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The Half-life for Maternally Derived Immunoglobulin G Anti-viral Antibodies in Data from an Observational Field Study[a]

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Abstract

Using data for 161 calves from a matched case-control study nested in an observational prospective cohort study, the half-life of virus-specific maternal serum antibodies was estimated. Each calf had virus-neutralizing antibody titers measured against bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis, parainfluenza-3, and bovine respiratory syncytial viruses determined postcolostrally, and at one or two time points later in life (but before 100 d of age). In this calf population, few calves were infected with viruses, because only 16 virus isolations from 15 animals were obtained and the population showed declining titers for viruses. The half-life for virus-specific antibodies was estimated as 23 d or less (95% confidence interval [CI], 21 to 26 d). This corresponds to the half-life for IgG found in many other studies with different study designs. If the three calves persistently infected (PI) with BVDV and/or five suspected-BVDV-PI-calves were excluded, the estimated half-life did not change. If samples from calves >30 d of age were excluded from the analysis, the half-life was determined to be 12 d (95% CI, 10 to 17 d).

Résumé

À l'aide de données provenant de 161 veaux d'une étude appariée de type cas-contrôle imbriquée dans une étude longitudinale prospective, la demi-vie des anticorps sériques maternels spécifiques à certains virus a été estimée. Les titres d'anticorps neutralisant les virus ont été mesurés chez chaque veau pour le virus de la diarrhée virale bovine (BVD) et de la rhino trachéite bovine infectieuse, le virus parainfluenza-3 et le virus respiratoire syncytial bovin après la prise du colostrum et à deux moments par la suite (avant l'âge de 100 jours). Dans cette population de veaux, peu d'individus étaient infectés par les virus comme l'indique l'isolement de seulement 16 cas de virus chez 15 veaux et la chute des titres viraux dans la population. La demi-vie des anticorps spécifiques aux virus était estimée à 23 jours ou moins (intervalle de confiance à 95% (IC), 21 à 26 jours). Cette valeur correspond à la demi-vie des IgG rapportée dans plusieurs autres études avec des plans expérimentaux différents. La demi-vie resta similaire même en excluant les trois veaux infectés avec le BVD de façon persistante et/ou les cinq cas soupçonnés d'infection persistante au BVD. Lorsque les échantillons des veaux âgés de plus de 30 jours étaient exclus, la demi-vie était de 12 jours (IC à 95%, 10 à 17 jours).

Introduction

Serum antibody concentrations immediately begin to decline through normal catabolic processes after absorption of maternally-derived immunoglobulin (Ig)

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ceases.[18] The estimate of the half-life for post-colostral IgG in calf serum varies, depending on the procedure of determination. Some sources suggest a half-life of 20 days for bovine IgG[2] whereas others suggest a range of nine to 21 days for IgG.[7]

The immune proteins in bovine colostrum are of the IgG class (primarily IgG_1) and so the half-life of specific antibodies would not be different from the half-life of total IgG.[18] Measurements of the decline of specific antibody activity should provide comparable data to total IgG measurements.

The first objective of this study was to use determinations of specific antibody titers to estimate the half-life of virus-specific post-colostral IgG in calf serum. The second objective was to compare the estimated half-life to estimates from previous studies to determine if an observational field study would be suitable for half-life estimations.

Material and Methods

The overall design of the observational field study was a matched case-control study nested in a prospective cohort study (i.e., cases and controls were selected from the calves in the cohort study).[21,22] The sampled population was a convenience sample of female dairy calves from 18 herds situated in the practice area of the study veterinarian (GDM). The first 35 calves that were alive at the first herd visit were systematically selected in birth-order starting from alternating ends of the herd-visiting route each month. The calves were enrolled between January 1, 1990, and December 31, 1990. From these calves, 105 calves contracted pneumonia (cases). Additionally, 59 age- (± 2 wk) and herd-matched control calves were chosen (there was a shortage of healthy calves in some herds). From this case-control study, 161 calves were used for the half-life determination. No calves were vaccinated during the study.

The first blood samples were taken at a mean 5.7 days of age (Table 1). The second and third blood samples were taken at 21-day intervals from the case and matched control calves after the veterinarian diagnosed a respiratory disease. Virus neutralizing (VN) antibodies to bovine viral diarrhea virus (BVDV), infectious bovine rhinotracheitis virus (IBRV), parainfluenza-3 virus (PI-3V), and bovine respiratory syncytial virus (BRSV) were determined in the Diagnostic Laboratory at the Cornell University College of Veterinary Medicine according to standard procedures.[5,14]

Transtracheal wash (TTW) samples were taken at the same time as the second blood sample collection following disinfection of the skin over the trachea from a lavage with 20 ml of sterile saline through a catheter.[b] The TTW samples were examined at the Diagnostic Laboratory of the College of Veterinary Medicine for the presence of viruses using standard procedures.[5] Cell cultures used in testing were selected to detect any of the common viral respiratory pathogens; primary bovine lung cells and primary bovine testicle cells were used. Individual calves with BVDV isolations from the TTW were further tested to determine existence of persistent

Table 1. Number of specific serum antibody determinations (from 161 female Holstein calves < 3 months old) that were used in IgG half-life calculations.

Specific serum antibody concentration against	Number of specific serum antibody determinations		
	1st determination (at mean 5.7 d of age) range 1-16 d	2nd determination (at mean 42.0 d of age) range 7-90 d	3rd determination (at mean 59.3 d of age) range 26-94 d
Bovine viral diarrhea virus	159	157	154
Infectious bovine rhinotracheitis virus	156	157	153
Parainfluenza-3 virus	152	152	152
Bovine respiratory syncytial virus	83	83	89

[b]Delmed I-Cath, Charter Med, Lakewood, NJ 08701

infections. There were three pneumonic calves persistently infected (PI) with BVDV (BVDV isolated from mononuclear cell preparations of whole blood on two occasions at least three weeks apart, regardless of serologic status) and five PI suspect calves.[23]

Numbers of specific serum antibody determinations from these 161 calves are presented in Table 1. The number of the BRSV determinations was smaller than the number of the other antibody determinations due to insufficient serum collected from some calves. The BRSV antibodies were determined four years after the study had ended.

In a linear multiple regression analysis using the procedure MIXED in SAS, natural logarithms of the reciprocal titers were used as the dependent (outcome) variables.[15] The independent variable was the age in days when each sample was taken. Each titer was one observation (one study unit) in these analyses; for example, each calf could contribute up to four observations for the first titer, four for the second titer, and four for the third titer. Because each calf was used in the regression several times (all virus titers were determined from all calves), calf was designated as a random effect in modeling to adjust for the interdependence between titers from the same animal.

The following criteria were used to exclude observations from the analysis:

1. First titers that were zero were excluded, because either they could not decline or they were incorrect measurements. The corresponding second and third titers against the same virus in the same calf were also excluded, because they most probably were not maternally derived in that calf.
2. If the age of the calf at the time of the second sample was the same as the age of the calf at the time of the first sample, we concluded that only one sample had been taken. As a consequence, the second titer was excluded so that there was only one observation in the analysis. In this case, these duplicate observations would have biased the results because, in real life, there was only one sample with one determination for each virus.
3. The second titer was excluded if it was greater than or equal to the corresponding first titer, because the second titer in that calf would most probably not be entirely maternally derived. The corresponding third titer was also excluded.
4. The third titer was excluded if it was greater than or equal to the corresponding second titer, because the third titer in that calf would most probably not be entirely maternally derived.
5. Only antibodies against viruses were used because this population of 161 calves did not, in general, seem to have contracted viral infections during the first three months of life. There were few virus isolations (16 isolations from 15 different calves in the population of 161 calves), and no seroconversions to viruses (as was shown previously with geometric mean titers[22]).

Two additional criteria to exclude observations were used:

6. The second titer was excluded if the age of the calf at sampling was > 30 d, because we assumed that antibody production had started by this age.[3]
7. The third titer was excluded if the age of the calf at sampling was > 30 d (for the same reason as the previous exclusion).

The procedure MEANS in SAS was used to calculate the age distributions.[16]

Results

The seroconversions and TTW results have been presented previously[21,22] and are not reproduced here to avoid duplication and to focus on the half-life determination. The age distributions of calves used in this study at first, second, and third specific antibody determination are presented in Table 2 in order to show the intersampling intervals.

Using exclusion criteria 1 to 5, there were 1,071 observations for virus antibodies. The model was $\log_e$(virus titer) = 4.607 (SE, 0.093) - 0.030 days (SE, 0.002). A half-life of 23 d was calculated (95% confidence interval [CI], 21 to 26 d). If the three calves persistently infected (PI) with BVDV and/or five suspected BVDV-PI calves were excluded, the estimated half-life did not change.

Using exclusion criteria 1 to 7, there were 617 observations for virus antibodies. The model was $\log_e$(virus titer) = 4.847 (SE, 0.109) - 0.056 days (SE, 0.008). A half-life of 12 d was calculated (95% CI, 10 to 17 d).

Discussion

The confidence interval for the estimated half-life of 23 d included the 21-d half-life reported from other studies, indicating that our method gave comparable results. McEwan *et al* estimated a half-life of 21.5 d for passively acquired Ig using 15 bull calves less than one week of age and sampling at 7-d intervals for 21 d.[11] Husband *et al* measured the concentration of IgG_1 and IgG_2 in the serum of seven calves at 0, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 d of age, finding the half-life between 16 and 32 d.[8] Logan *et al* examined serum samples taken

Table 2. Age distributions of 161 female Holstein calves < 3 months old used in viral antibody half-life calculations.

Serum antibody determination	N*	Age at antibody determination (d)				
		Mean	Quartile			Range
			First	Second	Third	
First	478	5.7	3.0	5.0	8.0	2 to 16
Second	302	42.0	25.0	35.5	61.0	12 to 92
	(128) †	(22.7)	(17.0)	(24.0)	(27.5)	(12 to 30)
Third	291	59.3	45.0	55.0	72.0	22 to 100
	(11)	(27.0)	(22.0)	(29.0)	(30.0)	(22 to 30)

* N = number of observations; † the values in brackets were obtained if calves > 30 d of age were excluded from the analysis.

from 16 calves at birth, 4 h, 24 h, 48 h, 1 wk, and thereafter at weekly intervals until the calves were 12 wk old.[10] Their estimate of the half-life of IgG was 21 d. Porter used 10 periodic assays over the first six weeks of life to determine that the half-life for IgG was 20 d for two Ayrshire calves.[13] Sasaki *et al* measured the decline of Ig in plasma of six female Holstein calves following a meal of iodine-125-labeled IgG_1 in colostrum derived from their dams, taking measurements before colostrum intake and thereafter once every three days for approximately three weeks.[17] The half-life, as measured by the decrease of IgG_1 in plasma concentration, was 19.9 d ± 1.9 d.

Brar *et al* determined (using 40 calves that were sampled when 48 h old and thereafter once a month) a half-life of 21 d for BVDV and IBRV antibodies.[4] Palfi *et al* obtained the same result, but they had only one calf that was not persistently infected with BVDV in their study for determination of BVDV antibody half-life.[12] Additionally, they calculated a half-life of 5 to 11 d for BVDV antibodies in 11 persistently BVDV-infected calves and speculated that the presence of BVDV due to persistent infection might have caused the more rapid disappearance of maternal BVDV antibodies from the serum. Baker *et al* observed the decline of maternally-derived BRSV antibody titer in 62 calves that were sampled once a month; at least 12 of the calves were sampled for the first time shortly after colostrum intake.[1] Using their decline figure, a rough half-life estimate of about 27 d for BRSV antibodies was calculated. In a recent study, Kirkpatrick *et al* reported half-life estimates of 12.7 d for IBRV, 20.5 d for BVDV, and 28.1 d for BRSV in 30 dairy calves sampled at two days of age and monthly thereafter.[9]

The VN tests are in general used as gold standards in virology. The most sensitive tests (in terms of the amount of antibody detectable) are the primary binding tests that directly measure interaction between antigen and antibody.[19,20] The VN tests used in this study for viral antibodies are highly specific and extremely sensitive[5,14]; neutralization tests detect the IgM isotype best, but they also detect the IgG and IgA isotypes well.[19] In this group of calves, viral antibodies could be assumed to be maternal or passively acquired IgG, because calves were not, in general, challenged naturally with viruses (as could be inferred from the declining geometric mean titers).[22] But, if there was endogenous antibody production in this calf population, exclusion criteria 1 to 5 did not necessarily detect and delete those calves. Calves could still have mounted antibody responses although the third titer was lower than the second titer or the second titer lower than the first titer; with endogenous production, the decline would seem to be slower than pure decline in passive antibody. The tests used would not have differentiated between these two types of antibodies. Additionally, we would acknowledge that titers are measured with some unknown degree of imprecision.

With criteria 3 and 4, observations were excluded where part of the titer was assumed to be a result of antibody production of the calf. Those second and third titers that were zero were included in the analysis, because although there was no information about the time when these titers originally reached zero, their exclusion would have excluded the shorter half-lives — thus biasing the results to a longer estimated half-life than with their inclusion. As a consequence, in our study the real half-life would be shorter than or equal to the estimated half-life.

If we assumed that there was some endogenous antibody production present in calves older than 30 d and these observations were excluded, the estimate for the half-life of virus antibodies was 12 d (or less, keep-

ing in mind that observations with zero titers in the second and third samples were included). This is surprisingly close to the half-life of 11.5 d ± 0.6 d as measured by the disappearance of iodine-125-labeled IgG_1 from the plasma by Sasaki.[17] In this latter analysis, we excluded longer half-lives that either were natural (in which case their exclusion was not justified) or were a consequence of endogenous Ig production. An argument against the need to use these additional exclusion criteria was the fact that there were only a few virus isolations. However, virus isolations are not necessarily successful from all virus infections. For example, isolation of BRSV is difficult unless the samples are from the first three or four days post infection (before respiratory signs appear).[2] Nevertheless, the lack of seroconversions[22] supports the interpretation that few viral infections were missed. Therefore, the exclusion of titers measured after age 30 d serves more to suggest a lower limit to the half-life than to "contradict" our estimate that included titers from calves more than 30 d old. Furthermore, because we are measuring agent-specific antibodies, we believe the analyses without exclusion criteria 6 and 7 are more valid. An alternative explanation for two different half-lives could be that a biphasic elimination curve exists that results in a more rapid elimination of antibody in the first month of life. The presence of a biphasic elimination curve could be related to different rates of metabolism or, alternatively, concentration effects of the antibody within the calf.

Only antibodies against viruses were used in the analysis, because many calves seemed to mount antibody responses against mycoplasma, and all of the calves clearly seroconverted against bacteria.[22] These actively acquired antibodies could not have been differentiated from the maternally derived antibodies with the tests used in this study. The long "half-lives" for bacterial and mycoplasmal antibodies (data not shown) confirmed the suspicion of "contamination" by active responses. Half-lives for antibodies against different viruses were not calculated separately because we assumed the most-dominant IgG-subclass for all viruses in calf serum to be the same as in dam's colostrum — namely IgG_1.[18] Additionally, half-lives for BVDV, IBRV, and BRSV as estimated in other studies corresponded to each other.[1,4,12]

Although each calf was sampled only three times, the total number of sampled calves was 161, which is much higher than sample sizes in previous half-life studies. Additionally, calves in our study were sampled at different times and in different intervals (which could compensate for the lack of complete sets of samples for each calf). However, we acknowledge that because each calf was sampled only three times and calves were not sampled at the same times and at short intervals, this approach is not ideal in half-life determination. Calves were from 18 different herds with different vaccination programs and colostrum management practices. These might affect the decay of antibodies. In this respect, an experimental design would be superior for half-life determination. However, all of the herds were in the same veterinary practice and all had about the same number of calves in the study. Our estimates across so many calves in several commercial herds are consistent with those from studies with conditions more internally homogeneous than we probably had; this suggests that the estimates from earlier studies and our study will generalize.

The two half-lives (23 and 12 d) obtained from the same data illustrate how the use of different exclusion criteria can change the results meaningfully (in this case even significantly) when using data that were not originally designed for half-life determination. On the other hand, both half-lives were in accordance with other studies; this suggests the usefulness of observational prospective studies even for such objectives as these.

From a practical perspective the half-life of colostral antibody provides us with some information as to when we could expect to see a measurable serological response to vaccination. The presence of passive immunity appears to block measurable serological responses to viral vaccination, but this should not imply that immune response is blocked. Measurable serological responses may not be the only beneficial aspects of early vaccination in the calf. Recent research with a MLV vaccine to IBRV and BRSV in calves with maternal antibody demonstrated that the vaccinated calves did not respond with a significant increase in serum antibody, but they responded with virus-specific T-cell responses.[6] This cellular response primes the calf for subsequent vaccinations or for pathogenic challenge.

The half-life estimates also provide practitioners with an estimate of the maximum duration of protection from colostrally-derived passive immunity. While the level of passive antibody required to provide protective immunity is difficult to speculate and subject to external variables such as challenge dose, if there is little or no antibody present it is reasonable to assume the calf is susceptible. Assuming first-order kinetics, we would not expect much protection from passive antibodies beyond six half-lives (138 or 72 days based on the data reported here) and the actual duration of protection is likely less. This provides clinically useful information on susceptibility, since exposed calves who have not benefited from active immune stimulation prior to loss of passive immunity will likely be at higher risk for infection and disease.

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Abstract

Relationships between the Shedding of *Coxiella burnetii,* Clinical Signs and Serological Responses of 34 Sheep

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Two abortions associated with *Coxiella burnetii* occurred in a group of 34 pregnant ewes. The seroprevalence of *C. burnetii* infection was studied by using an ELISA and the immunofluorescence (IF) assay was applied to the contents of vaginal swabs. In addition, a PCR assay, with primers based on a transposon-like repetitive region of the *C. burnetii* genome (trans-PCR), was used for the highly sensitive and specific detection of *C. burnetii* in vaginal swabs, milk and faeces. Of the 34 animals tested at parturition, eight (24 percent) were positive by ELISA, 11 (32 percent) were positive by IF, and 15 (44 percent) were positive when the vaginal swab extract was subjected to the trans-PCR assay. *C. burnetii* was therefore detected by PCR in the vaginal swabs of seven seronegative ewes. However, five weeks after lambing, 16 (47 percent) of the animals tested were ELISA positive but only two animals (6 percent) were positive by PCR. Among the ELISA- and PCR-positive animals, eight (25 percent) shed coxiella in their milk and six (18 percent) did so in their faeces.