

Evaluation of treatment of colostrum-deprived kittens with equine IgG

P. Cynda Crawford, DVM, PhD; Rita M. Hanel, DVM; Julie K. Levy, DVM, PhD

Objective—To evaluate equine IgG as a treatment for kittens with failure of passive transfer of immunity (FPT).

Animals—13 specific pathogen-free queens and their 77 kittens.

Procedure—Kittens were randomized at birth into 9 treatment groups. One group contained colostrum-fed (nursing) kittens; the other groups contained colostrum-deprived kittens that were administered supplemental feline or equine IgG PO or SC during the first 12 hours after birth. Blood samples were collected at serial time points from birth to 56 days of age for determination of serum IgG concentrations. The capacity of equine IgG to opsonize bacteria for phagocytosis by feline neutrophils was determined via flow cytometry.

Results—Kittens that received feline or equine IgG SC had significantly higher serum IgG concentrations than those of kittens that received the supplements PO. In kittens that were administered supplemental IgG SC, serum IgG concentrations were considered adequate for protection against infection. The half-life of IgG in kittens treated with equine IgG was shorter than that in kittens treated with feline IgG. Feline IgG significantly enhanced the phagocytosis of bacteria by feline neutrophils, but equine IgG did not.

Conclusions and Clinical Relevance—Serum concentrations of equine IgG that are considered protective against infection are easily attained in kittens, but the failure of these antibodies to promote bacterial phagocytosis in vitro suggests that equine IgG may be an inappropriate treatment for FPT in kittens. (*Am J Vet Res* 2003;64:969–975)

Prior to development of adaptive immunity, passive transfer of maternal antibodies plays an important role in providing neonates with protection against infectious diseases during the first few weeks of life.^{1,3} Similar to neonates of many mammalian species, neonatal kittens rely on ingestion of colostrum for passive transfer of maternal antibodies.^{4,7} During the first 24 hours after birth, a nonselective macromolecular transport mechanism in the small intestine of neonates transfers intact colostrum immunoglobulins and other proteins from the intestinal lumen into the circula-

tion.⁸⁻¹⁰ Thereafter, closure of this absorption pathway occurs, which effectively terminates passive acquisition of humoral immunity. In addition to this nonselective mechanism for passive transfer of immunity, neonatal enterocytes possess a receptor-mediated mechanism specific for IgG transport.¹¹⁻¹⁶ This receptor (FcγRn) selectively binds the Fc portion of IgG for transport from the intestinal lumen into the circulation without degradation.¹⁷

Neonates that fail to acquire adequate amounts of antibodies prior to cessation of intestinal absorption are at significant risk for infection. Failure of passive transfer of immunity (FPT) is a well-documented cause of infection-related illness and death in large animal neonates.¹⁸⁻²⁰ The economic impact of FPT has led to the development of several immunoglobulin supplements for prevention and treatment of FPT in large animal neonates. In contrast, there are few published reports^{21,22} of studies involving administration of immunoglobulins to small animal neonates. In 1 study,²³ parenteral administration of adult cat serum successfully corrected serum IgG deficiencies in newborn kittens with FPT resulting from colostrum deprivation. However, the use of adult cat serum as a source of supplemental immunoglobulins requires collection of large volumes of blood from multiple donors that have been screened for infectious diseases and tested for compatibility of blood types. A more ideal supplement would be a commercial source of concentrated IgG, such as those used for large animal neonates,²⁴⁻²⁶ that is available in large quantities, is relatively inexpensive, has a long shelf life, and is effective when administered in small volumes.

The findings of several studies²⁷⁻³³ indicate that oral administration of antibodies from another species (ie, foreign) to large animal neonates within 24 hours of birth results in absorption of those antibodies; moreover, protection against diseases common to the donor and recipient species is provided. In newborn kittens, the absorption of foreign antibodies after oral administration has also been reported.⁶ Collectively, these findings suggest the feasibility of administering a commercial preparation of foreign IgG to neonatal kittens to provide supplemental IgG as an alternative to administration of adult cat serum. The purpose of the study reported here was to evaluate the efficacy of a commercial preparation of equine IgG that is licensed for treatment of FPT in foals as a source of supplemental immunoglobulin for neonatal kittens with FPT. Passive transfer of immunity in colostrum-deprived newborn kittens that received either a feline or equine IgG supplement was compared with that in colostrum-fed (nursing) kittens. In addition, the capacity of equine IgG to opsonize bacteria for phagocytosis by feline neutrophils was determined in vitro to

Received September 9, 2002.

Accepted March 17, 2003.

From the Department of Small Animal Clinical Sciences (Crawford, Hanel, Levy) and the Comparative Clinical Immunology Program (Crawford, Levy), College of Veterinary Medicine, University of Florida, Gainesville, FL 32608.

Funded in part by the Harold H. Morris Trust Fund for Research in Diseases of Small Animals.

The authors thank Alex Trapp and Debbie Malcolm for technical support.

Address correspondence to Dr. Levy.

assess the potential immune function of the foreign IgG in kittens.

Materials and Methods

Reagents—Commercial preparations^a of propidium iodide (PI; P4170), 0.4% trypan blue (T8154), paraformaldehyde (P6148), and tryptic soy broth (T8907) were used in our study. Commercial preparations^b of fluorescein isothiocyanate (FITC)-conjugated polyclonal anti-feline IgG and FITC-conjugated polyclonal anti-equine IgG were used in the indirect immunofluorescence assay. These antibodies recognize IgG heavy and light chains. Magnesium- and calcium-free phosphate-buffered saline solution (PBSS; pH, 7.2) was prepared by conventional techniques.

Animals—Thirteen blood type-A specific pathogen-free queens and their 77 kittens (in 16 litters) were included in the study. Queens were under constant observation during the final days of gestation, and all deliveries were attended. The research protocol was approved by the Institutional Animal Care and Use Committee and was conducted in facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Treatment groups—Kittens were removed from the queens immediately after birth to prevent suckling and ingestion of colostrum. Kittens in each litter were randomly assigned to 1 of 9 treatment groups; kittens in these groupings were colostrum fed ($n = 12$), colostrum deprived but given a supplement of 400 mg of feline IgG PO (8), colostrum deprived but given a supplement of 800 mg of feline IgG PO (9), colostrum deprived but given a supplement of 400 mg of feline IgG SC (8), colostrum deprived but given a supplement of 800 mg of feline IgG SC (7), colostrum deprived but given a supplement of 400 mg of equine IgG PO (8), colostrum deprived but given a supplement of 800 mg of equine IgG PO (8), colostrum deprived but given a supplement of 400 mg of equine IgG SC (8), or colostrum deprived but given a supplement of 800 mg of equine IgG SC (9). Kittens in the colostrum-fed group were returned to their queens to nurse after blood samples were obtained. All other kittens were fed a milk replacer^c for 48 hours to assure colostrum deprivation before being returned to their queens and allowed to suckle normally for the duration of the study.

Feline and equine IgG supplements—A pooled collection of serum (2,100 mg of IgG/dL) was prepared from blood samples obtained from blood type-A adult cats in the research colony. Aliquots of lyophilized serum containing 400 mg of IgG each were prepared from the serum pool by a commercial manufacturer.^d The final product was endotoxin-free as determined by the Limulus amoebocyte lysate assay, sterilized by gamma irradiation, and safety tested in mice. A commercial preparation^e of lyophilized purified equine IgG was obtained for use as an immunoglobulin supplement. The lyophilized feline serum (hereafter referred to as feline IgG) and the lyophilized equine IgG were each reconstituted in sterile physiologic saline (0.9% NaCl) solution to a concentration of 200 mg of IgG/mL. Kittens receiving treatment with 400 mg of IgG were administered 2 mL of feline or equine IgG (PO or SC) within 6 hours of birth; kittens receiving treatment with 800 mg of IgG were administered 2 mL of feline or equine IgG (PO or SC) within 6 hours of birth and an additional 2-mL dose 4 hours later.

Sample collection—At parturition, samples of blood (1 mL) and colostrum (500 μ L) were collected from each queen. Blood samples (0.2 mL) were obtained from each kitten via jugular venipuncture at birth (day 0) and 0.5, 1, 1.5, 2, 7, 14, 28, 42, and 56 days of age. Sera were obtained from all blood samples and stored at -80°C pending serum IgG analysis.

Measurement of IgG—The concentrations of feline IgG in colostrum and feline and equine IgG in sera were determined by radial immunodiffusion assay,^b as described.²³ The concentration of feline IgG standards ranged from 32 to 2,000 mg of IgG/dL of serum. The concentration of equine IgG standards ranged from 200 to 1,600 mg of IgG/dL of serum. For statistical purposes, serum samples with < 32 mg of feline IgG/dL and < 200 mg of equine IgG/dL were recorded as having a concentration of 0 mg of IgG/dL. Both feline and equine IgG concentrations were determined in sera from colostrum-deprived kittens that received supplemental equine IgG.

Determination of apparent efficiency of IgG absorption—For each kitten that received supplemental IgG, the apparent efficiency of absorption (% AEA) of IgG was calculated by multiplying the estimated blood volume (mL) by the peak serum IgG concentration (mg/mL), dividing the product by the amount of IgG (mg) administered, and multiplying the resulting value by 100.³⁴ The blood volume of kittens was estimated on the basis of 75 mL of blood/kg of body weight.³⁵ To calculate the % AEA, it was assumed that there were equal proportions of IgG in the intravascular and extravascular pools, as determined in neonatal foals after colostrum ingestion.³²

Determination of the half-life of IgG—The biological half-life ($T_{1/2}$) of feline or equine IgG in each kitten was determined from linear regression curves generated by plotting the natural logarithm (Ln) of serum IgG concentrations against time. For colostrum-fed kittens and colostrum-deprived kittens that received supplemental feline IgG, the time points extended from the time of the highest concentration (peak) to the time of the lowest concentration (nadir) of feline IgG in the serum. For colostrum-deprived kittens that received supplemental equine IgG, the time points extended from the peak concentration to the lowest detectable concentration of equine IgG in the serum. The $T_{1/2}$ value was calculated as $-\text{Ln } 2$ divided by the slope of the regression line (b).³⁶

Preparation of bacterial targets—*Staphylococcus aureus* was obtained from a kitten with sepsis; after growth in tryptic soy broth for 18 hours at 37°C , the concentration of bacteria was determined by colony counts on serial dilutions of the broth culture. The bacteria were heat killed at 70°C for 60 minutes, washed, and resuspended to the original volume in sterile PBSS containing 100 μ g of PI/mL. After incubation at 23°C for 60 minutes, the PI-labeled bacteria were washed and resuspended in sterile PBSS to a concentration of 1×10^9 cells/mL. Aliquots of the labeled bacteria were protected from light exposure during storage at 4°C and warmed to 23°C prior to use.

Indirect immunofluorescence assay—An indirect immunofluorescence assay was used to verify the presence of *S aureus*-specific antibodies in the feline and equine IgG supplements. Each IgG supplement was diluted in sterile PBSS to a concentration of 20 mg of IgG/mL; 100- μ L aliquots were added to tubes containing 100 μ L (1×10^8 cells) of PI-labeled *S aureus*. Control tubes contained bacteria only. After incubation at 4°C for 30 minutes, the samples were washed with sterile PBSS and the cell pellets resuspended in 20 μ L of either undiluted FITC-conjugated anti-feline IgG or FITC-conjugated anti-equine IgG. After incubation at 4°C for 30 minutes, the samples were washed; cell pellets were resuspended in 100 μ L of sterile PBSS, and aliquots were removed for immunofluorescent microscopy.

Opsonophagocytosis assay—The bacterial opsonization capacities of the feline and equine IgG supplements were determined by exposing donor neutrophils from an adult cat to PI-labeled bacteria that had been preincubated with feline or equine IgG, followed by flow cytometric measurement of bac-

terial phagocytosis by the neutrophils. Starting at a concentration of 20 mg of IgG/mL, serial 2-fold dilutions of the feline and equine IgG supplements were prepared in an agammaglobulinemic serum pool obtained from blood from colostrum-deprived kittens. Fifteen μ L of each dilution was added to 15 μ L of PI-labeled *S aureus* bacteria (1.5×10^7 bacteria) and incubated at 37°C for 30 minutes. Control tubes contained bacteria with sterile PBSS or agammaglobulinemic serum. Donor leukocytes were prepared by density gradient centrifugation of heparinized blood that was collected from an adult cat in the research colony. The donor leukocytes were suspended in sterile PBSS to a concentration of 5×10^6 cells/mL, and 100- μ L aliquots (5×10^5 cells) were added to the opsonized bacteria to achieve a bacteria-to-leukocyte ratio of 30:1. The samples were incubated with continuous mixing for 30 minutes at 37°C, placed on ice to stop phagocytosis, and fixed by the addition of 500 μ L of ice-cold 0.5% paraformaldehyde in PBSS. Extracellular fluorescence was quenched by the addition of 10 μ L of 0.4% trypan blue. The samples were protected from light exposure and stored on ice; within 2 hours of preparation, flow cytometric analysis was performed.

Analysis via flow cytometry—The samples from the opsonophagocytosis assay were acquired on a flow cytometer⁸ equipped with a 488-nm argon-ion laser for excitation and 15 mW of power. Density cytograms were generated by linear amplification of the signals in the forward scatter and side scatter channels. Fluorescence cytograms were based on a 4-decade logarithmic amplification of signals in the FL2 channel (585-nm filter with a 42-nm band). Neutrophils were selectively analyzed on the basis of their size and complexity in the density cytogram, and data from acquisition of 10,000 events/sample were processed by computer software.^h

Statistical analyses—Data are reported as mean \pm SD. Proportions of male or female kittens were compared among groups with the χ^2 test. Among treatment groups, the Kruskal-Wallis test was used for comparisons of birth weights, IgG doses, serum IgG concentrations at each time point, % AEA, and IgG $T_{1/2}$ values; Mann-Whitney *U* rank sum tests were used for comparisons of the percentage of neutrophils that phagocytosed bacteria. Simple linear regression was used to calculate correlation coefficients (*r*) for colostrum IgG concentration in the queens versus serum IgG concentration in the colostrum-fed kittens. For all tests, *P* values < 0.05 were considered significant.

Results

Serum and colostrum IgG concentrations in the queens—At parturition, mean \pm SD serum IgG concentration of the queens was $1,574 \pm 472$ mg/dL (range, 800 to 2,500 mg/dL). Mean colostrum IgG concentration was $6,995 \pm 2,279$ mg/dL (range, 2,760 to 10,000 mg/dL).

Treatment groups—Birth weights of kittens ranged from 77 to 143 g (mean, 111 ± 12 g). There were no significant differences among treatment groups with regard to the proportions of male and female kittens (*P* = 1.0) or mean birth weights (*P* = 0.6). Because all of the kittens that were given a supplement received a fixed dose of feline or equine IgG, the differences in birth weights resulted in differences in the amount of IgG administered per kg of body weight. However, the mean doses of IgG were not significantly different between groups that received 400 mg of feline ($3,466 \pm 209$ mg/kg) or equine IgG ($3,537 \pm 492$ mg/kg) PO (*P* = 0.7) or between groups that received 400 mg of feline ($3,582 \pm 333$ mg/kg) or

equine IgG ($3,736 \pm 562$ mg/kg) SC (*P* = 0.5). Similarly, the mean doses of IgG were not significantly different between groups that received 800 mg of feline ($7,504 \pm 601$ mg/kg) or equine IgG ($7,227 \pm 612$ mg/kg) PO (*P* = 0.4) or between groups that received 800 mg of feline ($7,254 \pm 289$ mg/kg) or equine IgG ($7,740 \pm 817$ mg/kg) SC (*P* = 0.2). None of the kittens had detectable serum IgG at birth. All kittens remained healthy for the duration of the study.

Comparison of variables among treatment groups—The minimum serum IgG concentration considered adequate for protection against infection in foals³⁷ and goat kids³⁸ is 800 mg/dL, whereas that for calves is 1,000 mg/dL.³⁹ The minimum serum IgG concentration necessary for protection of neonatal kittens against infection is unknown. For the purposes of our study, adequate passive transfer of immunity in kittens was defined as peak serum IgG concentration > 800 mg/dL. The following comparisons were made among treatment groups: highest concentration of IgG in the serum (peak); percentage of kittens with peak serum IgG concentration > 800 mg/dL; interval during which serum IgG concentrations were > 800 mg/dL; time interval at which the lowest concentration of IgG in the serum (nadir) was detected; $T_{1/2}$ of maternally derived and supplement-derived serum IgG; and efficiency of absorption of equine and feline IgG via oral or parenteral administration.

Colostrum-fed kittens—Peak serum IgG concentrations were detected 12 to 24 hours after parturition in all of the colostrum-fed kittens. Mean peak value for this group at 24 hours of age was $3,523 \pm 571$ mg of IgG/dL. All of the kittens had peak serum IgG concentrations that were > 800 mg/dL; however, peak serum IgG concentrations were not significantly correlated with the colostrum IgG concentration in the queens (*r* = 0.01, *P* = 0.1). The serum IgG concentration remained above 800 mg/dL for 7 days in all kittens and for 14 days in 7 of the 12 kittens. Serum IgG concentrations steadily declined to a nadir at 28 days in 6 of the 12 kittens; the nadir was reached at 42 days in the other 6 kittens (Fig 1). The $T_{1/2}$ of the maternally derived IgG was 11.8 ± 2.6 days.

Serum IgG concentrations in the groups receiving supplement SC—Peak serum IgG concentrations occurred between 24 and 36 hours of age in colostrum-deprived kittens that received supplemental IgG SC, and they exceeded 800 mg/dL in all kittens. The % AEA for feline IgG was comparable (*P* = 0.1) to that for equine IgG at both the 400-mg ($23.3 \pm 5.8\%$ vs $22.0 \pm 4.1\%$, respectively) and 800-mg ($20.3 \pm 2.3\%$ vs $23.8 \pm 5.5\%$, respectively) doses. Consequently, peak serum IgG concentrations were similar (*P* = 0.06) for kittens that received 400 mg of supplemental feline or equine IgG ($1,081 \pm 330$ mg of IgG/dL vs $1,011 \pm 180$ mg of IgG/dL, respectively) and for kittens that received 800 mg of supplemental feline or equine IgG ($1,703 \pm 405$ mg of IgG/dL vs $2,245 \pm 594$ mg of IgG/dL, respectively). Kittens that were administered 800 mg of IgG SC had significantly (*P* = 0.004) higher peak serum IgG concentrations as well as higher serum IgG concentrations during the first week of life, compared

with concentrations detected in kittens that were administered 400 mg SC (Fig 1).

In all kittens treated SC with feline IgG, the nadir in serum IgG concentration was detected at 28 days of age; the $T_{1/2}$ of the supplement-derived feline IgG was similar ($P = 0.2$) for the 400-mg (11.5 ± 1.0 days) and 800-mg (10.6 ± 1.3 days) doses and was comparable ($P = 0.3$) to the $T_{1/2}$ of the maternally derived IgG (11.8 ± 2.6 days) in colostrum-fed kittens.

The nadir in serum IgG concentration occurred between 14 and 28 days of age in kittens treated SC with 400 mg of equine IgG and at 28 to 42 days of age in kittens treated SC with 800 mg of equine IgG. The $T_{1/2}$ of equine IgG was similar ($P = 0.2$) for the 400-mg (7.9 ± 2.7 days) and 800-mg (8.4 ± 1.6 days) doses, but was significantly shorter than the $T_{1/2}$ of feline IgG in colostrum-fed kittens ($P = 0.02$) and kittens receiving feline IgG SC ($P = 0.04$).

Serum IgG concentrations in the groups receiving supplement PO—Peak serum IgG concentrations were detected at 12 hours of age in all of the colostrum-deprived kittens that received supplemental IgG PO. The % AEA for feline IgG was comparable ($P = 0.3$) to that for equine IgG at both the 400-mg ($15.1 \pm 4.7\%$ vs $14.9 \pm 5.3\%$, respectively) and 800-mg ($10.3 \pm 3.3\%$ vs $11.7 \pm 1.9\%$, respectively) doses. Thus, peak IgG concentrations were similar ($P = 0.5$) in kittens that received 400 mg of feline or equine IgG (698 ± 294 mg of IgG/dL vs 703 ± 208 mg of IgG/dL, respectively) and in kittens that received 800 mg of feline or equine IgG ($1,026 \pm 345$ mg of IgG/dL vs $1,116 \pm 153$ mg of IgG/dL, respectively). Compared with peak serum IgG concentration in kittens receiving 400 mg of supplemental IgG PO, those administered 800 mg of IgG PO had significantly ($P = 0.02$) higher peak serum IgG concentrations. Fifteen of 17 kittens receiving 800 mg of IgG PO had peak serum IgG concentrations > 800 mg/dL; only 4 of 16 kittens treated with 400 mg of IgG PO had peak serum concentrations of that magnitude.

In contrast to colostrum-fed kittens and kittens that were administered IgG SC,

none of the kittens that were given IgG PO maintained serum IgG concentrations of ≥ 800 mg/dL after 24 hours, and their serum IgG concentrations quickly declined to a nadir at 14 days of age (Fig 2). Via the oral route, the $T_{1/2}$ values of feline and equine IgG were similar ($P = 0.06$); $T_{1/2}$ values at the 400-mg doses were 6.3 ± 1.3 and 4.7 ± 1.8 days, respectively, and at the 800-mg doses were 7.4 ± 3.0 and 5.4 ± 0.8 days, respectively.

Comparison of PO and SC administration of IgG

Peak serum IgG concentration was significantly ($P < 0.001$) higher in colostrum-fed kittens, compared with that of colostrum-deprived kittens that were administered supplemental IgG (regardless of IgG source, dose, or route of administration). Kittens that received supplemental IgG SC had significantly ($P = 0.02$) higher peak serum IgG concentration than that of kittens that received supplemental IgG PO. The absorption of sup-

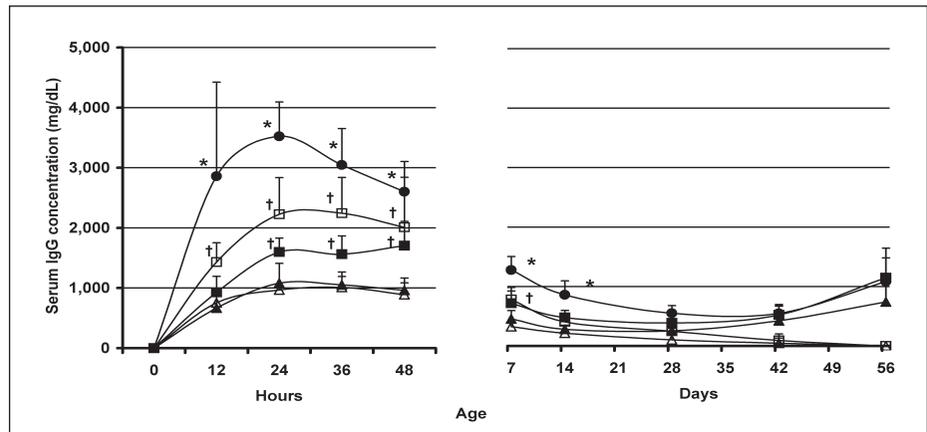


Figure 1—Mean \pm SD serum IgG concentrations from birth to 56 days of age in colostrum-fed kittens and colostrum-deprived kittens administered feline or equine IgG SC. Plots illustrate serum feline IgG concentrations in colostrum-fed kittens (closed circles; $n = 12$) and colostrum-deprived kittens administered 400 (closed triangles; 8) or 800 mg of feline IgG SC (open triangles; 7) and serum equine IgG concentrations in colostrum-fed kittens administered 400 (closed squares; 8) or 800 mg of equine IgG SC (open squares; 9). *Significant ($P < 0.05$) difference among values for colostrum-fed kittens, compared with values for all other kittens. †Significant ($P < 0.05$) difference among values for colostrum-deprived kittens administered 800 mg of feline or equine IgG, compared with values for those administered 400 mg of feline or equine IgG.

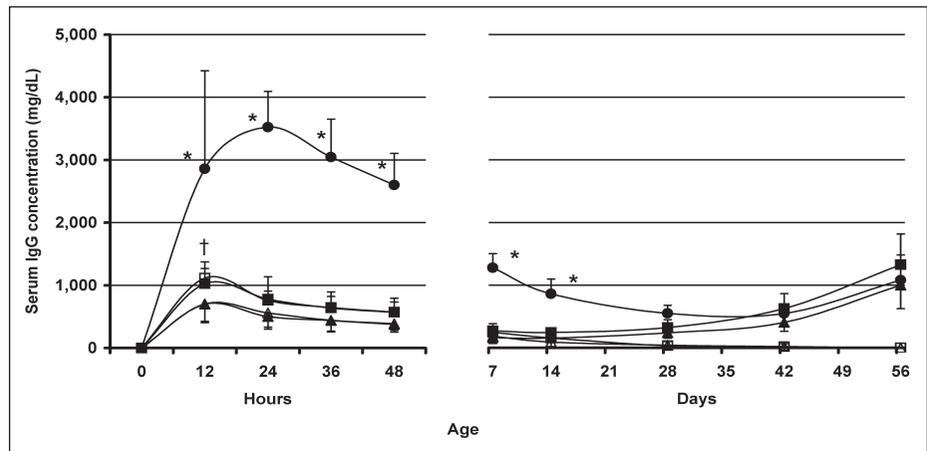


Figure 2—Mean \pm SD serum IgG concentrations from birth to 56 days of age in colostrum-fed kittens and colostrum-deprived kittens administered feline or equine IgG PO. Plots illustrate serum feline IgG concentrations in colostrum-fed kittens (closed circles; $n = 12$) and colostrum-deprived kittens administered 400 (closed triangles; 8) or 800 mg of feline IgG (open triangles; 9) and serum equine IgG concentrations in colostrum-fed kittens administered 400 (closed squares; 8) or 800 mg of equine IgG (open squares; 8). See Figure 1 for key.

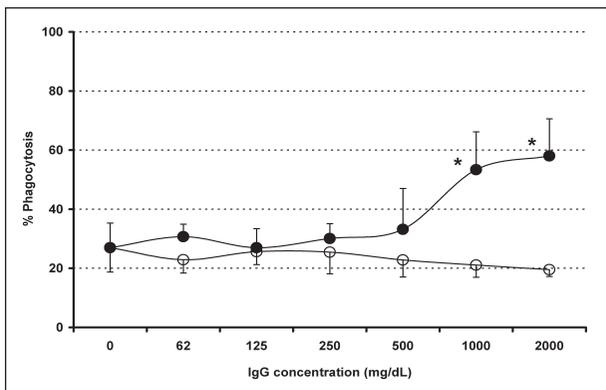


Figure 3—Comparison of bacterial opsonic activity in the feline and equine IgG preparations used as immunoglobulin supplements for treatment of colostrum-deprived kittens. Propidium-labeled *Staphylococcus aureus* were opsonized in agammaglobulinemic serum containing increasing concentrations of feline or equine IgG then added to feline neutrophils; mean \pm SD percentage of neutrophils that phagocytosed bacteria was determined by flow cytometry. Plots illustrate percentage of neutrophils that phagocytosed bacteria opsonized by feline (closed circles; $n = 20$) or by equine IgG (open circles; 11). *Significant ($P < 0.05$) difference between percentage of neutrophils that phagocytosed bacteria opsonized by feline IgG, compared with that for bacteria opsonized by equine IgG.

plemental IgG was more efficient ($P = 0.009$) via the SC route, compared with that achieved via the PO route.

For 2 weeks after achieving peak serum IgG concentration, the serum IgG concentration remained significantly ($P < 0.001$) higher in colostrum-fed kittens, compared with that in colostrum-deprived kittens that received supplemental IgG (Fig 1 and 2). During the first 2 weeks after birth, kittens that were administered IgG SC had significantly ($P = 0.03$) higher serum IgG concentration, compared with that of kittens that were administered IgG PO. The $T_{1/2}$ of IgG in the sera of kittens administered IgG SC was significantly ($P = 0.01$) longer than that detected in kittens administered IgG PO. Consequently, the nadir in serum IgG concentration occurred 2 weeks earlier in kittens administered IgG PO than it did in kittens administered IgG SC. As a result of endogenous synthesis, the concentration of feline IgG was equivalent in all treatment groups by 6 weeks of age.

Comparison of bacterial opsonization by feline and equine IgG—The capacity of the feline and equine IgG supplements to opsonize bacteria for phagocytosis by feline neutrophils was determined in vitro. Each of the IgG supplements contained *S aureus*-specific antibodies in the indirect immunofluorescence assay. Opsonization of *S aureus* with serum containing 2,000 mg of feline IgG/dL resulted in significantly ($P < 0.001$) greater phagocytosis by feline neutrophils, compared with opsonization achieved with agammaglobulinemic serum (Fig 3). With serial dilution of the feline IgG, the opsonic activity decreased and below a concentration of 1,000 mg/dL was not significantly different from that achieved with agammaglobulinemic serum (Fig 3). In contrast to the effects of feline IgG, opsonization of *S aureus* with serum containing 2,000 mg of equine IgG/dL did not enhance phagocytosis to a greater extent than that detected for agammaglobulinemic serum alone.

Discussion

Provision of supplemental feline or equine IgG to colostrum-deprived kittens was more effective via the SC route than via PO administration; IgG supplements given SC resulted in greater absorption, higher peak serum IgG concentrations, serum IgG concentrations considered adequate for protection against infection in a greater number of kittens, and longer duration of these protective serum concentrations.

Some investigators have speculated that the less efficient absorption of IgG administered PO (compared with that achieved via SC administration) was the result of saturation of the macromolecular transport mechanism by nonimmunoglobulin proteins in the supplements.⁴⁰⁻⁴² This does not entirely explain the lower absorption efficiency of IgG administered PO in kittens of the study reported here, because the purified equine IgG supplement was devoid of proteins that were contained in the feline IgG supplement, yet there was no difference in absorption. A more likely explanation for less efficient absorption of IgG administered PO was associated with transit time in the intestinal tract. Via PO administration, transit time for a bolus of IgG may limit the exposure time to the enterocytes, thereby limiting the amount of absorption by the nonselective and Fc γ Rn transport mechanisms. Another explanation for less efficient absorption of IgG administered PO is the absence in that preparation of trypsin inhibitors that are naturally present in colostrum. These trypsin inhibitors protect colostrum IgG from proteolytic degradation and thereby contribute to greater intestinal uptake, resulting in high serum levels of IgG.⁴³⁻⁴⁵ The processing of feline and equine IgG into a lyophilized supplement may also have damaged the molecules and contributed to decreased absorption. The efficiency of absorption of IgG in different commercial supplements for calves varied according to the type of processing used for preparation of each supplement.⁴⁶

In colostrum-fed kittens and those supplemented with feline IgG SC, the $T_{1/2}$ of feline IgG (11 days) was similar to that reported for passively acquired IgG in puppies (10 days),⁴⁷ fox cubs (9 days),⁴⁸ crias (16 days),⁴⁹ calves (16 days),⁵⁰ foals (11 to 26 days),⁵¹⁻⁵³ and piglets (8 to 13 days)⁵⁴⁻⁵⁶ but was longer than that previously reported^{5,6} for nursing kittens. This discrepancy may be due to differences in time points selected for calculation of the $T_{1/2}$ value. Compared with the $T_{1/2}$ of species-specific IgG, the administration of foreign IgG in foals^{32,33} and piglets³⁴ results in shorter $T_{1/2}$ values; similarly, the $T_{1/2}$ of equine IgG (supplemented SC) was significantly shorter than that of feline IgG (supplemented SC) in kittens. From analysis of our data, the $T_{1/2}$ values for feline and equine IgG administered PO were shorter than those measured after SC administration. Structural damage of the IgG molecules resulting from unprotected exposure to proteolytic enzymes in the intestinal tract could account for the shorter $T_{1/2}$ values of IgG administered PO; once absorbed, the damaged molecules may be more susceptible to catabolic processes.

In several studies^{24,25,40,41,57-61} in large animal neonates, administration of IgG supplements did not

achieve serum IgG concentrations comparable to those in colostrum-fed animals; in general, this was considered to be the result of underestimation of the amounts of supplement necessary to produce such serum concentrations. Similarly, treatment of colostrum-deprived kittens with feline or equine IgG did not produce serum IgG concentrations equal to those achieved by nursing kittens. However, correction of serum IgG to concentrations found in nursing neonates may not be necessary for protection; partial correction of FPT in calves and foals was considered clinically beneficial because of the significantly lower risk of death, compared with neonates with uncorrected FPT.^{24,26,57-59,62}

Many of the bacteria that cause neonatal septicemia are not species specific; antibodies derived from 1 species may protect other species from infection. Results of several studies²⁷⁻³³ have indicated that neonates may be protected from multiple pathogen challenges by administration of foreign IgG. In the study of this report, the feline IgG supplement significantly enhanced the phagocytosis of *S aureus* by feline neutrophils in vitro, but equine IgG did not (even though the equine IgG contained *S aureus*-specific antibodies). This finding suggested that equine IgG may opsonize bacteria, but the immune complexes may not bind to Fcγ receptors on feline neutrophils, or the binding is not effective in triggering the cascade of events necessary for inducing phagocytosis. Although there is evidence for conservation of Fcγ receptor structure and function in some species,⁶³ neither feline neutrophil Fcγ receptors nor their affinity for IgG from other species has been characterized. The binding of equine IgG to feline and equine neutrophil Fcγ receptors requires further elucidation.

Although concentrations of equine IgG considered sufficiently protective are easily attained in kittens, failure of equine IgG to promote bacterial phagocytosis in vitro suggests that these antibodies may not achieve the therapeutic goal of preventing infection. The therapeutic efficacy may best be determined by challenging colostrum-fed kittens and colostrum-deprived kittens treated with feline or equine IgG with common bacterial pathogens.

^aSigma Chemical Co, St Louis, Mo.

^bVMRD Inc, Pullman, Wash.

^cEukanuba milk replacer formula for kittens, Iams Co, Dayton, Ohio.

^dBioqual Inc, Rockville, Md.

^eLymphomune, Bioqual Inc, Rockville, Md.

^fHistopaque-1077, Sigma Chemical Co, St Louis, Mo.

^gFACSort flow cytometer, Becton-Dickinson Immunocytometry Systems, San Jose, Calif.

^hCELLQuest, version 3.3, Becton-Dickinson Immunocytometry Systems, San Jose, Calif.

References

- Mason JH, Dalling T, Gordon WS. Transmission of maternal immunity. *J Pathol* 1930;33:783-797.
- Brambell FWR. The transmission of immunity from mother to young and the catabolism of immunoglobulins. *Lancet* 1966;ii:1087-1093.
- Simpson-Morgan MW, Smeaton TC. The transfer of antibodies by neonates and adults. *Adv Vet Sci Comp Med* 1972;16:355-386.
- Harding SK, Bruner DW, Bryant IW. The transfer of antibodies from the mother cat to her newborn kittens. *Cornell Vet* 1961;51:535-539.
- Yamada T, Nagai Y, Matsuda M. Changes in serum

immunoglobulin values in kittens after ingestion of colostrum. *Am J Vet Res* 1991;52:393-396.

6. Casal ML, Jezyk PF, Giger U. Transfer of colostrum antibodies from queens to their kittens. *Am J Vet Res* 1996;57:1653-1658.

7. Pu R, Yamamoto JK. Immunology of the cat: passive transfer of maternal immunity. In: Pastoret PP, Griebel P, Bazin H, et al, eds. *Handbook of vertebrate immunology*. San Diego: Academic Press Inc, 1998;305-308.

8. Jeffcott LB. Duration of permeability of the intestine to macromolecules in the newly born foal. *Vet Rec* 1971;32:340-341.

9. Stott GH, Marx DB, Menefee BE, et al. Colostral immunoglobulin transfer in calves. I. Period of absorption. *J Dairy Sci* 1979;62:1632-1637.

10. Staley TE, Bush LJ. Receptor mechanisms of the neonatal intestine and their relationship to immunoglobulin absorption and disease. *J Dairy Sci* 1985;68:184-194.

11. Jakoi ER, Cambier J, Saslow S. Transepithelial transport of maternal antibody: purification of IgG receptor from the newborn rat intestine. *J Immunol* 1985;135:3360-3364.

12. Benlounes N, Chedid R, Thuillier F, et al. Intestinal transport and processing of immunoglobulin G in the neonatal and adult rat. *Biol Neonate* 1995;67:254-263.

13. Mayer B, Zolnai A, Frenyo LV, et al. Redistribution of the sheep neonatal Fc receptor in the mammary gland around the time of parturition in ewes and its localization in the small intestine of neonatal lambs. *Immunology* 2002;107:288-296.

14. Kacsokovics I, Wu Z, Simister NE, et al. Cloning and characterization of the bovine MHC class I-like Fc receptor. *J Immunol* 2000;164:1889-1897.

15. Wild AE, Burrows TD, Brand J. IgG transport across the gut of the suckling opossum (*Monodelphis domestica*). *Dev Comp Immunol* 1994;18:75-88.

16. West AP Jr, Bjorkman PJ. Crystal structure and immunoglobulin G binding properties of the human major histocompatibility complex-related Fc receptor. *Biochemistry* 2000;39:9698-9708.

17. Ghetie V, Ward ES. Transcytosis and catabolism of antibody. *Immunol Res* 2002;25:97-113.

18. McGuire TC, Crawford TB, Hallowell AL, et al. Failure of colostrum immunoglobulin transfer as an explanation for most infections and deaths of neonatal foals. *J Am Vet Med Assoc* 1977;170:1302-1304.

19. Donovan GA, Dohoo IR, Montgomery DM, et al. Associations between passive immunity and morbidity and mortality in dairy heifers in Florida, USA. *Prev Vet Med* 1998;34:31-46.

20. Virtala AM, Grohn YT, Mechor GD. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during their first 3 months of life. *Prev Vet Med* 1999;39:25-37.

21. Poffenbarger EM, Olson PN, Chandler ML, et al. Use of adult dog serum as a substitute for colostrum in the neonatal dog. *Am J Vet Res* 1991;52:1221-1224.

22. Bouchard G, Plata-Madrid H, Youngquist RS, et al. Absorption of an alternate source of immunoglobulin in pups. *Am J Vet Res* 1992;53:230-233.

23. Levy JK, Crawford PC, Collante WR, et al. Use of adult cat serum to correct failure of passive transfer in kittens. *J Am Vet Med Assoc* 2001;219:1401-1405.

24. Holloway NM, Tyler JW, Lakritz J, et al. Serum immunoglobulin G concentrations in calves fed fresh colostrum or a colostrum supplement. *J Vet Intern Med* 2002;16:187-191.

25. Crawford ML, Quigley JD III, Martin KR. Immunoglobulin concentrations in serum in response to injectable immunoglobulin in neonatal dairy calves. *J Dairy Sci* 1995;78:1567-1572.

26. Franz LC, Landon JC, Lopes LA, et al. Oral and intravenous immunoglobulin therapy in neonatal foals. *J Equine Vet Sci* 1999;19 (suppl 1):7-13.

27. Pierce AE, Smith MW. The intestinal absorption of pig and bovine lactoglobulins and human serum albumin by the newborn pig. *J Physiol* 1967;190:1-18.

28. Logan EF, Foster WH, Irwin D. A note on bovine colostrum as an alternative source of immunoglobulin for lambs. *Anim Prod* 1978;26:93-96.

29. Bridger JC, Brown JF. Development of immunity to porcine rotavirus in piglets protected from disease by bovine colostrum. *Infect Immun* 1981;31:906-910.

30. Clarkson MJ, Faull WB, Kerry JB. Vaccination of cows with clostridial antigens and passive transfer of colostrum antibodies from bovine colostrum to lambs. *Vet Rec* 1985;116:467-469.
31. Frenyo VL. Studies on the absorption of homologous and heterologous IgG in artificially reared newborn pigs. *Vet Res Commun* 1987;11:23-30.
32. Lavoie J-P, Spensley MS, Smith BP, et al. Absorption of bovine colostrum immunoglobulins G and M in newborn foals. *Am J Vet Res* 1989;50:1598-1603.
33. Holmes MA, Lunn DP. A study of bovine and equine immunoglobulin levels in pony foals fed bovine colostrum. *Equine Vet J* 1991;23:116-118.
34. McEwan AD, Fisher EW, Selman IE. An estimation of the efficiency of the absorption of immunoglobulin from colostrum by newborn calves. *Res Vet Sci* 1970;11:239-243.
35. Post GS. Hemapheresis. In: Feldman BF, Zinkl JG, Jain NC, eds. *Schalm's veterinary hematology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2000;871.
36. Paul PS, Mengeling WL, Pirtle EC. Duration and biological half-life of passively acquired colostrum antibodies to porcine parvovirus. *Am J Vet Res* 1982;43:1376-1379.
37. LeBlanc MM. Immunologic considerations. In: Koterba AM, Drummond WH, Kosch PC, et al, eds. *Equine clinical neonatology*. Philadelphia: Lea & Febiger, 1990;275-294.
38. Nandakumar P, Rajagopalaraja CA. Growth and mortality in relation to serum immunoglobulin level in neonatal kids. *Kerala J Vet Sci* 1983;14:49-52.
39. Center for Animal Health Monitoring. Dairy herd management practices focusing on preweaned heifers. Fort Collins, Colo: USDA Animal and Plant Health Inspection Services, Veterinary Services, 1993.
40. Constant SB, LeBlanc MM, Klapstein EF, et al. Serum immunoglobulin G concentration in goat kids fed colostrum or a colostrum substitute. *J Am Vet Med Assoc* 1994;205:1759-1762.
41. Quigley JD III, Fike DL, Egerton MN, et al. Effects of a colostrum replacement product derived from serum on immunoglobulin G absorption by calves. *J Dairy Sci* 1998;81:1936-1939.
42. Besser TE, Osborn D. Effect of bovine serum albumin on passive transfer of immunoglobulin G1 to newborn calves. *Vet Immunol Immunopathol* 1993;37:321-327.
43. Carlsson LC, Westrom BR, Karlsson BW. Intestinal absorption of proteins by the neonatal piglet fed on sow's colostrum with either natural or experimentally eliminated trypsin-inhibiting activity. *Biol Neonate* 1980;38:309-320.
44. Jensen PT, Pedersen KB. The influence of sow colostrum trypsin inhibitor on immunoglobulin absorption in newborn piglets. *Acta Vet Scand* 1982;23:161-168.
45. Quigley JD III, Martin KR, Dowlen HH, et al. Addition of soybean trypsin inhibitor to bovine colostrum: effects on serum immunoglobulin concentrations in Jersey calves. *J Dairy Sci* 1995;78:886-892.
46. Quigley JD III, Strohhahn RE, Kost CJ, et al. Formulation of colostrum supplements, colostrum replacers and acquisition of passive immunity in neonatal calves. *J Dairy Sci* 2001;84:2059-2065.
47. Pollock RVH, Carmichael LE. Maternally derived immunity to canine parvovirus infection: transfer, decline, and interference with vaccination. *J Am Vet Med Assoc* 1982;180:37-42.
48. Muller T, Selhorst T, Schuster P, et al. Kinetics of maternal immunity against rabies in fox cubs (*Vulpes vulpes*). *BMC Infect Dis* 2002;2:10-16.
49. Weaver DM, Tyler JW, Scott MA, et al. Passive transfer of colostrum immunoglobulin G in neonatal llamas and alpacas. *Am J Vet Res* 2000;61:738-741.
50. Husband AJ, Brandon MR, Lascelles AK. Absorption and endogenous production of immunoglobulins in calves. *Aust J Exp Biol Med Sci* 1972;50:491-498.
51. Mattheeuws DRG, Kaneko JJ, Loy RG, et al. Compartmentalization and turnover of ¹³¹I-labelled albumin and gamma globulin in horses. *Am J Vet Res* 1966;27:699-705.
52. Reilly WJ, MacDougall DF. The metabolism of IgG in the newborn foal. *Res Vet Sci* 1973;14:136-137.
53. Jeffcott LB. Studies on passive immunity in the foal. 1. γ -Globulin and antibody variations associated with the maternal transfer of immunity and the onset of active immunity. *J Comp Pathol* 1974;84:93-101.
54. Curtis J, Bourne FJ. Half-lives of immunoglobulins IgG, IgA and IgM in the serum of new-born pigs. *Immunology* 1973;24:147-155.
55. Prochazka Z, Franek M, Krejcl J. Duration of the persistence of ¹³¹I-labelled colostrum and serum IgG in the blood of newborn piglets. *Zentralbl Veterinarmed [B]* 1979;26:366-370.
56. Klobasa F, Werhahn W, Butler JE. Regulation of humoral immunity in the piglet by immunoglobulins of maternal origin. *Res Vet Sci* 1981;31:195-206.
57. Zaremba W, Guterbock WM, Holmberg CA. Efficacy of a dried colostrum powder in the prevention of disease in neonatal Holstein calves. *J Dairy Sci* 1993;76:831-836.
58. Todd AG, Whyte PBD, Carroll PD. A comparison of serum immunoglobulin concentrations in neo-natal calves fed substitute colostrums. *Aust Vet J* 1993;70:154-155.
59. Garry FB, Adams R, Cattell MB, et al. Comparison of passive immunoglobulin transfer to dairy calves fed colostrum or commercially available colostrum-supplement products. *J Am Vet Med Assoc* 1996;208:107-110.
60. Arthington JD, Hauptert JC, Weaver EM, et al. Serum-derived globulin proteins enhance the attainment of passive transfer immunity in newborn calves diagnosed with failure of passive transfer (FPT). *J Dairy Sci* 1996;74(suppl 1):250.
61. Morin DE, McCoy GC, Hurley WL. Effects of quality, quantity, and timing of colostrum feeding and addition of a dried colostrum supplement on immunoglobulin G1 absorption in Holstein bull calves. *J Dairy Sci* 1997;80:747-753.
62. Shideler RK, Squires EL, Mifflin RE, et al. Evaluation of lyophilized IgG as an oral supplement for failure of passive transfer in foals. In: *Proceedings*. 33rd Am Assoc Equine Pract 1987;763-767.
63. Lilliehook I, Johannisson A, Hakansson L. Expression of adhesion and Fc γ -receptors on canine blood eosinophils and neutrophils studied by anti-human monoclonal antibodies. *Vet Immunol Immunopathol* 1998;61:181-193.