

Passive transfer of colostral immunoglobulin G in neonatal llamas and alpacas

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Objective—To evaluate precolostral hypogammaglobulinemia in neonatal llamas and alpacas, to determine when postcolostral peak serum IgG concentrations develop, to determine whether differences in postcolostral serum IgG concentrations between llamas and alpacas exist, and to determine postcolostral half-life of serum IgG in llamas and alpacas.

Design—Prospective observational study.

Animals—29 llama and 10 alpaca crias.

Procedure—Blood samples were collected prior to suckling and on days 1, 2, and 3 after parturition and analyzed for serum IgG concentration by use of a commercial radial immunodiffusion assay. Additional samples were collected on days 8, 13, and 18 from 8 crias to determine mean half-life of IgG.

Results—Llamas and alpacas are born severely hypogammaglobulinemic. Mean serum IgG concentrations for day-1, -2, and -3 samples for llamas were 1,578 mg/dl, 1,579 mg/dl, and 1,401 mg/dl, respectively, and for alpacas were 2,024 mg/dl, 1,806 mg/dl, and 1,669 mg/dl, respectively. Peak serum immunoglobulin concentration developed between days 1 and 2. Mean half-life of IgG for all crias was 15.7 days.

Conclusions and Clinical Relevance—Although increased mortality has been linked to failure of passive transfer, it is clearly possible to raise crias that have low serum immunoglobulin concentrations. Llamas and alpacas do not differ significantly with respect to immunoglobulin absorption or IgG concentration in neonates. The optimal sampling time for passive transfer status is between 1 and 2 days. (*Am J Vet Res* 2000;61:738–741)

In most domestic large animals, the placenta prevents the parturient transfer of immunoglobulins from the dam to the fetus. The placenta of llamas is diffuse, microcotyledonary, and epitheliochorial, similar to that of mares.¹ Consequently, transplacental transfer of immunoglobulins is presumed to be negligible.

Transfer of immunoglobulins from the dam to the neonate is a critical factor determining survival of the neonate. Failure of passive transfer (FPT) is

not a disease but rather a condition that predisposes neonates to the development of disease. Failure of passive transfer has been linked to increased morbidity and mortality in calves, lambs, foals, and alpaca crias.²⁻⁷ Various studies have demonstrated FPT rates of up to 35% in cattle and 19 to 24% in foals.^{5,6,8,9} This renders FPT a major economic consideration for producers.

There is significant intraspecies variation in colostral IgG concentrations, peak serum immunoglobulin concentrations after ingestion of colostrum, time interval required to reach peak, and immunoglobulin half-life in neonatal serum. In calves, peak serum immunoglobulin concentrations develop by 32 hours after parturition; the reported half-life of IgG is approximately 23 days.^{8,10} Studies have been performed in calves quantitating the increased mortality risk associated with low immunoglobulin concentrations. In calves < 7 days old, an increased risk of mortality is associated with IgG concentrations < 1,000 mg/dl.¹¹ Increases in serum IgG concentration above this threshold are not associated with substantially decreased mortality risk.¹¹ Consequently, a serum IgG concentration of 1,000 mg/dl for neonatal calves is a reasonable goal for producers. In sheep, IgG half-life is 10.6 to 14.5 days.¹² In horses, reported IgG half-life varies from 11.5 to 23 days.¹² Failure of passive transfer in horses is commonly considered to develop when serum IgG concentration is < 400 mg/dl.^{5,6} However, it has been suggested that a concentration of < 800 mg/dl may be a more appropriate cut-off value.¹³ Reported IgG half-lives are more variable in pigs than in other species: 6.5 to 22.5 days.¹² Peak serum IgG concentrations in neonatal pigs are seen at 12 hours, despite the fact that immunoglobulins are absorbed across gastrointestinal tract epithelial cells until 36 hours postpartum in this species.^{14,15}

Information regarding immunoglobulin concentrations in neonatal llamas and alpacas is sparse. It has been demonstrated that similar to cattle, sheep, and horses, llamas and alpacas are born hypogammaglobulinemic and have a significant increase in immunoglobulin concentrations after the ingestion of colostrum.^{7,16,17} In one study, serum IgG concentrations in neonatal, juvenile, and adult llamas were examined.¹⁸ However, this study did not examine serum IgG concentrations during the first week of life of neonatal llamas to assess passive transfer.¹⁸ One study of alpacas in Peru demonstrated that IgG is the major immunoglobulin transferred from the dam to the cria through colostrum.¹⁶ Immunoglobulin M is also transferred, but to a lesser degree.¹⁶ Prior studies have

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investigated the possibility that IgG subisotypes exist in alpacas; however, subisotypes were not identified.¹⁶

Failure of passive transfer has been associated with increased mortality in neonatal alpacas in Peru.⁷ However, hygienic conditions, acceptable mortality goals, and management intervention are expected to differ dramatically between American and Peruvian farms. Failure of passive transfer, defined as serum IgG concentrations < 1,000 mg/dl at 48 hours of age, was found in 9% of crias sampled in the Peruvian study.⁷ Of the crias with serum IgG concentrations < 1,000 mg/dl at 48 hours of age, 7 of 9 (77%) died within the first 30 days of life. Mortality was significantly lower in crias that had IgG concentrations ranging from 1,000 to 1,999 mg/dl (2 of 27 [7%] crias died), and only 1 of 46 (2%) crias with an IgG concentration \geq 2,000 mg/dl died by 30 days of age. Serum IgG concentrations < 1,000 mg/dl in crias < 3 days old were significantly associated with subsequent neonatal mortality ($P = 0.001$).⁷ In crias > 20 days old, a correlation between serum IgG and mortality was not detected. Consequently, a 48-hour threshold concentration of 1,000 mg/dl appears to be reasonable in llama and alpaca crias.

The purposes of the study reported here were to obtain an estimate of rate of FPT in neonatal llamas (*Lama glama*) and alpacas (*Lama pacos*) that were housed under typical conditions in the United States, to evaluate precolostral hypogammaglobulinemia in neonatal llamas and alpacas, to determine when postcolostral peak serum IgG concentrations develop, to determine whether differences in postcolostral serum IgG concentrations between llamas and alpacas exist, and to determine postcolostral half-life of serum IgG in llamas and alpacas. This information is critical to the development of programs to monitor passive transfer in llamas and alpacas.

Materials and Methods

Animals—Serum IgG concentrations were monitored in crias from 5 farms in central Missouri. All dams were housed on their farms of origin under the standard management protocol for that farm. Dams were considered by their owners to be healthy, and no abnormalities were detected during routine physical examinations. Other than routine vaccinations and worming, drugs or other compounds were not administered to dams during gestation or parturition. Only crias resulting from observed parturitions were included in this study. Crias were restrained from nursing until after collection of initial blood samples. Initial blood samples were obtained within 1 hour of parturition in all crias. Thereafter, owners encouraged crias to stand and nurse. All crias received colostrum only from their dam. Crias that received immunoglobulin from an alternate source (caprine or bovine colostrum, or oral plasma) were excluded from the study, as were crias that received whole blood or plasma transfusions. Only crias that survived until 72 hours of age were included in the study (1 cria was excluded by these criteria). Thirty-nine crias met the criteria for inclusion; these included 29 llama and 10 alpaca crias. Hydration, adequacy of colostrum intake, health status, management intervention, and dam or cria behavior were not considered as independent variables.

Sample collection—Whole blood was collected by jugular venipuncture. Blood samples were collected just after parturition (presuckling samples; time 0) and at 1, 2, and 3 days of age. Presuckling samples were collected within 1 hour of birth. Day 1, 2, and 3 samples were collected at 24 ± 3 hours, 48 ± 3 hours, and 72 ± 3 hours, respectively. In a subset of 8 crias (6 llamas and 2 alpacas), additional blood samples were collected on days 8, 13, and 18; these samples were used to calculate serum IgG half-life. All samples were centrifuged; serum was obtained and stored at 4 C for further analyses.

Determination of serum IgG concentration—A radial immunodiffusion assay for camelid immunoglobulin^a was performed according to manufacturer's specifications. Briefly, .05 ml of serum was added to 1 well of a 24-well plate containing antiserum to camelid IgG in agarose. Three standards supplied by the manufacturer were run concurrently with each test sample. The plate was incubated at room temperature (23 C) for 24 hours. The diameter of the diffusion ring was measured and compared to a log graph generated from the standards. For statistical comparisons, all samples with serum IgG concentrations lower than the assay's lowest detectable concentration, 144 mg/dl, were designated as having 0 mg/dl of IgG.

Statistical analyses—Mean (\pm SD) IgG concentrations were calculated, and a Kolmogorov-Smirnov normality test for serum IgG concentration for each time point was performed by use of commercially available software.^b Day 1, 2, and 3 serum IgG concentrations were compared between llama and alpaca crias by use of 1-way ANOVA. In a subset of 8 crias, IgG half-life was calculated for each cria as follows: a log linear regression line was calculated from the data by use of commercially available software.^b The half-life ($t_{1/2}$) of IgG in days was then calculated, using the following equation:

$$t_{1/2} = [\log_{10} (10^9/2) - a] / b$$

where a is the y-intercept and b is the slope of the regression line.¹⁹ Mean, median, SD, and 95% confidence interval of the half-life were calculated. The calculated mean half-life was used to generate a theoretical decay curve for serum IgG concentrations. This curve was premised on a day-2 serum IgG concentration of 1,000 mg/dl, which was considered threshold for FPT. Prevalence of FPT and mortality were compared with those of 2 prior studies by use of a *t*-test for sample proportions.^{7,20}

Results

Precolostral serum IgG concentrations in all crias were below the assay's lowest detectable limit of 144 mg/dl. Serum IgG concentrations at all time periods passed a Kolmogorov-Smirnov test for normality.²¹ Mean (\pm SD) serum IgG concentrations for day 1, 2, and 3 samples were $1,718 \pm 1,194$ mg/dl, $1,655 \pm 1,197$ mg/dl, and $1,500 \pm 1,051$ mg/dl, respectively. Serum IgG concentration ranges on days 1, 2, and 3 were 0 to 4,153 mg/dl, 0 to 4,425 mg/dl, and 0 to 3,377 mg/dl, respectively, with medians of 1,631 mg/dl, 1,499 mg/dl, and 1,315 mg/dl. Mean serum IgG concentrations on days 1, 2, and 3 were not significantly different between llamas (day 1, 1,578 mg/dl; day 2, 1,579 mg/dl; day 3, 1,401 mg/dl) and alpacas (day 1, 2,024 mg/dl; day 2, 1,806 mg/dl; day 3, 1,669 mg/dl; P values = 0.332, 0.569, and 0.520, respectively). The calculated half-life of IgG ranged from 10.1 to 27.7 days (mean

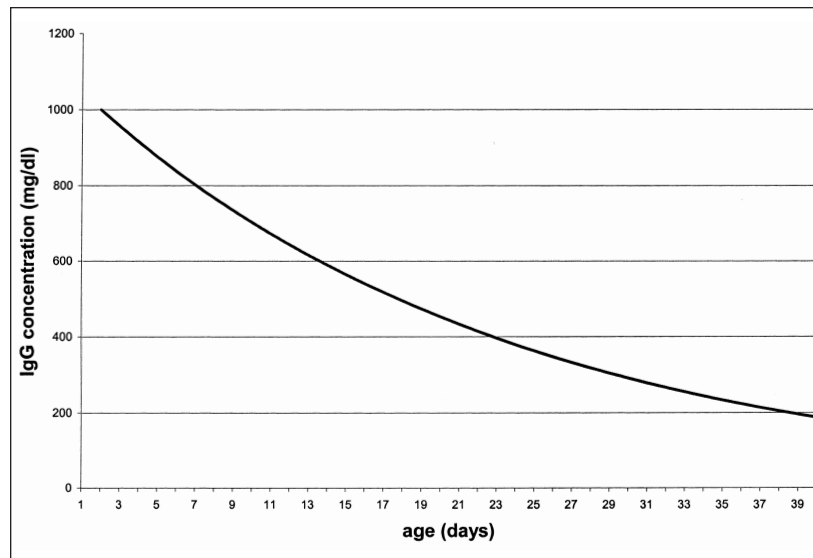


Figure 1—Theoretical decay curve of serum IgG concentrations for llama and alpaca crias, based on a serum IgG concentration of 1,000 mg/dl at 48 hours of age and an IgG half-life of 15.7 days.

\pm SD, 15.7 ± 5.6 ; 95% confidence interval, 10.7, 20.7). A serum IgG decay curve based on a half-life of 15.7 days was generated (Fig 1).

Peak serum concentrations of IgG in neonatal llamas and alpacas developed after 24 hours; 15 crias had peak concentrations on day 1, 6 crias had the same IgG concentration on days 1 and 2, and 12 crias had increased concentrations on day 2. Of the 39 crias examined, 20.5% (8/39) had serum IgG concentrations $<$ 1,000 mg/dl on day 2. None of the crias were treated for infectious diseases within the first 60 days of life. Only 2 deaths occurred in the study population; one at 16 months and the other at 24 months of age. Neither death was attributable to infectious disease.

Discussion

Similar to other studies, precolostral serum IgG concentration in all crias was $<$ 144 mg/dl, which was the lowest concentration measurable by the assay used in our study. The half-life of serum IgG in llamas and alpacas is similar to that for other domestic species.

No cria with serum IgG concentration \geq 1,000 mg/dl on day 1 had a serum IgG concentration $<$ 1,000 mg/dl on day 2. However, 7.7% (3/39) of crias would have been incorrectly classified as having inadequate passive transfer if the day 1 sample alone had been used to determine passive transfer status. Consequently, we recommend that sampling for passive transfer in llama and alpaca crias be delayed until crias are at least 36 hours old. Samples obtained later than 2 days of age may be evaluated on the basis of the decay curve generated in this study (Fig 1) to determine passive transfer status. The curve we generated is premised on an IgG concentration of 1,000 mg/dl at 48 hours of age; these values were chosen because they were considered to define FPT in llamas and alpacas. Serum IgG concentrations were not significantly differ-

ent between llamas and alpacas at any of the sample times. Consequently, recommendations that are made for llamas regarding passive transfer of IgG can most likely be extrapolated to alpacas.

Monitoring programs for passive transfer status in crias can be incorporated into standard farm management protocols. Because crias are born with negligible serum IgG concentrations, ingestion and absorption of colostrum immunoglobulin is critical to achieving adequate serum concentrations of immunoglobulins during the neonatal period.

The rate of FPT in our study was 20.5%. This rate was not significantly different from rates recorded in other studies; however, a significant difference was detected between rates of FPT in 2 reports.^{7,20} In 1 study, the population consisted of crias that were evaluated because of illness at a teaching hospital (in the United States).²⁰ Consequently, a high rate of FPT was expected. In another study, performed in Peru, a significantly higher mortality rate was detected than the rate observed in our study.⁷ This difference most likely reflects differences in management practices or different economic practicalities of therapeutic intervention between American and Peruvian herds.

Results of our study confirmed that llamas and alpacas are born severely hypogammaglobulinemic. The optimal sampling time for passive transfer status is between 1 and 2 days. Llamas and alpacas do not differ significantly with respect to either immunoglobulin absorption or IgG concentrations in neonates. Although increased mortality has been linked to failure of passive transfer, it is clearly possible to raise crias that have low serum concentrations of immunoglobulins.

^aCamelid IgG radial immunodiffusion assay, Triple J Farms, Redmond, Wash.

^bSigma-Stat version 2.0, Jandel Scientific Software, San Rafael, Calif.

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