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Human salmonellosis associated with young poultry from a contaminated hatchery in Michigan and the resulting public health interventions, 1999 and 2000

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SUMMARY

Although approximately 95% of disease caused by nontyphoidal salmonella is transmitted by foodborne vehicles, four documented salmonella outbreaks in the 1990s have been traced to contact with young poultry. No environmental studies of source hatcheries were completed. This case-control study was performed by comparing culture-confirmed *Salmonella* Infantis in Michigan residents, identified between May and July 1999, with two age- and neighbourhood-matched controls. Eighty environmental and bird tissue samples were collected from an implicated hatchery; all salmonella isolates underwent pulsed-field gel electrophoresis (PFGE) analysis. The study included 19 case-patients sharing the same PFGE subtype and 37 matched controls. Within 5 days before illness onset, 74% of case-patients resided in households raising young poultry compared with 16% of controls (matched OR 19·5; 95% CI 2·9, 378·1). Eight hatchery samples yielded *Salmonella* Infantis with PFGE subtypes matching the patients' isolates. This investigation identified birds from a single hatchery as the source of human illness and confirmed the link by matching PFGE patterns from humans, birds and the hatchery environment. Subsequent public health interventions reduced, but did not eliminate, transmission of poultry-associated salmonellosis. Five additional PFGE-linked cases were identified in Spring 2000, necessitating quarantine of the hatchery for depopulation, cleaning and disinfection.

INTRODUCTION

Nontyphoidal salmonellosis is an important cause of human illness in the United States, resulting in an estimated 1·4 million illnesses and approximately 600 deaths annually [1]. About 95% of human salmonellosis cases are caused by foodborne sources [1], often contaminated foods of animal origin. Although less common, contact with animals, birds and reptiles in particular, remains an important source of salmonella infection for humans [2–4]. *Salmonella enterica* serotype Infantis has been recovered in humans and animals of many species as well as animal products such as pig ear dog treats [5]. According to Centers for Disease Control and Prevention (CDC) data collected through the Public Health Laboratory Information System, *Salmonella* Infantis has been one of the top 15 most frequently reported human serotypes in the last decade, although it has contributed only 1–2% of the human salmonella isolates reported [6–13]. In animals, *Salmonella* Infantis similarly contributes 1–2% of the nonhuman salmonella isolates reported to CDC from

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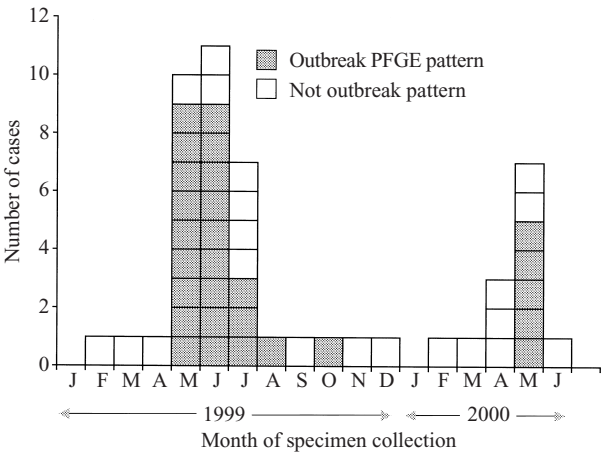

Fig. 1. Laboratory isolates of *Salmonella* Infantis by specimen collection date, January 1999–June 2000, Michigan.

the National Veterinary Services Laboratory, according to CDC's nonhuman salmonella database for 1990–9.

The mandatory reporting of salmonella isolates by laboratories in Michigan was established in 1965. A 1999 evaluation of Michigan's salmonella surveillance system indicated approximately 90% of the Michigan laboratories performing enteric cultures forward their isolates to the state public health laboratory for serotyping (MJ Wilkins, CDC, personal communication, 1999). The Michigan Department of Community Health Laboratory participates in PulseNet, a national network of public health laboratories using standardized pulsed-field gel electrophoresis (PFGE) procedures to subtype bacteria commonly involved with foodborne outbreaks [14]. This study details the investigation of a *Salmonella* Infantis outbreak that occurred in Michigan in 1999 and recurred in 2000.

Preliminary investigation

In May 1999, 10 *Salmonella* Infantis laboratory isolates were reported to the Michigan Department of Community Health, well above the expected number of 0–2 cases per month. The rise in the number of monthly cases triggered a request for PFGE analysis of the 10 isolates; 9 had indistinguishable or closely related PFGE patterns, suggesting a common source.

The 9 PFGE-related patients resided in 8, geographically dispersed, rural counties in Michigan. Review of enteric case investigation forms revealed no common factors except rural residence and/or animal exposure. Six of nine patients reported direct or indirect contact with domestic poultry within 6 days before illness onset. Cases continued to be reported through the summer, prompting a case-control study in June 1999. The distribution of cases over time is shown in Figure 1.

MATERIALS AND METHODS

Case-control study

A case-patient was defined as a Michigan resident with culture confirmed *Salmonella* Infantis infection, sharing the PFGE outbreak patterns, between 1 May and 31 July, 1999. Twenty-one case-patients from 19 counties had illness that met the case definition.

In June 1999, a matched case-control study was initiated by the Michigan Department of Community Health. Persons were interviewed by telephone using a standard questionnaire detailing animal exposure history. Two healthy controls were sought for each patient: using sequential digit dialing within area code and prefix, controls were matched by age and place of residence. Controls were considered healthy if they reported no gastrointestinal illness in the month before or after the symptom onset date of their corresponding case-patient. The age match scheme was based on a tier ranging from ± 2 years for case-patients under 5 years of age, to a maximum of ± 10 years for case patients 20 years and older.

Statistical analysis

Mantel–Haenszel matched and unmatched odds ratios were calculated for poultry exposures on the questionnaire using Epi-Info, version 6.04c (CDC, Atlanta, Georgia).

Environmental investigation

Because young poultry were the suspected route of exposure, efforts to determine the source of the birds and bird feed were initiated. The Michigan Department of Agriculture assisted in the investigation by tracing the source of the young poultry through the reported points of purchase (usually farm and feed retail outlets). Michigan Department of Agriculture also assisted in searching for a common feed source for the young birds.

A single Michigan hatchery was identified as the origin for many of the traceable birds; the site was visited in September 1999 by Michigan Department of Community Health epidemiologists, along with staff

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from the Michigan Department of Agriculture and Michigan State University. Forty-seven environmental and 33 bird tissue samples were collected. The environmental sampling focused on the incubation and hatching areas. The United States Department of Agriculture National Poultry Improvement Plan and Auxiliary Provisions [15] environmental sampling protocol was followed. Tissues were obtained from 12 chicks collected on the day of environmental sampling and 21 hens that died during a forced molt 1 month later. No other types of birds remained on the farm during the time of sample collection. Bird necropsies and tissue sample collection were completed at the Michigan State University Animal Health Diagnostic Laboratory, East Lansing, Michigan. A portion of the tissue samples and all salmonella isolates were forwarded to the state public health laboratory for serotyping and PFGE analysis.

Microbiologic investigation

Human isolates of salmonella species were submitted to the Michigan Department of Community Health Laboratory for confirmatory culture and serotyping. Upon receipt, each isolate was plated on MacConkey and trypticase soy agar plates. Complete biochemical identification was performed on screen-positive bacterial colonies according to published protocols [16] to confirm isolates as salmonella.

Environmental swab samples were directly plated onto MacConkey and Hektoen agar plates and also enriched in tetrathionate broth overnight. After 18 h of incubation at 35 °C, the broth cultures were plated onto MacConkey and Hektoen agar plates and the previously inoculated plates were examined for the presence of hydrogen sulphide producing or non-lactose fermenting enteric bacilli. The methodologies of Ewing [16] were followed for identification of salmonella species.

Bird tissue samples were macerated in trypticase soy broth in a Stomacher (Seward, London, England). Enriched tissue samples were plated on both MacConkey and Hektoen agars. Cultures were incubated at 35 °C and inspected daily for 2 days for non-lactose fermenting or hydrogen sulphide producing enteric bacilli. Suspect colonies were screened on triple sugar iron agar and for the production of urease, and identified as salmonella according to previously mentioned standard methods.

Human, environmental, and tissue isolates confirmed to be salmonella were serotyped according to the biochemical procedures specified by the Kauffman–White scheme [16].

Pulsed-field gel electrophoresis

The genetic relatedness of the *Salmonella* Infantis isolates was determined through PFGE analysis as follows: chromosomal DNA from isolates of *Salmonella* Infantis was prepared in 1% Seakem Gold agarose (FMC Corporation, Rockland, MA) as previously described [17]. Enzymatic digestion of agarose embedded DNA was performed using either 50 units of *Xba*I (Boehringer-Mannheim, Indianapolis, IN) or 30 units *Bln*I (Boehringer-Mannheim) for a minimum of 2 h at 37 °C.

Electrophoresis was performed at 6 volts per centimeter for 18 h with an initial switch time of 2·2 sec and a final switch time of 64 sec, using either the CHEF Mapper or CHEF DR-II (Bio-Rad Laboratories, Hercules, CA). The molecular size marker used was *Salmonella* serotype Newport am01144. Image analysis was performed on the GelDoc 2000 System (Bio-Rad Laboratories, Hercules, CA), and PFGE patterns were evaluated with Molecular Analyst Plus software (Bio-Rad Laboratories, Hercules, CA). Strain relatedness was determined using criteria as previously described [18]. Upon completion of pattern analysis, digital images of individual banding patterns were posted on the PulseNet listserve to all participating PulseNet laboratories for comparison with their identified patterns.

RESULTS

Case characteristics

Between May and July 1999, 21 cases of *Salmonella* Infantis were identified in Michigan residents. Ages of the case-patients ranged from 8 days to 82 years with a median of 19 years. Eight (38%) were less than 10 years of age and 12 (57%) were female. Of the 21 patients, 17 reported diarrhoea (5 specifying bloody diarrhoea), 12 reported fever, and 3 reported vomiting. Five patients reported household members with symptoms similar to their own, three patients were hospitalized, and no patients died. None of the reportedly ill household members was included in this study as they were not known to be culture-confirmed, and further details of their illnesses were unknown. Many patients or members of their household reported raising 'backyard' or non-commercial flocks of poultry for meat or eggs.

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Table 1. Salmonella *Infantis cases and control study participants: poultry exposures and associated risks* (*odds ratios*) *of illness, Michigan, 1999*

	Cases		Controls				
Poultry exposure	No.	%	No.	%	Odds ratio	95% CI	*P*-value
Direct/Indirect*	14	73·7	6	16·2	19·5	2·89, 378·10	0·0003
Chicks	5	26·3	3	8·1	4·1	0·66, 28·86	0·10
Ducklings	1	5·2	0	0·0	†	†	†
Pheasants	1	5·2	0	0·0	†	†	†
Turkeys	0	0·0	1	2·7	†	†	†
Multiple species‡	7	36·8	2	5·4	10·2	1·57, 108·34	0·005

* Matched analysis.
† Not calculated.
‡ May include chicks, ducklings, goslings, turkeys, or pheasants.

Matched case-control study

Matched case-control analysis included 19 of 21 total patients and 37 controls. Two matched controls were found per eligible case-patient, except one for whom only a single control was obtained. The two case-patients excluded from further analysis had either an uncertain exposure history or the absence of any matched controls. Fourteen (74%) of the 19 cases and 6 (16%) of the 37 controls had either direct contact with young poultry, or resided in a household raising young poultry, within 5 days before illness onset (matched odds ratio (OR) = 19·5; 95% confidence interval (CI) 2·9, 378·1). The poultry exposures of cases and controls are summarized in Table 1. Although small sample size limited the extent of stratification, exposure to multiple species of young birds was found to be significantly associated with illness (OR = 10·2; 95% CI 1·6, 108·3).

Environmental investigation

Of the 21 total case-patients, 16 were able to provide specific information regarding the source of their birds. Bird source traces for 14 patients led directly to a single Michigan hatchery. The birds traced to the common hatchery included chicks, ducklings, goslings, young turkeys and pheasants. There was no common feed source for the young birds at the hatchery or after they were purchased. The hatchlings were not fed at the hatchery, as they can survive for up to 3 days on their residual yolk sacs [19].

The implicated hatchery was a small, family business, primarily a mail-order supplier to farm and feed stores and private individuals. The hatchery provides birds for backyard (non-commercial) egg or meat flocks as well as fancy fowl, ducks, geese, and turkeys. During the springtime peak in production, the facility can hatch and ship up to 100000 young birds per week. The overall sanitation and biosecurity on the farm was considered poor by current agricultural standards. The facility was not meeting the minimum hatchery sanitation requirements as described in the National Poultry Improvement Plan of which it was a registered participant [15]. Enforcement of the National Poultry Improvement Plan standards are delegated from the United States Department of Agriculture to each state's Department of Agriculture. In Michigan, this facility was not required to meet the sanitation and biosecurity standards of a facility involved with food production.

Microbiologic investigation

Three of the 47 environmental isolates collected from the hatchery were culture positive for *Salmonella* Infantis. All 12 chicks sampled were negative for *Salmonella* Infantis; 5 of 21 hens were positive. Table 2 portrays the source for all positive salmonella isolates for the bird and environmental samples collected.

In addition to Infantis, other salmonella serotypes isolated from the environmental samples were Montevideo, Chester, and Mbandaka.

Pulsed-field gel electrophoresis

The PFGE patterns of 15 human *Salmonella* Infantis outbreak isolates were indistinguishable using *Xba*I and *Bln*I restriction enzymes, and this pattern was considered to be the primary outbreak pattern. The PFGE patterns of 5 of the remaining human isolates

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Table 2. *Number and percentages of non-human samples collected for hatchery environmental investigation, Michigan, 1999*

Sample source	No. collected	Culture positive for salmonella		Salmonella serotype	
		No.	%	No.	Serotype
Chicks (from facility)	12	0	0·0		
Hens (from facility)	21	5	20·0	5	Infantis
Facility isolates					
Hatching area	18	7	38·9	5	Montevideo
				1	Infantis
				1	Mbandaka
Incubator area	15	5	33·3	3	Montevideo
				1	Infantis
				1	Chester
Hen coop	4	1	25·0	1	Infantis
Miscellaneous*	10	0	0·0		
Total	80	18	22·5		

* Includes transport vans, investigator boots, and de-beaking machine.

differed from the primary pattern by 1–2 DNA fragments and were considered to be closely related genetically to the primary outbreak strain [18]. A final isolate differed from the primary pattern by three bands plus the shift of a band and was considered to be possibly related genetically, although there was a strong epidemiologic link to the outbreak.

The *Salmonella* Infantis isolates obtained from the five hen samples, the hatching-rack environmental sample, and the incubator prep table environmental sample had PFGE patterns indistinguishable from the primary human outbreak pattern. The single isolate obtained from the hen coop had a PFGE pattern indistinguishable from the primary human outbreak pattern with *Bln*I although it showed a two-band difference with *Xba*I (Figure 2). This environmental sample was considered genetically closely related to the primary human outbreak pattern.

No participating PulseNet laboratories reported the identification of the Michigan outbreak patterns in their states during the outbreak period.

Public health interventions

After the 1999 investigation, interventions were implemented at three levels. First, poultry experts from Michigan State University evaluated the hatchery in October 1999 and provided formal written recommendations designed to interrupt the circulation of salmonella serotypes, improve sanitation, and instill basic biosecurity precautions on the farm. Examples include: change coveralls and disinfect boots when moving between groups of poultry and before entering the hatchery, clean and disinfect wire egg baskets each day, keep tops of incubators and hatchers clean, and restrict visitors from poultry areas.

Second, a safety insert for the consumer was designed by the Michigan Department of Agriculture. The insert highlighted the importance of thorough hand washing after handling poultry and/or any equipment that may be contaminated with poultry faeces and recommended the supervision of children handling young poultry. The inserts were included in the hatchery's mail-order catalogue and distributed to 77 Michigan retail farm and feed stores selling chicks in the springtime. In total, 14500 inserts were distributed during the spring of 2000.

Finally, for the general public, two statewide press releases were issued by the Michigan Department of Community Health in the spring of 2000 to discourage poultry as pets for children and to raise awareness about the risk posed by young poultry to children, the elderly and the immunocompromised. The releases were widely published in the newspapers and covered by several Michigan radio and television stations.

The sequel, 2000

Despite the aforementioned interventions, transmission of *Salmonella* Infantis from young poultry to humans recurred in May of 2000; five new cases were reported to the Michigan Department of Community Health, all with PFGE patterns indistinguishable from the 1999 primary outbreak pattern. The patient

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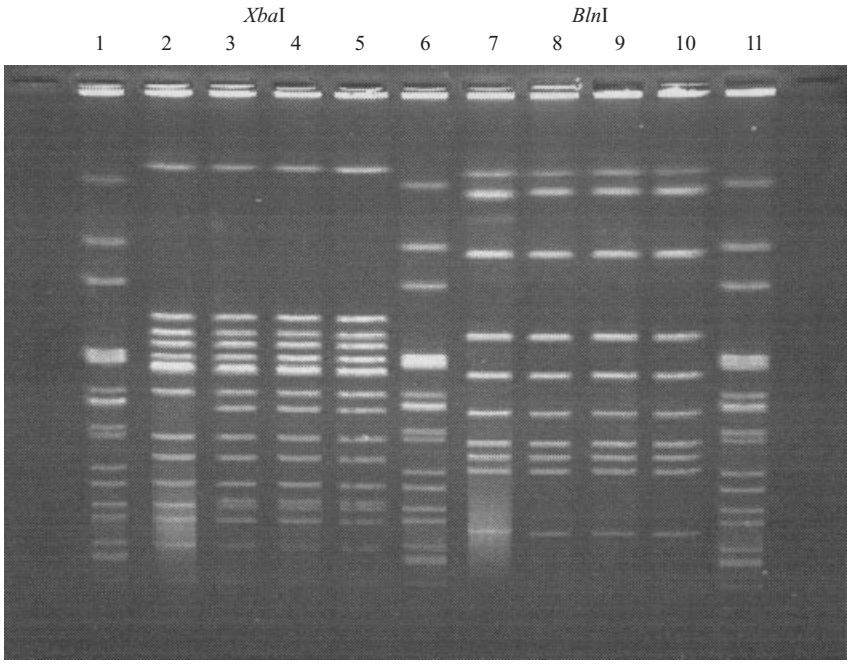

Fig. 2. PFGE profiles of genomic DNA from environment isolates of *Salmonella* Infantis, from a hatchery in Michigan, 2000. The outbreak pattern from a human isolate is shown in lane 5 (*Xba*I) and lane 10 (*Bln*I). The pattern from the hen house isolate is shown in lanes 2 (*Xba*I) and 7 (*Bln*I). The pattern from the hatching rack isolate is shown in lanes 3 (*Xba*I) and 8 (*Bln*I). The PFGE pattern from the incubator room prep table isolate is shown in lanes 4 (*Xba*I) and 9 (*Bln*I). Lanes 1, 5 and 11 contain the molecular size marker, *Salmonella* Newport am01144.

ages ranged from 16 months to 45 years with a median age of 11 years. All lived in rural areas and two were hospitalized. Four of the five patients reported direct exposure to chicks or ducklings originating from the previously implicated hatchery.

In June 2000, the Michigan Department of Community Health purchased 20 chicks from a farm and feed retail store supplied solely with chicks from the implicated hatchery. Faecal samples were cultured at the state public health laboratory and the chicks were found to be shedding *Salmonella* Infantis in their faeces at the time of purchase. The PFGE pattern of these isolates matched the 1999 primary human outbreak pattern.

Under the Michigan Public Health Code, the Michigan Department of Community Health had the authority to close the facility 'upon a determination that an imminent danger to the health or lives of individuals exists' [20]. Presented with evidence of ongoing transmission to humans, the Michigan Department of Agriculture offered an alternative option that included a state-imposed quarantine and availability of indemnity funds.

On 10 July 2000, the Michigan Department of Agriculture, with the support of the Michigan Department of Community Health, placed the farm under state quarantine. The terms of the quarantine required the depopulation of all birds and destruction of all unhatched eggs on the farm, followed by thorough cleaning and disinfection. Michigan Department of Agriculture offered indemnity for the birds and fertile eggs, and provided personnel, equipment, and supplies for the depopulation and disinfection. In total, 6100 chickens, geese, and ducks were euthanized and over 60000 fertilized eggs destroyed.

The farm was revisited in August 2000, following the depopulation, cleaning and disinfection of the facility. Forty-four composite environmental samples were collected from throughout the facility by Michigan Department of Community Health personnel, using the United States Department of Agriculture National Poultry Improvement Plan and Auxiliary Provisions sampling protocol [15]; all were culture negative for salmonella. During the same period, the producers purchased 6000 day-old birds to raise offsite and use as the breeding flock for 2001. Although not required under the terms of the quarantine, the producers wished to have these purchased birds sampled upon arrival to prevent re-infection of the facility via this route. Michigan Department of Community Health personnel collected 21 composite

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faecal samples from the shipping boxes of the day-old chicks, again using the United States Department of Agriculture, National Poultry Improvement Plans and Auxiliary Provisions sampling protocol; the samples were culture negative for all salmonella species.

The quarantine was lifted on 12 September 2000 and the hatchery was expected to be back in full production for the 2001 springtime peak. A Michigan Department of Agriculture veterinarian would visit the farm on a monthly basis to ensure adherence to the sanitation and biosecurity improvements recommended by the Michigan Department of Agriculture and Michigan State University.

DISCUSSION

Our investigation is the first to epidemiologically identify birds from a single hatchery as the source of human infection and confirm the link by producing matching PFGE patterns from humans, birds, and the hatchery environment. The combined body of clinical, epidemiologic, and laboratory evidence justified the intensity of the public health interventions implemented.

Contributing factors

We identified four factors that facilitated the transmission of *Salmonella* Infantis from young poultry to humans. First, 1999 was a record sales year for this hatchery. According to the producers, the increase in business was thought to be due to the public's attempts to prepare for the impending 'Y2K' crisis. Many new customers expressed the desire to raise poultry to become more self-sufficient and less reliant on commercial sources for food. Thus, 'Y2K' preparations may have led to an increase in the number of inexperienced or first-time bird owners. Improper care-taking noted in this investigation often involved housing birds in inappropriate locations, such as chicks being raised in a cage or aquarium indoors, chicks kept in a box in the garage, and ducklings and goslings brought indoors when it rained. In addition, some children were assigned as the primary caretakers of young birds, and in many instances, the hatchlings were extensively handled.

Second, the biosecurity standards and sanitation level of this facility allowed the organism to circulate throughout the facility and infect most, if not all species of birds. Generally, lack of biosecurity allows the introduction of pathogens onto a facility from numerous sources. Poor sanitation allows the pathogens to circulate freely throughout the facility and be transmitted from one generation of birds to the next [21]. This was verified by the variety of poultry species from which patients were infected and the multiple locations within the facility from which positive environmental samples were collected. The initial introduction of *Salmonella* Infantis to this facility could have occurred via many different pathways, including: contaminated people, equipment, animals, feed or bedding, as well as rodents, wildlife, eggs routinely hatched from outside sources, and the annual purchase of new breeding flocks with no isolation or routine testing regimen [22].

Third, the primary method of hatchling delivery was United States Postal Service, which likely ensured the birds would be exposed to ambient air temperatures far below the ideal. The chicks were being shipped throughout Michigan starting in March, when the temperatures average around freezing. Newly hatched chicks should be maintained at 32–35 °C for their first week of life [23]. The cold stress placed on these birds during shipping would increase their susceptibility to infection with salmonella and encourage the shedding of salmonella organisms already present in their gut [24].

Finally, the traditional public health message about salmonella has focused on the safe handling and cooking of raw poultry meat and eggs. Although this message is appropriate and necessary, it largely fails to include warnings about handling young poultry and the inappropriateness of young birds as pets for children.

The combination of potentially inexperienced bird owners, the presence of the organism in a high variety of bird species, the stressing of the birds during shipping, and lack understanding of the importance of hand-washing practices, led to an outbreak of *Salmonella* Infantis in Michigan during the Spring of 1999. When several of these issues were addressed through public health interventions, the number of human cases associated with birds from the implicated hatchery dropped dramatically in 2000. The Michigan Department of Community Health continues to monitor both the number of cases and the PFGE patterns of poultry-associated salmonellosis cases in Michigan to evaluate the effectiveness of the recent intervention efforts.

There are several references in the literature establishing the handling of chicks and/or ducklings

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as the source of exposure to various salmonella serotypes [4, 25–27]. Finding salmonella in a poultry facility is far from surprising with the percentage of infected facilities ranging from 42–77% [21, 28, 29] based on environmental sample collection. During the 1999 investigation, we did not test birds owned by the case-patients or perform environmental testing of their premises. Other limitations of our study include a small sample size, which necessitated the inclusion of both direct and indirect exposure to young poultry in the exposure definition. We did not investigate specific behaviours associated with handling of the birds, such as frequency of contact, nuzzling or facial contact, or handwashing practices, which may influence the likelihood of becoming ill following contact with young poultry. Finally, accuracy of exposure recall may have been affected by delays in interviewing some case-patients (up to 2 months) and controls (up to 3·5 months).

This study details an extensive investigation that ultimately led to the implication of a single hatchery as the source of infection for multiple species of young birds; these birds in turn were the source of infection for the human outbreak in Michigan. Human acquisition of disease through contact with live animals is probably under-recognized and consequently underreported to public health authorities. Documenting a confirmed chain of transmission from live animals to humans on a population basis has not been frequently accomplished. Physicians and other clinicians need to consider animal exposure when taking a patient history, especially with clients who are in settings or situations with high probability of animal contact. Only then can human disease of animal origin be included in the differential diagnosis and a linkage between the two explored.

This study also highlights the important role of regular collaboration between state departments of Public Health and Agriculture. The responsibilities of these two departments may place them at odds over interventions necessary to protect the public's health; interventions that may have economic implications for the agricultural community. The need for successful partnership is likely to grow as food and animal production becomes more centralized with greater potential for widespread contamination, as the use of antibiotics in food animals enhances antibiotic resistance in humans, and as zoonotic illnesses increasingly make their way into human populations.

The wider availability and use of molecular and other laboratory technologies are now making the identification of epidemiologic linkages between animals and humans easier and more certain. PFGE analysis was crucial in establishing the genetic relatedness of isolates associated with this outbreak. It is essential that our ability to communicate and collaborate with each other across sometime competing missions and interests keep pace with the advancement in technology and the rapidly expanding need.

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