

A comparison of *Salmonella enteritidis* phage types from egg-associated outbreaks and implicated laying flocks

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SUMMARY

Infections due to *Salmonella enteritidis* are increasing worldwide. In the United States, between 1985 and 1989, 78% of the *S. enteritidis* outbreaks in which a food vehicle was identified implicated a food containing raw or lightly cooked shell eggs.

Under a US Department of Agriculture regulation published in 1990, eggs implicated in human food-borne *S. enteritidis* outbreaks were traced back to the source flock. The flock environment and the internal organs of a sample of hens were tested for *S. enteritidis*. We compared the *S. enteritidis* phage types of isolates from 18 human, egg-associated outbreaks and the 15 flocks implicated through traceback of these outbreaks. The predominant human outbreak phage type was recovered from the environment in 100% of implicated flocks and from the internal organs of hens in 88% of implicated flocks we tested. The results support the use of phage typing as a tool to identify flocks involved in human *S. enteritidis* outbreaks.

INTRODUCTION

World Health Organization surveillance data on human salmonellosis for 1979–87 indicate that *Salmonella enteritidis* infection increased in the Americas and Europe [1]. In 1990, *S. enteritidis* became the predominant serotype of *Salmonella* in human infections in the United States, surpassing *S. typhimurium* [2]. The 8591 isolates of *S. enteritidis* reported to the Centers for Disease Control (CDC) in 1990 [2] are estimated to represent between 1% and 10% of all cases [3]. The reported rate of *S. enteritidis* infection in humans was highest in the northeastern United States; however, rates have been rising in other regions [4].

The increase in reported *S. enteritidis* infection in humans is associated in part with shell eggs [5, 6]. Hens with subclinical infection of the reproductive tract can transmit the bacteria in intact shell eggs [7]. Between 1985 and 1989, 78% of *S. enteritidis* outbreaks in the United States in which a food vehicle was identified implicated foods containing raw or lightly cooked eggs [8]. A case-control study

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in Minnesota reported a strong association between sporadic *S. enteritidis* infection in humans and the consumption of raw or lightly cooked eggs and a lesser association with raw ground beef [9].

In response to the *S. enteritidis* epidemic, the US Department of Agriculture (USDA) developed a traceback programme to identify the source flock for shell eggs implicated in egg-associated *S. enteritidis* outbreaks [10]. In this study, we compared the phage type of *S. enteritidis* isolates recovered from human patients in egg-associated outbreaks with those from the environment and from internal organs of hens in the implicated poultry operations.

METHODS

Human isolates included in this study were recovered from 18 *S. enteritidis* outbreaks epidemiologically associated with shell eggs in early 1990 through late 1991, by using isolation methods that have been previously described [11]. These outbreaks are a subset of approximately 35 documented egg-associated *S. enteritidis* outbreaks reported to the Enteric Diseases Branch of CDC by state health department epidemiologists during this period.

When CDC was informed that an outbreak had occurred with an egg-containing food as the implicated vehicle, epidemiological information about the outbreak was collected and human *S. enteritidis* isolates were phage typed by the Enteric Diseases Laboratory, CDC using phages developed by Ward and colleagues [12, 13].

The USDA *Salmonella enteritidis* Task Force initiated a traceback when it received a report from a state epidemiologist that eggs were the most probable source of infection in an *S. enteritidis* outbreak. Purchase and shipping records, packing date, and descriptive information, including egg size, grade, and package markings, were used to trace the implicated eggs back through market channels to the source flock. After an egg traceback was completed, flock owners were notified that eggs from a flock under their supervision had been implicated in an *S. enteritidis* outbreak. The typical egg operation in these investigations had multiple houses, with approximately 70000 hens per house.

Swabs from manure and egg belts in implicated poultry houses were examined for *S. enteritidis* by using previously described methods [14, 15]. In poultry houses where environmental tests recovered *S. enteritidis*, 300 hens were serologically tested with the use of a rapid plate test for pullorum antigen. The internal organs (heart, liver, spleen, ovary, and oviduct) of all serological reactors and a random sample of hens to yield 60 per house were harvested for bacteriological examination. A separate pooled sample of internal organs was collected from each of the 60 hens. These specimens were examined for salmonella. If *S. enteritidis* was not recovered from the initial sample of hens, the flock was retested [10]. All *S. enteritidis* isolates from the poultry environment and organs of hens were characterized by phage typing at the National Veterinary Services Laboratory (NVSL), using the phages developed by Ward and colleagues [12]. We compared the phage types of human *S. enteritidis* isolates from outbreaks with *S. enteritidis* phage types from the egg production flock implicated in the outbreak through egg traceback.

Table 1. Phage type (PT) of *Salmonella enteritidis* isolates from human outbreaks and the egg operation implicated by traceback

Flock	Outbreak location	Month (1990)	Human outbreak PT	Poultry environment PT	Internal organ PT
A	Nashville, TN	June	8	8	Negative
B	Delmar, DE	Sept.	8	8,23	8
C	Chicago, IL	Dec.	13a	13a,23,28	13
D	Versailles, KY	Aug.	8	8,13a,23,28	8,13a,13
E	Chicago, IL	Oct.	8	8,13,23	8
F	Jefferson Co., TN	Oct.	8	8,13a,23,28	8,23,28
G	Woburn, MA	Jan.	14b	14b	14b
G	Suffield, CT	Feb.	14b	14b	14b
H	Tarreyton, NY	Mar.	8	3,8,13,13a,23	8,23
J	Linthicum, MD	Mar.	8	8,23,14b,13,34	8
J	Nazareth, PA	May	8	8,23,14b,13,34	8
J	Fallston, MD	July	8	8,23,14b,13,34	8
K	Bristol, CT	June	8	8,13a	2,8,13a,28
L	Eastern Pennsylvania	June	8	*	8,23
M	Eatonton, NJ	Sept.	8,34	8,23	†
N	Dauphin, PA	Dec. (1991)	8	2,8,23	8
P	Gaithersburg, MD	June	8	8,13a,22,23,34	8,13a,28
Q	Syracuse, NY	May	2	2	2

* Hens' organs were tested without environmental sampling because the flock was implicated in three outbreaks.

† Flock slaughtered before the hens were tested.

RESULTS

In the first 15 months of the traceback programme, eggs from 18 egg-associated *S. enteritidis* outbreaks were traced back to 15 egg-laying operations (Table 1). The mean distance between the location of the *S. enteritidis* outbreak and the implicated egg production farm was 148 miles (range, 35–380 miles). Of these 18 outbreaks, 13 (72%) were associated with eggs produced in another state.

Salmonella enteritidis phage types from human outbreaks and from the environment and organs of hens from the implicated egg production operations are presented in Table 1. Flock J was associated with three outbreaks and flock G with two outbreaks. Both flocks were associated with outbreaks in more than one state. Flock L was implicated in 70 cases of *S. enteritidis* that occurred over a 5-week period in 9 franchises of a restaurant chain in eastern Pennsylvania. This flock was restricted without environmental testing. The remaining 12 flocks that we investigated were associated with one outbreak each.

Of 14 flocks in which the environment was tested for *S. enteritidis*, all 14 (100%) were culture-positive for *S. enteritidis*. *S. enteritidis* was recovered from the organs of hens in 13 (93%) of 14 flocks in which organs were tested. The phage type responsible for the human outbreak was recovered from the environment of the implicated flock in all 17 (100%) tracebacks for which this comparison was possible and from the internal organs of hens for 15 (88%) of 17 investigations for which this comparison was possible.

Salmonella enteritidis phage type 8 (PT 8) was the predominant phage type

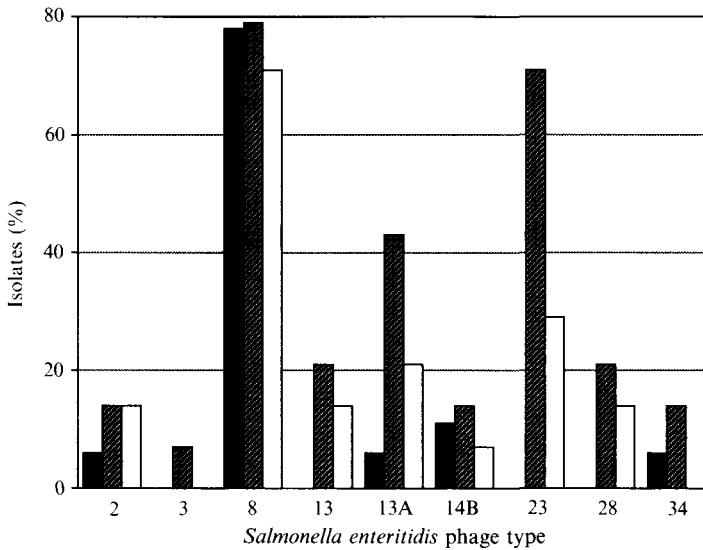


Fig. 1. Frequency of *Salmonella enteritidis* phage types isolated in human outbreaks and poultry facilities. Black bars, outbreaks ($n = 18$); hatched bars, environment ($n = 14$); and internal organs, white bars ($n = 14$).

isolated from both humans and poultry (Fig. 1). This phage type was recovered from stool specimens of humans in 14 (78%) of 18 outbreaks. It was detected in the poultry environment in 11 (79%) of 14 flocks tested and in the internal organs of 10 (71%) of 14 flocks that we tested. *S. enteritidis* PT 8 was recovered from the internal organs of hens in 9 (91%) of 10 flocks that were environmentally positive for this phage type. All flocks in which *S. enteritidis* PT 8 was recovered from the internal organs of hens had been implicated in a human outbreak involving this phage type. In one flock implicated in an *S. enteritidis* PT 8 outbreak, the phage type was present in the environment but not in the internal organs of hens that were sampled.

Salmonella enteritidis PT 14b was recovered from humans in 2 (11%) of 18 outbreaks. Both outbreaks were traced to flock G. *S. enteritidis* PT 14b was the only phage type recovered from the environment and organs of flock G: it was also found in the environment of one other flock.

Salmonella enteritidis PT 13a was recovered from humans in 1 (6%) of 18 outbreaks, which was traced back to flock C. *S. enteritidis* PT 13a was found only in the environment of this flock. A different phage type, *S. enteritidis* PT 13, was recovered from the internal organs of flock C hens. Overall, *S. enteritidis* PT 13a was present in the environment of 6 (43%) of the flocks and in the organs of hens in 3 (21%) of the flocks.

DISCUSSION

In this series of investigations, the phage type responsible for the human outbreaks was consistently isolated from the environment of the implicated flock (100%) and from the internal organs of hens of the implicated flock (86%). These results suggest that traceback is likely to identify the source flock for an *S. enteritidis* outbreak.

Multiple phage types were recovered from only one human outbreak: both *S. enteritidis* PT 8 and *S. enteritidis* PT 34 were isolated from humans during the Eatonton, NJ, outbreak (Table 1). Of the 4 confirmed *S. enteritidis* cases in this outbreak, 3 restaurant patrons were infected with *S. enteritidis* PT 8, while 1 employee with minimal exposure to the implicated food was infected with *S. enteritidis* PT 34. *S. enteritidis* PT 34 may have been unrelated to the outbreak or may reflect undetected polyclonal infection in the suspect flock.

Multiple phage types were present in the environment of 11 (79%) of the 14 flocks that were tested (range, 2–5 phage types). Multiple phage types were isolated from the internal organs of hens in 6 (46%) of the 13 flocks with organ infection (range 2–4 phage types). In three flocks, phage types recovered from the internal organs were not detected in the farm environment, but in none of these cases was the phage type responsible for the human outbreak.

In the United States, the predominant phage type is *S. enteritidis* PT 8. Surveys of human isolates [13, 16], isolates from hens at slaughter and liquid eggs [17], and from animal species [18], found a 39% or higher prevalence for *S. enteritidis* PT 8. *S. enteritidis* PT 4, the predominant phage type in Europe [1], was not recovered from either outbreaks or poultry operations.

The predominance of *S. enteritidis* PT 8 in both human outbreaks and poultry flocks was a limitation in this study, decreasing the ability to discriminate between related and unrelated infections. Other bacterial typing methods, such as plasmid profile or pulsed field gel electrophoresis, might be used to further categorize *S. enteritidis* isolates of the same phage type.

Another deficiency in this study was the lack of a control group for the flocks that were tested. It is not possible to determine whether the distribution of phage types presented here is different from what would have been found in the general egg-laying poultry population. However, the less common outbreak phage types were consistently recovered from both the environment and organs of hens on the implicated farms in the absence of *S. enteritidis* PT 8. These data suggest that traceback led the investigators back to the correct farm.

In this study, phage typing supported the epidemiological link between infected poultry flocks and outbreaks of human illness. As illustrated by the outbreak involving flock M, phage typing may also permit the identification of unrelated sporadic cases occurring within an outbreak. Although the results of this investigation support the validity of the traceback as a means of identifying *S. enteritidis*-infected flocks responsible for human outbreaks, the efficacy of this approach in controlling *S. enteritidis* infections in humans has not been proven. Currently, no long-term follow-up data exist that show the traceback programme is reducing the number of *S. enteritidis* outbreaks among consumers of shell eggs. Production of *S. enteritidis*-contaminated eggs by infected hens may peak within weeks of exposure, then rapidly decline [19]. Thus, implicated flocks may constitute a comparatively low risk for production of *S. enteritidis*-contaminated eggs by the time of flock testing. A proactive voluntary risk-reduction programme for controlling *S. enteritidis* in eggs is being explored.

The US Department of Health and Human Services has set a goal of reducing the incidence of human *S. enteritidis* outbreaks by two-thirds by the year 2000 [20]. Controlling *S. enteritidis* infection in humans will require public education on

safe food handling practices and integrated efforts by government, retailers, and the poultry industry to reduce *S. enteritidis* contamination in shell eggs.

REFERENCES

1. Rodrigue DC, Tauxe RV, Rowe B. International increase in *Salmonella enteritidis*: a new pandemic? *Epidemiol Infect* 1990; **105**: 21–7.
2. Centers for Disease Control. *Salmonella* Surveillance Report 1990. Atlanta, GA: United States Department of Health and Human Services, Public Health Service, 1991.
3. Chalker RB, Blaser MJ. A review of human salmonellosis. III. Magnitude of *Salmonella* infection in the United States. *Rev Infect Dis* 1988; **10**: 111–21.
4. Centers for Disease Control. Outbreak of *Salmonella enteritidis* infection associated with the consumption of raw shell eggs, 1991. *MMWR* 1990; **38**: 909–12.
5. St Louis ME, Morse DL, Potter ME, et al. The emergence of grade A eggs as a major source of *Salmonella enteritidis* infections. New implications for the control of salmonellosis. *JAMA* 1988; **259**: 2103–7.
6. Telzak EE, Budnick LD, Greenberg MSZ, Blum S, Shayegani M, Benson CE. A nosocomial outbreak of *Salmonella enteritidis* infection due to the consumption of raw eggs. *N Engl J Med* 1990; **323**: 394–7.
7. Humphrey TJ, Baskerville A, Mawer S, Rowe B, Hopper S. *Salmonella enteritidis* phage type 4 from the contents of intact eggs: a study involving naturally infected hens. *Epidemiol Infect* 1989; **103**: 415–23.
8. Tauxe R, Lee L, Rodrigue D, Farmer JJ III, Blake PA. *Salmonella enteritidis* outbreaks in the United States 1985–1989: the epidemic expands. Abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. Atlanta, GA: American Society for Microbiology; 1990: 914.
9. Medberg CW, David MJ, White KE, MacDonald KI, Osterholm MT. Sporadic *Salmonella enteritidis* and *S. typhimurium* infections in Minnesota and consumption of undercooked eggs. Abstracts of the 30th Interscience Conference on Antimicrobial Agents and Chemotherapy. Atlanta, GA: American Society for Microbiology; 1990: 820.
10. US Dept of Agriculture, Animal and Plant Health Inspection Service. Code of Federal Regulations, 9 CFR Parts 71 and 82, Chickens affected by *Salmonella enteritidis*. Washington, DC: US Govt Printing Office; 1991: 3730–42.
11. Ewing WH. Genus *Salmonella*. In: Edwards PR, Ewing WH, eds. *Identification of Enterobacteriaceae*, 4th ed. New York: Elsevier Science, 1986: 181–318.
12. Ward LR, deSa JDH, Rowe B. A phage-typing scheme for *Salmonella enteritidis*. *Epidemiol Infect* 1987; **99**: 291–4.
13. Hickman-Brenner FW, Stubbs AD, Farmer III JJ. Phage typing of *Salmonella enteritidis* in the United States. *J Clin Microbiol* 1991; **29**: 2817–23.
14. Mallinson ET, Tate CR, Miller RG, Bennett B, Russek-Cohen E. Monitoring poultry farms for *Salmonella* by drag-swab sampling and antigen capture immunoassay. *Avian Dis* 1989; **33**: 684–90.
15. Miller RG, Tate CR, Mallinson ET, Scherrer JA. Xylose-lysine tergitol 4: an improved selective enrichment medium for the isolation of *Salmonella*. *Poultry Sci* 1991; **70**: 2429–32.
16. Rodrigue DC, Cameron DN, Puhf ND, et al. Comparison of plasmid profiles, phage types, and antimicrobial resistance patterns of *Salmonella enteritidis* isolates in the United States. *J Clin Microbiol* 1992; **30**: 854–7.
17. Mason J, Ebel E. APHIS *Salmonella enteritidis* control program. In: Snoeyenbos GH, ed. *Proceedings of the Symposium on the Diagnosis and Control of Salmonella*. Richmond, VA: US Animal Health Association 1992: 78.
18. Ferris K, Miller D. *Salmonella* serotypes from animals and related sources reported during July 1988–1989. *Proceedings of the 93rd Annual Meeting of the US Animal Health Association* 1989: 521–38.
19. Gast RK, Beard CW. Isolation of *Salmonella enteritidis* from internal organs of experimentally infected hens. *Avian Dis* 1990; **34**: 438–46.
20. US Department of Health and Human Services, Public Health Service. *Healthy People 2000: National Health Promotion and Disease Prevention Objectives*. DHHS Publication No. (PHS)91–50212. Washington, DC: US Govt Printing Office, 1991.