Vaccination Schedules to Raise Antibody Concentrations Against ϵ-Toxin of *Clostridium perfringens* in Ewes and Their Triplet Lambs[1]

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ABSTRACT: The objective of this experiment was to compare vaccination schedules for ewes and their lambs to raise antibody concentrations to ϵ-toxin of *Clostridium perfringens*, the causative agent of enterotoxemia. Half of 200 Finnsheep × Dorset ewes were vaccinated with *C. perfringens* type D toxoid vaccine 3 wk before lambing. Serum samples were obtained from 20 ewes that were to be vaccinated and 20 ewes that would remain unvaccinated before treatment and at wk 2, 1, and 0 before the start of lambing. Antibody concentrations in sera of unvaccinated ewes remained at 2 IU/mL, but they peaked in vaccinated ewes at 15 IU/mL by wk 1 before lambing. Lambs from each of the first 13 and the first 14 sets of triplets from vaccinated and unvaccinated ewes, respectively, received one of three vaccination treatments: no vaccine (control), vaccination on d 1 and 21 of age, or vaccination on d 21 and 42 of age. Antibody concentrations declined in sera of vaccinated ewes from 8.5 IU/mL immediately after lambing to 3 IU/mL 12 wk later. Vaccination of lambs did not increase sera antibody concentration. However, prepartum vaccination of ewes significantly increased lamb antibody concentrations (19 IU/mL) compared with lambs reared by unvaccinated ewes (2 IU/mL). Vaccination of ewes resulted in lambs with higher antibody concentrations until wk 10 postpartum. Concentrations declined to .6 IU/mL in all lambs at 12 wk. Because concentrations of .2 IU/mL may be protective, these results indicate that vaccination of ewes before lambing imparts passive protection in lambs to 12 wk of age, whereas vaccination of young lambs provides no added protection.

Key Words: Enterotoxemia, Ewes, Immunization, Lambs

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Introduction

Enterotoxemia originates in the intestine and affects all species of domestic animals throughout the world (Jensen, 1974; Fleming, 1985). *Clostridium perfringens* type D-producing ϵ-toxin is the most common cause in sheep and goats worldwide, and younger animals usually are the most affected (Blackwell, 1983; Hughes and Reuter, 1986; Itodo and Ike, 1990). Under normal conditions, *Clostridium perfringens* do not damage the host and may stimulate immunity against some of the toxins they produce (Bullen, 1954; Fleming, 1985). But, under conditions of high carbohydrate and protein substrates arriving undigested at the small intestine, *Clostridium perfringens* multiply rapidly and produce lethal toxins (Blackwell, 1983; Fleming, 1985).

Before a commercial vaccine was available, enterotoxemia caused more economic losses among feedlot- and pasture-reared lambs than all other diseases combined (Jensen, 1974). Lambs managed under the highly productive STAR system at Cornell traditionally have been vaccinated against enterotoxemia at 1 to 2 d of age and at weaning (50 to 60 d). Considering the high amount of grain that the ewes and lambs consume during their lifetime under this system, there is no extraordinary incidence of enterotoxemia at the Cornell sheep production centers, but it is not clear that the time and expense of early vaccination help to prevent the disease.

Present recommendations (ASIA, 1992) are that lambs should be vaccinated twice at less than 6 wk of age to prevent enterotoxemia even when their mothers were vaccinated in late pregnancy. The objective of this research was to compare vaccination schedules for

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ewes and their lambs to raise antibody concentrations to ϵ-toxin of *Clostridium perfringens*, the causative agent of enterotoxemia.

Materials and Methods

The experiment was conducted at the Cornell University Mount Pleasant sheep farm beginning 3 wk before the March lambing season. Two hundred Finnsheep × Dorset ewes ranging in age from 3 to 7 yr and bred to Suffolk or Finnsheep × Dorset rams were used. One group of 100 ewes with odd eartag numbers was vaccinated with 3 mL of Electroid D, a *Clostridium perfringens* type D toxoid vaccine (Coopers Animal Health, Mundelein, IL). Another group of 100 ewes with even eartag numbers was not vaccinated. Before vaccination, 20 ewes were randomly selected from each control and vaccinated group to obtain jugular blood samples. Blood samples (6 mL) from these ewes were also collected at 2, 1, and 0 wk before lambing.

Before beginning the experiment no grain was fed and the ewes had ad libitum access to mixed grass hay. Thus, the first blood samples obtained constituted the base sera antibody titer against ϵ-toxin. After the initial blood sample was collected, in addition to ad libitum access to hay the ewes were fed daily an average of 454 g of High Energy Lamb Pellets (16% CP, Agway, Syracuse, NY) and 227 g of barley.

The first 14 ewes from each group that lambed and reared triplets were used in the second part of the experiment. Coincidentally, 13 of these ewes were among the 40 ewes sampled before lambing. The ewes lambed between March 21 and April 1, 1994. After the lambs had suckled and within 24 h after lambing, jugular blood samples were obtained from each ewe (6 mL) and her lambs (1.5 mL). Each lamb from a set of triplets received one of three vaccination treatments: no vaccine, vaccination on d 1 and 21 of age, or vaccination on d 21 and 42 of age. Vaccinated lambs were given 2 mL of Electroid D. Blood samples were obtained from the ewes (6 mL) and lambs (4 mL) every other week until the lambs were 12 wk old. The average daily feed intake of the lactating ewes was 3.1 kg of High Energy Lamb Pellets and 1.4 kg of mixed grass hay. Lambs were offered High Energy Lamb Pellets for *ad libitum* consumption in a creep area. One lamb that came from a vaccinated ewe died of pneumonia early in the experiment. Data from the other two lambs and the ewe were not included in the analysis of results, so there were 13 sets of triplets from vaccinated ewes and 14 sets of triplets from unvaccinated ewes.

Preparation of Serum

Blood samples were placed in an incubator at 37°C for 30 min and then left overnight at 4°C. The next day, serum was transferred from the tubes to 1.5-mL microcentrifuge tubes and stored at –20°C.

Competitive Enzyme Linked Immunosorbent Assay

A competitive ELISA was validated to measure serum concentrations of antibodies to ϵ-toxin. Samples were incubated in a humid chamber at 37°C on an orbital shaker unless otherwise indicated.

Epsilon (ϵ)-toxin (USDA National Veterinary Services Laboratory, Ames, IA) was provided in a concentrated culture supernatant that had been treated sequentially with trypsin, then a trypsin inhibitor. The culture supernatant was diluted in 15% glycerol to contain approximately 300,000 mouse LD_{50} units per milliliter. The ϵ-toxin was further diluted 1:100 in .06 *M* carbonate buffer (pH 9.6) and used to coat modular microwell plates (Nunc-F-16) overnight at 4°C (100 μL/well). The plates containing the antigen solution were stored at –70°C until used.

Plates were thawed and washed eight times (Ultra wash II, Dynatech, Chantilly, VA) with PBS containing .05% (vol/vol) Tween 20 (**PBS-T**). Wells were blocked for 30 min with 1% gelatin in PBS-T (200 μL/well) then washed eight times with PBS-T. The serum samples were randomly selected and diluted 1:25 in PBS-T.

An international standard for *C. perfringens* type D antitoxin (Central Veterinary Laboratory, Weybridge, U.K.) was used to establish a standard curve for each plate. It consisted of a pepsin-refined antitoxin prepared from the serum of a horse immunized against the toxin. The concentration of the standard was 1,020 IU/mL. Serum samples (20), appropriate controls (see below), and standards (.4, .2, .1, .05, .025, .0125, .00625, and .00313 IU/mL in PBS-T) were added in triplicate (100 μL/well) and incubated overnight at 4°C.

Monoclonal antibodies (**mAb**) of murine origin (Central Veterinary Laboratory, Weybridge, U.K.) were used in the competition assay. The original preparation was lyophilized from a serum-free tissue culture supernatant. The antibodies were reconstituted to contain 20 μg of IgG1 per milliliter (10 IU/mL) in sterile PBS and stored in aliquots at 4°C. After the overnight incubation of serum or standards with antigen, the wells were incubated with a 1:60 further dilution of the mAb (50 μL/well) in PBS-T for 1 h, then washed eight times with PBS-T.

The detection of bound mAb used a peroxidase-conjugated, affinity-purified sheep anti-mouse IgG (H+L) (Jackson Immunoresearch Laboratories, West Grove, PA). The reporter antibody was diluted to 32 μg/mL in sterile PBS-T containing 1% BSA, and aliquots were stored at –70°C. The reporter antibody was added to the wells (100 μL/well, optimum dilution, 1:3,000), incubated for 1 h, and then plates were washed eight times with PBS-T.

Substrate reagent (.39 m*M* 2-2′azino-D_1 [3-ethyl benzthiazoline sulfonic acid] diamonium salt [**ABTS**];

Table 1. Example absorbance values obtained for the control samples of the competitive ELISA assay

Control sample	Mean	SD	CV
ϵ-Toxin + reporter antibody	.028	.0025	9.1
ϵ-Toxin + mAb[a] + reporter antibody	2.395	.0807	3.4
ϵ-Toxin + positive control serum + mAb + reporter antibody	.345	.03873	11.2
ϵ-Toxin + fetal lamb serum + mAb + reporter antibody	2.247	.04888	2.2

[a]mAb = monoclonal antibody.

2 m*M* hydrogen peroxide; 49.3 m*M* citrate buffer, pH 4) was added (100 μL/well) and incubated at room temperature for 10 min on a Micro Shaker II (Dynatech). The reaction was stopped with a solution of sodium azide (.004 m*M*), and absorbance values were read at 405 and 490 nm with a Microplate Autoreader EL311 (Bio-Tek Instruments, Winooski, VT) that accounted for background by reporting the difference between the two readings.

Controls were included in triplicate in each plate (Table 1). The first included toxin and reporter antibody and resulted in essentially no nonspecific reaction. The second included toxin, mAb, and reporter antibody to give the maximal reactivity, and thus the maximal absorbance. The third included toxin, serum from a hyperimmunized ram that had been vaccinated on three occasions at 15-d intervals with 5 mL of Electroid D, mAb, and reporter antibody. This was the positive control that competed with the mAb for binding sites on the antigen. The fourth included toxin, fetal lamb serum, mAb, and reporter antibody and served as the negative control.

The mean, standard deviation, and coefficient of variation were calculated for the triplicate determinations of standards, controls, and experimental sera. Of the 803 triplicate serum sample sets, 725 had CV less than 15%. Samples that had CV between 15 and 29% had no obvious outliers, so reassaying them was not expected to change the treatment means. Experimental samples with CV $\geq$ 30% had obvious outliers that were discarded, and new means based on the duplicate replicates were calculated for those samples.

The mean absorbance values for the eight standards and their respective $\log_{10}$ IU were graphed, and the least squares method was used to calculate a straight line that best fitted the data. Standard curve equations for the 48 plates had r^2 values ranging from .92 to .99, most being from .96 to .99. The concentrations of antibodies against ϵ-toxin in the experimental sera were interpolated from the equation of the standard curve using the mean absorbance for replicates, corrected for dilution used, and expressed in international units per milliliter.

The mAb used in this assay neutralized ϵ-toxin in the mouse lethality test and reacted with three proteins in a trypsinized preparation of ϵ-prototoxin (Boarer et al., 1988). The advantage of using this antibody in a competitive assay is that it measures protective antitoxin in the experimental sera. The cELISA assay could detect concentrations of antibody to ϵ-toxin as low as .00625 IU/mL. This sensitivity is comparable to that of .004 IU/mL for a solid-phase immunoassay developed to detect ϵ antitoxin (Bernath, 1976).

Statistical Analysis

The antibody concentrations were transformed to natural logarithms (ln) because the raw data were not normally distributed.

Prepartum Ewes. Data were analyzed as a split plot with the main plot effect of vaccination (1 df) tested against variation among ewes within vaccination group (38 df), and the subplot effects of week (3 df) and week $\times$ vaccination (3 df) tested against residual error (113 df).

Postpartum Ewes and Their Triplet Lambs. Ewes lambed on different dates but were sampled thereafter on the same day each week so that there were different days postpartum for each ewe when the blood samples were taken. Therefore, the natural log of antibody concentration was regressed against days postpartum for each ewe, and the individual ewe coefficients were analyzed. The effect of vaccination on each coefficient was determined by one-way analysis of variance. To allow for a nonlinear relationship, quadratic equations were also computed for each ewe, but the quadratic coefficients were not significant. The data for lambs were analyzed similarly except that the quadratic coefficient was significant, and the coefficients were analyzed as a split plot with the main plot effect of ewe vaccination (1 df) tested against variation among ewes within vaccination group (25 df), and the subplot effects of lamb vaccination (2 df) and ewe vaccination $\times$ lamb vaccination (2 df) tested against residual error (50 df).

The significant quadratic component for the ln lamb data implied that two or more compartments of decay were present for the antibodies, so a curve-peeling analysis was performed based on Brown and Manno (1978). The equation, $y = Ae^{-kt} + Be^{-jt}$, was fitted to the data, with k and j being fractional rate constants

Table 2. Serum antibody concentrations in ewes before lambing[a]

	Weeks before lambing[b]			
Ewe vaccination[c]	−3	−2	−1	0
Unvaccinated (n = 20)				
ln value	.5501	.7233	.3900	.2197
Antilog (ln), IU/mL	1.7	2.1	1.5	1.2
Vaccinated (n = 20)				
ln value	.3659	2.1401	2.7056	2.1222
Antilog (ln), IU/mL	1.4	8.5	15.0	8.3

[a]SEM = .1864 for the vaccination × week interaction, $P < .0001$.
[b]SEM = .1318 for the effect of week, $P < .0001$.
[c]SEM = .3082 for the effect of vaccination, $P < .0015$.

for a two-pool model. A MINITAB (MINITAB, 1994) macro for finding the best two-pool model was written to perform the analysis. There were not enough data to fit this model for each lamb. Therefore, antibody concentrations for each lamb were expressed as percentages of the starting value and pooled with data from other lambs in the same vaccination group. The natural logarithm-transformed data from d 5 to the end of the experiment were fitted to a simple linear equation (for compartment B). The equation was then used to predict antilog values for days before 5 d of age. The natural logarithms of these differences were then fitted to a simple linear equation (for compartment A) and the predicted antilog values computed. Predicted antilog values for the two equations were summed to obtain predicted values for each lamb. The residual sum of squares (**RSS**) were calculated. This completed one round of computations. The procedure was repeated, diminishing the days for component B by units of 5 each iteration so that the next round started with data from d 10 until the end of the experiment, and so on. The combined equation set that gave the minimum RSS was selected as the one that best fitted the data. The half-lives of the antibodies for each pool were calculated independently using the exponent for each pool: $t_{1/2} = \ln(2)/k$ (pool A) and $\ln(2)/j$ (pool B).

The two-pool model equation was fitted to the pooled data sets for 1) unvaccinated lambs from vaccinated ewes, 2) lambs vaccinated at d 1 and 21 from vaccinated ewes, and 3) lambs vaccinated at d 21 and 42 from vaccinated ewes. There was not enough change in antibody level from the beginning to the end of the experiment to fit the two-pool model to data from lambs from ewes that had not been vaccinated.

Results and Discussion

Ewes in the Cornell STAR sheep management system have a potential lambing frequency of 1.67 times a year (Lewis et al., 1996). The Finnsheep × Dorsets in this experiment have a potential rate of two to three lambs per ewe per lambing. The lambs are weaned early and fed for rapid growth. Because the ewes are immediately rebred to lamb more frequently than in most traditional production systems, they are never in recovery or maintenance periods after weaning and before rebreeding. This means that during lactation the ewes are fed to produce enough milk to rear twins or triplets, while maintaining body condition because they are expected to breed soon after weaning. To maintain this level of production, these sheep are fed high-energy and -protein diets during the lactation period (Hogue, 1994).

Bullen (1954) showed that many sheep experience a subclinical intoxication to ϵ-toxin. Sublethal doses of ϵ-toxin stimulate the production of specific antibodies (Jensen, 1974). It has been reported that up to 30% of the individuals in an unvaccinated flock have protective immunity due to subclinical infection (Blackwell, 1983). Thus, ewes managed under the STAR system may receive sufficient amounts of starch in their small intestine to present a subclinical level of ϵ-toxin. This could stimulate specific antibody production and protect them from a more acute form of the disease.

Prepartum Ewes

Antibody titers in ewes before lambing are shown in Table 2 and described in a complete graphical representation of the experiment in Figure 1. Vaccination dramatically increased antibody levels until wk −1. Antibody levels in vaccinated ewes then declined by wk 0. The average antibody concentration of the unvaccinated ewes remained the same, except for a slight decrease at wk 0. The decreases for vaccinated and unvaccinated ewes at wk 0 probably resulted from extraction of serum antibodies into colostrum.

There was no significant increase in antibody concentration in the unvaccinated ewes. Thus, it seems unlikely that a possible subclinical intoxication with ϵ-toxin due to increased grain feeding stimulated the immune system of the ewes. Vidal et al. (1969) found that the amounts of starch reaching the ileum of sheep were 10, 33, 52, and 120 g/kg of diet for 100:0, 75:25, 50:50, 25:75 forage to concentrate ratios, respectively. Lactating ewes in this experiment consumed a diet with approximately a 30:70 forage to

Table 3. Regression coefficients of equations relating natural log of antibody concentrations to days after lambing for ewes with triplets

Ewe vaccination	Regression coefficients	
	b_0	b_1 (× age)
Unvaccinated	.140	.00128
Vaccinated	2.249	–.01562
SE	.02953	.00346
P-value	<.002	<.002

concentrate ratio, so significant amounts of starch must have been present in the small intestine.

Antibody concentrations of .15 to .2 IU/mL have been reported to be protective in sheep (Freichs and Gray, 1975; Blackwell et al., 1991). Based on the geometric mean antibody level of 1.6 IU/mL (Table 2), most of the unvaccinated ewes were protected. The ewes in this experiment were at least 3 yr old. The protective antibody levels in the unvaccinated ewes may have been developed in response to vaccinations for enterotoxemia as lambs and subsequent periodic boosters. A possible subclinical infection with *C. perfringens* ϵ-toxin may boost or maintain antibody levels initially produced by vaccination. This effect could be useful because the vaccines available for the prevention of enterotoxemia are alum preparations that supposedly do not maintain immunity in sheep for longer than 6 mo (Blackwell, 1983). Alternatively, the high-grain diet may have increased local gut antibody secretion that was not reflected in the peripheral serum (Unanue and Benacerraf, 1984).

Postpartum Ewes

Regression coefficients of equations relating ln(antibody concentration) to days postpartum for ewes that had triplets are given in Table 3. The equation for vaccinated ewes was different ($P < .002$) from the equation for unvaccinated ewes. A graphical representation of the equations obtained by taking the anti-log of the predicted values from the linear equations of the ln-transformed data is shown in Figure 1.

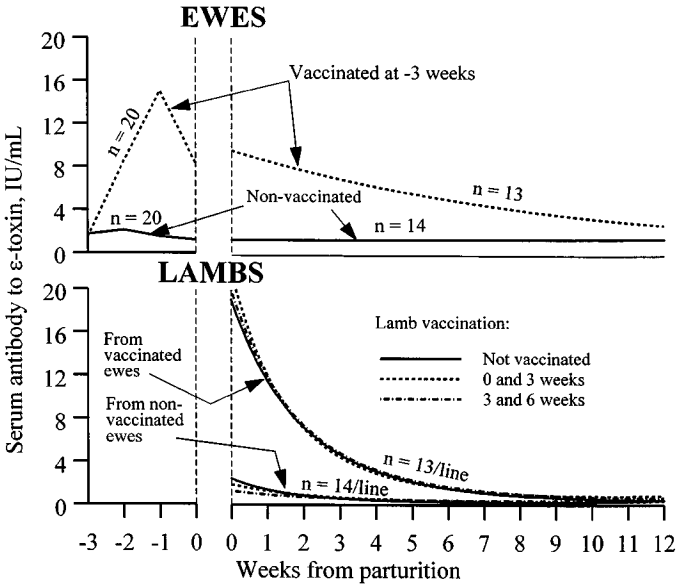

Figure 1. Effect of vaccination schedule of ewes and their triplet lambs on serum antibodies to ϵ-toxin of *Clostridium perfringens* type D.

After lambing, vaccinated ewes started with high values similar to those in ewes sampled just before lambing, but the antibody concentrations in the vaccinated ewes progressively declined with time. The control ewes had almost no change in antibody concentrations.

Triplet Lambs

Regression coefficients of the quadratic equations relating ln(antibody concentration) to days of age for lambs are given in Table 4. The equations for lambs from vaccinated ewes had higher ($P < .0002$) intercepts than equations for lambs from unvaccinated ewes. Lamb vaccination at either 1 and 21 d or at 21 and 42 d did not increase antibody levels. We expected

Table 4. Regression coefficients of quadratic equations relating natural log of antibody concentrations to days of age for triplet lambs

Ewe vaccination (EV)	Lamb vaccination (LV)	b_0[a]	b_1[c] (× age)	b_2[d] (× age^2)
Unvaccinated	Unvaccinated	.8982	–.08266	.00074
	1, 21 d	.6429	–.05601	.00043
	21, 42 d	.2592	–.04490	.00042
Vaccinated	Unvaccinated	2.9463	–.07354	.00033
	1, 21 d	3.0765	–.08785	.00059
	21, 42 d	2.9912	–.07624	.00042

[a]SE_{EV} = .4377, $P < .0002$; SE_{LV} = .1334, $SE_{EV \times LV}$ = .1887.
[c]SE_{EV} = .0089, SE_{LV} = .0090, $SE_{EV \times LV}$ = .0127.
[d]SE_{EV} = .0001, SE_{LV} = .0001, $SE_{EV \times LV}$ = .0002.

Table 5. Exponential decay equations, $y = Ae^{-kt} + Be^{-jt}$, for serum antibodies to ϵ-toxin in lambs from vaccinated ewes

Lamb vaccination	A	k	B	j	$t_{1/2}$(A), d	$t_{1/2}$(B), d
Unvaccinated	62.4	.006	46	.035	11.6	19.8
1, 21 d	100.2	.095	22.6	.023	7.3	30.0
21, 42 d	93.7	.057	12.6	.012	12.1	57.8

lambs from unvaccinated ewes to respond better to the vaccine than lambs from vaccinated ewes because the amount of antibodies transferred through colostrum was considerably less. Unvaccinated ewes had antibody concentrations against ϵ-toxin that may be considered protective and could have been sufficient to suppress a specific immune response. Fleenor and Stott (1983) found that maternally derived antibodies suppressed neonatal antibody production in newborn calves after primary immunization. This could explain the similar lack of response to the vaccine for lambs from unvaccinated and vaccinated ewes.

A graphical representation of the equations obtained by taking the anti-log of the predicted values from the equations of the ln-transformed data is shown in Figure 1. Lambs from the unvaccinated ewes had circulating antibody concentrations similar to those of their dams. Lambs from vaccinated ewes had greater titers of circulating antibodies than lambs from unvaccinated ewes, and immediately after colostrum intake, their antibody titers were even greater than those in the sera of their dams. The levels declined dramatically by about 6 wk of age. Thus, vaccinating the ewes before parturition greatly enhanced the passive transfer of pathogen-specific antibodies against ϵ-toxin. A similar response was reported for calves from cows vaccinated 124 d before calving (Troxel et al., 1997).

The significant quadratic component for the relationship of the ln(antibody concentrations) to days of age implied that the exponential equation $y = Ae^{-kt} + Be^{-jt}$ could describe the data. The method of curve-peeling used to solve this equation assumed that pool A had a fast rate of decay and pool B had a slow rate of decay (Brown and Manno, 1978). For example, pool A could be antibodies in serum and pool B could be antibodies in interstitial fluids. Thus, antibody decay could be explained by the sum of a direct disappearance from serum (pool A) and by transfer of antibodies from interstitial fluids (pool B) to serum.

The curve-peeling results for lambs from vaccinated ewes are shown in Table 5. The half-lives of the antibodies in pool A among the control lambs and the lambs vaccinated at 21 and 42 d were similar and comparable to the reported metabolic half-life of 14.5 d for IgG1 in sheep (Tizard, 1982), the subisotype measured in this project. The half-life of the antibodies in pool A for the lambs vaccinated on d 1 and 21 was slightly lower than the half-lives of the antibodies in pool A for the control lambs and lambs vaccinated at 21 and 42 d. This may have resulted from a faster drop in the antibody concentration due to the toxoid of the first vaccination being complexed with the pathogen-specific circulating antibodies. The half-lives for pool B varied among the groups. In increasing order of half-life duration, first was the control group, next was for the group vaccinated on d 1 and 21 of age, and the longest was for the group vaccinated on d 21 and 42 of age.

Further studies are necessary to determine an appropriate vaccination schedule for lambs after weaning. In highly productive systems, however, lambs can be ready for market before 5 mo of age so that vaccination may not be necessary if the ewes were vaccinated 3 to 4 wk before parturition.

Implications

The results of this experiment indicate that ewes should be vaccinated 3 to 4 wk before parturition and that there is no benefit of vaccinating lambs before 6 wk of age. Further studies are needed to recommend an adequate immunization schedule for lambs after weaning or whether fast-growing lambs from vaccinated ewes need to be immunized.

Literature Cited

ASIA. 1992. SID Sheep Production Handbook. pp 210–211. American Sheep Industry Association, Englewood, CO.

Bernath, S. 1976. Solid-phase radioimmunoassays for quantitative antibody determination of bacterial exotoxins. Measurement of *Clostridium perfringens* type D ϵ antitoxin. Dev. Biol. Stand. 32:77–83.

Blackwell, T. E. 1983. Current status of enterotoxemia in sheep and goats. In: Proc. AASRP Annu. Mtg., Springfield, IL. pp 41–51.

Blackwell, T. E., D. G. Butler, J. Prescott, and B. Wilcox. 1991. Differences in signs and lesions in sheep and goats with enterotoxemia induced by intraduodenal infusion of *Clostridium perfringens* type D. Am. J. Vet. Res. 52:1147–1152.

Boarer, C.D.H., M. G. Sojka, V. J. White, and P. L. Roeder. 1988. The production and evaluation of monoclonal antibodies to Clostridium perfringens type D epsilon toxin. J. Biol. Stand. 16: 207–218.

Brown, R. A., and J. E. Manno. 1978. ESTRIP, a BASIC computer program for obtaining initial polyexponential parameter estimates. J. Pharm. Sci. 67:1687–1691.

Bullen, J. J. 1954. Enterotoxemia of sheep: Clostridium welchii type D in the alimentary tract of normal animals. J. Pathol. Bacteriol. 64:201–206.

Fleenor, W. A., and G. H. Stott. 1983. Quantification of bovine IgG, IgM and IgA antibodies to *Clostridium perfringens* β-toxin by enzyme immunoassay II. Systemic effects of maternally derived antibodies on immunization of newborn calves. Vet. Immunol. Immunopathol. 4:663–654.

Fleming, S. 1985. Enterotoxemia in neonatal calves. Food Anim. Pract. 1:509–514.

Freichs, G. N., and A. K. Gray. 1975. The relation between the rabbit potency test and the response of sheep to sheep clostridial vaccines. Res. Vet. Sci. 18:70–75.

Hogue, D. E. 1994. Further observations on feeding highly productive sheep. In: Proc. Cornell Nutr. Conf., Ithaca, NY. pp 140–142.

Hughes, K. L., and R. E. Reuter. 1986. Enterotoxaemia in Sheep, Goats and Cattle. CSIRO, Melbourne, Australia.

Itodo, A. E., and R. O. Ike. 1990. Enterotoxemia in sheep and cattle in the Jos Plateau, Nigeria. Trop. Vet. 8:120–122.

Jensen, R. 1974. Diseases of Sheep. Lea & Febiger, Philadelphia, PA.

Lewis, R. M., D. R. Notter, D. E. Hogue, and B. H. Magee. 1996. Ewe fertility in the STAR accelerated lambing system. J. Anim. Sci. 74:1511–1522.

MINITAB. 1994. MINITAB Reference Manual. MINITAB, State College, PA.

Tizard, I. 1982. Veterinary Immunology. W. B. Saunders Company, Philadelphia, PA.

Troxel, T. R., G. L. Burke, W. T. Wallace, L. W. Keaton, S. R. McPeake, D. Smith, and I. Nicholson. 1997. Clostridial vaccination efficacy on stimulating and maintaining an immune response in beef cows and calves. J. Anim. Sci. 75:19–25.

Unanue, R., and B. Benacerraf. 1984. Textbook of Immunology. Williams and Wilkins, Baltimore, MD.

Vidal, H. M., D. E. Hogue, J. M. Elliot, and E. F. Walker, Jr. 1969. Digesta of sheep fed different hay-grain ratios. J. Anim. Sci. 29: 62–68.