Enterotoxemia in the Goat: The Humoral Response and Local Tissue Reaction Following Vaccination with Two Different Bacterin-Toxoids

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ABSTRACT

A vaccination trial involving 72 goats was designed to compare the epsilon antitoxin titres and local reactions at the injection sites, of two commercial enterotoxemia vaccines. Three dosage regimes were used for each vaccine (12 goats per group). Although no significant differences were noted in humoral immune response between the two vaccines (P = 0.05), one vaccine regime resulted in low titres (P = 0.05) on two occasions. Local tissue reactions at injection sites persisted for six months in 53% of the goats regardless of vaccine used or dosage administered. No immunological basis for the reported differences in vaccine efficacy between sheep and goats was observed in this trial.

Key words: *Clostridium perfringens* type D, enterotoxemia, vaccination, goats.

RÉSUMÉ

Cette expérience de vaccination impliquait 72 chèvres et elle visait à comparer les titres d'antitoxine epsilon et les réactions aux sites d'injection, suite à l'administration sous-cutanée de deux vaccins commerciaux contre l'entérotoxémie. Chacun des six groupes expérimentaux comptait 12 chèvres qui reçurent trois doses différentes de chacun de deux vaccins. Même si on n'enregistra aucune différence appréciable dans les taux d'anticorps attribuables à chacun des vaccins (P = 0,05), une des doses donna des titres faibles (P = 0,05), à deux occasions. Les réactions tissulaires qui se développèrent aux sites d'injection persistèrent pendant six mois, chez 53% des chèvres, indépendamment du vaccin ou de la dose. Cette expérience ne permit pas de trouver une raison immunologique susceptible d'expliquer les différences déjà rapportées entre les moutons et les chèvres, relativement à l'efficacité de la vaccination contre l'entérotoxémie.

Mots clés: *Clostridium perfringens* type D, entérotoxémie, vaccination, chèvres.

INTRODUCTION

Enterotoxemia is a major disease of dairy goats (1). It can present either as a sudden death syndrome or as a chronic diarrhea culminating in death or spontaneous recovery (2). The use of vaccines specifically developed for sheep has been considered effective in preventing enterotoxemia in that species (3). Currently no vaccines are manufactured specifically for use in goats. When vaccines developed for sheep are used in goats, they appear to decrease the incidence and severity of the disease but do not prevent the disease. In addition, vaccination often results in unsightly reactions at the site of inoculation. These reports of poor efficacy and unsightly local reactions (4) have led to much confusion and disagreement with regard to the efficacy of various vaccines, ideal vaccination regimes and the immunocompetency of goats as compared to sheep. In this report we present the antibody response and local inflammatory reaction of goats to repeated vaccination with either of two commercial sheep enterotoxemia vaccines.

MATERIALS AND METHODS

ANIMALS

Seventy-two goats, 68 female and four male, ranging in age from two to eight years were used in this trial. The goats were selected from four herds in which no history of enterotoxemia or vaccination for enterotoxemia existed. Purebred Nubians, Toggenburgs and Saanens and crossbred Alpines, Nubians, Toggenburgs and Saanens were represented in this trial.

VACCINES

Two commercial multi-component clostridial vaccines were used. Vaccine A (Clostroid C-D; Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) is a formalin

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This study was supported by a grant from the Ontario Ministry of Agriculture and Food.

Submitted July 12, 1982.

TABLE I. Vaccination Protocol for Groups A1, A2, A3, B4, B5, B6 at 0 Time, 6, 30 and 56 Weeks

Groups	0 Time No. of Goats	0 Time Dosage (mL)	6 Weeks No. of Goats	6 Weeks Dosage (mL)	30 Weeks No. of Goats	30 Weeks Dosage (mL)	56 Weeks No. of Goats	56 Weeks Dosage (mL)	Total Dose of Vaccine
A1 sheep dosage	12	2.5	12	2.5	11	2.5	11	2.5	10
A2 cattle dosage split[a]	12	5	12	5	12	5	11	5	20
A3 arbitrary dosage	12	5	11	2.5	10	2.5	8	2.5	12.5
B4 sheep dosage	12	5	12	2	12	2	12	2	11
B5 cattle dosage split[a]	12	5	12	5	12	5	9	5	20
B6 cattle dosage	12	5	12	5	12	5	11	5	20

[a]5 mL dosage divided into half and injected concommitantly at sites approximately 10 cm apart

inactivated bacterin-toxoid prepared from *Clostridium perfringens* Types C and D cultures. Vaccine B (Covexin-8; Burroughs Wellcome Ltd., Beckenham, Kent, England) contains the specific formol treated toxoids of *Clostridium perfringens* types C and D, *Clostridium tetani*, the specific formol treated toxoid of *Clostridium septicum* together with the products of lysed organisms and formolized whole cultures of *Clostridium chavoei*, *Clostridium oedematiens* types B and D and *Clostridium perfringens* type B.

VACCINATION PROTOCOL

The vaccination protocol is shown in Table I. Thirty-six goats were vaccinated on four occasions with vaccine A as follows: 12 goats (group A1) were vaccinated each time using the recommended dosage for sheep (2.5 mL); 12 goats (group A2) were vaccinated each time using the recommended dosage for cattle (5 mL). This dosage was split into two equal parts and administered concommitantly at injection sites approximately 10 cm apart. The third group of 12 goats (group A3) was vaccinated using an arbitrary dose which consisted of a primary inoculation of 5 mL followed by three booster inoculations of 2.5 mL each.

Thirty-six goats were vaccinated on four occasions with vaccine B as follows: 12 goats (group B4) were vaccinated each time using the recommended dosage for sheep (5 mL primary inoculation followed by 2 mL booster inoculations); 12 goats (group B5) were vaccinated each time using the recommended dosage for cattle (5 mL). This dosage was split into two equal parts and administered concommitantly at injection sites approximately 10 cm apart. The third group of 12 goats (group B6) was vaccinated each time using the recommended dosage for cattle (5 mL).

All vaccinations were administered subcutaneously on the lateral thorax at sites previously swabbed with 70% alcohol. Twenty gauge, one inch needles were used and discarded after each injection. The first vaccination was administered at time 0 and subsequent booster injections were administered at six, 30 and 56 weeks. Vaccinations at 0 time and 30 weeks were given on the right thorax and injections at six and 56 weeks were given on the left thorax.

BLOOD COLLECTION AND TESTING

Blood samples were collected before the first vaccination (time 0), three weeks following the second vaccination (nine weeks), prior to (30 weeks) and two weeks following the third vaccination (32 weeks) and prior to (56 weeks), four days after (56.5 weeks) and three weeks after (59 weeks) the fourth vaccination. Serum was separated by centrifugation and stored at -20°C for one to four months. The samples were then thawed and heat inactivated at 56°C for 30 minutes and stored at -20°C until tested by standard procedures to determine epsilon antitoxin titres (5).

STATISTICAL EVALUATION

The mean logarithmic serum epsilon antitoxin titres of all goats receiving vaccine A (groups A1, A2, A3), all goats receiving vaccine B (groups B4, B5, B6) and all six vaccination groups (A1, A2, A3, B4, B5, B6) were compared. The mean logarithmic serum epsilon antitoxin titres of all goats with epsilon antitoxin titres at time 0 were compared to the mean logarithmic serum epsilon antitoxin titres of all goats without epsilon antitoxin titres at time 0. The above comparisons were done at nine, 30, 32, 56, 56.5 and 59 weeks after the initial vaccination using the Student t-test (Tables II, III and IV).

TABLE II. Mean Serum Epsilon Antitoxin Titres of all Goats Receiving Vaccine A (Groups A1, A2, A3) and all Goats Receiving Vaccine B (Groups B4, B5, B6) at 9, 30, 32, 56, 56.5 and 59 Weeks After the Initial Vaccination

Group	9 Weeks Titre	9 Weeks Sig.	30 Weeks Titre	30 Weeks Sig.	32 Weeks Titre	32 Weeks Sig.	56 Weeks Titre	56 Weeks Sig.	56.5 Weeks Titre	56.5 Weeks Sig.	59 Weeks Titre	59 Weeks Sig.
Vaccine A (Groups A1,A2,A3)	2.41 (0.05)	NS.	0.14 (-1.09)	N.S.	5.65 (.68)	N.S.	0.41 (-0.81)	N.S.	1.18 (-0.31)	N.S.	15.79 (0.79)	N.S.
Vaccine B (Groups B4,B5,B6)	2.83 (0.11)	N.S.	0.14 (-1.09)	N.S.	5.19 (0.64)	N.S.	0.42 (-0.82)	N.S.	0.77 (-0.41)	N.S.	10.38 (0.61)	N.S.

N.S. No significant difference ($P>0.05$)
( ) mean logarithmic titre

TABLE III. Mean Serum Epsilon Antitoxin Titres of Groups A1, A2, A3, B4, B5, B6 at Nine, 30, 32, 56 and 56.5 and 59 Weeks Postvaccination

Group	9 Weeks Titre	9 Weeks Stat. sig.	30 Weeks Titre	30 Weeks Stat. sig.	32 Weeks Titre	32 Weeks Stat. sig.	56 Weeks Titre	56 Weeks Stat. sig.	56.5 Weeks Titre	56.5 Weeks Stat. sig.	59 Weeks Titre	59 Weeks Stat. sig.
A1	3.51 (0.13)	N.S.	0.20 (-0.96)	N.S.	5.00 (0.62)	N.S.	0.20 (-0.97)	N.S.	0.63 (-0.40)	N.S.	16.59 (0.74)	++
A2	2.20 (0.09)	N.S.	0.13 (-1.12)	N.S.	6.67 (0.78)	++	0.68 (-0.61)	N.S.	1.43 (-0.26)	N.S.	19.59 (0.94)	++
A3	1.43 (0.10)	N.S.	0.09 (-1.20)	N.S.	5.15 (0.63)	N.S.	0.30 (-0.89)	N.S.	1.60 (-0.28)	N.S.	9.47 (0.65)	N.S.
B4	1.83 (0.35)	N.S.	0.10 (-1.11)	N.S.	3.08 (0.45)	+	0.16 (-1.14)	N.S.	0.64 (-0.51)	N.S.	2.93 (0.17)	+
B5	4.32 (0.23)	N.S.	0.19 (-1.04)	N.S.	6.92 (0.79)	++	0.96 (-0.51)	N.S.	1.22 (-0.19)	N.S.	21.11 (0.90)	++
B6	2.26 (0.22)	N.S.	0.13 (-1.13)	N.S.	5.42 (0.65)	N.S.	0.26 (-0.76)	N.S.	0.55 (-0.49)	N.S.	9.79 (0.83)	++

N.S. No significant difference ($P<0.05$)
+Statistically smaller than ++ ($P<0.05$)
( ) mean logarithmic titre

ASSESSMENT OF LOCAL REACTIONS

Local reactions at injection sites were determined by palpation by the researcher at three, six, nine, 30, 32, 56, 56.5 and 59 weeks postvaccination.

RESULTS

ANIMALS

Ten goats were either culled or died during the experiment. Three goats were eliminated between time 0 and 30 weeks (two died, one culled) and seven were culled between 32 and 56 weeks. None of these goats were eliminated from the trial due to enterotoxemia or as a result of vaccination.

SERUM EPSILON ANTITOXIN TITRES

Serum epsilon antitoxin titres were observed in 54% (38 of 72) of the goats prior to the first vaccination although no history of clinical disease or vaccination was reported in any of the goats used in the trial (Table V). At 30 weeks Group B4 had the lowest percentage of goats with prevaccination serum epsilon antitoxin titres of all six groups (Table VI).

Each goat, regardless of group, was placed into one of three categories at each of the times tested, based on serum epsilon antitoxin titres. The classification scheme is as follows: Less than 0.1 IU of epsilon antitoxin per mL of serum, unprotected, between 0.1 IU and 1.0 IU of epsilon antitoxin per mL of serum at risk; (poor responders at risk of becoming unprotected in the near future) and greater than 1.0 IU of epsilon antitoxin per mL of serum, protected (Fig. 1).

The mean titres of all goats at

TABLE V. Serum Epsilon Antitoxin Titres (IU/mL) of Goats Prior to Vaccination

Serum Epsilon Antitoxin Titres IU/mL	Number of Goats	Classification of Immune Status[a]
0.100	1 (1.0)	At Risk
0.021-0.050	6 (8.0)	Unprotected
0.020	2 (3.0)	Unprotected
0.011-0.019	26 (38)	Unprotected
0.010	3 (4)	Unprotected
<0.010	34 (46)	Unprotected

( )Percentage of goats with serum epsilon antitoxin titres prior to vaccination
[a]Based on classification scheme used by Burroughs-Wellcome in sheep

TABLE VI. Percentage of Goats With Prevaccination Serum Epsilon Antitoxin Titres in Each Vaccination Group at 32 Weeks

Group	Percentage of Goats
A1 — Sheep dose	55% (6/11)
A2 — Cattle dose split	67% (8/12)
A3 — Arbitrary dose	50% (5/10)
B4 — Sheep dose	33% (4/12)
B5 — Cattle dose split	50% (6/12)
B6 — Cattle dose whole	67% (8/12)

( ) No. of goats with titre / No. of goats in group at 32 weeks

TABLE IV. Mean Serum Epsilon Antitoxin Titres at 9, 30, 32, 56, 56.5 and 59 Weeks After the Initial Vaccination of Goats With and Without Prevaccination Serum Epsilon Antitoxin Titres

Group	9 Weeks[a] No. of Goats	9 Weeks[a] Titre	30 Weeks[b] No. of Goats	30 Weeks[b] Titre	32 Weeks[b] No. of Goats	32 Weeks[b] Titre	56 Weeks[a] No. of Goats	56 Weeks[a] Titre	56.5 Weeks[a] No. of Goats	56.5 Weeks[a] Titre	59 Weeks[a] No. of Goats	59 Weeks[a] Titre
Goats with prevaccination titres	37	3.02 (0.05)	37	0.19 (-1.00)	37	6.03 (0.71)	35	0.41 (-0.75)	35	0.89 (-0.37)	35	16.00 (0.75)
Goats without prevaccination titres	32	2.27 (-0.15)	32	0.08 (-1.2)	32	4.48 (0.58)	27	0.40 (-0.94)	27	0.88 (-0.37)	27	8.98 (0.62)

[a]No statistical difference in column ($P>0.05$)
[b]Statistical difference in column ($P<0.05$)
( ) mean logarithmic titre.

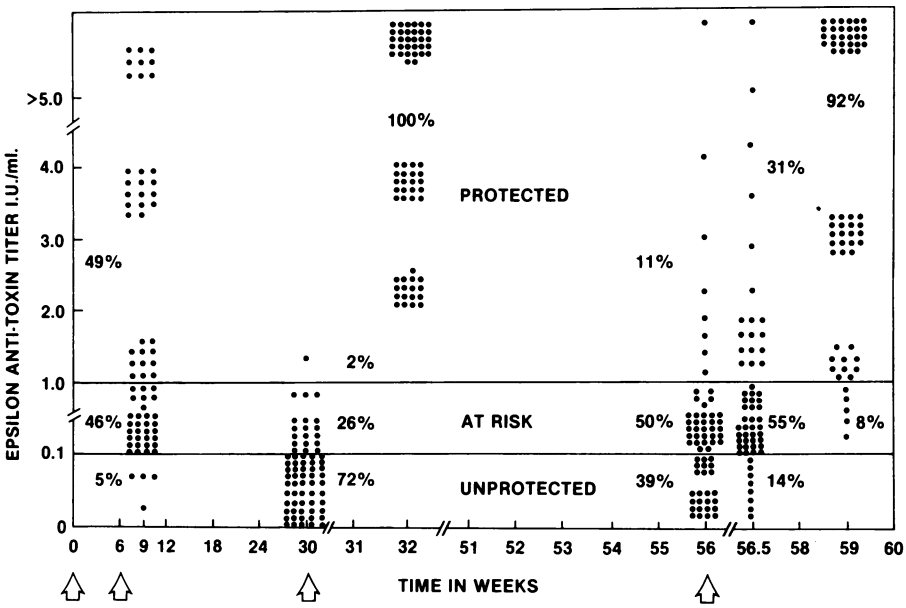

Fig. 1. Response of mature goats following vaccination at 0 time, 6, 30 and 56 weeks (△) for the prevention of enterotoxemia. Each dot approximates the epsilon antitoxin titre in IU/mL for one goat. The goats were vaccinated with either Clostroid C-D (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) or Covexin-8 (Burroughs Wellcome Ltd., Beckenham, Kent, England).

nine, 30, 32 and 56 weeks were compared to the mean of the reported titres of 20 sheep which had been vaccinated and assayed for serum epsilon antitoxin at comparable intervals (3). These results are illustrated in Fig. 2.

STATISTICAL EVALUATION

No significant difference existed between the mean logarithmic titre of all goats receiving vaccine A when compared to the mean logarithmic titre of all goats receiving vaccine B at all times tested (Table III). No significant difference in mean logarithmic titres existed between any of the six vaccination groups at nine, 30, 56 and 56.5 weeks. However, the titre for group B4 at 32 weeks was significantly lower than the titres for groups A2 and B5. Furthermore, the titre for group B4 at 59 weeks was significantly lower than the titres for groups A1, A2, B5 and B6 (Table III).

No significant differences were found between mean logarithmic titres of goats with prevaccination serum epsilon antitoxin titres when compared to goats without prevaccination serum epsilon antitoxin titres at nine, 56, 56.5 and 59 weeks, regardless of vaccine used or dosage administered. However, goats with serum epsilon antitoxin titres prior to vaccination had significantly higher titres at 30 and 32 weeks (Table IV).

The individual titres from the 72 goats are shown in Fig. 1. There was a large range of vaccination response at nine, 26 and 59 weeks although the titres were short-lived. An anamnestic response occurred within four days after the booster injection at 56 weeks.

When the mean titres of all goats at nine, 30, 32 and 56 weeks were compared to the reported mean serum epsilon antitoxin titres of 20 sheep which had received a similar schedule of vaccination with a comparable vaccine marked similarities in titre fluctuations were observed (Fig. 2).

LOCAL REACTIONS

The incidence of local reactions following vaccination is shown for selected times in Table VII. The most commonly observed reaction was a firm raised area approximately 2 cm in diameter. Because reactions were assessed at varying intervals relative to injection and tended to increase or decrease in severity with time, no attempt was made to relate severity of reaction to specific vaccination regimes. However, at 56 weeks or approximately six months after the third vaccination, 53% of the goats had persistent local reactions at one or

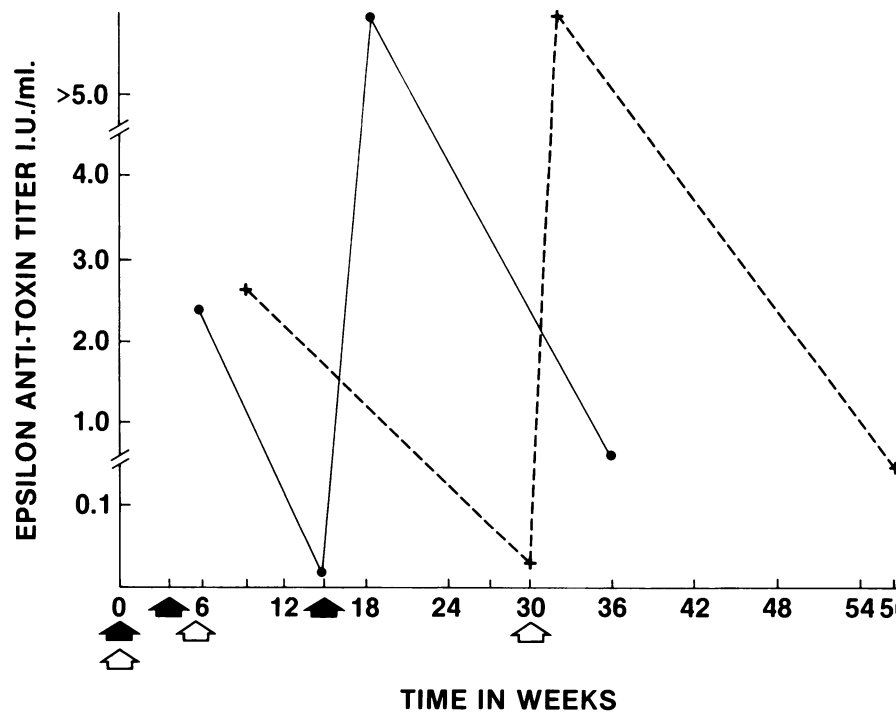

Fig. 2. Mean epsilon antitoxin titres in goats (----) versus sheep [_____, Jansen BC. (7)] following three vaccinations for the prevention of enterotoxemia. △ — vaccine administered to goats, ▲ — vaccine administered to sheep.

TABLE VII. Percentage of Goats with Local Reactions at the Vaccination Sites at 3, 9, 32, 56, and 59 Weeks After the Initial Vaccination

Time \ Groups	A1	A2	A3	B4	B5	B6	Totals
3 weeks (3 weeks after the 1st vaccination)	67% (8/12)	92% (11/12)	83% (10/12)	83% (10/12)	100% (12/12)	100% (12/12)	88% (63/72)
9 weeks (3 weeks after the 2nd vaccination)	92% (11/12)	100% (12/12)	91% (10/11)	100% (12/12)	100% 12/12)	100% (12/12)	97% (69/71)
32 weeks (2 weeks after the 3rd vaccination)	91% (10/11)	100% (12/12)	100% (10/10)	100% (12/12)	100% (12/12)	100% (12/12)	99% (68/69)
56 weeks (26 weeks after the 3rd vaccination)	45% (5/11)	73% (8/11)	63% (5/8)	50% (6/12)	44% (4/9)	45% (5/11)	53% (33/62)
59 weeks (3 weeks after the 4th vaccination)	73% (8/11)	91% (10/11)	50% (4/8)	100% (12/12)	100% (9/9)	100% (11/11)	87% (54/62)
Totals	74% (42/57)	91% (53/58)	80% (39/49)	87% (52/60)	91% (49/54)	90% (52/58)	85% (287/336)

more of the injection sites (Table VII).

DISCUSSION

Although the goats studied had no history of either vaccination for enterotoxemia or clinical disease resembling enterotoxemia, over half of them had serological evidence of exposure to the toxin prior to vaccination. Based on values established in sheep, prevaccination titres were not considered sufficient to confer immunity except in one goat. This finding is consistent with reports that natural subclinical infection with *Clostridium perfringens* type D caused seroconversion in sheep (6). Because the assay for serum epsilon antitoxin was not readily available it was impossible to preselect a population of seronegative goats for this study. Nonetheless, it was felt that this population represented the field situation.

The similarity of the titres in all goats, whether induced by vaccine A or B may reflect the basic similarity in the antigenicity of the two vaccines used. However, the differences between subgroups identified at 32 weeks (group B4 lower than groups A2 and B5) and at 59 weeks (group B4 lower than groups A1, A2, B5 and B6) may have been due to factors such as: 1) the difference in prevaccination serum epsilon antitoxin status of the goats in each group, 2) the variation in immunocompetency between the goats in each group and 3) the difference in the amount of antigen administered.

With regards to the prevaccination immune status, group B4 had the lowest percentage of seropositive goats of the six groups at 32 weeks (Table VI). Therefore, the prevaccination immune status may have contributed to the differences observed at this time. Variations in immunocompetency to enterotoxemia vaccination have been reported in sheep where wide ranges in titres were observed following vaccination (7). Such variations were also observed in the goats in this study (vaccination responses ranging from 2-100 IU epsilon antitoxin/mL of serum within vaccination groups) and may account for the differences observed between groups of goats which otherwise are difficult to explain. For example, at 59 weeks group B4 had a lower titre than group A1 but not lower than group A3 although group A1 received a lower dose of the vaccine than group A3. This apparent lower response to a higher dose of antigen may be a result of this wide variation in vaccination response. The role of vaccine dosage could not be ascertained because total antigenic content relative to *Clostridium perfringens* type D was not determined for either vaccine.

Based on the half-life of IgG in goats (8), the poorer response of group B4 at 32 weeks accounts for only a two week decrease in duration of immunity. For instance, the goats in group A2 at 32 weeks had a mean titre of 6.67 IU of epsilon antitoxin/mL of serum and would have been "unprotected" (<0.1 IU of epsilon antitoxin/mL of serum) roughly 14 weeks later while the goats in group B4 with a mean titre of 3.08 IU of epsilon antitoxin/mL of serum would have been "unprotected" roughly 12 weeks later. Thus, although the titres were significantly different in these two groups, that difference in terms of the duration of protection was short. Likewise, at 59 weeks, the maximum immunological difference between group B4, which had the lowest mean titre, and groups A2 and B5 which had the highest mean titres, represented only a five week difference in duration of immunity. Consequently, it is the authors' opinion that the poorer response of group B4, although statistically significant, was not clinically significant.

The differences in titres of goats with and without prevaccination serum epsilon antitoxin titres at 30 and 32 weeks reinforce the impression that subclinical infection may affect vaccination response. It is possible that goats with prevaccination titres maintain high titres for extended periods of time or have an enhanced anamnestic response to booster vaccinations.

The presence of local reactions in over half the goats six months after a series of three vaccinations (Table VII) are of clinical importance because such lesions may be confused with those of caseous lymphadenitis. Accordingly, it is suggested that clostridial vaccines in goats be administered at sites distinct from regional lymph nodes in order to avoid confusing such reactions with the lesions of caseous lymphadenitis.

Because no difference was observed in epsilon antitoxin titres of goats receiving vaccine A as compared to goats receiving vaccine B, and because the differences observed in subgroups were biologically of minimal clinical significance, all goats regardless of vaccine used or dosage administered were plotted on a scattergram (Fig. 1). From this it is apparent

that in order to maintain epsilon antitoxin titres at protective levels it is necessary to vaccinate goats three to four times a year. However, this practice may be time consuming and costly, and as was demonstrated in this study, result in unsightly reactions at the injection sites.

The close similarity in the titre fluctuations observed in this study and that of Jansen (7) in sheep suggests a comparable ability of the two species to respond to vaccination for enterotoxemia. The similar rate of decline in serum epsilon antitoxin is consistent with the reported half-life of IgG in the two species; sheep, 14 days (9) and goats, 14-17 days (8).

Despite reports that goats respond less effectively than sheep to vaccination for the prevention of enterotoxemia (4, 10), the results of this study do not support an immunological basis for this difference. Variation in species susceptibility to infection or management differences between the two species might be the explanation for this variation in vaccination response.

Given a susceptible host, enterotoxemia in sheep is dependent on the presence of *Clostridium perfringens* type D in the small bowel along with large amounts of poorly digested feed at the same site due to overeating. Assuming that the same conditions as affecting sheep are necessary for disease in the goat, then management practices designed to prevent overeating together with vaccination at times when overeating opportunities are difficult to avoid should minimize the occurrence of enterotoxemia in a herd of goats.

ACKNOWLEDGMENTS

The authors wish to thank the Burroughs Wellcome Laboratories for epsilon antitoxin analysis, Marylin Taylor, Margaret Shorthill, Douglas Andrews and Yvonne Matchett for allowing their goats to be used in this trial and Chris Lewis and Helen Rooyakkers for their technical assistance.

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