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Short communication

Evaluation of two commercial agarose gel kits for estimating IgG concentrations in neonatal calves: A pilot study

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Abstract

This study characterized agarose gel electrophoresis (AGE) profiles of calf serum and evaluated the accuracy of two commercial AGE kits (J711 and J718) for estimating immunoglobulin G (IgG) concentrations. Samples were collected from 10 calves at birth (Day 0), 10-12 h later (Day 0.5), and 2 days after colostrum ingestion (Day 2). AGE was performed with both kits, and IgG concentrations were measured via ELISA. After colostrum ingestion, $\beta_2$globulin (Glb) and γGlb fractions merged electrophoretically and were analyzed together as β_2γGlb. On Day 0, γglobulin was nearly absent, confirming no transplacental Ig transfer. By Day 2, β_2γGlb had markedly increased. Linear mixedeffects models showed that β_2γGlb was a strong predictor of IgG levels, with marginal R^2 values of 0.960 (J711) and 0.958 (J718). The results suggest that the AGE kits have potential as a practical alternative for evaluating passive immunity in calves.

Keywords: agarose gel electrophoresis (AGE), β2- and γ-globulins, immunoglobulin G (IgG), neonatal calf, passive transfer of immunity

Introduction

Neonatal calves must obtain maternal immunoglobulins (Igs) by consuming colostrum, which is known as passive transfer of immunity. In bovine colostrum, IgG is the predominant Ig, accounting for more than 75% of total Igs (Hurley and Theil 2011). The assessment of passive immunity in calves is typically based on serum concentrations of IgG measured between 24 and 48 h after birth (Quigley and Drewry 1998). In this study, we focused on serum protein electrophoresis using agarose gel electrophoresis (AGE) as an alternative to direct IgG measurement methods – such as radial immunodiffusion and enzyme-linked immunosorbent assay (ELISA) – which may provide faster clinical utility. In bovine serum, AGE separates six fractions: albumin (Alb), and α_1, α_2, β_1, β_2 and γglobulins (Glb) (Nagy et al. 2015). The distribution of IgG is primarily within the γGlb fraction and extends across the β_2 to γGlb regions (Nagyová et al. 2017). We investigated the AGE profiles of serum from newborn calves before and after colostrum ingestion using two commercial gels (J711 and J718), evaluated the effect of colostrum on β_2 and γGlb fractions by comparing AGE results with measured IgG concentrations.

Materials and Methods

This study used surplus frozen (-60°C) serum samples from 10 neonatal calves (4 Holstein males, 5 Holstein females, and 1 Japanese Black female), originally part of a larger experimental group in a previous project on blood biochemical profiling during the neonatal and pre-weaning periods. All procedures for the original project were approved by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine (approval no. 18-93; approved April 10, 2018). This retrospective approach enabled analysis without new animal trials, promoting animal welfare by reducing animal use. All calves were reared following the farm's standard protocol to ensure proper colostrum and nutrition management. Briefly, Holstein females received frozen colostrum, while Holstein males and the Japanese Black calf were given a commercial colostrum replacer within 1–6 hours of birth, followed by regular feeding with milk replacers from Day 2 onward. Blood samples were collected from the jugular vein at three time points: within 30 min of birth and before colostrum intake (Day 0), 10-12 h after birth (Day 0.5), and on Day 2.

AGE was performed using QuickGel SP (J711) and QuickGel βsplit SP plate (J718) agarose gel kits (Helena Laboratories Japan, Saitama, Japan) with an electrophoresis analyzer (Epalyzer2; Helena Laboratories Japan), following the previous reports (Yamagishi et al. 2025). Total protein concentrations were determined using a clinical biochemistry analyzer (DriChem NX600; Fujifilm Medical, Tokyo, Japan). Absolute concentrations (g/L) of the fractions were calculated based on the total protein concentration. IgG concentrations were measured using a sandwich ELISA kit (E11118; Bethyl Laboratories, TX, USA).

Because the β_2-Glb and γ-Glb fractions were often merged, they were combined as the $\beta_2\gamma$-Glb fraction for subsequent statistical analysis. Statistical analysis was performed using jamovi (version 2.7.24) with the GAMLj module. The relationship between IgG concentration and $\beta_2\gamma$Glb concentrations from each AGE kit was evaluated using linear mixedeffects models (LMMs). $\beta_2\gamma$Glb concentration and calf age were included as fixed effects, and calf ID was used as a random effect. To derive practical predictive equations, covariates were retained in their original scale (*i.e.*, not centered). Model fit was assessed using marginal R^2. Significance was assessed at $p<0.05$.

Results and Discussion

Pre-colostral profiles (Day 0) typically showed six fractions, with a nearly absent γ-Glb region in 9 of 10 samples with J711 and all 10 samples with J718 (Fig. 1A and D). After colostrum intake, a marked shift to a five-fraction pattern occurred, featuring a merged $\beta_2\gamma$-Glb peak observed in most samples on Day 0.5 (8/10 with J711; 10/10 with J718) and in all 10 samples by Day 2 (Fig. 1B, C, E and F). For both J711 and J718, data points clustered tightly around the regression lines, demonstrating a strong positive linear correlation between $\beta_2\gamma$-Glb concentration and IgG levels (Fig. 1G and H). The marginal R^2 was 0.960 for J711 and 0.958 for J718, indicating that either reagent explained approximately 96% of the variance in IgG concentration. Based on fixedeffects estimates, predictive equations for estimating IgG concentration (y, g/L) from $\beta_2\gamma$Glb concentration (x, g/L) were derived using the linear form $y = ax + b$:

$$\text{J711: } y = 1.234x - 1.622$$
$$\text{J718: } y = 1.226x - 1.583$$

Regression slopes were nearly identical and highly significant (J711: $t = 10.37$, $p<0.001$; J718: $t = 11.05$, $p<0.001$). Calf age did not significantly affect IgG concentration in either model ($p>0.05$), indicating that accurate IgG estimation is possible from Day 0 to Day 2 regardless of agerelated physiological changes.

Immunoglobulins and complement proteins migrate across the β- and γ-Glb regions (Nagyová et al. 2017).

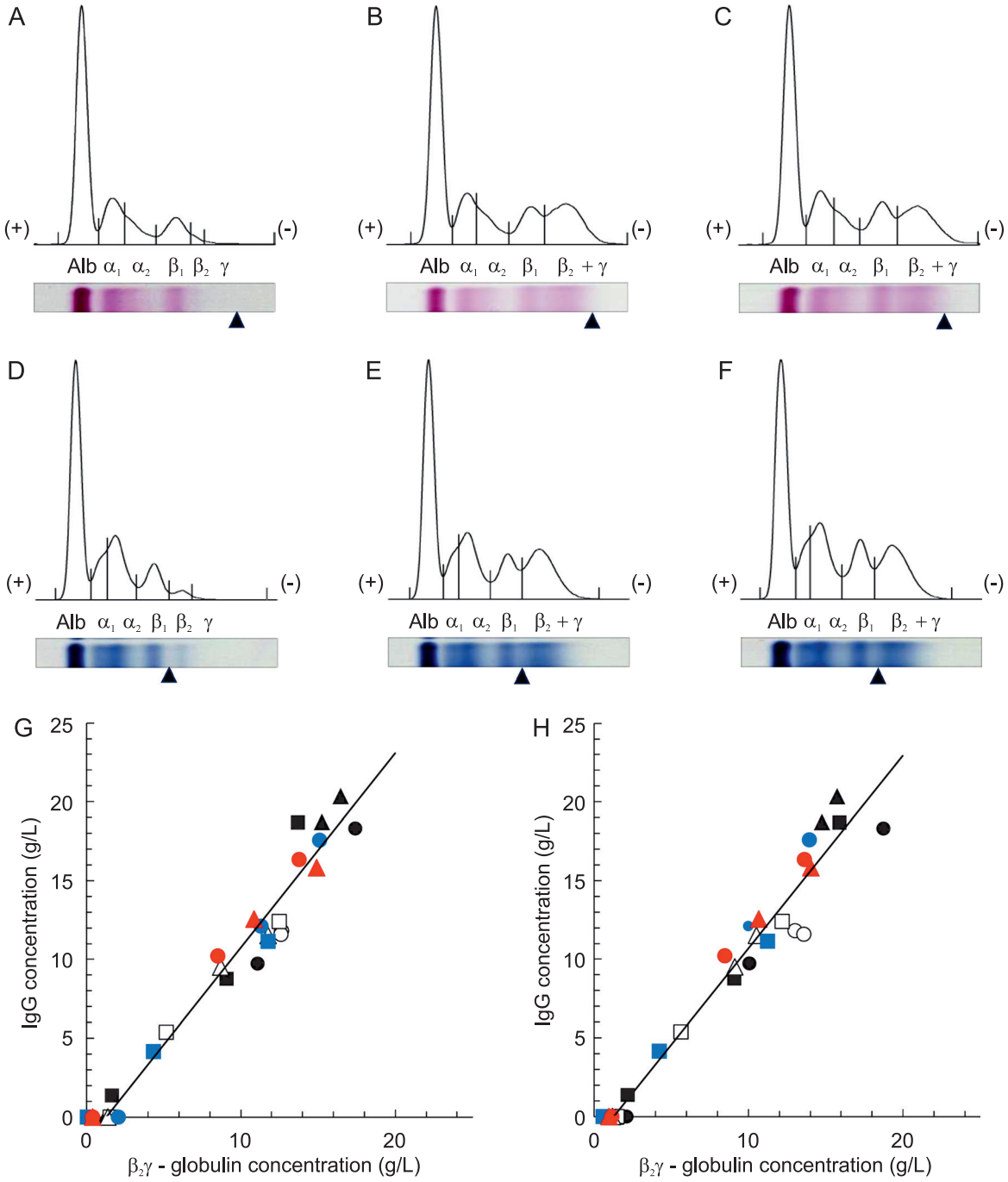

Fig. 1. Representative densitometric tracings (electrophoretograms) from AGE (A - F) and relationship between IgG concentration measured via ELISA and $\beta_2\gamma$-globulin concentration measured using two AGE kits (J711 and J718). Samples were collected from calves on ages Day 0 (A, D), Day 0.5 (B, E), and Day 2 (C, F). Note the distinct merging of β_2- and γ-globulin fractions (designated as β_2+γ) observed after colostrum ingestion (Day 0.5 and Day 2). The triangles indicate the application point (origin). Alb: albumin; α_1: α_1-globulin; α_2: α_2-globulin; β_1: β_1-globulin; β_2: β_2-globulin; γ: γ-globulin. (G) J711 and (B) J718: Each point represents an individual measurement on Day 0, 0.5, or 2 (n = 30). Different marker shapes and colors represent individual calves. The solid lines represent the regression lines derived from the LMMs (A: $y = 1.234x - 1.622$ for J711; B: $y = 1.226x - 1.583$ for J718).

In human and veterinary medicine, β-γ bridging is often associated with chronic liver disease and increased immunoglobulin production (Tothova et al. 2016). Although the merging of the β_2- and γ-globulin fractions observed in this study resembles a β-γ bridging pattern electrophoretically, the calves in this study were clinically healthy neonates and the change appeared immediately after colostrum ingestion, corresponding with a marked increase in serum IgG concentrations. Therefore, the observed pattern is more likely attributable to passive transfer of maternal immunoglobulins than to pathological β-γ bridging.

In addition, the increase in the $\beta_2\gamma$ region was characterized by a broad-based electrophoretic pattern rather than a discrete monoclonal peak. This broad pattern is consistent with the passive transfer of a heterogeneous population of maternal immunoglobulins (Nagyová et al. 2017) and contrasts with monoclonal gammopathies that produce narrow electrophoretic peaks (Tothova et al. 2016). Because endogenous immunoglobulin synthesis is minimal immediately after birth, the observed electrophoretic changes are most likely attributable to absorbed maternal antibodies.

This study has several limitations. The sample size was small, calves differed in breed, sex and colostrum source, and archived frozen serum samples were used retrospectively. Therefore, these findings should be interpreted as preliminary observations and require confirmation in larger prospective studies. In conclusion, the results provide preliminary evidence that AGE-derived $\beta_2\gamma$-Glb concentrations may serve as a surrogate marker for IgG concentration in neonatal calves.

Author Declarations

Ethics approval

The present study used residual frozen serum samples collected in a previous project and did not involve any new animal experiments. The original project was approved by the Animal Care and Use Committee of Obihiro University of Agriculture and Veterinary Medicine (approval no. 18-93; approved April 10, 2018).

Use of generative artificial intelligence

Generative AI tools (Microsoft Copilot) were used only at the study design stage to help explore possible statistical analysis approaches.

Conflict of interest

The authors have no conflicts of interest to declare.

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