need for alternative methods that can be used under field conditions to predict whether colostral IgG concentration is adequate. Refractometry,[1] hydrometry,[2,3] and measuring the weight of the first milking[4] have all been reported as potential methods for estimating IgG concentration in bovine colostrum. To our knowledge, however, sensitivity and specificity of using these methods to predict whether colostral IgG concentration is adequate have not been compared. The purpose of the study reported here therefore was to determine sensitivity and specificity of using a commercially available refractometer, 2 commercially available hydrometers, and weight of the first milking to predict adequacy of IgG concentration in bovine colostrum.

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Materials and Methods

Animals—One hundred sixty Holstein cows housed at the University of Missouri Foremost Dairy were included in the study. At the time of the study, cows in the herd that were not lactating were housed in a single free-stall barn and fed a complete mixed ration, although they also had access to a grass pasture. When cows developed signs of impending parturition, they were moved to another barn for observation. During the nonlactating period, all cows received a commercially available intramammary treatment that incorporated a latex teat sealant[a] to minimize the potential for colostrum to leak from the mammary gland. The experimental protocol was approved by the University of Missouri–Columbia Animal Care and Use Committee.

Experimental protocol—Composite colostrum samples obtained from 2004 through 2006 were used in the study. Individual colostrum samples were used only if parturition had been observed and colostrum had been obtained within 2 hours after parturition. Composite colostrum samples were collected with a portable bucket milking machine.

Weight of the colostrum (ie, weight of the first milking) was determined,[b] and temperature of the colostrum was measured with a digital thermometer.[c] Specific gravity was then measured with 2 commercially available hydrometers,[d,e] and refractivity was measured with an electronic refractometer.[f] All methods were performed in accordance with the manufacturers' recommendations.

For the first hydrometer (hydrometer 1),[d] a portion of the colostrum was transferred to a graduated cylinder and cooled in a refrigerator until the temperature was between 14° and 30°C. The hydrometer was then lowered into the graduated cylinder until it floated freely, and a reading was obtained. The hydrometer had been calibrated by the manufacturer to report estimated colostral IgG concentration in units of grams per liter.

For the second hydrometer (hydrometer 2),[e] a portion of the colostrum was cooled in a refrigerator until the temperature was between 20° and 25°C. The hydrometer was inserted into a glass tube with a plastic tip on 1 end and a squeeze bulb on the other, and the glass tube was inserted into the colostrum. When pressure was released on the squeeze bulb, colostrum was drawn into the glass tube, allowing the hydrometer to float. After the hydrometer had stabilized, a reading was obtained. The hydrometer had been calibrated by the manufacturer to report estimated colostral IgG concentration in units of grams per liter.

For the refractometer,[f] temperature of the colostrum was checked to ensure that it was between 10° and 40°C. An aliquot (0.3 mL) was placed on the prism of the refractometer, and a reading was obtained. The refractometer had been calibrated by the manufacturer to report refractivity in Brix units (%). The remaining portion of each colostrum sample was frozen at –20°C until IgG concentration could be determined by means of radial immunodiffusion.

Measurement of colostral IgG concentration—Colostral IgG concentration was determined by means of radial immunodiffusion, performed as described with minor modifications.[5] Radial immunodiffusion plates were prepared by dissolving 1% agarose[g] in sodium barbital buffer[g] containing 0.1% sodium azide.[g] Rabbit anti-bovine IgG (1%)[g] was added to the agarose solution, and 11 mL of the final solution was added to 10-cm-diameter Petri dishes. After the agarose had solidified, 3-mm-diameter wells were cut in the agar. Colostrum samples were diluted 1:120 with barbital buffer, and 5 µL of each sample was placed in a well. The diameter of the zone of precipitation was recorded after 72 hours of incubation at 23°C. Sample IgG concentrations were determined by comparing diameters of zones of precipitation with a standard curve generated with serial dilutions of a bovine IgG standard.[g]

Data analysis—Samples for which any values were missing were excluded from the study. Normality of the data was confirmed by use of statistical software.[h] Descriptive statistics (mean, SD, and percentage of samples with IgG concentration < 50 g/L) were calculated for results of radial immunodiffusion. One-way ANOVA[h] was used to compare mean colostral IgG concentration among cows grouped on the basis of lactation (ie, first vs second vs third or later lactation).

For each of the 4 test methods (hydrometer 1, hydrometer 2, refractometer, and weight of the first milking), sensitivity, specificity, and their 95% confidence intervals were calculated at various potential cutpoints. For these calculations, sensitivity was defined as the probability of a test result indicative of an inadequate colostral IgG concentration for a sample with an IgG concentration < 50 g/L, as determined by means of radial immunodiffusion, and specificity was defined as the probability of a test result indicative of an adequate colostral IgG concentration for a sample with an IgG concentration ≥ 50 g/L, as determined by means of radial immunodiffusion. In addition, for each potential cutpoint, the proportion of colostrum samples that would have been classified as having adequate IgG concentration was calculated. Finally, for each of the 4 test methods, the optimal cutpoint was determined by choosing the endpoint which maximized both sensitivity and specificity. Estimates of sensitivity and specificity at these optimal cutpoints were then compared among the 4 test methods by means of the z test for a difference between population proportions.[6] Linear regression was used to test for linear associations between colostral IgG concentration as determined by means of radial immunodiffusion and results of each of the 4 test methods.

All analyses were performed with standard software.[h] Values of $P < 0.05$ were considered significant.

Results

One hundred seventy-one colostrum samples were collected during the 3-year study period. Seventy-seven samples were from cows in their first lactation, 40 were from cows in their second lactation, and 54 were from cows in their third or later lactation. For 11 cows in the study, 2 colostrum samples in consecutive lactations were obtained. For the remaining 160 cows in the study, only a single colostrum sample was obtained.

Mean ± SD weight of the first milking was 7.4 ± 3.9 kg (16.3 ± 8.6 lb), and mean temperature of the fresh colostrum was 34.6 ± 2.6°C (94.3 ± 4.7°F). For all samples, mean IgG concentration as determined by means of radial immunodiffusion was 68.5 ± 32.4 g/L, and 55 of the 171 (32%) samples had colostral IgG concentration < 50 g/L. Mean IgG concentration for colostrum samples from cows in their third or later lactation (73.9 ± 34.6 g/L) was significantly higher than concentrations for samples from cows in their first lactation (65.8 ± 32.0 g/L) and for samples from cows in their second lactation (66.3 ± 30.2 g/L).

For hydrometer 1, the optimal cutpoint was determined to be 70 g/L; at this cutpoint, sensitivity of hydrometer 1 was 0.75 and specificity was 0.78 (**Table 1**). For hydrometer 2, the optimal cutpoint was determined to be 87.5 g/L; at this cutpoint, sensitivity of hydrometer 1 was 0.76 and specificity was 0.66 (**Table 2**). For the refractometer, the optimal cutpoint was determined to be 22%; at this cutpoint, sensitivity of the refractometer was 0.75 and specificity was 0.78 (**Table 3**). For weight of the first milking, the optimal cutpoint was 8.5 kg; at this cutpoint, sensitivity of weight of the first milking was 0.42 and specificity was 0.74 (**Table 4**). At the op-

Table 1—Sensitivity and specificity of using a hydrometer (hydrometer 1) to screen 171 bovine colostrum samples for low IgG concentration (< 50 g/L).

Test result (g/L)	Sensitivity (95% CI)	Specificity (95% CI)	No. (%) of samples classified as adequate	Total volume of samples classified as adequate (L)
≤ 10	0.05 (0.00–0.11)	0.99 (0.81–1.00)	171 (100)	1,258.7
≤ 20	0.11 (0.03–0.19)	0.98 (0.95–1.00)	167 (97)	1,220.1
≤ 30	0.21 (0.11–0.33)	0.97 (0.94–1.00)	163 (95)	1,193.1
≤ 40	0.29 (0.17–0.41)	0.95 (0.92–0.99)	156 (91)	1,126.1
≤ 50	0.47 (0.34–0.60)	0.93 (0.88–0.98)	150 (88)	1,052.0
≤ 60	0.61 (0.49–0.75)	0.83 (0.76–0.90)	137 (80)	960.4
≤ 70	0.75 (0.63–0.86)	0.78 (0.71–0.86)	119 (70)	810.2
≤ 80	0.80 (0.69–0.91)	0.66 (0.58–0.75)	105 (61)	697.3
≤ 90	0.93 (0.86–0.99)	0.52 (0.43–0.62)	88 (51)	582.0
≤ 100	0.96 (0.91–1.00)	0.40 (0.32–0.49)	65 (38)	417.5
≤ 110	1.00 (NA)	0.29 (0.21–0.38)	49 (29)	303.0
≤ 120	1.00 (NA)	0.25 (0.18–0.34)	34 (20)	188.2
≤ 130	1.00 (NA)	0.09 (0.04–0.14)	20 (12)	117.7
≤ 140	1.00 (NA)	0.00 (NA)	10 (6)	55.5

Sensitivity was defined as the probability of a test result indicative of an inadequate colostral IgG concentration for a sample with an IgG concentration < 50 g/L (determined by means of radial immunodiffusion). Specificity was defined as the probability of a test result indicative of an adequate colostral IgG concentration for a sample with an IgG concentration ≥ 50 g/L.

CI = Confidence interval. NA = Not applicable; confidence interval was not calculated.

Table 2—Sensitivity and specificity of using a hydrometer (hydrometer 2) to screen 171 bovine colostrum samples for low IgG concentration (< 50 g/L).

Test result (g/L)	Sensitivity (95% CI)	Specificity (95% CI)	No. (%) of samples classified as adequate	Total volume of samples classified as adequate (L)
≤ 25	0.11 (0.03–0.19)	0.99 (0.97–1.00)	171 (100)	1,258.2
≤ 37.5	0.15 (0.05–0.24)	0.97 (0.93–1.00)	164 (96)	1,198.7
≤ 50	0.35 (0.22–0.47)	0.94 (0.90–0.98)	159 (93)	1,163.0
≤ 62.5	0.36 (0.24–0.49)	0.88 (0.85–0.91)	145 (85)	1,076.8
≤ 75	0.67 (0.54–0.80)	0.74 (0.66–0.82)	135 (79)	991.9
≤ 87.5	0.76 (0.65–0.88)	0.66 (0.58–0.75)	104 (61)	692.9
≤ 100	0.89 (0.81–0.97)	0.53 (0.44–0.63)	90 (53)	607.2
≤ 112.5	0.92 (0.86–1.00)	0.40 (0.31–0.49)	69 (40)	452.1
≤ 125	1.00 (NA)	0.00 (NA)	50 (29)	318.7

See Table 1 for key.

Table 3—Sensitivity and specificity of using a refractometer to screen 171 bovine colostrum samples for low IgG concentration (< 50 g/L).

Test result (%)	Sensitivity (95% CI)	Specificity (95% CI)	No. (%) of samples classified as adequate	Total volume of samples classified as adequate (L)
≤ 14	0.07 (0.04–0.11)	1.00 (NA)	167 (97)	1,214.9
≤ 15	0.13 (0.04–0.21)	0.99 (0.97–1.00)	163 (95)	1,176.6
≤ 16	0.16 (0.07–0.26)	0.97 (0.93–1.00)	160 (94)	1,158.4
≤ 17	0.22 (0.11–0.33)	0.97 (0.93–1.00)	155 (91)	1,107.1
≤ 18	0.31 (0.19–0.43)	0.97 (0.92–0.99)	150 (88)	1,062.4
≤ 19	0.40 (0.27–0.53)	0.93 (0.88–0.98)	141 (82)	1,003.7
≤ 20	0.52 (0.40–0.66)	0.92 (0.87–0.97)	135 (79)	929.3
≤ 21	0.64 (0.51–0.76)	0.90 (0.84–0.95)	125 (73)	854.5
≤ 22	0.75 (0.63–0.86)	0.78 (0.70–0.85)	107 (63)	730.1
≤ 23	0.80 (0.69–0.91)	0.65 (0.56–0.73)	87 (51)	591.9
≤ 24	0.84 (0.74–0.93)	0.58 (0.49–0.67)	76 (44)	500.6
≤ 25	0.87 (0.78–0.96)	0.47 (0.38–0.57)	62 (36)	393.9
≤ 26	0.91 (0.83–0.99)	0.42 (0.33–0.51)	55 (32)	353.4
≤ 27	0.93 (0.86–1.00)	0.33 (0.24–0.41)	44 (26)	293.8
≤ 28	0.93 (0.86–1.00)	0.27 (0.19–0.35)	35 (20)	226.3
≤ 29	0.96 (0.91–1.00)	0.20 (0.13–0.27)	26 (15)	161.8
≤ 30	0.96 (0.91–1.00)	0.16 (0.10–0.23)	22 (13)	134.0
≤ 31	0.98 (0.95–1.00)	0.10 (0.05–0.16)	13 (8)	81.9
≤ 32	1.00 (NA)	0.07 (0.02–0.13)	8 (5)	48.9

See Table 1 for key.

Table 4—Sensitivity and specificity of using weight of the first milking to screen 171 bovine colostrum samples for low IgG concentration (< 50 g/L).

Test result (kg)	Sensitivity (95% CI)	Specificity (95% CI)	No. (%) of samples classified as adequate	Total volume of samples classified as adequate (L)
> 3	0.80 (0.69–0.91)	0.12 (0.06–0.18)	154 (90)	1,225.4
> 4	0.72 (0.61–0.84)	0.27 (0.19–0,35)	127 (74)	1,133.0
> 5	0.61 (0.49–0.75)	0.34 (0.26–0.43)	117 (68)	1,071.0
> 6	0.54 (0.41–0.68)	0.46 (0.37–0.55)	95 (56)	974.4
> 8	0.42 (0.35–0.48)	0.69 (0.61–0.77)	62 (36)	741.4
> 8.5	0.42 (0.28–0.55)	0.69 (0.61–0.77)	56 (33)	692.1
> 9	0.36 (0.23–0.49)	0.81 (0.74–0.88)	46 (27)	606.4
> 10	0.31 (0.19–0.43)	0.86 (0.80–0.92)	34 (20)	494.4
> 15	0.11 (0.03–0.19)	0.97 (0.94–1)	11 (6)	214.5
> 20	0.07 (0.04–0.11)	1.00 (NA)	4 (2)	77.3

See Table 1 for key.

Table 5—Results of linear regression of results of 4 methods for estimating IgG concentration in bovine colostrum and actual concentration as determined by means of radial immunodiffusion.

Test method	Regression equation	R^2
Hydrometer 1	Colostral IgG = 15.3 + (0.63 × test result)	0.41
Hydrometer 2	Colostral IgG = 14.4 + (0.59 × test result)	0.30
Refractometer	Colostral IgG = –24.7 + (3.96 × test result)	0.41
Weight of first milking	Colostral IgG = 77.6 – (1.2 × test result)	0.03

timal cutpoint, sensitivity for weight of the first milking was significantly lower than sensitivity for each of the other 3 methods, but no significant differences were identified among the other 3 methods with regard to sensitivity. Specificities of hydrometer 1, the refractometer, and weight of the first milking were not significantly different, but specificities of hydrometer 1 and the refractometer were significantly higher than specificity of hydrometer 2. Specificity of weight of the first milking was not significantly different from specificity of hydrometer 2. Significant direct linear relationships were identified between colostral IgG concentration, as determined by radial immunodiffusion, and results for hydrometer 1, hydrometer 2, and the refractometer (**Table 5**). A significant inverse linear relationship was identified between colostral IgG concentration and weight of the first milking. Regression analysis of colostral IgG concentration versus weight of the first milking after square or logarithmic transformation did not improve the fit.

Discussion

Results of the present study suggested that either of the 2 hydrometers or the electronic refractometer could be used to screen bovine colostrum to identify samples with inadequate IgG concentration. However, optimal cutpoints varied among instruments, even for the 2 hydrometers, indicating that instrument-specific cutpoints were required. Use of weight of the first milking as a screening test to identify bovine colostrum with inadequate IgG concentration could not be justified because of the low sensitivity associated with this method.

On the basis of recommendations from previous studies,[2,7] colostrum samples in the present study with an IgG concentration < 50 g/L, determined by means of radial immunodiffusion, were considered to have an inadequate IgG concentration, whereas samples with an IgG concentration ≥ 50 g/L were considered to have an adequate IgG concentration. On the basis of this cutoff, 55 of the 171 (32%) samples in the present study were considered to have an inadequate IgG concentration. Mean colostral IgG concentration in the present study was substantially higher than that reported in a previous study[3] but lower than the concentration reported in another study.[8] Several explanations exist for differences observed among studies. First, although timing of colostral collection was not reported in a previous study,[3] colostrum collected > 2 hours after parturition has a significantly lower IgG concentration than colostrum collected earlier.[9] Second, colostral IgG concentration may vary among herds because of differences in management, nutrition, and environment.[5] Third, IgG concentration varies throughout the colostrum obtained during the first milking, with lower IgG concentration in the last fractions obtained.[10] Hence, collection method will have an effect on IgG concentration. In the present study, IgG concentration in colostrum from cows in their first lactation was not significantly different from concentration in colostrum from cows in their second lactation, which was consistent with results of a previous study.[5]

In choosing the optimal endpoint for each of the 4 test methods in the present study, we considered not only sensitivity and specificity but also whether a particular endpoint would yield sufficient colostrum to feed calves. Feeding of at least 100 g of colostral IgG is recommended for adequate passive transfer,[11] and in a previous study,[11] only 36% of colostrum samples had an IgG concentration ≥ 50 g/L, whereas 66% had an IgG concentration ≥ 33 g/L and 85% had an IgG concentration ≥ 25 g/L. Hence, feeding of 3 to 4 L of colostrum

is recommended to minimize failure of passive transfer in dairy calves. For the present study, we assumed that feeding a minimum of 3 L of colostrum was a reasonable goal. Thus, the minimum total amount of colostrum required for the 171 calves produced by cows in the present study was 513 L, and optimal cutpoints for each of the 4 methods were chosen to ensure that at least 513 L of colostrum would be classified as having an adequate IgG concentration. Although we calculated a single optimal cutpoint for each of the test methods in the present study, from a practical point of view, it may be better to use a range of potential values. Thus, for hydrometer 1, we recommend test values of 60 to 90 g/L be used to identify samples with adequate IgG concentration; for hydrometer 2, we recommend test values of 75 to 100 g/L; and for the refractometer, we recommend test values of 20% to 23%. Use of values in the lower end of each of these ranges would increase the amount of colostrum classified as adequate, but would also increase the chance that colostrum samples with IgG concentration < 50 g/L would be classified as adequate. Use of values in the higher end of each of these ranges would decrease the amount of colostrum classified as adequate, but would also decrease the chance that colostrum samples with IgG concentration < 50 g/L would be classified as adequate. Limiting availability of colostrum through the use of rigorous screening protocols will increase the need for colostrum storage facilities.

The 2 hydrometers and the electronic refractometer used in the present study are sensitive to temperature, and colostrum must be within specified temperature ranges to obtain repeatable readings with these instruments. Mean ± SD temperature of fresh colostrum in the present study was 34.6 ± 2.6°C. Hence, the electronic refractometer had the advantage of not requiring cooling of the colostrum, whereas for both hydrometers, cooling was required before a reading could be obtained.

Recently, results of using an immunoassay to estimate IgG concentration in colostrum were reported.[12] Reported sensitivity of the immunoassay for identifying colostrum samples with inadequate IgG concentration (ie, < 50 g/L) was higher (0.93) than sensitivities reported for methods used in the present study. However, the per-test cost of the immunoassay was substantially higher than the per-test cost for methods used in the present study. Furthermore, results of the immunoassay are qualitative, whereas results for methods used in the present study were quantitative. Nevertheless, the immunoassay could be recommended for use on farms with a high proportion of colostrum samples with inadequate IgG concentration.

The manufacturers of hydrometers 1 and 2 have recommended that test results of 50 g/L and 100 g/L, respectively, be used to identify colostrum samples with inadequate IgG concentration. Substituting these values in regression equations obtained in the present study yielded colostral IgG concentrations of 46.8 g/L and 73.4 g/L, respectively. This suggested that both hydrometers systematically overestimated colostral IgG concentration.

Sensitivities of the 2 hydrometers in the present study were higher than values reported previously.[3] Although colostral immunoglobulin concentration is related to colostral specific gravity,[2] colostral specific gravity is more closely correlated with colostral protein concentration than with colostral IgG concentration.[2,13,14] Also, colostral specific gravity is affected by various factors, including month of calving, season, lactation number,[14] and colostral temperature.[15,16] Factors affecting colostral specific gravity vary from herd to herd, and this likely accounts for the difference in sensitivity between the present and previous[3] studies. In addition, all colostrum samples in the present study were collected within 2 hours after parturition, whereas collection times for samples in the previous study[3] were not reported, and a decrease in IgG concentration has been reported for colostrum samples collected > 2 hours after parturition.[9] Finally, colostrum was equilibrated to room temperature (18° to 22°C) prior to testing in the previous study,[3] whereas samples in the present study were cooled to manufacturer-recommended temperatures prior to testing.

Because predictive values of positive and negative test results are dependent on prevalence and the prevalence of colostrum with low IgG concentration likely varies from farm to farm, we did not calculate predictive values in the present study. A test method that maximizes sensitivity would be expected to yield low numbers of false-negative results and thus would identify most colostral samples with low IgG concentration. For practical purposes, colostral samples identified as having low IgG concentration with a screening test would typically be retested with a method with high specificity. Thus, for screening tests, maximizing sensitivity is considered more critical.

a. Orbeseal, Pfizer Animal Health, Exton, Pa.
b. Healthometer, Sunbeam Products Inc, Boca Raton, Fla.
c. Traceable thermometer, Control Co, Friendswood, Tex.
d. Colostrometer, Biogenics, Mapleton, Ore.
e. Milking tube colostrum scale, Waukee, Iowa.
f. PAL-1 refractometer, Atago USA Inc, Bellevue, Wash.
g. Sigma-Aldrich Co, St Louis, Mo.
h. SAS for Windows, version 9.13, SAS Institute Inc, Cary, NC.

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