J. Dairy Sci. 98:1–6
http://dx.doi.org/10.3168/jds.2014-8491

Technical note: Comparison of radial immunodiffusion and ELISA for quantification of bovine immunoglobulin G in colostrum and plasma

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ABSTRACT

Historically, radial immunodiffusion (RID) has been the only method that directly measures IgG; however, recent studies have reported IgG concentrations in colostrum, milk, and plasma as measured using an ELISA. To our knowledge no comparison between RID and ELISA methods has been made for bovine colostrum or plasma. The objective of this study was to compare IgG concentrations measured by both methods in samples of bovine colostrum before and after heat treatment and bovine plasma. Concentration of IgG was quantified using a commercially available RID kit and a modified ELISA. Samples of bovine colostrum and plasma were collected from individual animals and colostrum was tested before and after heat treatment at 60°C for 30 min. All samples were tested using both methods. Pearson correlation coefficients were determined for RID and ELISA values from unheated colostrum, heat-treated colostrum, and plasma samples. Mixed models were used to determine the effect of assay on IgG measurement in colostrum and plasma and effect of heat treatment on IgG concentration in colostrum. A weak correlation was found between ELISA and RID results in plasma and unheated colostrum. Concentration of IgG was significantly lower in all sample types when measured by ELISA compared to RID. Thus, direct comparison of ELISA and RID results is not recommended. Colostrum IgG concentration significantly decreased after heat treatment as measured by ELISA, but means were not different when measured by RID. Correlation plots between colostrum values measured before and after heat treatment indicated changes in the colostrum protein matrix due to heat affected RID and ELISA assays differently. This investigation compared RID and ELISA results, but no conclusions could be drawn as to the accuracy of either assay.

Key words: radial immunodiffusion, ELISA, IgG, calf, colostrum

Technical Note

Absorption from colostrum is the only method by which bovine neonates receive the immunoglobulins that defend against disease in the first weeks of life. The most prevalent immunoglobulin in bovine colostrum is IgG (Butler, 1969). Concentration of IgG is routinely measured in colostrum and in the blood of calves 24 to 48 h after birth to determine colostrum quality and assess success or failure of passive transfer of immunity. To ensure that calves receive an adequate mass of IgG, at least 4 L of colostrum containing at least 50 g/L of IgG should be fed as soon as possible after birth (Godden, 2008). A radial immunodiffusion (**RID**) method has been developed and successfully used for determining IgG concentration in milk, colostrum, and blood plasma; RID results have been used in the development of other methods correlating IgG concentration with specific gravity, refraction index, or plasma total protein to estimate IgG concentration (Pritchett et al., 1994, Tyler et al., 1996; Weaver et al., 2000; Bielmann et al., 2010). Historically, RID has been the only method that directly measures IgG; however, recent studies have reported IgG concentrations in colostrum, milk, and plasma as measured using an ELISA (Vetter et al., 2013; Baumrucker and Bruckmaier, 2014; Baumrucker et al., 2014; Gelsinger et al., 2015). To our knowledge no comparison between RID and ELISA methods has been made for bovine colostrum or plasma. Considerable cost may be accrued by routine performance of RID due to limited commercial availability. An ELISA may be an economical alternative method.

Heat treatment of colostrum is a method developed to reduce calf exposure to pathogens. Several studies have reported no effect of heat treatment on colostrum IgG concentration (Godden et al., 2006; Elizondo-Salazar et al., 2010). However, these studies used RID for measuring colostrum IgG concentration. The effect of heat treatment has not been assessed using ELISA, which may be a more sensitive method.

The objective of the present experiment was to measure IgG concentration in colostrum samples before and after heat treatment and also in plasma using both

Received June 16, 2014.
Accepted February 19, 2015.
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RID and ELISA methods and to compare results from each method. We hypothesized that results from each method would be positively correlated and that the ELISA procedure would give more precise estimates of IgG concentration.

Colostrum samples (n = 58) were collected from individual cows at the first milking after calving and frozen until needed for analysis. Blood samples were obtained 48 h after birth from 104 bull calves that were fed either unheated or heat-treated colostrum. Each calf received 4 L of colostrum within 8 h after birth. Blood samples were centrifuged (1,500 × *g* at 4°C) and plasma was collected and frozen until needed for analysis.

The RID procedure was performed according to manufacturer instructions (#728411; Triple J Farms, Bellingham, WA). One RID plate with anti-bovine IgG antibody within an agarose gel (0.1 *M* phosphate buffer, 0.1% sodium azide, 1 ug/mL of amphotericin B, 0.002 *M* EDTA; pH 7.0) and 3 standard solutions of bovine serum (1.96, 14.02, and 27.48 g of IgG/L) were included in each kit. Standard solutions were used as provided, without dilution, for the plate with which they originated. A plasma sample of known IgG concentration was included in each assay as a positive control.

To ensure that colostrum IgG concentration fell within the range of the standards provided, samples were diluted 1:10 in a 0.85% saline solution. The full process was duplicated for each sample. Precipitin ring diameters were measured after a 24-h incubation at 20°C using a peak scale loupe 7× (#1975; GWJ Company, Peak Optics, Hacienda Heights, CA). Mean IgG concentration and standard deviation were calculated from results of duplicated tests and used to calculate a coefficient of variation for each sample. Inter and intra-assay coefficients of variation were ≤10.5%. Samples with coefficient of variation >10.5% were retested. Plasma samples were tested in duplicate wells but without dilution as plasma IgG concentrations were expected to be within the range of the provided standards.

The ELISA procedure used for colostrum and plasma was adapted from instructions provided by the manufacturer (Bethyl Laboratories, Montgomery, TX) and the protocol described by Vetter et al. (2013). Purified IgG (1 mg IgG/mL; #P10–115; Bethyl Laboratories) was diluted using Tris buffer saline with Tween 20 (TBS-Tween; 50 m*M* Tris, 0.14 *M* sodium chloride, 0.05% Tween 20, pH 8.0) to create 7 standard solutions (1,000, 500, 250, 125, 62.5, 31.25, and 15.125 ng of IgG/mL). Standard solutions were made in test tubes, thoroughly mixed by vortexing individual tubes, and transferred into wells at the appropriate time in the ELISA protocol. Duplicate standard dilution series were created for each assay and TBS-Tween served as a negative control.

Whole colostrum was diluted using TBS-Tween with final dilution factors of 1×10^6, 2×10^6, 4×10^6, and 8×10^6. Dilution series were created in separate test tubes and final solutions were transferred to individual wells at the appropriate time in the ELISA protocol. All dilutions were duplicated so that a total of 8 wells (2 wells per dilution factor) contained solutions representing the same colostrum sample. Wells were coated with 100 μL of affinity-purified IgG (2 μg/mL; #A10–118A; Bethyl Laboratories). A 0.05% solution of gelatin from cold water fish skin (#G7041; Sigma-Aldrich Inc., St. Louis, MO) was used as a blocking agent and 150 μL were added to each well. Horseradish peroxidase conjugated antibody produced in sheep against bovine IgG heavy chain (A10–118P; Bethyl Laboratories) was used to detect IgG concentration in standard and sample solutions. A working solution containing 5 ng/mL of detection antibody was created using TBS-Tween. Each well received 100 μL of the detection antibody working solution. The enzyme reaction was carried out using 100 μL of 3,3′,5,5′-tetramethylbenzidine ELISA peroxidase substrate (Rockland Immunochemicals Inc., Gilbertsville, PA) and terminated with 100 μL of 0.2 *M* sulfuric acid. Individual plates were incubated at 20°C for 60 min after each step and wells were washed 5 times with TBS-Tween before adding the reagent for the subsequent step. The only exception occurred in the enzyme reaction and addition of 0.2 *M* sulfuric acid; the enzyme reaction was allowed to proceed for 10 to 30 min and wells were not washed before adding sulfuric acid.

Plasma was diluted in TBS-Tween to a final dilution factor of 5×10^5. All dilutions were duplicated and tested in 4 separate wells such that a total of 8 wells contained solutions representing the same plasma sample. Reagents and protocols are described above, except that standard solutions ranged from 3.125 to 200 ng of IgG/mL. This narrower range of standards and a single final dilution factor (50,000×) were used for plasma because plasma IgG concentration was expected to be less variable than colostrum.

Absorbance was read using a plate reader (Victor^3 Multilabel Counter model 1420, PerkinElmer Life Sciences, Waltham, MA) at a wavelength of 450 nm. Absorbance values from wells containing negative controls were averaged and subtracted from standard and sample absorbance values. Duplicate standard values were averaged and used to create a standard curve relating absorbance to IgG concentration. Results from individual assays were used only when the coefficient of determination value from the standard curve exceeded