Prevention of fatal salmonellosis in neonatal calves, using orally administered chicken egg yolk *Salmonella*-specific antibodies

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Objective—To protect neonatal calves against fatal salmonellosis within the first 2 weeks after birth, using chicken egg yolk antibodies specific against *Salmonella typhimurium* or *S dublin*.

Animals—38 neonatal Holstein calves from *Salmonella*-free farms.

Procedure—After removal of the lipid components with hydroxypropylmethylcellulose phthalate, egg yolk antibodies were spray dried. At 4 days of age, calves were challenge exposed by oral inoculation with 10^{11} virulent *S typhimurium* (experiment 1) or *S dublin* (experiment 2). Starting from the challenge-exposure day, egg yolk antibody preparations were administered orally 3 times a day for 7 to 10 days.

Results—In passive immunization trials, the orally administered antibodies conferred dose-dependent protection against infection with each of the homologous strains of *Salmonella*. Within 7 to 10 days after challenge exposure, all control calves died, whereas low-titer antibody-treated calves had 60 to 100% mortality. Only fever and diarrhea, but no deaths ($P < 0.01$), were observed in calves given the highest titer of antibody.

Conclusions and Clinical Relevance—Compared with that in control calves, survival was significantly higher among calves given antibodies with titers of 500 ($P < 0.05$) and 1,000 ($P < 0.01$) homotypic for *S typhimurium* and with titer of 5,000 ($P < 0.01$) for *S dublin*. Egg yolk antibodies specific for whole cell *S typhimurium* or *S dublin* are protective against fatal salmonellosis when given in sufficiently high concentration, and may be clinically useful during a salmonellosis outbreak. (*Am J Vet Res* 1998;59:416–420)

Salmonella infections in calves are still a worldwide problem, and the bacteria have established themselves as enzootic in several regions. Two serovars, *S typhimurium* and *S dublin*, account for most salmonellosis cases. The *Salmonella* serotypes most commonly associated with bovine salmonellosis are *S typhimurium* and *S dublin*. Results of a number of experiments suggest that live and killed organism vaccines may protect calves.[1-10] However, killed *S dublin* vaccines have been used to protect calves against *S dublin* infection with variable but usually unsatisfactory results.[11-12]

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In other studies, bacterial and virus virulence was reduced by passive immunization with chicken egg yolk antibodies.[13-14] Chicken egg yolk antibodies are cheaper to produce in large volume than are antibodies derived from serum or colostrum or by use of monoclonal techniques, and their efficacy has been reported.[13] Passive immunization with egg yolk antibodies has indicated some degree of efficacy in the control of experimentally induced intestinal colonization by salmonellae in mice.[15] It was, therefore, necessary to establish the efficacy of egg yolk antibody as the sole preventive agent in a controlled trial in calves, preferably those with severe salmonellosis, before it can be integrated in combination with other established methods of prevention and treatment of cattle with the disease. The study reported here was, therefore, undertaken to evaluate the efficacy of *Salmonella*-specific egg yolk antibodies as the sole agent in preventing fatal salmonellosis in calves within the first 2 weeks after birth.

Materials and Methods

Calves—Thirty-eight neonatal Holstein calves from *Salmonella*-free farms were studied in protection experiments with antibody powder preparations. Five-month-old White Leghorn chickens (strain Hyline W36) from *Salmonella*-free farms were used for immunization and later as the source of egg yolk antibodies.

Bacteria and growth conditions—*Salmonella typhimurium* strain 234 and *S dublin* strain 256 were obtained elsewhere.[a] They were grown in Luria broth for 18 hours at 37 C with shaking. After incubation, bacteria were harvested by centrifugation; the bacterial pellet was suspended in phosphate-buffered saline solution (PBSS), and thereafter, was inoculated into calves or inactivated for use as a vaccine by addition of 0.5% formalin in PBSS at 37 C for 18 hours.

Preparation of antibody from egg yolk—The vaccine contained 10^{11} colony-forming units of inactivated *Salmonella* antigen/ml of oil adjuvant,[b] administered IM in the breast of chickens. Eight weeks after the initial injection, chickens were given a booster in similar manner, and the eggs were harvested 2 weeks later. Serum samples from inoculated hens were obtained for a positive control during antibody titration. The yolk was carefully separated from the egg white and yolk membrane and was mixed with 8 volumes of distilled water. An aqueous suspension of 5% hydroxypropylmethylcellulose phthalate[c] in 80% ethyl alcohol was added to the diluted egg yolk gradually, while mixing evenly; the mixture was then incubated at 10 C for 18 hours.[16] The supernatant was collected, passed through a 0.45-μm membrane filter, and applied to a spray dry ma-

chine.[d] Application of the filtrate with a pump was done at an air-inlet temperature of 140 C. At the bottom of the dryer, the dried material was transported by a flow of air at 72 C to the collection vat. The dried antibody powder was stored in a dessicator at room temperature until use.

Titration of antibodies by ELISA—Whole cell lysate antigen was prepared by ultrasonicatione of broth-cultivated *S typhimurium* or *S dublin* cells. Microtitration plate[f] wells were coated with 100 µl of a 5 µg/ml solution of the whole cell lysate antigen in 0.05*M* carbonate buffer (pH 9.6) at 4 C for 18 hours. Sample antibody powders were reconstituted in PBSS (1:100 dilution) to make working antibody solutions. Colostrum and milk were treated with rennin to separate casein from the antibody-containing fraction before titration. A twofold serial dilution in 0.05% Tween 20-PBSS of test sample (antibody, serum, colostrum, or milk) homotypic for the respective *Salmonella* species was then added (100 µl/well) and incubation proceeded at 37 C for 1 hour. Rabbit anti-chicken or anti-bovine IgG conjugated with horseradish peroxidase[g] diluted 1:8,000 was applied, followed by incubation at 25 C for 30 minutes; thereafter, the *o*-phenylenediamine dihydrochloride substrate was added. After 20 minutes, the color reaction was inhibited by addition of $3N H_2SO_4$, and the OD at 490 nm was determined by use of a microtitration plate reader.[h] The antibody dilution giving an absorbance value of 0.2 (OD_{490}) in the linear twofold dilution curve was taken as the sample titer.

Challenge exposure of calves to salmonellae, oral administration of antibodies, and clinical observations—Calves were randomly grouped as follows: experiment 1 with groups 1–4, where group 1 is the control, and experiment 2 with groups 5–7, where group 5 is the control. Experiment-1 calves were later challenge exposed with *S typhimurium*, and experiment-2 calves were challenge exposed with *S dublin*. Control calves of both experiments were given egg yolk antibody from nonimmunized hens with < 1:10 ELISA titer against *Salmonella* sp. Calves of groups 2 4 of experiment 1 were given egg yolk antibody with respective ELISA titer of 1:250, 1:500, and 1:1,000 against *S typhimurium*, and those of groups 6 and 7 of experiment 2 received yolk extract with 1:2,500 and 1:5,000 antibody titer, respectively, against *S dublin*. Antibody treatment was done by dissolving the antibody (about 1 g for the full dose of either antibody experiment product or egg yolk powder from nonimmunized hens) in 50 ml of commercial bovine milk product[i] without additives that was delivered orally via a nipple fed from the inside by a silicon tube attached to a 50-ml syringe. This was followed by consumption of 1 L of milk without antibody from a feeding bottle. This method allowed intake of allocated antibody even by weak calves, because milk consumption varied according to appetite.

Immediately after birth, each calf was fed 2 L of colostrum obtained from its dam. The anti-*Salmonella* ELISA titer was < 1:20 in the various colostrum pools. At 12 and 24 hours after birth, calves were fed milk (*Salmonella* ELISA titer < 1:20) to dilute and wash out remaining colostrum in the gastrointestinal tract. In the controlled clinical trial for prevention of fatal salmonellosis, egg yolk powder from nonimmunized hens and antibody powder from the egg yolk of *Salmonella*-inoculated hens were given orally as described previously for the control and treated groups, respectively. At 4 days of age, about 2 hours after the final suckling period, calves were orally challenge exposed with a virulent strain of *Salmonella* suspended in 50 ml of milk administered through a 50-ml syringe. The infective dose contained 10^{11} viable virulent *Salmonella* organisms per calf. At 2 hours after *Salmonella* administration, calves in treatment groups were given antibody as described previously, followed by 1 L of milk. At each feeding time, each calf was treated similarly 3 times/d for 7 or 10 days after infection. The clinical response of each calf was recorded throughout the experiment and was evaluated in terms of fecal consistency score (daily), rectal temperature change (daily), weight gain (at the time of death or at necropsy), and mortality. The onset of diarrhea or fever was determined as the day calves were first observed after challenge exposure. Fecal scoring was done by a veterinarian member of the group who did not know the grouping of calves before and during the trial. Feces were given numerical scores as follows: 0 = normal, 1 = soft consistency, 2 = mild diarrhea, 3 = severe watery diarrhea. Testing of rectal and intestinal swab and internal organ specimens for *Salmonella* sp was done by culturing the specimens on trypticase soy agar[j] with 5% defibrinated sheep blood, DHL agar,[k] and SS agar,[k] and in selenite broth.[l] The *Salmonella* strain was detected by use of the slide agglutination test with *Salmonella* O4 and O9 antisera.[m] Calves were euthanatized at 7 (*S typhimurium*-challenge exposed) or 10 (*S dublin*-challenge exposed) days after infection because these respective days corresponded to the day that control nonantibody-treated calves in each experiment died. *Salmonella dublin* infection had a generally milder clinical outcome, which accounts for the slightly longer observation period.

Statistical analysis—The Student or Welch *t*-test was used to assess the significance of differences in fecal consistency scores, rectal temperature change, survival period, and bacterial count between antibody-treated and control calves, whereas the Fischer exact test was used to assess differences in clinical response, mortality, and positive bacterial isolation frequency between antibody-treated and control calves.

Results

Titers of spray-dried antibody powders specific for *Salmonella* sp—The spray-dried antibody powder was tested for immunoreactivity with *Salmonella* sp by use of an ELISA. The titer of the *S typhimurium* antibody solution obtained from egg yolk was 1,000, and correlated with titer obtained from the serum of immunized hens; that of the *S dublin* antibody solution was 5,000. The ELISA titer of antibody powder produced from egg yolk of nonimmunized hens was < 10 against either *Salmonella* antigen. A cross-reactivity test, using immunized chicken egg yolk antibody preparation, indicated a low serologic cross-reaction between the *S typhimurium* and *S dublin* antibody products (data not shown). In vivo, the antibody was not protective when the heterologous *Salmonella* species was used for challenge exposure (data not shown).

Experiment 1: Clinical evaluation of calves after challenge exposure with *S typhimurium* and passive immunization with *S typhimurium* antibody powder—The clinical responses of calves after challenge exposure and treatment with antibodies were compared (Tables 1 and 2). Calves of group 4 (high antibody titer) had the highest survival (100%) from *S typhimurium* infection. There was a significant ($P < 0.01$) difference in survival between the control group and group 4. Calves of group 2 (low antibody titer) and control calves given egg yolk antibodies from nonimmunized hens did not survive the infection. Calves given the low (group 2)- and intermediate (group 3)-titer antibodies had 100 and 33% mortality, respectively. Thus, survival of calves was antibody dose dependent. The difference in time of onset of diarrhea and fever (> 40 C) between control and antibody-treated groups was generally dose dependent, with diarrhea or fever somewhat more delayed as antibody concentration increased. The difference in time of onset of diarrhea and fever between group-4 (high-antibody group) and control calves was significant

Table 1—Clinical response of calves to challenge exposure with *Salmonella typhimurium* and treatment with antibody powders of various titers

Group	Antibody titer	No. of calves with diarrhea/total on day 1	3	5	7	Onset of diarrhea	Onset of fever (40 C over)	Survival period	Survival rate on day 7 (%)
1	< 10	3/6 (1.5)[a]	5/5 (2.6)	2/2 (3.0)	0/0	1.8 ± 0.6	1.0 ± 0.6	3.8 ± 1.1	0
2	250	5/6 (2.3)	4/4 (3.0)	2/2 (3.0)	0/0	1.2 ± 0.4	1.3 ± 0.5	3.6 ± 1.5	0
3	500	0/6 (0.2)	4/6 (2.0)	3/4 (2.3)	2/4 (1.5)	3.2 ± 1.1	1.2 ± 1.1	5.8 ± 1.7*	67*
4	1,000	0/6 (0)	4/6 (1.8)	4/6 (2.0)	4/6 (2.0)	3.5 ± 1.3*	2.2 ± 0.9*	7.0 ± 0.0†	100†

*$P < 0.05$, †$P < 0.01$, relative to group 1 (control).
[a]Mean fecal score on clinical observation day.

Table 2—Isolation rate and titer of *S typhimurium* from daily rectal swab and internal organ specimens from challenge-exposed calves

Group	Antibody titer	No. of rectal swab specimens positive/total on day 1	3	5	7		No. of internal organ or swab specimens positive/total Duodenum	Jejunum	Ileum	Cecum	Colon	Mesenteric lymph node	Liver	Spleen	Lungs	Gall bladder
1	<10	6/6 (7.5)[a]	5/5 (7.7)	2/2 (8.1)	0/0	Died	4/6 (4.3)	5/6 (5.6)	6/6 (7.4)	6/6 (8.0)	6/6 (8.0)	6/6	6/6	6/6	6/6	6/6
2	250	6/6 (6.6)	4/4 (7.3)	2/2 (7.7)	0/0	Died	4/6 (3.8)	6/6 (6.4)	6/6 (7.0)	6/6 (7.2)	6/6 (7.3)	6/6	6/6	6/6	6/6	6/6
3	500	6/6 (6.7)	6/6 (6.8)	3/4 (4.4)	2/4 (3.0)	Died	2/2 (5.5)	2/2 (7.7)	2/2 (7.7)	2/2 (6.9)	2/2 (7.1)	2/2	2/2	2/2	2/2	2/2
						Survived	1/4 (1.9)	2/4 (2.7)	3/4 (3.0)	3/4 (3.9)	3/4 (4.0)	3/4	2/4	2/4	2/4	2/4
4	1,000	6/6 (6.9)	4/6 (4.1)*	4/6 (3.8)	3/6 (3.0)	Survived	2/6 (1.3)	2/6 (1.6)*	2/6* (1.6)†	4/6 (2.9)†	4/6 (2.9)†	4/6	1/6†	1/6†	1/6†	2

[a]Mean $\log_{10}$ colony-forming units (CFU)/g of sample/group.
See Table 1 for key.

($P < 0.05$). The degree of severity of diarrhea (on the basis of fecal score) among groups with surviving calves tended to be lower as antibody concentration increased. Mean survival tended to be longer with increasing antibody concentration. It must be noted that the difference in survival of calves within each group and between groups precludes direct comparison of group means in terms of frequency and duration of diarrhea, duration of fever, and body weight gain. For this reason, these variables could not be used as an accurate measure of protection by antibody. Thus, specific protection against salmonellosis attributable to orally administered egg yolk antibodies could be concluded from these data by taking into account the dose-dependent nature of survival rate, duration of survival period, delayed onset of diarrhea and fever, and reduced intensity of diarrhea (on the basis of fecal score). All survivor calves of groups 3 and 4 at necropsy were physically alert and were consuming the entire allocated milk ration at feeding time.

Three of 6 calves in group 4 (high-antibody titer) and 2 of 4 survivors in group 3 were excreting the bacteria in the feces at the end of the study (Table 2). The infective *Salmonella* counts recovered per gram of feces were generally lower in group 4, compared with group 1. Calves that died before the end of the observation period, regardless of group, were excreting the bacteria, and almost all were culture positive on the basis of isolations from organ swab specimens. In these calves, the counts recovered from organs were high, compared with those for survivors in groups 3 and 4. Among survivors at necropsy, the isolation rate and counts of salmonellae recovered from interned organs were lower in group-4 calves ($P < 0.01$–0.05), compared with values for control calves. Among the internal organs examined, mesenteric lymph node, cecum, and colon had the highest frequency of culture positivity. From the organ isolation data, egg yolk antibody specifically reduced intestinal colonization and severity of septicemia, correlating with higher survival rate and better clinical outcome after challenge exposure.

Experiment 2: Clinical evaluation of calves after challenge exposure with *S dublin* and passive immunization with *S dublin* antibody powder—Clinical responses of calves after challenge exposure and treatment with antibodies were compared (Tables 3 and 4). Calves of group 7 (high-antibody titer) had the highest survival (100%) from *S dublin* infection, compared with those of groups 5 (control 5) and 6 (low-antibody titer). None of the control calves survived the infection, whereas 3 of 5 (60%) group-6 calves were protected from fatal salmonellosis. The delay in the onset of diarrhea or fever tended to increase with increased antibody concentration, but the degree of severity of diarrhea was lower in the high-antibody titer group. Mean survival period was longest in the high-antibody titer group; all survived until 10 days. Similar to experiment 1, the difference in survival of calves between groups precludes direct comparison of group means in terms of frequency and duration of diarrhea, duration of fever, and body weight gain. Thus, specific protection from *S dublin* infection by orally administered egg yolk antibodies could be concluded from the dose-dependent survival rate, duration of survival period, delayed onset of diarrhea and fever, and reduced intensity of diarrhea. Similar to calves of experiment 1, all surviving calves regardless of groups were physically alert with full appetite.

The pattern of isolation and recovery counts from rectal and internal organ swab specimens reflect those of experiment 2 (Table 4). Thus, at the end of study, 3 of 5 calves of the high-antibody titer group and 3 of 3 calves of the low-antibody titer group were excreting the bacteria. Counts from culture-positive rectal swab specimens were generally lower in group-7 calves (high-antibody titer), compared with those of group-5 calves. Calves that died before the end of the observation period, regardless of group, were excreting the bacteria. All organs examined from these calves were culture positive, and counts were high, compared with results for group-7 antibody-treated calves that survived challenge exposure. Among group-7 calves, pos-

Table 3—Clinical response of calves to challenge exposure with *S dublin* and treatment with antibody powders of various titers

Group	Antibody titer	No. of calves with diarrhea/total on that day 1	3	5	7	10	Onset of diarrhea	Onset of fever (40 C over)	Survival period	Survival rate on day 10
5	< 10	1/4 (0.5)[a]	4/4 (2.3)	4/4 (2.8)	2/2 (3.0)	0/0	2.0 ± 0.7	1.5 ± 0.5	6.8 ± 1.5	0
6	2,500	1/5 (0.0)	1/5* (1.2)	4/5 (2.0)	1/3 (0.7)	1/3 (0.7)	3.0 ± 1.3	1.4 ± 0.8	8.2 ± 2.2	60
7	5,000	0/5 (0.0)	3/5 (1.2)	2/5 (1.2)	2/5 (1.0)	2/5 (1.0)	3.6 ± 2.0	2.6 ± 1.4	10.0 ± 0.0*	100†

*$P < 0.05$, †$P < 0.01$, relative to group 5 (control).
[a]Mean fecal score on clinical observation day.

Table 4—Isolation rate and titer of *S dublin* from daily rectal swab and internal organ specimens from challenge-exposed calves

Group	Antibody titer	No. of rectal swab specimens positive/total on day 1	3	5	7	10		No. of internal organ or swab specimens positive/total Duodenum	Jejunum	Ileum	Cecum	Colon	Mesenteric lymph node	Liver	Spleen	Lungs	Gall bladder
5	<10	4/4 (6.1)[a]	4/4 (7.0)	4/4 (7.8)	2/2 (7.9)	0/0	Died	4/4 (5.7)	4/4 (7.1)	4/4 (7.5)	4/4 (7.5)	4/4 (7.5)	4/4	4/4	4/4	4/4	4/4
6	2,500	5/5 (6.1)	5/5 (6.4)	5/5 (6.7)	3/3 (6.7)	3/3 (6.5)	Died	2/2 (5.7)	2/2 (6.5)	2/2 (7.4)	2/2 (7.4)	2/2 (6.7)	2/2	2/2	2/2	2/2	2/2
							Survived	1/3 (0.8)	1/3 (1.1)	1/3 (1.4)	3/3 (4.5)	3/3 (5.0)	3/3	3/3	2/3	3/3	2/3
7	5,000	5/5 (6.3)	3/5 (4.0)	3/5 (4.0)	3/5 (3.9)	3/5 (3.8)	Survived	1/5* (0.8)†	2/5 (2.2)*	3/5 (3.8)	3/5 (3.9)	3/5 (3.8)	3/5	3/5	2/5	3/5	2/5

*$P < 0.05$, relative to group 5 (control).
[a]Mean $\log_{10}$ (CFU)/g of sample/group.

itive isolation rate and counts recovered from internal organs at the time of necropsy were lower than those of calves in group 5. The mesenteric lymph node, liver, lungs, ileum, cecum, and colon specimens were most frequently positive for viable salmonellae. From the internal organ isolation data, egg yolk antibody specifically reduced intestinal colonization and severity of septicemia, similar to results of experiment 1.

Discussion

We evaluated the efficacy of the antibody preparations derived from egg yolks of hens inoculated with *Salmonella* antigens in preventing experimentally induced fatal salmonellosis in neonatal calves within the first 2 weeks of life. Because our objective was to evaluate efficacy under severe challenge exposure conditions, we used a challenge dose higher than those in the field to induce a repeatable fatal outcome after infection and, thus, clearly establish efficacy under these controlled conditions. Our results point out the feasibility of preventing mortality by oral administration of egg yolk antibody as passive protection during a *Salmonella* outbreak to protect calves facing serious risk of contracting the disease. The experiments were conducted under usual conditions prevailing in intensively reared cattle farms (access to colostrum, adequate milk intake, and minimal stress). Severely stressed calves under field conditions may succumb to a lower challenge dose, but whether they will respond similarly to passive antibody treatment remains to be determined.

Our data indicated that antibodies prepared from the egg yolks of immunized hens, when used at a sufficiently high concentration, may appreciably protect calves against challenge exposure with homologous *Salmonella* strains, in contrast to antibodies extracted from nonimmunized hens. On the basis of postchallenge exposure mortality as the ultimate measure of protection, we documented that 100% of calves treated with the highest titer of antibody used in this experiment (1,000 for *S typhimurium* or 5,000 for *S dublin*) had survived the infection 7 to 10 days after challenge exposure. Although some calves still had diarrhea, it was mild (score ≤ 2.0 in our system) and mean titer excreted was within values ($10^{3-3.8}$ colony-forming units/g of feces) consistent with mild infection of the gastrointestinal tract. In the case of the *S dublin* experiment, the frequency of diarrhea was on the wane by day 10. On the basis of isolation of infective salmonellae from internal organs from calves of groups 4 and 7 (high-antibody titer), only 1 of 6 and 3 of 5 calves had positive *S typhimurium* and *S dublin* results, respectively, for the liver, spleen, and lungs. Of those that were culture positive, mean bacterial load in the gastrointestinal tract was low.

Although the frequency of morbidity (diarrhea) was not significantly reduced by antibody treatment, the resulting diarrhea was generally mild, consistent with the low bacterial load in the gastrointestinal tract at necropsy. Other variables, such as body weight gain, could not accurately determine the efficacy of the antibody because mean survival was different among groups. Likewise, in a severe challenge-exposure model, we observed that mortality may not correlate with mean body weight because some calves die early, which does not allow much time for weight reduction. Protection from fatal challenge exposure, however, was quantifiable and allowed group comparison.

Comparison of isolation of *Salmonella* sp from the intestines and internal organs of calves with diarrhea that died and surviving calves revealed a correlation between the degree of intestinal and internal organ colonization and mortality. Whereas swab specimens from the intestines and internal organs of calves treated with high-titer antibodies had a low count, swab specimens from dead calves almost always yielded a high count of the *Salmonella* challenge strain. It appears that death resulted after an overwhelming *Salmonella* infection. If the antibodies could prevent death of susceptible animals, the critical time for antibody treatment would be at the early stages of infection when initial colonization of *Salmonella* organisms is impaired by these antibodies, thereby preventing full-blown systemic infection.

On the basis of this observation, use of *Salmonella*-specific egg yolk antibodies as a preventive acquires particular importance when there is risk of salmonellosis for any reason, especially during outbreaks, with the expressed purpose of preventing further deaths among calf populations at risk. Here, clinical use of antibody involving 3 times/d treatment to preclude mortality or

to tide the calves over the critical period would be mandatory. Although the 3 times/d regimen may not be possible under range conditions for obvious reasons, this may be particularly suitable under intensive rearing conditions in regions where pasture land is quite limited, such as in Japan. Although it was intended as a prophylactic agent, the yolk antibody was most probably also partially therapeutic, because its continued oral use after challenge exposure may have lessened the numbers of proliferating bacterial cells adhering to intestinal colonization sites.

Although the antibody trial product was successful in preventing mortality when used singly under conditions of the study reported here, it is, nevertheless, compatible with antimicrobial treatment and current methods of dam vaccination as disease preventive tools on the farm. In fact, the antibody product when used in the field, is intended as an adjunct to other methods of prevention and treatment. In instances where antimicrobial resistance has emerged, which has been the trend for recent *Salmonella* field isolates, egg yolk antibody can be the next best alternative to dam vaccination while taking advantage of the unlikely emergence of resistance to the antibody. In the field, use of antibody in combination with other preventive measures is expected to greatly improve survivability of calves during *Salmonella* epizootics. The question of whether an antibody-treated healthy calf will survive beyond 7 or 10 days after infection (as delineated in this study) becomes less important because other proven methods of prevention and treatment could be implemented at the same time. Surviving calves examined at the end of this study had only mild infection (on the basis of intestinal and internal organ isolation data). The observation that these calves at 7 or 10 days after challenge exposure were consuming the entire milk ration at feeding time should improve the prognosis, especially when other clinical supports, such as antimicrobials, are to be provided. The value of oral antibody administration when used in a combination treatment lies in the fact that it acts as a first line of defense in mucosal protection involving fast elimination of invading bacteria, thereby preventing or lessening the severity of morbidity arising from infection.

In this study, we documented that homotypic antibodies against *S typhimurium* or *S dublin* are protective against fatal experimentally induced salmonellosis when delivered orally before and repeatedly after severe challenge exposure. It is, therefore, recommended that oral passive immunization, using egg yolk antibodies, be considered as an adjunct to vaccination or antimicrobial treatment, or both, when there is real danger of exposure to salmonellae. Egg yolk antibodies specific for other *Salmonella* species can be produced in sufficient amounts by inoculating hens, which involves less cost, compared with large-scale antibody production in cattle or other animal species. The emergence of highly resistant *Salmonella* strains or any other bacterial pathogen causing neonatal diarrhea, such as enterotoxigenic *Escherichia coli*, only emphasizes the suitability of passive immunization as a practical approach to combating diseases of the neonatal intestinal tract.

[a] Chiba Prefectural Institute of Animal Health, Sakura City, Japan.
[b] Maine Biological Laboratories, Waterville, Me.
[c] Shinetsu Chemical Co, Tokyo, Japan.
[d] Model L-12, Ohkawara Kakohki, Kanagawa, Japan.
[e] Ohtake Works, Tokyo, Japan.
[f] Immulon 2, Dynatech Laboratories Inc, Alexandria, Va.
[g] Cappel, Organon Teknika Corp, West Chester, Pa.
[h] Model MR 5000, Dynatech, City, State, Country.
[i] Meiji Milk Products, Tokyo, Japan.
[j] BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.
[k] Eiken Chemical Co, Tokyo, Japan.
[l] Nissui Pharmaceutical Co, Tokyo, Japan.
[m] Denka Seiken, Tokyo, Japan.

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