INFECTION AND IMMUNITY, Dec. 2001, p. 7950–7954 Vol. 69, No. 12
0019-9567/01/$04.00+0 DOI: 10.1128/IAI.69.12.7950–7954.2001

Salmonella DNA Adenine Methylase Mutants Elicit Protective Immune Responses to Homologous and Heterologous Serovars in Chickens

E. L. DUEGER,[1,2]* J. K. HOUSE,[1,2] D. M. HEITHOFF,[3] AND M. J. MAHAN[3]

Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California 95616[1]; Remedyne Corporation, Santa Barbara, California 93105[2]; Department of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, California 93106[3]

Received 26 June 2001/Returned for modification 4 September 2001/Accepted 21 September 2001

***Salmonella* DNA adenine methylase (Dam) mutants that lack or overproduce Dam are highly attenuated for virulence in mice and confer protection against murine typhoid fever. To determine whether vaccines based on Dam are efficacious in poultry, a *Salmonella* Dam$^-$ vaccine was evaluated in the protection of chicken broilers against oral challenge with homologous and heterologous *Salmonella* serovars. A *Salmonella enterica* serovar Typhimurium Dam$^-$ vaccine strain was attenuated for virulence in day-of-hatch chicks more than 100,000-fold. Vaccination of chicks elicited cross-protective immune responses, as evidenced by reduced colonization (10- to 10,000-fold) of the gastrointestinal tract (ileum, cecum, and feces) and visceral organs (bursa and spleen) after challenge with homologous (Typhimurium F98) and heterologous (Enteritidis 4973 and *S. enterica* O6,14,24: e,h-monophasic) *Salmonella* serovars that are implicated in *Salmonella* infection of poultry. The protection conferred was observed for the organ or the maximum CFU/tissue/bird as a unit of analysis, suggesting that Dam mutant strains may serve as the basis for the development of efficacious poultry vaccines for the containment of *Salmonella*.**

Salmonellosis resulting from the consumption of contaminated eggs and poultry meat poses a significant public health risk to consumers worldwide. The Centers for Disease Control and Prevention has estimated that there are approximately 2 million cases of human nontyphoid salmonellosis per year in the United States, resulting in up to 2,000 deaths (1). Most cases of salmonellosis in developed countries are zoonotic in origin and not due to person-to-person contamination. This disease is caused by exposure to products contaminated with *Salmonella*, e.g., animal products (such as eggs, milk, poultry), or the ingestion of food products that have been exposed to animal feces. Economic constraints associated with improved management of production and slaughter facilities suggest that on-farm control of *Salmonella* via a combination of antibiotics, competitive exclusion products, and/or vaccination may be the most practical and economically feasible methods toward reducing contamination of poultry products (34). Such a reduction in preharvest pathogen load may provide a means for decreasing the potential for transmission to humans.

Dam$^-$ *Salmonella* is attenuated for virulence in day-of-hatch chicks. *Salmonella* DNA adenine methylase (Dam) mutants are attenuated for virulence in mice and elicit protective (10, 16) and cross-protective (15) immune responses against murine typhoid fever. To examine whether Dam$^-$ *Salmonella* cells were attenuated for virulence, we challenged day-of-hatch chicks with either Dam$^-$ or Dam$^+$ *Salmonella enterica* serovar Typhimurium UK-1 (Table 1). All chicks (15 out of 15) survived that were infected on the day of hatch with 10^{10} Dam$^-$ UK-1 (MT2313) cells (Table 2). In contrast, 8 of 15 chicks survived after challenge with 10^5 Dam$^+$ UK-1 (MT2315) cells. These data indicate that a mutation in *dam* attenuated the virulence of serovar Typhimurium UK-1 in day-of-hatch chickens by ≥100,000-fold.

Immunization with Dam$^-$ *Salmonella* elicits protective immunity. To determine whether Dam$^-$ serovar Typhimurium elicited protective immune responses, chicks were orally vaccinated with 10^7 CFU of Dam$^-$ Typhimurium UK-1 (MT2313) within 10 h of hatching and again at 1 week of age. Chicks were challenged at 5 weeks of age with 10^8 CFU of serovar Typhimurium F98 (MT2318). Vaccine efficacy was determined by comparison of vaccinate ($n = 62$) and control ($n = 62$) quantitative cultures of the spleen, bursa of Fabricius, ileum, cecum, and feces; cultures were performed at 2, 3, 5, 7, 9, 12, and 14 days postchallenge. The mean $\log_{10}$ CFU of homologous challenge with Typhimurium F98 by organ and day of termination for vaccinated birds relative to controls are presented in Fig. 1A. Vaccination with Dam$^-$ UK-1 (MT2313) resulted in significantly lower CFU ($P < 0.05$) in the spleen and feces of vaccinates on all 7 postchallenge days examined. Significantly lower CFU in vaccinates relative to controls were observed in the bursa on 4 out of 7 termination days, in the ileum on 5 of 7 days, and in the cecum on 3 of 7 days.

Vaccinated birds also had significantly lower CFU than controls following homologous challenge with serovar Typhimurium F98 (MT2318) with maximum CFU/tissue/bird as the unit of analysis (Fig. 2). All control birds had at least 40 CFU of challenge organism in at least one organ following challenge. However, no salmonellae were isolated in any organ from 7 out of 62 (11%) vaccinates; an additional 7 out of 62 (11%) vaccinates had only 10 CFU isolated from at least one organ. In vaccinates, 32 out of 62 (52%) birds had ≤10^3 CFU

* Corresponding author. Mailing address: Dept. of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA 95616. Phone: (530) 752-7407. Fax: (530) 752-0414. E-mail: eldueger@ucdavis.edu.

TABLE 1. Bacterial strains and phage used in this study[a]

Strain	Genotype	Source and/or reference(s)
S. enterica serovar Typhimurium		
UK-1	Wild-type χ3761	Curtiss (12)
F98	Wild-type χ4433	Curtiss (2, 12)
MT2057	ATCC 14028 *zif-7504*::Mu*dJ*	5
MT2116	ATCC 14028 *dam102*::Mu*d*-Cm	John Roth; 16
MT2313	UK-1 *dam102*::Mu*d*-Cm	This work
MT2315	UK-1 *zif-7504*::Mu*dJ*	This work
MT2318	F98 *zif7-504*::Mu*dJ*	This work
S. enterica serovar Enteritidis		
4973	Wild-type χ3700	Curtiss (12, 19)
MT2314	4973 *zif7-504*::Mu*dJ*	This work
S. enterica serovar O6,14,24		
K00-670	Serogroup H, O6,14,24:e,h-monophasic	California Animal Health and Food Safety Laboratory
MT2339	K00-670 Δ*zif7-506*::Kn	20; this work
Bacteriophage P22	HT105/1 *int*-201	31

[a] *S. enterica* serovars Typhimurium UK-1 (χ3671, serogroup B), Typhimurium F98 (serogroup B [2]), and Enteritidis 4973 (serogroup D [19]) were obtained from Roy Curtiss III (12); the *S. enterica* serovar O6,14,24:e,h-monophasic (serogroup H) strain was obtained from the California Animal Health and Food Safety Laboratory (strain K00-670) and was derived from a recent virulent poultry outbreak. Kanamycin-resistant (Kn^r) derivatives of *Salmonella* challenge strains were constructed by transducing a Kn^r insertion element, Mu*dJ*, from strain MT2057 (16) or by integrating a suicide plasmid containing a deletion associated with a kanamycin resistance determinant, Δ*zif-7506*::Kn, into all challenge strains (20); these Kn^r mutations were previously shown not to affect the virulence of serovar Typhimurium in mice (5, 20). The Dam$^-$ UK-1 vaccine strain was constructed by transducing the insertion element, *dam102*::Mu*d*-Cm (encoding chloramphenicol [Cm^r] resistance), from strain MT2116 into UK-1 (16). Strains used in infection studies were grown overnight in Luria broth at 37°C with shaking. The high-frequency generalized transducing bacteriophage P22 mutant HT105/1 *int*-201 was used for all transductional crosses (31), and phage-free, phage-sensitive transductants were isolated as previously described (4). Luria broth (9) and 1% lactose MacConkey agar (Difco) were the laboratory media used in these studies. Final concentrations of antibiotics (Sigma) were as follows: kanamycin, 50 μg/ml; chloramphenicol, 20 μg/ml.

in at least one organ, compared to 2 (3%) out of 62 controls in this category. Birds with $\leq 10^4$ CFU in at least one organ included 46 (74%) out of 62 vaccinates compared to only 7 (11%) out of 62 controls. Taken together, these data indicate that immunization of chicks with Dam$^-$ serovar Typhimurium confers protection against homologous challenge with the organ or maximum CFU/tissue/bird as the unit of analysis.

Immunization of chicks with Dam$^-$ *Salmonella* elicits cross-protective immunity. Next, we examined the cross-protective capacity of chicks immunized with Dam$^-$ *Salmonella*. Sixty chicks were orally vaccinated with 10^7 Dam$^-$ UK-1 (MT2313) cells within 10 h of hatching and again at 1 week of age; 60 additional chicks remained as nonvaccinated controls. All chicks were challenged at 5 weeks of age with either 10^8 CFU of serovar Enteritidis 4973 (serogroup D; MT2314) or 10^9 CFU of serovar *S. enterica* O6,14,24:e,h-monophasic (serogroup H; MT2339). Figure 1B and C show the mean $\log_{10}$ CFU of serovars Enteritidis 4973 and *S. enterica* O6,14,24:e,h-monophasic challenge organisms by organ and day of termination for vaccinated birds relative to nonvaccinated controls. Six days postchallenge with serovar Enteritidis 4973 (MT2314), vaccinates had significantly lower CFU in the spleen, bursa, cecum, and feces: no challenge organisms were recovered from any vaccinated bird organs, whereas 50 to 60% of control organs were positive for *Salmonella*. No challenge organisms were recovered from the spleens of vaccinates on day 6 or 7 postchallenge with *S. enterica* serovar O6,14,24:e,h-monophasic (MT2339); in contrast, salmonellae were recovered from 19 out of 20 control spleens on these 2 days.

TABLE 2. Dam$^-$ *Salmonella* is attenuated for virulence in chickens[a]

Strain	Relevant genotype	Challenge dose (CFU)	No. of survivors
MT2313	Dam$^-$ UK-1	10^{10}	15/15
MT2315	Dam$^+$ UK-1	10^5	8/15

[a] Chicks within 10 h of hatching were infected with the *S. enterica* serovar Typhimurium strain indicated. Survival was assessed until 3 weeks of age. Strains used in infection studies were grown overnight in Luria broth at 37°C, and serial dilutions were performed in 0.2 M Na_2HPO_4 buffered to pH 8.0 to the desired cell density for infection. Fertile eggs obtained from White Leghorn chickens (Specific Pathogen Free Avian Services, Charles River) were incubated and hatched in several Marsh Automatic incubators (Lyon Electric Co., Inc., Chula Vista, Calif.).

Vaccinated birds had significantly lower CFU than controls following heterologous challenge when the maximum CFU/tissue/bird was used as the unit of analysis (Fig. 2). No serovar Enteritidis 4973 was recovered from 18 out of 30 (60%) challenged vaccinates compared to only 7 out of 30 (23%) noninfected control birds. Vaccinated birds also showed protection against challenge with serovar *S. enterica* O6,14,24:e,h-monophasic, as 7 out of 30 (23%) vaccinates had ≤ 100 CFU in at least one organ; no control birds were in this category. Taken together, these data indicate that chicks vaccinated with Dam$^-$ serovar Typhimurium UK-1 elicited cross-protective immune responses to challenge with serovars Enteritidis 4973 and *S. enterica* O6,14,24:e,h-monophasic for the organ or maximum CFU/tissue/bird as the unit of analysis.

The safety of the food supply can be compromised by large-scale animal husbandry, agricultural methods, and distribution practices that are prone to microbial contamination. This public health problem has been recently exacerbated by the emergence of pathogens that are resistant to multiple antibiotics and/or cause more debilitating forms of disease (e.g., *Esche-*

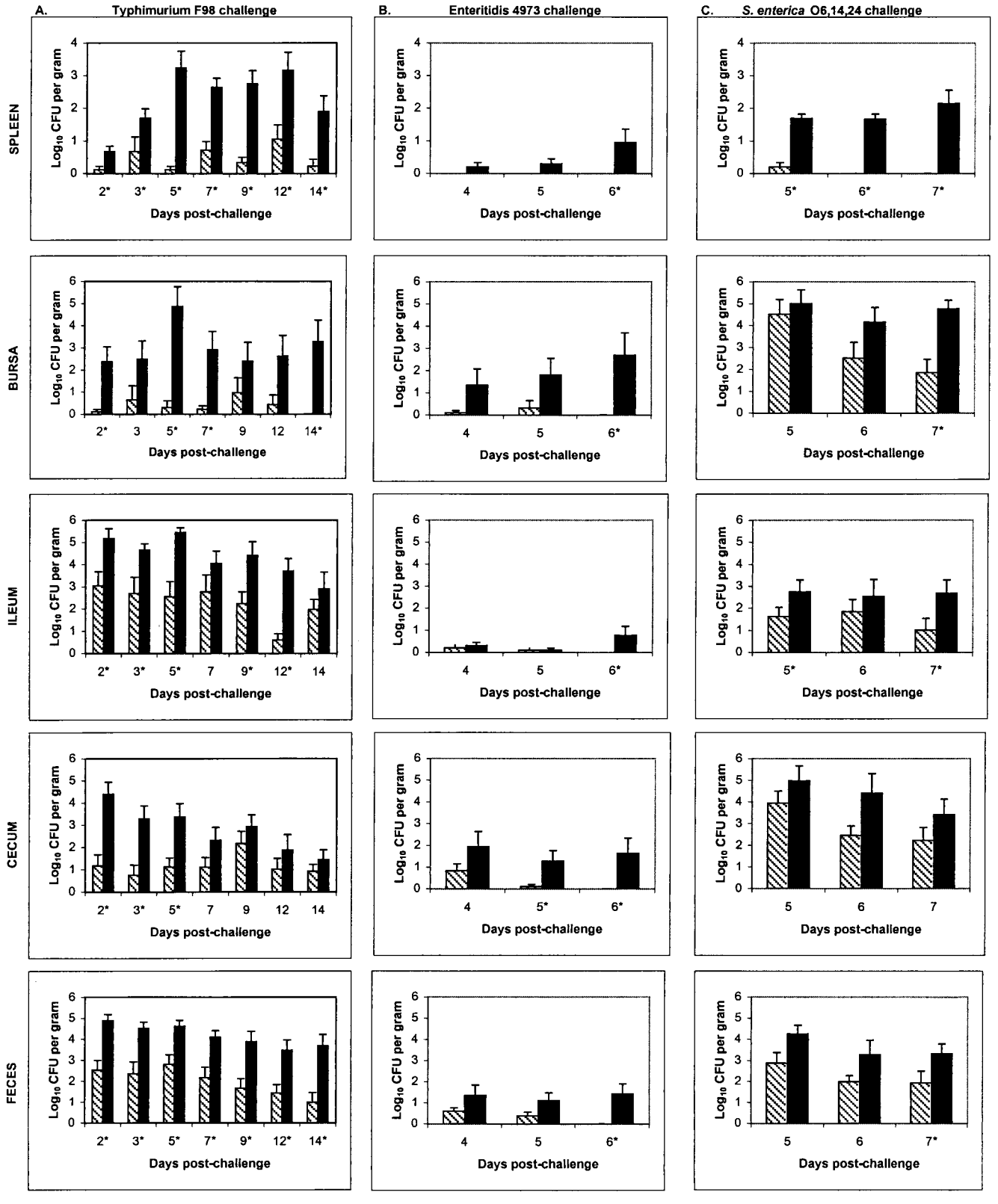

FIG. 1. Dam$^-$ *Salmonella* elicits protective responses in chicken tissue sites. The protective capacity of Dam$^-$ serovar Typhimurium was assessed by orally immunizing 62 chicks with 10^7 CFU of Dam$^-$ UK-1 (MT2313) within 10 h of hatch and boosted with the same dose at 1 week of age (hatched bars); 62 additional chicks remained as nonvaccinated controls (filled bars). All chicks were challenged at 5 weeks of age with 10^8 CFU of serovar Typhimurium F98 (MT2318). Data are depicted as mean $\log_{10}$ CFU by organ. Nine control and nine vaccinated chickens were terminated at 2, 3, 5, 7, 9, and 12 days postchallenge; 8 birds per group were terminated 14 days postchallenge. Cross-protective immunity was

richia coli O157:H7 and *Salmonella* serovars Enteritidis and Typhimurium DT104). Vaccination of chickens offers a practical and economically feasible approach to reducing contamination of poultry products. Here, we show that an *S. enterica* serovar Typhimurium Dam$^-$ mutant was severely attenuated for virulence in day-of-hatch chicks. Additionally, chicks immunized with this *Salmonella* Dam$^-$ vaccine strain exhibit protective immune responses against homologous and heterologous *Salmonella* serotypes that are implicated in *Salmonella* infection of poultry. Vaccines based on altered levels of Dam activity may prove effective in controlling *Salmonella* contamination of poultry, meat, and dairy products derived from animals susceptible to *Salmonella* infection and colonization.

Enumeration of salmonellae isolated from the visceral organs and intestinal tract of vaccinated and nonvaccinated chickens challenged with virulent serovars Typhimurium or Enteritidis or *S. enterica* O6,14,24:e,h-monophasic was used to determine the degree of protection associated with vaccination. Significantly lower mean $\log_{10}$ CFU were observed in visceral organs and the gastrointestinal tract of vaccinates versus nonvaccinates. Comparison of these results with challenge studies for other vaccines is problematic as the outcome of infection varies greatly with challenge strain, inoculation and immunization dose, use of multiple (booster) immunizations, the age of the birds at vaccination and challenge, statistical analysis of the data, etc. (11, 17, 18). Previous studies using live attenuated Salmonella *aroA* (11, 17, 18) and Δ*cya* Δ*crp* mutants (12) showed reduced visceral invasion and colonization of the gastrointestinal tract in chickens by homologous and, to a lesser extent, heterologous challenge strains. Oral vaccination with attenuated *aroA* mutants of serovar Typhimurium (3) or Enteritidis (6, 7) reduced fecal shedding following homologous challenge, but not heterologous challenge (8). Vaccination with serovar Typhimurium Δ*cya* Δ*crp* conferred protection against intestinal and visceral invasion by both homologous and heterologous challenge serotypes (12). Moreover, this vaccine also provided protection against intestinal, visceral, reproductive tract and egg colonization by *Salmonella* for at least 11 months postvaccination, with no effect on egg production (13).

Results of this study are promising in that significant protection was observed following homologous and heterologous challenge at high challenge doses. It should be noted that a single challenge may not reflect the field situation wherein animals can be exposed to various doses of several virulent serovars alone and in combination. That said, multiple and/or continuous exposures to several serovars in the field situation do not necessarily result in susceptibility of immunized animals to disease: repeated exposures may contribute to the maintenance of heightened levels of protection in vaccinated hosts.

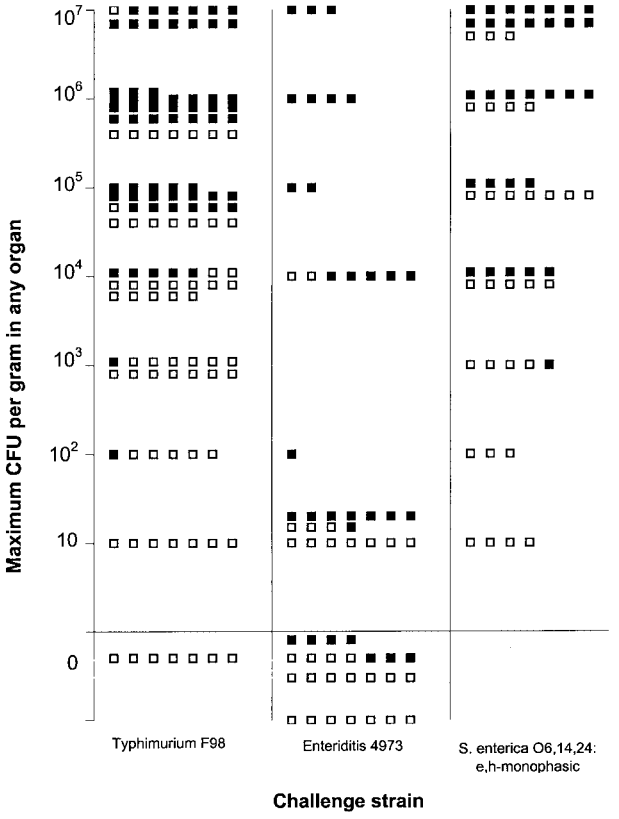

FIG. 2. *Salmonella* Dam$^-$ vaccine elicits protective responses as determined by the maximum CFU/tissue/bird analysis. Vaccinated (open boxes) and nonvaccinated (closed boxes) chicks were challenged as described in the Fig. 1 legend. The maximum CFU from the five tissues examined for each bird determined the category into which the bird was included. For example, if no CFU were found in the spleen, bursa, ileum, or cecum but 10^6 CFU were found in feces, the bird was included in the $\leq 10^6$ CFU category.

The data presented here suggest that vaccines based on altered DNA methylation may reduce preharvest *Salmonella* contamination in poultry, ultimately decreasing the potential for food-borne transmission of this pathogen to humans. DNA

assessed as follows. Sixty chicks were orally vaccinated with 10^7 Dam$^-$ UK-1 (MT2313) cells within 10 h of hatching and again at 1 week of age; 60 additional chicks remained as nonvaccinated controls. All chicks were challenged at 5 weeks of age with either 10^8 CFU of serovar Enteritidis 4973 (MT2314) or 10^9 CFU of *S. enterica* serovar O6,14,24:e,h-monophasic (MT2339). Ten control and 10 vaccinated chickens were terminated at 4, 5, and 6 days postchallenge for the serovar Enteritidis challenge or 5, 6, or 7 days postchallenge for the *S. enterica* serovar O6,14,24:e,h-monophasic challenge. For each experiment, a separate cohort of 8 to 14 nonvaccinated, nonchallenged negative control birds were maintained and necropsied as described below. Approximately 1 g of each organ was obtained in the following order: spleen, bursa of Fabricius, ileum and ileal contents, cecum and cecal contents, and rectum and feces. Organs were weighed, homogenized, and serially diluted, and 100 μl of each dilution was plated on 1% lactose MacConkey agar plates containing kanamycin. For detection of salmonellae at concentrations below 40 CFU/g, the 1:4 dilution homogenate sample was incubated for 24 h in 9 ml of tetrathionate solution, streaked for single colonies on lactose MacConkey agar, and incubated for 24 h at 37°C. Samples positive by selective enrichment in tetrathionate broth were recorded as 10 CFU, and negative samples were recorded as 0 CFU. For tissue experiments, bacterial titers were confirmed via serial dilutions plated on 1% lactose MacConkey agar; colony serotype was confirmed via standard biochemical tests (urea, triple sugar iron, and ONPG [*O*-nitrophenyl-β-D-galactopyranoside]) and agglutination with appropriate antisera on two randomly selected colonies from each organ of each bird. ∗, significant difference ($P < 0.05$) between vaccinates and controls as assessed by the Mann-Whitney test.

methylation plays a role in the virulence of a wide variety of pathogens of the gamma subdivision of proteobacteria, including *Salmonella* (murine typhoid; 10, 15), *Yersinia pseudotuberculosis* (murine bacteremia; 21), and *Vibrio cholerae* (cholera; 21). Additionally, DNA methylation is required for the virulence of *Brucella abortus* (fetal calf abortion; 30) via CcrM, a cell-cycle regulated DNA adenine methyltransferase present in members of the alpha group of proteobacteria (28, 33). Since Dam and CcrM affect the virulence of such distantly related pathogens, the function of DNA methylation in virulence may emerge as a general theme in bacterial pathogenesis.

The role of DNA methylation in virulence and the elicitation of protective immune responses may rely on its capacity as a global regulator of gene expression (16, 22, 24, 25, 26, 28, 29). Dam regulates the production of a number of adhesins in *E. coli* (23, 32) and *Salmonella* (27), as well as several genes required for *Salmonella* infection (14, 16). Such ectopic gene expression may result in the production of an expanded repertoire of antigens that contribute to the heightened immunity seen in vaccinated animals (15, 16, 21). Thus, dysregulation of Dam activity may be a means to elicit protective immune responses directed against diverse pathogens that infect a wide variety of animal hosts (24, 25).

We thank Steve Julio for critically reviewing the manuscript.

This work was supported by USDA grant 2000-02539 (to J.K.H and M.J.M.), a grant from Remedyne Corporation (to J.K.H.), private donations from Jim and Deanna Dehlsen, the Santa Barbara Cottage Hospital Research Program (to M.J.M.), and a postdoctoral grant from the Cancer Center of Santa Barbara (to D.M.H.).

REFERENCES

1. **Altekruse, S. F., M. L. Cohen, and D. L. Swerdlow.** 1997. Emerging food borne diseases. Emerg. Infect. Dis. **3:**285–293.
2. **Barrow, P. A., H. W. Smith, and J. F. Tucker.** 1984. The effect of feeding diets containing avopracin on the excretion of Salmonella by chickens experimentally infected with natural sources of Salmonella organisms. J. Hyg. **93:**439–444.
3. **Barrow, P. A., J. O. Hassan, and A. Berchieri, Jr.** 1990. Reduction in faecal excretion of *Salmonella typhimurium* strain F98 in chickens vaccinated with live and killed *S. typhimurium* organisms. Epidemiol. Infect. **104:**413–426.
4. **Chan, R. K., D. Botstein, T. Watanabe, and Y. Ogata.** 1972. Specialized transduction of tetracycline resistance by phage P22 in *Salmonella typhimurium*. II. Properties of a high-frequency-transducing lysate. Virology **50:** 883–898.
5. **Conner, C. P., D. M. Heithoff, S. M. Julio, R. L. Sinsheimer, and M. J. Mahan.** 1998. Differential patterns of acquired virulence genes distinguish Salmonella strains. Proc. Natl. Acad. Sci. USA **95:**4641–4645.
6. **Cooper, G. L., L. M. Venables, R. A. Nicholas, G. A. Cullen, and C. E. Hormaeche.** 1992. Vaccination of chickens with chicken-derived Salmonella enteritidis phage type 4 aroA live oral Salmonella vaccines. Vaccine **10:**247–254.
7. **Cooper, G. L., L. M. Venables, R. A. Nicholas, G. A. Cullen, and C. E. Hormaeche.** 1993. Further studies of the application of live Salmonella enteritidis *aroA* vaccines in chickens. Vet. Rec. **133:**31–36.
8. **Cooper, G. L., L. M. Venables, M. J. Woodward, and C. E. Hormaeche.** 1994. Vaccination of chickens with strain CVL30, a genetically defined Salmonella enteritidis *aroA* live oral vaccine candidate. Infect. Immun. **62:**4747–4754.
9. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
10. **Garcia-Del Portillo, F., M. G. Pucciarelli, and J. Casadesus.** 1999. DNA adenine methylase mutants of *Salmonella typhimurium* show defects in protein secretion, cell invasion, and M cell cytotoxicity. Proc. Natl. Acad. Sci. USA **96:**11578–11583.
11. **Harrison, J. A., B. Villarreal-Ramos, P. Mastroeni, R. Demarco de Hormaeche, and C. E. Hormaeche.** 1997. Correlates of protection induced by live Aro-*Salmonella typhimurium* vaccines in the murine typhoid model. Immunology **90:**618–625.
12. **Hassan, J. O., and R. Curtiss III.** 1994. Development and evaluation of an experimental vaccination program using a live avirulent *Salmonella typhimurium* strain to protect immunized chickens against challenge with homologous and heterologous Salmonella serotypes. Infect. Immun. **62:**5519–5527.
13. **Hassan, J. O., and R. Curtiss III.** 1997. Efficacy of a live avirulent *Salmonella typhimurium* vaccine in preventing colonization and invasion of laying hens by *Salmonella typhimurium* and *Salmonella enteritidis*. Avian Dis. **41:**783–791.
14. **Heithoff, D. M., C. P. Conner, P. C. Hanna, S. M. Julio, U. Hentschel, and M. J. Mahan.** 1997. Bacterial infection as assessed by in vivo gene expression. Proc. Natl. Acad. Sci. USA **94:**934–939.
15. **Heithoff, D. M., E. Y. Enioutina, R. A. Daynes, R. L. Sinsheimer, D. A. Low, and M. J. Mahan.** 2001. *Salmonella* DNA adenine methylase mutants confer cross-protective immunity. Infect. Immun. **69:**6725–6730.
16. **Heithoff, D. M., R. L. Sinsheimer, D. A. Low, and M. J. Mahan.** 1999. An essential role for DNA adenine methylation in bacterial virulence. Science **284:**967–970.
17. **Hormaeche, C. E., H. S. Joysey, L. Desilva, M. Izhar, and B. A. D. Stocker.** 1991. Immunity conferred by AroA- Salmonella live vaccines. Microb. Pathog. **10:**149–158.
18. **Hormaeche, C. E., P. Mastroeni, J. A. Harrison, R. Demarco de Hormaeche, S. Svenson, and B. A. D. Stocker.** 1996. Protection against oral challenge three months after i.v. immunization of BALB/c mice with live Aro *Salmonella typhimurium* and *Salmonella enteritidis* vaccines is serotype (species)-dependent and only partially determined by the main LPS O antigen. Vaccine **14:**251–259.
19. **Jones, F., R. C. Axtell, F. R. Tarver, D. V. Rives, S. E. Scheideler, and M. J. Wineland.** 1991. Experimental factors contributing to Salmonella colonization of chickens, p. 3–21. *In* L. C. Blankenship, J. S. Bailey, N. A. Cox, N. J. Stern, and R. J. Meinersmann (ed.), Colonization control of human bacterial enteropathogens in poultry. Academy Press, Inc., New York, N.Y.
20. **Julio, S. M., C. P. Conner, D. M. Heithoff, and M. J. Mahan.** 1998. Directed formation of chromosomal deletions in *Salmonella typhimurium:* targeting of specific genes induced during infection. Mol. Gen. Genet. **258:**178–181.
21. **Julio, S. M., D. M. Heithoff, D. Provenzano, K. E. Klose, R. L. Sinsheimer, D. A. Low, and M. J. Mahan.** 2001. DNA adenine methylase is essential for viability and plays a role in the pathogenesis of *Yersinia pseudotuberculosis* and *Vibrio cholerae*. Infect. Immun. **69:**7610–7615.
22. **Kahng, L. S., and L. Shapiro.** 2001. The CcrM DNA methyltransferase of *Agrobacterium tumefaciens* is essential, and its activity is cell cycle regulated. J. Bacteriol. **183:**3065–3075.
23. **Krabbe, M., N. Weyand, and D. Low.** 2000. Environmental control of pilus gene expression, p. 305–321. *In* G. Storz and R. Hengge-Aronis (ed.), Bacterial stress responses. ASM Press, Washington, D.C.
24. **Low, D. A., N. J. Weyand, and M. J. Mahan.** 2001. The roles of DNA methylation in the control of bacterial gene expression and virulence. Infect. Immun. **69:**7197–7204.
25. **Mahan, M. J., and D. A. Low.** 2001. DNA methylation regulates bacterial gene expression and virulence. ASM News **67:**1–7.
26. **Marinus, M. G.** 1996. Methylation of DNA, p. 782–791. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.
27. **Nicholson, B., and D. A. Low.** 2000. DNA methylation-dependent regulation of pef expression in Salmonella typhimurium. Mol. Microbiol. **35:**728–742.
28. **Reisenauer, A., L. S. Kahng, S. McCollum, and L. Shapiro.** 1999. Bacterial DNA methylation: a cell cycle regulator? J. Bacteriol. **181:**5135–5139.
29. **Reisenauer, A., K. Quon, and L. Shapiro.** 1999. The CtrA response regulator mediates temporal control of gene expression during the *Caulobacter* cell cycle. J. Bacteriol. **181:**2430–2439.
30. **Robertson, G. T., A. Reisenauer, R. Wright, R. B. Jensen, A. Jensen, L. Shapiro, and R. M. Roop II.** 2000. The *Brucella abortus* CcrM DNA methyltransferase is essential for viability, and its overexpression attenuates intracellular replication in murine macrophages. J. Bacteriol. **182:**3482–3489.
31. **Schmeiger, H.** 1972. Phage P22-mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119:**75–88.
32. **van der Woude, M., B. Braaten, and D. Low.** 1996. Epigenetic phase variation of the *pap* operon in *Escherichia coli*. Trends Microbiol. **4:**5–9.
33. **Wright, R., C. Stephens, and L. Shapiro.** 1997. The CcrM DNA methyltransferase is widespread in the alpha subdivision of proteobacteria, and its essential functions are conserved in *Rhizobium meliloti* and *Caulobacter crescentus*. J. Bacteriol. **179:**5869–5877.
34. **Zhang-Barber, L., A. K. Turner, and P. A. Barrow.** 1999. Vaccination for control of Salmonella in poultry. Vaccine **17:**2538–2545.

Editor: D. L. Burns