SERUM GAMMA-GLUTAMYLTRANSFERASE AS A PROGNOSTIC INDICATOR OF NEONATAL VIABILITY IN NONDOMESTIC RUMINANTS

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SERUM GAMMA-GLUTAMYLTRANSFERASE AS A PROGNOSTIC INDICATOR OF NEONATAL VIABILITY IN NONDOMESTIC RUMINANTS

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Abstract: Rapid assessment of immune status in neonatal ruminants of endangered species facilitates early intervention in cases of inadequate passive transfer of maternal immunoglobulins. Serum gamma-glutamyltransferase (GGT) was used to evaluate suspected passive transfer status in 25 North Indian muntjac (*Muntiacus muntjak vaginalis*), 45 Cretan goats (*Capra algagrus cretica*), 20 white-lipped deer (*Cervus albirostris*), 25 Mhorr gazelles (*Gazella dama mhorr*), and 31 Soemmerring's gazelles (*Gazella soemmerringi soemmerringi*). Serum GGT, measured within 48 hr of birth, was compared with clinical condition at 5 days of age. Neonatal Soemmerring's and Mhorr gazelles with GGT >600 U/L were likely to survive without medical intervention, whereas GGT <400 U/L was a good indicator that the gazelle neonate would need medical intervention. Neonatal muntjac with GGT >200 U/L were also likely to survive without medical intervention. Because there is no gold standard for evaluating passive transfer status in neonatal nondomestic ruminants, it is recommended to evaluate the results of more than one diagnostic test, as well as clinical condition, in considering health status and disposition of neonatal ruminants of endangered species.
Key words: Failure of passive transfer, gamma-glutamyltransferase, ruminant, neonate.

INTRODUCTION

Ruminants are born with extremely low serum immunoglobulin (Ig) levels, and absorption of colostral antibodies after birth is essential to provide passive immunity during the neonatal period. The importance of colostrum to the health of neonatal domestic ruminants has been commonly reported in the literature.[1,18] Assessment of appropriate suckling, colostral intake, and maternal care can be challenging in nondomestic species in a zoo or wild animal park, where groups of animals are housed together, and natural exhibits create blind spots and hiding places for neonates. Rapid assessment of immune status would facilitate early intervention in cases of failure of passive transfer (FPT) of maternal antibodies and ensure these animals the best chance of survival.

Several studies have investigated methods to establish immunoglobulin status in a variety of neonatal nondomestic ungulates, including zinc sulfate turbidity,[11] total protein refractometry,[4,11,16] glutaraldehyde coagulation,[4,5,11,14] and serum electrophoresis.[11,14,20] Some assays used in domestic animal medicine, such as radioimmunodiffusion (RID) and enzyme-linked immunosorbent assay, require species-specific reagents and are not practical for multispecies zoological collections.[6]

Gamma-glutamyltransferase (GGT) is a membrane-associated protein involved in amino acid transport. Activity of GGT is high in secretion products such as seminal fluid, bile, and colostrum.[21] The GGT molecule is readily absorbed by the neonatal ruminant, and serum activity of GGT is high in domestic calves and lambs that consume colostrum.[17,21] Colostrum secretion and neonatal absorption of GGT decrease rapidly after the onset of lactation in cows. Absorption of GGT and colostral immunoglobulins is limited to the same 24-hr window. Consequently, serum GGT levels may be used to indicate successful passive transfer when immunoglobulin levels cannot be measured directly.[23] Measurement of GGT levels in serum can be performed by most standard chemistry analyzers and does not require specialized equipment or processing of the sample. Although measurement of total protein by refractometry can be altered by the hydration status of the neonate, GGT is not.[15] In neonatal llamas (*Llama llama*) and alpacas (*Llama pacos*), GGT was not predictive of passive transfer status.[9,22] The GGT levels compared with RID results in bongo (*Tragelaphus euryceros isaaci*) and Java banteng (*Bos javanicus*) were considered helpful but not clinically significant.[7] Significant differences in GGT between normal and abnormal neo-

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nates (determined by total protein value) were found in slender-horned gazelles (*Gazella leptoceros*) and giraffe (*Giraffa camelopardalis*) but not in Addra gazelles (*Gazella dama ruficollis*) or bontebok (*Damaliscus dorcas dorcas*).[7]

No single, reliable method for assessment of FPT in neonatal nondomestic ruminants has been determined. The purpose of this investigation is to determine whether a predictive relationship exists between serum GGT and neonatal survivability.

MATERIALS AND METHODS

A retrospective study design was used to evaluate the ability of serum GGT to predict neonatal prognosis for survival without medical intervention.

Blood samples were collected from five species of newborn nondomestic ruminants housed at the San Diego Zoo (SDZ), born between January 1996 and March 2003, with complete medical records including serum biochemistry analysis. Twenty-five north Indian muntjac (''muntjac,'' *Muntiacus muntjak vaginalis*), 45 Cretan goats (*Capra algagrus cretica*), 20 white-lipped deer (''deer,'' *Cervus albirostris*), 25 Mhorr gazelles (*Gazella dama mhorr*), and 31 Soemmerring's gazelles (*Gazella soemmerringi soemmerringi*) were included in the study. All ruminants born at SDZ receive a neonatal examination at 18–48 hr of age, which includes physical examination, weight, gender verification, blood collection from the jugular vein, permanent identification, and administration of prophylactic antibiotic (procaine penicillin G, MWI Veterinary Supply, Highland, California 92346, USA) and tetanus toxoid (Tetanus toxoid, Fort Dodge Laboratories, Fort Dodge, Iowa 50501, USA). All laboratory procedures were performed on-site at the SDZ clinical pathology laboratory. Whole blood in serum separator tubes was centrifuged and the serum harvested for a standard biochemical panel. GGT was analyzed by the Ciba Corning Express 550 Plus (Bayer Corporation, Tarrytow, Indiana 10591-5097, USA), measured by the modified Szasz method (Bayer Corporation).[19]

Neonate medical records were evaluated retrospectively and compiled into a database including species, gender, birth date, date of blood collection, weight at exam, GGT results, abnormal physical exam findings, treatments, and medical care administered. The clinical condition of the neonate was evaluated within 5 days of age and was divided into either ''normal'' or ''abnormal.'' Normal neonates required no medical follow-up after their neonatal exam or had a single recheck performed on exhibit for minor problems such as worn hooves, long umbilicus, or a transient heart murmur. Abnormal neonates required medical care and were hospitalized, transferred to the hand-rearing facility, or died. Necropsy reports of neonates that died within 2 days of birth were reviewed for evidence of nursing activity, indicated by presence of milk in the abomasum, and for predominant postmortem findings. After being analyzed separately, neonatal Mhorr gazelles and Soemmerring's gazelles were grouped together in a gazelle group (''gazelles'') for analysis because of their close taxonomic relationship. GGT results were compared between normal and abnormal neonates in the gazelle, muntjac, deer, and Cretan goat groups.

In addition, clinically healthy adult muntjac (n = 16), Cretan goats (n = 25), deer (n = 24), Mhorr gazelles (n = 10), and Soemmerring's gazelles (n = 16) greater than 9 mo of age, examined for preshipment or quarantine, with complete medical records, were evaluated for serum GGT values. Adult gazelles were grouped together in an adult gazelle group for analysis. Adult GGT values in the four ruminant groups were compared with GGT values in normal neonates.

Statistical analysis

The GGT was not normally distributed and was analyzed for need for medical care in all four ruminant groups sampled using the Wilcoxon signed ranks test (JMP IN, Version 4, SAS Institute, Cary, North Carolina 27513, USA). The GGT results of neonates and adults in each ruminant group were not normally distributed and were compared using the Wilcoxon signed ranks test (JMP IN, Version 4, SAS Institute). Values of $P < 0.05$ were considered statistically significant. Data are presented as mean values and ranges.

RESULTS

Neonatal GGT results are shown in Table 1. The The GGT levels were significantly higher in normal neonatal gazelles than in abnormal neonatal gazelles. Six of 24 abnormal neonatal gazelles had GGT values >400 U/L. Five of these gazelles were hand-raised because the dam had a poor history of raising offspring, and the sixth gazelle was treated at the hospital overnight but returned to the exhibit to be raised by the dam. GGT levels were significantly different between normal and abnormal neonatal muntjacs. The GGT levels in the normal neonates were significantly higher than adult GGT levels in all four groups of ruminants evaluated (Table 2).

Postmortem evaluations of abnormal neonates that died within 2 days of age demonstrated an

Table 1. GGT values in neonatal gazelles (Soemmerring's gazelles and Mhorr gazelles), Cretan goats, North Indian muntjac (muntjac), and white-lipped deer (deer) between 18 and 48 hr of age, compared with clinical condition at 5 days of age.

		Normal neonates		Abnormal neonates	
Group	*N*	Mean ± SD (range) (U/L)	*N*	Mean ± SD (range) (U/L)	*N*
Gazelles	56	1,519 ± 1,081 (217–4,220)[b]	32	452 ± 907 (2–3,915)[b,c]	24
Cretan goats	45	75 ± 42 (22–240)	39	64 ± 29 (22–97)	6
Muntjac	25	950 ± 656 (239–2,620)[b]	23	118 ± 55 (79–157)[a]	2
Deer	20	224 ± 115 (119–527)	19	43	1

[a] GGT, gamma-glutamyltransferase.
[b] Significant difference between normal and abnormal neonates, $P < 0.05$.
[c] Six abnormal gazelle neonates had GGT > 400 U/L, these gazelles were removed from the dam because of poor maternal history not because of clinical condition of the neonate.

empty abomasum in the deer (GGT = 43 U/L), one munjtac (GGT = 79 U/L), one Cretan goat (GGT = 24 U/L), and two gazelles (GGT = 23 and 23 U/L). Postmortem evaluations of abnormal neonates that died between 4 and 21 days of age identified two gazelles with inflammation in the gastrointestinal or respiratory tracts, or both (GGT = 6 and 283 U/L).

DISCUSSION

The artiodactyls included in this study are all ruminants; yet, each has evolved to occupy a different ecological niche and has developed a different survival strategy.[13] Although all five species have the same cotyledonary, epitheliochorial placentation,[8,12] other aspects of their reproductive biology may be different, leading to differences in the composition and production of colostrum by the dam and differences in the absorption of colostrum, GGT, and immunoglobulins by the neonate.

In the gazelle and muntjac groups, GGT levels were significantly higher in normal neonates, indicating that assessment of neonatal serum GGT levels at 24–48 hr after birth may be a reliable positive prognostic indicator for neonatal survival in these species. In a study of 12 domestic calves, postsuckling serum GGT levels were 800 times higher than maternal GGT serum levels. These elevated neonatal GGT levels took 5 wk to return to baseline levels.[21] An investigation in Holstein heifer calves established that 1-day-old calves with successful passive transfer should have serum GGT > 200 U/L and that calves with GGT < 50 U/L should be classified as having FPT.[15] In this study, the mean GGT value of normal gazelle neonates was significantly higher than that of abnormal gazelles, but the ranges of normal and abnormal gazelles overlap. Of the six abnormal gazelles that had GGT > 400 U/L, five were hand-raised because of the dam's poor reproductive history not because the neonates appeared overtly ill. The sixth gazelle was taken to the hospital because of the dam's poor reproductive history and inclement weather but was released back to the exhibit after overnight treatment. These six animals may have consumed some amount of colostrum before being removed from the exhibit. On the basis of the distribution of our data, postsuckling neonatal gazelles have greater GGT elevations than neonatal domestic calves, and neonatal gazelles with GGT < 400 U/L appear likely to require medical care. Neonatal gazelles with

Table 2. Comparison of neonatal and adult GGT values in gazelles (Soemmerring's gazelles and Mhorr gazelles), Cretan goats, North Indian muntjac (muntjac), and white-lipped deer (deer).[a]

	Normal neonates (<3 days of age)		Adults (>9 mo of age)	
Group	Mean ± SD (range) (U/L)	*N*	Mean ± SD (range) (U/L)	*N*
Gazelles	1,519 ± 1,081 (217–4,220)[b]	32	19 ± 9 (4–36)	26
Cretan goats	75 ± 42 (22–240)[b]	39	35 ± 8 (23–53)	25
Muntjac	950 ± 656 (239–2,620)[b]	23	117 ± 27 (82–176)	16
Deer	224 ± 115 (119–527)[b]	19	32 ± 4 (22–39)	24

[a] GGT, gamma-glutamyltransferase.
[b] Significant difference between neonatal values and adult values, $P < 0.0001$.

GGT > 600 U/L are likely to be successfully raised by the dam without medical intervention.

In neonatal muntjac and deer, the ranges for normal and abnormal neonates were more distinct, possibly because all three abnormal cervid neonates were clinically ill and two demonstrated an empty gastrointestinal tract on necropsy at 2 days of age. It is likely that these three abnormal neonates were ill, at least in part, because of immunosuppression from having inadequate maternal antibodies. Because of the low number of abnormal deer neonates ($n = 1$), no statistical comparison of GGT was performed between normal and abnormal neonatal deer. On the basis of the distribution of our data, muntjac with GGT > 200 U/L appear likely to be successfully raised by the dam without medical intervention. Because of the small number of abnormal muntjac and deer in this study, however, further investigation is needed before recommendations can be made.

The duration of GGT elevation is not consistent across domestic species investigated. In neonatal domestic foals, GGT was ineffective in evaluating FPT because levels rose slowly during the first 5 days of life.[2] Conversely, GGT levels in domestic calves were elevated for 5 wk postpartum.[21] The GGT levels increased immediately after suckling in neonatal domestic goat kids[2] and lambs[17] but began to decrease by 24 hr of life. A similar, rapid decrease in GGT levels may explain why GGT results for the neonatal Cretan goats included in this study were not significantly associated with clinical findings. At the SDZ, neonates were examined on the second calendar day of life, which could have been between 18 and 48 hr of life, depending on the time of birth and the time of the neonatal exam. If Cretan goats were examined in the latter half of this time period, their maternally derived GGT elevations may have already been declining, as demonstrated in domestic neonatal kids. Serum GGT levels in postsuckling neonatal domestic kids were reported to be only 6.5 times higher than serum GGT values of their dams,[2] suggesting that the overall increase in postsuckling GGT in goats is not as dramatic as is observed in cows. In neonatal nondomestic goats, further investigation into GGT within the first 24 hr of life is required to determine the usefulness of this enzyme as an indicator of successful passive transfer.

In this study, GGT levels of normal neonates were significantly higher than adult GGT levels in all four groups of ruminants studied. These data implicate absorption of colostrum as the cause of increased neonatal GGT values and are consistent with findings of highly elevated GGT levels in domestic calves.[21] Depending on the age of the patient, the primary consideration for elevated GGT in healthy neonatal ruminants should be colostral passive transfer. In adult ruminants, hepatic disease should be considered the most likely cause of elevated GGT.[17,21]

The GGT results in the five abnormal neonates that died within 2 days of age support the postmortem discovery of empty gastrointestinal tracts, consistent with lack of colostrum ingestion. Two abnormal neonates that died between 4 and 21 days of age had evidence of inflammation on postmortem evaluation and had low serum GGT levels. These findings support the suspicion of infection or inflammation associated with inadequate maternal antibodies. Evaluation of postmortem findings in this study was limited to neonates that died within approximately 3 wk of age because studies of domestic calves have demonstrated neonatal mortality associated with FPT to be highest during the second and third week of life.[3,10]

In domestic ruminants, the gold standard for evaluating successful passive transfer of immunity is measurement of immunoglobulins using RID.[11,15] Unfortunately, RID requires species-specific reagents and has not been developed for nondomestic ungulates, other than llamas and alpacas.[6] Because there is no quantitative measurement for FPT, we elected to evaluate presumed successful passive transfer on the basis of the clinical condition of the neonate within 5 days of birth. Although FPT is a very common cause of illness in the neonatal ruminant, it is possible that neonates that required medical attention or that died in this study had successful passive transfer of immunoglobulins and became ill or died for other reasons. Clinicians at SDZ used total protein values, glutaraldehyde results, and reproductive history of the dam to guide management decisions regarding the neonates included this study. Consequently, some neonates that may have had successful passive transfer may have been identified as abnormal in this study, when they might have survived normally in the exhibit with the dam. This appears to be the case in five gazelles with GGT > 400 U/L that were identified as abnormal by the methods of this study. Designation of all neonates that required any medical attention as abnormal was elected to develop the most conservative recommendations possible, with regard to suspected FPT in these endangered species. The objective method of designating normal neonates as those that did not require medical care after their neonatal exams was chosen because it was less subjective than examining detailed individual medical histories. Although this method may have misidentified some animals, significant differences were still observed between the normal and the abnormal animals in each taxonomic group and general recommendations could be made.

This study has demonstrated significant differences in the reliability of one test as a prognostic indicator of neonatal viability in four different groups of ruminants. Because there is no single gold standard for FPT in neonatal nondomestic ruminants, it is recommended to evaluate results from more than one test for FPT, as well as clinical condition, in considering health status and disposition of an endangered neonate.

CONCLUSIONS

In conclusion, in evaluation of neonates between 18 and 48 hr of life, neonatal gazelles with GGT > 600 U/L were likely to be successfully raised by the dam without need for medical intervention, whereas GGT < 400 U/L was a good indicator that the neonate would need medical intervention. In neonatal muntjac, GGT > 200 U/L was associated with successful neonatal survival without need for medical intervention. In neonatal Cretan goats, serum GGT levels were not significantly associated with clinical outcome.

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