Evaluation of serum enzyme activities as predictors of passive transfer status in lambs

Domenico Britti, DVM, PhD; Genesio Massimini, DVM; Angelo Peli, DVM, PhD;
Alessia Luciani, DVM; Andrea Boari, DVM

Objective—To determine the associations between serum IgG concentration and serum activities of γ-glutamyltransferase (GGT), alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and pseudocholinesterase for the potential use of these serum enzymes as predictors of passive transfer status in neonatal lambs.

Design—Prospective observational study.

Animals—47 Sardinian lambs from birth to 2 days old.

Procedure—Serum enzyme activities were measured by use of commercially available kits and a clinical biochemical analyzer. Serum IgG concentration was determined by single radial immunodiffusion. Associations between serum IgG concentration and the activity of each serum enzyme were established by use of regression analysis.

Results—A significant correlation was detected between serum IgG concentration and serum GGT activity in 1- and 2-day-old lambs. Minimal correlations were detected between serum IgG concentration and serum alkaline phosphatase activity in 1-day-old lambs and serum pseudocholinesterase activity in 1- and 2-day-old lambs. No significant associations were detected between serum IgG concentration and serum activities of aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase. A multiple linear regression model was accurate for the estimation of the natural logarithm of serum IgG concentration as a function of the natural logarithm of serum GGT activity and of the age of lambs at the time of sampling (adjusted R^2 = 0.89). This model was then used to calculate the serum GGT activity equivalent to various serum IgG concentrations for 1- and 2-day-old lambs.

Conclusions and Clinical Relevance—Results suggested that passive transfer status in neonatal lambs can be successfully predicted by measurement of serum GGT activity but not by measurement of the other enzymes tested. (*J Am Vet Med Assoc* 2005; 226:951–955)

In ruminants, syndesmochorial placentation prevents the in utero transfer of maternal immunoglobulins. For this reason, newborn ruminants rely on the ingestion and absorption of maternal immunoglobulins from colostrum. This process, termed passive transfer, is important for subsequent protection against neonatal infectious diseases.[1-4] **Failure of passive transfer (FPT)** and resulting hypogammaglobulinemia have been linked to increased risk of neonatal illness and death.[1-4] The most common causes for FPT include a sick or injured neonate, injury or death of the dam, inadequate quality or production of colostrum, and failure of the neonate to suckle or absorb ingested colostrum.[1,2,4-6] The prevalence of FPT in lambs varies, occurring in 3.4% to 20% of lambs, with mortality rates ranging from 45% to 50% in lambs from the first few days of age to 2 to 3 weeks after birth.[5-10] This makes FPT a major economic concern for breeders.

Several methods are presently available for detection of FPT in newborn ruminants. **Single radial immunodiffusion (SRID)**, developed by Mancini et al[11] and modified by Fahey and McKelvey,[12] is the most accurate test for direct measurement of serum IgG concentration. Other tests, including the determination of serum total protein concentration by refractometry, the sodium sulfite turbidity test, the zinc sulfate turbidity test, and the serum glutaraldehyde coagulation test, indirectly estimate serum IgG concentration on the basis of concentration of total globulins or other proteins that are associated with IgG during passive transfer.[1,2,13,14] These tests vary in accuracy, cost, and their suitability to veterinary practice. Although several studies have been performed in calves to quantify the increased risk of death associated with low serum IgG concentrations, few studies concerning this association have been performed in lambs. In neonatal calves, an increased risk of death is associated with serum concentrations of IgG < 1,000 mg/dL as determined by SRID.[4,13-15] The dividing line between hypogammaglobulinemia and normal serum IgG concentration in neonatal lambs has not yet been universally accepted. In clinically normal 1- and 2-day-old lambs, serum IgG concentrations measured by SRID vary widely, with mean values ranging from 2,000 to 3,000 mg/dL.[1,6,10,16] Previously, partial FPT has been defined as 1 SD below the normal mean and FPT as 2 SD below the mean for clinically normal 24-hour-old lambs.[6] In the latter study, a serum IgG value of approximately 800 mg/dL was > 2 SD from the mean.[6]

Concentrations of IgG_1, the main IgG subisotype in lamb's serum after colostrum intake,[1,2] were measured in an observational study of lambs by McGuire et al,[10] who calculated a mortality rate of 45% in 20 of 590 lambs 24 to 36 hours old with serum IgG_1 concentra-

From the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, University of Teramo, Viale F. Crispi 212, I-64100 Teramo, Italy (Britti, Massimini, Luciani, Boari); and the Department of Veterinary Clinical Sciences, School of Veterinary Medicine, University of Bologna Alma Mater Studiorum, Via Tolara di Sopra 50, I-40064 Ozzano Emilia, Italy (Peli). Dr. Massimini's present address is San Rocco Private Veterinary Center, Via delle Pesche 9, I-00060 Capena, Rome, Italy.
Supported by the School of Veterinary Medicine, University of Teramo.
Address correspondence to Dr. Britti.

tions < 600 mg/dL. In another study[16] in 36-hour-old lambs, the mortality rate was 3 to 4 times higher in lambs with serum IgG_1 concentrations < 1,000 mg/dL, compared with lambs with higher serum IgG_1 concentrations. Consequently, it appears that a serum IgG concentration > 1,000 mg/dL for 1- to 2-day-old lambs may be a reasonable goal for producers to decrease the risk of death associated with FPT.

Because intestinal absorption is nonselective in neonatal ruminants, calves and lambs have the capability to absorb many proteins, including macromolecular substances, within the first 24 to 48 hours after birth.[1,2,17,18] If colostrum is ingested within an appropriate time, colostral enzymes can pass through the intestinal barrier by use of the same mechanism as immunoglobulins and could thus be used as markers of passive transfer status. In neonatal calves, serum **γ-glutamyltransferase (GGT)** activity is directly correlated with serum IgG concentration; consequently, low serum GGT activity is consistent with FPT.[14,15,17,19-23] Several threshold cutoff points in serum GGT activity, ranging from 50 to 300 U/L, have been suggested for the identification of FPT in calves.[4,15,20,21] The association between serum GGT activity and serum immunoglobulin concentration has also been observed in buffalo calves,[24] lambs,[25-27] kids,[28] and puppies[29] but not in crias[30] or foals.[28,31] After colostrum intake, a transient increase in serum **alkaline phosphatase (ALP)** activity was detected in young calves at the time of colostrum absorption[17,32] and a significant correlation was detected between serum γ-globulin concentration and serum ALP activity in 1-day-old lambs.[26] Serum **aspartate aminotransferase (AST)** activity also reportedly increases concurrently with the absorption of colostral γ-globulin in lambs[26] but not always in neonatal calves.[17,32-34] Serum **lactate dehydrogenase (LDH)** activity increases in the first 24 hours after birth in lambs[35] and calves.[33,34] To the authors' knowledge, the activities of **alanine aminotransferase (ALT)** and **pseudocholinesterase (PCE)** in ewe colostrum and serum from neonatal lambs has not yet been investigated.

The purpose of the study reported here was to evaluate the associations between serum IgG concentration and serum activities of GGT, ALP, AST, ALT, LDH, and PCE for the potential use of these serum enzymes as predictors of passive transfer status in neonatal lambs.

Materials and Methods

Sample collection and processing—Serum samples were obtained from 47 Sardinian lambs immediately after birth (presuckling samples; day 0) and at 1 (24 hours) and 2 days (48 hours) of age. The serum activities of GGT, ALP, ALT, AST, LDH, and PCE were determined by use of commercially available kits[a] and an automated analyzer.[b] Serum IgG concentration was measured by SRID.[c] Enzyme activities were also measured in casein-deprived presuckling colostrum samples (obtained by addition of 2.5 mg of rennin/mL of whole colostrum) from 32 dams at parturition, as described for serum samples.

Statistical analyses—Mean ± SD for serum IgG concentration, serum enzyme activities, and colostrum enzyme activities was calculated. Serum IgG concentration and serum enzyme activities on days 0, 1, and 2 were compared by use of 1-way repeated-measures ANOVA. When results of the *F* test were significant, time-dependent differences were localized by use of the Tukey HSD test. Simple linear regression analysis was performed to evaluate the correlation between serum IgG concentration and serum activity of each enzyme (GGT, ALP, AST, ALT, LDH, and PCE) in lambs. If the linearity assumption between the related variables was not satisfied, an appropriate transformation of the data was performed. Equations having large coefficients of determination (R^2) were selected for further analysis. Multiple linear regression models were fitted to predict the mean value of the serum IgG concentration in lambs (continuous dependent variable) as a function of the serum enzyme activity (continuous independent variable) and age of lambs at the time of sampling (categorical independent variable). Independent variables (serum enzyme activity and age of lambs) were permitted to enter the model when nonzero coefficients ($P < 0.05$) and insignificant collinearity were present. The mathematical equation that related the variables in the developed models was expressed by the following formula: $Y = \alpha + \beta_1 x_1 + \beta_2 x_2$, where Y is the predicted measurement of IgG, x_1 is the serum activity for a specific enzyme, x_2 is the age of the lamb at the time of sampling (day 1 = 0 and day 2 = 1), α is the y-intercept, and β_1 and β_2 are the partial regression coefficients. To assess the goodness of fit of the models that contained 2 independent variables, an adjusted coefficient of determination (adjusted R^2) was calculated to determine the proportion of the total variance in the dependent variable that was accounted for by variation in the independent variables. Coefficients of determination were multiplied by 100 and expressed as a percentage to indicate the total variation of Y, which was explained by regression. For all analyses, values of $P < 0.05$ were considered significant. Calculations were performed by use of a statistical software package.[d]

Results

For the 47 lambs, mean ± SD serum IgG concentrations on days 0, 1, and 2 were 28.4 ± 6.6 mg/dL, 2,695 ± 2,290 mg/dL, and 2,634 ± 1,980 mg/dL, respectively. On day 1, mean serum IgG concentration increased significantly ($P < 0.05$) after colostrum intake. Presuckling serum activity was highest for ALP (mean, 1,042 ± 345 U/L), followed by LDH (mean, 821 ± 274 U/L), PCE (mean, 58.9 ± 10.9 U/L), GGT (mean, 54.8 ± 14.9 U/L), AST (mean, 27.8 ± 11.8 U/L), and ALT (mean, 5.1 ± 2 U/L). On day 1, a significant increase ($P < 0.05$) in the serum activities of GGT (mean, 4,077 ± 4,567 U/L), ALP (mean, 2,169 ± 1,259 U/L), LDH (mean, 1,399 ± 415 U/L), AST (mean, 128 ± 37 U/L), and ALT (mean, 7.6 ± 2.6 U/L) was detected. On day 2, a significant ($P < 0.05$) decrease in the serum activities of GGT (mean, 1,994 ± 2,091 U/L), ALP (mean, 1,349 ± 487 U/L), LDH (mean, 1,052 ± 285 U/L), and AST (mean, 95.6 ± 55.3 U/L) was detected. Serum PCE activities on day 0 (mean, 58.9 ± 10.9 U/L), day 1 (mean, 57.4 ± 7.2 U/L), and day 2 (mean, 57.3 ± 12 U/L) were not significantly different ($P = 0.714$). The enzyme activity in ewe colostrum was highest for GGT (mean, 11,311 ± 5,300 U/L), followed by LDH (mean, 2,304 ± 1,794 U/L), ALP (mean, 226 ± 85 U/L), AST (mean, 118 ± 55 U/L), PCE (mean, 111 ± 58 U/L), and ALT (mean, 17.2 ± 5.7 U/L).

A significant correlation was detected between serum IgG concentration and serum GGT activity in 1- ($P < 0.01$; $R^2 = 0.60$) and 2- ($P < 0.01$; $R^2 = 0.58$) day-old lambs. Minimal correlations were detected between serum IgG concentration and serum ALP activity ($P <$

0.01; R^2 = 0.33) in 1-day-old lambs and serum PCE activity in 1- ($P < 0.01$; R^2 = 0.34) and 2- ($P < 0.05$; R^2 = 0.28) day-old lambs. Significant associations between serum IgG concentration and serum activities of AST, ALT, and LDH were not detected. Because preliminary scatter diagrams of the data suggested a positive curvilinear association between serum GGT activity and serum IgG concentration, this association was linearized by taking the **natural logarithm** (**ln**; to base *e*) of each variable (**Figure 1**). As a result, the strength of the association between the **ln of serum GGT activity (ln[GGT])** and the **ln of serum IgG concentration (ln[IgG])** in 1- ($P < 0.01$; R^2 = 0.88) and 2- ($P < 0.01$; R^2 = 0.77) day-old lambs was substantially improved.

A multiple linear regression model was developed to estimate the ln[IgG] as a function of the ln[GGT] and of lamb age (24 and 48 hours of age). The partial regression coefficients indicated that ln[GGT] and lamb age were significantly ($P < 0.05$) associated with the estimated serum IgG concentration. The interaction between these independent variables was not significant. Serum IgG concentration for 1- and 2-day-old lambs could be successfully predicted ($P < 0.001$; adjusted R^2 = 0.89) by use of the following formula: ln[IgG] (mg/dL) = 2.251 + 0.700 × ln[GGT] (U/L) + 0.378 × lamb age, where the categorical independent variable, lamb age, was equal to zero if the lamb was sampled at 1 day of age and was equal to 1 if the lamb was sampled at 2 days of age. The derived formula was then used to estimate the serum GGT activity equivalent to different serum IgG concentrations for 1- and 2-day-old lambs (**Table 1**).

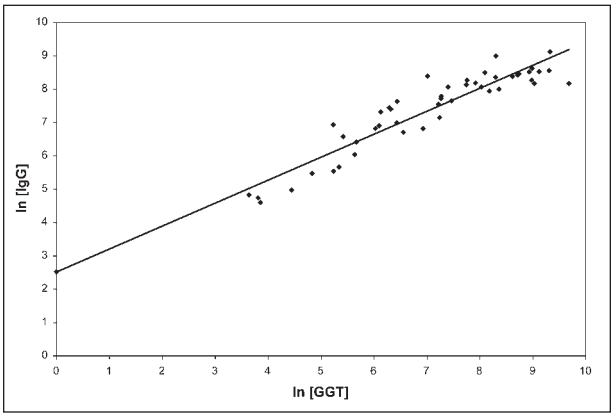

Figure 1—Scatter diagram illustrating the association between the natural logarithm of serum γ-glutamyltransferase activity (ln[GGT]) and the natural logarithm of serum IgG concentration (ln[IgG]) observed in 47 neonatal lambs sampled at 1 day of age. After log transformation, the distance between the points and the identity line has been decreased, and as a result of the linearized association, the approximating curve is a straight line.

Table 1—Values for serum γ-glutamyltransferase (GGT) activity equivalent to various serum IgG concentrations for 1- and 2-day-old lambs, calculated by use of a multiple linear regression model.

	Serum GGT activity (U/L) equivalent	
IgG (mg/dL)	**Day 1**	**Day 2**
500	288	168
1,000	775	451
1,500	1,383	806
2,000	2,085	1,215

Discussion

Results of this study confirm the high activity of GGT in ewe colostrum and the correlation between serum GGT activity and serum IgG concentration in newborn lambs, similar to results of other studies for calves[14,15,17,19-23] and lambs.[25-27] Results of 1 study[26] indicated that serum GGT activity in newborn lambs after colostrum intake was 140 times higher than the serum GGT activity in healthy adult sheep. On the contrary, serum GGT activity in presuckling lambs is similar to that in healthy adult sheep.[26] In our study, serum GGT activity in 1-day-old lambs (mean, 4,077 U/L) was as much as 70 times higher than serum GGT activity in lambs at birth (mean, 54.8 U/L) and was strongly correlated with serum IgG concentration, suggesting a transfer of GGT from maternal colostrum to serum of young lambs through the same mechanism as IgG. In previous studies, regression models were generated to predict passive transfer status as a function of age and serum GGT activity for 1-day-old lambs[27] and for lambs < 15 days of age.[25] In our study, the regression model that was developed was accurate in predicting serum IgG concentration as determined by the adjusted R^2 value of 0.89; therefore, approximately 90% of the variation in serum IgG concentration was explained by its association with serum GGT activity and lamb's age. Note that this model is only applicable in the specified range of the independent variables used in development of the regression analysis (from 40 to 16,000 U of GGT/L and in 1- and 2-day-old lambs) and should not be extrapolated beyond these limits. On the other hand, it is well known that the lamb's small intestine rapidly loses its ability to absorb intact maternal globulins and colostrum enzyme activities such as GGT between 24 and 48 hours after birth.[1,2,18] The decrease in serum GGT activity is rapid probably because of its degradation or deactivation.[17,26] This large decrease in serum GGT activity, as seen in the study reported here, suggests that a strength correlation between serum GGT activity and serum IgG concentration is only to be detected within 24 hours of birth.[17,6]

In agreement with results of other studies in calves[17,32] and lambs,[26] we detected an increase in serum ALP activity after colostrum intake and a positive association with serum IgG concentration in 1-day-old lambs. Although significant, the coefficient of determination (R^2 = 0.33) indicated that the serum ALP activity in 1-day-old lambs explained only 33% of the total variance of serum IgG concentration in 1-day-old lambs. In our study, the activity of ALP in ewe colostrum was lower than the serum ALP activity in lambs and insufficient to account for the increase in serum ALP activity of approximately 1,100 U/L detected between 0 and 1 day of life. Results of similar studies[26,36-38] suggest that the serum ALP activity in lambs is derived from the brush-border of the intestinal mucosa during protein transmission because ALP activity in ewe colostrum is inadequate to account for the increase in serum ALP activity in lambs and serum ALP activity increases in lambs after intake of milk substitute. Results of our study are in agreement with the findings of those studies, indicating that the increase in ALP activity in serum of neonatal lambs is

associated with feeding but not necessarily with ingestion of colostrum; hence, serum ALP activity cannot be considered a predictor enzyme of passive transfer status.

Two types of cholinesterase have been described in blood from mammals: acetylcholinesterase and PCE, which are found in RBCs and in plasma or serum, respectively. In ruminants, acetylcholinesterase is reportedly a better sentinel than PCE for detection of exposure to organophosphates or carbamates in whole blood samples,[39] whereas serum PCE activity, which is of hepatic origin, varies during liver disorders.[40] However, the reference range and diagnostic value of these enzymes in ruminants have not been determined. In our study, the activity of PCE in ewe colostrum was approximately 2-fold greater than the serum activity in neonatal lambs; however, the reason for this was not clear. Although the correlation between serum PCE activity and serum IgG concentration was significant, this enzyme only accounted for 34% and 28% of the variation in serum IgG concentration in 1- and 2-day-old lambs, respectively. Thus, we concluded that serum PCE activity is a poor indicator of passive transfer status in neonatal lambs.

Concentrations of other enzymes, such as AST,[32-34] ALT,[33] and LDH[33,34] in calves and AST[26] and LDH[35] in lambs, reportedly increase in serum within the first 24 hours of life. In our study, the activities of AST, ALT, and LDH in colostrum appeared adequate to account for their significant increase in lamb's serum between 0 and 1 day of life. However, the lack of association between serum IgG concentration and serum activities of AST, ALT, and LDH suggested that the postsuckling increase of these enzymes was the consequence of enhanced endogenous production (ie, physiologic response, intestinal origin, or muscle damage caused by parturition) rather than colostrum intake.

Overall, results of our study indicated that the measurement of serum GGT activity in the early neonatal period may be used to assess passive transfer status in neonatal lambs. The advantages of the GGT test are that it is inexpensive, readily available to many practitioners, and should not be affected by dehydration[21]; therefore, it could represent a valuable alternative to the SRID method, which is usually time-consuming, laborious, and expensive for routine use in veterinary clinical practice. The availability of a rapid method for the detection of lambs with FPT gives the clinician an opportunity to begin treatment before the intestine's ability to absorb IgG decreases and thus reduces the lamb's potential exposure to infectious agents. Additionally, evidence that GGT activity remains stable in serum after refrigeration or frozen storage[41,42] provides the opportunity to collect, store, and process samples at a later time for farm screening and passive transfer monitoring programs.

a. Roche reagent, Roche Diagnostic Systems, Branchburg, NJ.
b. BM/Hitachi 917 analyser, Boehringer Mannheim Corp, Indianapolis, Ind.
c. Sheep IgG VET-RID kit, Bethyl Laboratories Inc, Montgomery, Tex.
d. GraphPad InStat version 3.05 for Windows, GraphPad Software, San Diego, Calif.

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