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The epidemiological relationship between salmonella isolated from poultry meat and sewage effluents at a long-stay hospital

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SUMMARY

Between February 1988 and March 1989 chicken carcases delivered to the kitchen of a long stay psycho-geriatric hospital were screened every week for salmonella contamination. While 214 of 477 (45%) individual carcases carried one or more salmonella types, every single consignment examined contained affected carcases.

Simultaneously sewers draining the residential accommodation and excluding kitchen effluent, were also monitored. Thirty out of 79 (38%) of Moore's swabs were positive for salmonella. There was a statistically significant association between the salmonella types isolated from chicken and those isolated from sewers the following week.

Following a change in kitchen policy to order only cooked chicken there was a significant reduction in the isolation of salmonella from the sewers.

INTRODUCTION

The increased awareness of the problems of foodborne infections has resulted in a large number of publications many of which indicate that salmonellae have become the most frequent cause of foodborne infection. The epidemiological relationship between human salmonellosis and isolations from meat and other food animal products has been demonstrated [1–3]. In the United Kingdom where poultry meat is a primary source of animal protein, poultry products have become increasingly incriminated as the source of most outbreaks and sporadic incidents in which the food vehicles were identified [4, 5]. In many incidents there is only circumstantial evidence to implicate poultry meat and there is a need to establish and clarify the epidemiological relationship between poultry meat and human salmonella infections.

Published reports of foodborne salmonellosis indicate that many salmonella-excreting persons involved in outbreaks do not manifest any overt clinical symptoms and others may remain excreters for varying periods of time after clinical recovery [6–8]. It is therefore important to be able to demonstrate an epidemiological association between consumption of poultry meat and human

salmonella infections – without relying totally on the investigation of clinical incidents.

Poultry meat used in a catering establishment can be screened to identify the salmonellae to which the consumers are exposed. Salmonella infection and salmonella excretion in the consuming population can be investigated by parallel monitoring of the sewers draining the defined population area. The purpose of this study was to compare the salmonella types isolated from both sources and to analyse the frequency of obtaining identical types during corresponding periods, thus demonstrating an epidemiological association.

MATERIALS AND METHODS

The study hospital

A long-stay psycho-geriatric hospital was selected to exclude the effluents of industry, retail shops, or from sewers of other residential areas. The patients (approximately 800) in the hospital constituted a cohort whose food sources were known and whose infection or transient carriage and excretion of salmonellae could be monitored by Moore's swabs.

Chicken carcase sampling

Batches of fresh and frozen eviscerated chicken delivered on contract to the hospital kitchen were sampled at weekly intervals. The chickens originated from different poultry producers in Scotland and England. A one-in-ten systematic sample (n = 10–17 carcases) was examined from each weekly batch, by the whole carcase rinse method [9]. The survey was designed to last for 52 weeks. However, after 43 weeks, because of a change in kitchen policy the supply of raw chicken carcases to the hospital was substituted with pre-cooked deboned whole-chicken. The change in policy coincided with general media publicity given to the problem of salmonella in poultry products. Over the 43-week period samples were collected on 38 occasions. For the other 5 weeks no chickens were used in the kitchen. A total of 477 fresh and frozen chicken carcases was examined.

Each carcase was thoroughly rinsed in a sterile polythene bag with 300 ml of sterile buffered peptone water (pre-enrichment broth) using the method described by Cox and co-workers [9]. The rinse fluid was carefully transferred back into a sterile plastic container.

To compare the incidence of salmonella in the pre-cooked chicken with the incidence in raw whole carcases the cooked meat was also sampled. The pre-cooked whole carcase deboned chickens were supplied in frozen vacuum-packs. A one-in-ten sample of the packs was examined each week. Meat was aseptically collected from each pack, using sterile scissors and forceps, macerated and diluted 1 : 10 in sterile buffered peptone water. Random samples were taken for 5 weeks and a total of 102 packs was examined.

Sewer sampling

The use of the Moore's gauze swabs in drains or sewers has been demonstrated to satisfactorily monitor salmonella excretion in a specific population [10, 11]. Moore's swabs were laid at two sewers (A and B) draining the residential wards of

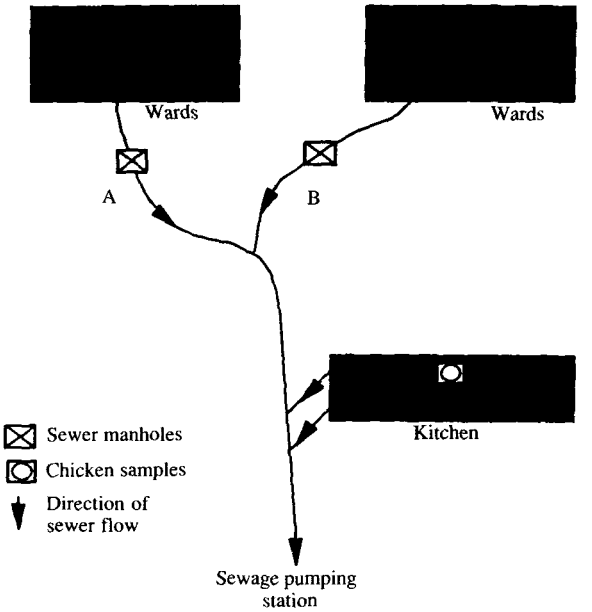

Fig. 1. Map of site.

the hospital patients. The two man-holes were located proximal to the entry of the kitchen effluent into the sewer (Fig. 1). This avoided the possibility of salmonellae from the kitchen effluents reaching the sampling sites and invalidating the results. Each sewer swab was left in place for 7 days; a replacement swab was placed every week, while a contaminated swab was collected into 300 ml of sterile buffered peptone water. A total of 79 swabs was recovered during the same period that raw chicken carcases were sampled. One of the swabs was lost in the sewer flow or eaten by rats. Following the introduction of pre-cooked chicken, sewer swabs were taken for a further 5 weeks (after an interval of 7 weeks), during which a total of 10 swabs was examined (Fig. 2).

Isolation and identification of salmonellae

For all samples, the buffered peptone water pre-enrichment was incubated at 37 °C for up to 48 h. Subcultures were made from the pre-enriched peptone water into enrichment media (at a ratio of 1 : 100) after 18–24 h, and again after 42–48 h. The enrichment procedure was based on standard methods, and was carried out in modified Rappaport–Vassaliadis and Selenite F broth (at temperatures of 43° and 37 °C respectively). Subcultures from each enrichment were made at 24 and 48 h, onto three selective and differential solid media – desoxycholate citrate agar, xylose-lysine-desoxycholate agar, and brilliant green agar. The above protocol was based on work carried out previously by the Scottish Salmonella Reference Laboratory (SRRL) [12]. The selective agar plates, incubated at 37 °C, were examined at 24 and 48 h, and colonies typical of salmonella were carefully picked and subcultured onto MacConkey agar. Purified isolates were then characterized, using the SSRL standard biochemical protocol. Each isolate was then serotyped by standard methods as modified by SSRL [13–15]. Since each carcase rinse fluid

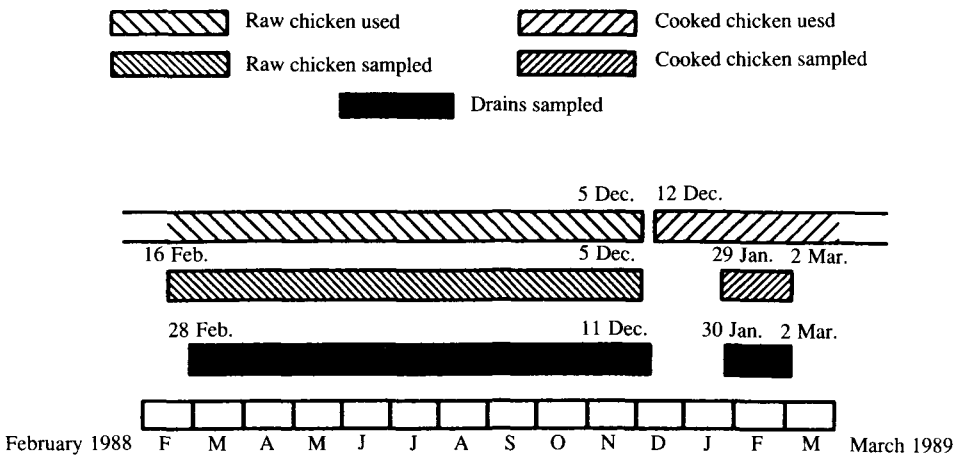

Fig. 2. Sampling and use of chickens.

and sewer swab may have contained more than one salmonella serotype [9, 11], the search for multiple serotypes was achieved by picking five typical colonies from each selective agar plate.

Phage-typing of *Salmonella typhimurium* and *S. enteritidis* isolates was performed by the SSRL according to standard methods. Some of the initial isolates of *S. enteritidis* were sent to the Division of Enteric Pathogens, Public Health Laboratory Service, Colindale, London for phage typing.

Analysis of data

The frequencies of different salmonellae recovered from chicken and sewers were determined. Strains isolated from both sources were compared chronologically. This gave an indication of the frequency of detection, in the sewage, the same salmonella isolated from chicken during the preceding week. Statistical tests for evidence and strength of this association were based on (i) the observed and expected numbers of identical serotypes or phage types detected in chicken and in sewers; and (ii) the observed and expected numbers of corresponding weeks during which the same salmonellae were isolated from chicken and sewers. The standard χ^2 analysis was performed.

RESULTS

Raw chicken carcases

Positive salmonella isolations were made from 214 of the 477 fresh and frozen chicken carcases sampled. This gave an overall contamination rate of 45% (Table 1). Salmonellae were isolated in each of the 38 weeks during which raw chicken carcases were sampled, indicating that every single batch of the chicken carcases examined contained individually contaminated carcases. The proportions of contaminated carcases for each of the 38 weekly batches ranged from 27–67%.

The 214 salmonella-positive carcases yielded a total of 231 salmonella isolates, comprising 19 different salmonella serotypes (Table 2). The most frequently occurring serotypes were *S. enteritidis* (51), *S. typhimurium* (41), *S. virchow* (21) and *S. hadar* (19). Multiple salmonella serotypes were isolated from 16 carcases.

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Table 1. *Salmonella contamination of raw chicken carcases and pre-cooked chicken*

	Raw chicken carcases	Pre-cooked chicken
Number examined	477	102
Number positive for salmonellae	214 (45%)	0
Range of incidence rate	27–67%	0
Median incidence rate	50%	0
Number of salmonella isolates	231	0
Number of serotypes	19	0

Table 2. *Salmonellae isolated from chicken carcases and sewer swabs*

Chicken			Sewer		
S. enteritidis			*S. enteritidis*		
pt 4	48		pt 4	6	
pt 7	1	51	pt 8	1	7
pt 11	2				
S. typhimurium			*S. virchow*		6
pt 2	3				
pt 49	13		*S. typhimurium*		
pt 104	15	41	pt 10	1	
pt 141	9		pt 49	2	4
pt RDNC	1		pt 104	1	
S. virchow		21	*S. clichy*		4
S. hadar		19	*S. thompson*		3
S. bredeney		13	*S. montevideo*		2
S. binza		11	*S. senftenberg*		1
S. eimsbuettel		7	*S. hadar*		1
S. schwarzengrund		7	*S. eimsbuettel*		1
S. minnesota		7	*S. minnesota*		1
S. mbandaka		7	*S. binza*		1
S. senftenberg		6	*S. heidelberg*		1
S. montevideo		6	*S. rough*: *gm*		1
S. indiana		3			
S. kinshasa		2			
S. thielallae		2			
S. livingstone		1			
S. rough: *gm*		14			
6, 7:-:1, 5 (monophasic)		9			
6, 7; 14:-:- (non-motile)		4			
Total		231			33

Pre-cooked chicken

None of the 102 packs of pre-cooked chicken examined yielded any salmonellae. All the samples were also negative for *Listeria* sp. organisms. However, coagulase-positive DNAase-positive *Staphylococcus aureus* was isolated from 29 (28·4%) of the 102 packs.

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Table 3. *The incidence of salmonellae in sewer swabs examined during periods when raw chicken carcases and pre-cooked chicken were prepared in the hospital kitchen*

	Period raw chicken was used	Period cooked chicken was used
Number of swabs examined	79	10
Number of swabs positive for salmonellae	30 (38%)	1 (10%)
Number of weeks examined	40	5
Number of weeks positive	28 (70%)	1 (20%)
Number of serotypes isolated	13	1

Table 4. *Corresponding (matching) weeks during which the same salmonella type was recovered from chicken and sewer drain*

Chicken	Week		Sewer
S. enteritidis 4			*S. enteritidis* 4
S. enteritidis 4 and *S. typhimurium* 104	5	6	*S. enteritidis* 4 and *S. typhimurium* 104
S. enteritidis 4	7	8	*S. enteritidis* 4
S. enteritidis 4	9	10	*S. enteritidis* 4
S. typhimurium 49	13	14	*S. typhimurium* 49
S. virchow	16	17	*S. virchow*
S. virchow	17	18	*S. virchow*
S. minnesota	18	19	*S. minnesota*
S. eimsbuettel	20	21	*S. eimsbuettel*
S. montevideo	26	27	*S. montevideo*
S. enteritidis 4	32	33	*S. enteritidis* 4
S. typhimurium 49	33	34	*S. typhimurium* 49
S. virchow	42	43	*S. virchow*

Sewers

A total of 89 sewer swabs was examined over the period of 45 weeks during which raw chicken carcases or pre-cooked chickens were also sampled. Salmonellae were detected from 30 (38%) of the swabs examined when raw chicken carcases were used in the hospital kitchen (Table 3). Only 1 (10%) of the 10 swabs examined after pre-cooked chicken had been introduced, was positive for salmonella. At least one of the pairs of swabs yielded salmonella during 28 (70%) of the 40 weeks when raw chicken carcases were used in the kitchen, whereas a positive swab was obtained only in 1 (20%) of the 5 weeks after the change to pre-cooked chicken.

A total of 33 isolates comprising 13 salmonella serotypes was detected from the sewers, during the period that raw chicken was being used. The most frequent serotypes were *S. enteritidis* (7), *S. virchow* (6) and *S. typhimurium* (4) (Table 2). All but 1 of the 7 *S. enteritidis* isolates were phage type 4. Multiple salmonella serotypes were detected from three individual sewer swabs.

A single isolate of *S. enteritidis* PT4 was obtained from the 10 swabs taken when pre-cooked chicken was being supplied.

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Comparison of chicken and sewer drain salmonella isolates

Eleven (69%) of 16 different salmonella serotypes and phage types isolated from drain swabs were also recovered from chicken carcases (Table 2). The exceptions were *S. clichy* (4 isolates), *S. thompson* (3) and single isolates of *S. enteritidis* PT8, *S. typhimurium* PT10 and *S. heidelberg*. Seven of the 11 salmonellae occurring in both chicken and sewers were recovered from both sources in corresponding or matching weeks (Table 4). *S. enteritidis* PT4 was observed in both chicken and the sewers during 5 matching weeks; *S. virchow* was observed from both sources during 3 matching weeks; while *S. typhimurium* PT49 was observed during 2 matching weeks.

During the period when raw chickens were sampled, sewers were monitored in 35 matching weeks (i.e. 1 week after the chicken). In 13 of these 35 week-pairs, the same salmonella type was detected in both chicken and sewers. A total of 30 salmonella serotypes and phage types was recovered from chicken or the sewers. Calculation of observed and expected frequencies of matching weeks in which each of the 30 salmonellae was isolated from both chicken and sewers show that the observed frequencies occurred more often than would be expected by chance ($\chi^2 = 15{\cdot}08$, $P < 0{\cdot}005$).

In both chicken carcases and sewers, *S. enteritidis*, *S. typhimurium* and *S. virchow* were the three most frequent serotypes detected. In chicken, these three serotypes constituted 49% of the 231 salmonella types isolated; while in the sewers, the three serotypes accounted for 52% of the 33 isolates. The three serotypes were observed in both chicken and sewer in 11 matching week-pairs, while the calculated expected frequency was 4·54. Thus, for the three most common serotypes, the observed frequency was again greater than would be expected ($\chi^2 = 9{\cdot}19$, $P < 0{\cdot}005$).

While salmonellae were isolated from raw chicken during each of 38 weeks that samples were taken, no salmonellae were recovered from pre-cooked chicken during the 5 weeks that cooked meat was examined. The change from raw to cooked chicken correlated in time with a marked drop in the recovery of salmonellae from the sewer. Thus, salmonella isolations were made from 38% of sewer swabs examined in 28 (70%) of 40 weeks when raw chicken was prepared in the hospital kitchen. After the change from raw to cooked chicken, salmonella isolation was made from only 1 (10%) of 10 swabs examined and only in 1 (20%) of the 5 weeks that samples were taken. This observed difference was statistically significant at 5% level ($P = 0{\cdot}0468$, Fisher's Exact Test).

DISCUSSION

Two hundred and fourteen (45%) of the 477 raw chicken carcases sampled in the hospital kitchen were positive for one or more salmonella types. Many surveys of eviscerated chicken in the United Kingdom during the past 15 years have reported similar contamination rates [16, 17]. Other surveys have recorded levels of carcase contamination higher than the 45% observed in this study [18–21]. A large number of carcases from different commercial sources in Scotland and

England were sampled in the present survey and this rate would seem to reflect the degree of salmonella contamination within the poultry industry during 1988.

Of most significance and particular concern was the fact that every single batch sampled contained individually contaminated carcases. The public health implication is that every single consignment of raw carcases delivered to the hospital and by implication to other private and public catering kitchens, contained salmonellae. If there is any lapse in hygiene, cross-contamination of the kitchen environment and/or other prepared foods is not only possible but likely. The consequence could be an outbreak of foodborne salmonella infection, with serious consequences [22].

The Moore's sewer swab is known to be a sensitive index of the excretion and passage of enteric organisms, as it allows continuous sampling to be taken for periods which can be related to dietary intake. Sewer swabs taken in parallel with sampling of the food sources have been shown to be of value in demonstrating salmonella excretion in hospital environments [23, 24]. By overcoming the problem of screening and identifying individual excreters in a closed population setting, the sewer swab technique is of value in relating salmonella excretion to contaminated food items. In surveys carried out in a small population at a university hospital Harvey and Price [25] recorded positive swabs ranging from 5–31% of the total swabs. In the present study, the sewer swabs proved a very useful method for monitoring salmonella excretion in the closed relatively static population.

No outbreaks of salmonellosis were reported in patients during the course of this investigation, despite the apparent salmonella excretion demonstrated. Although access to clinical records was not possible, retrospective scrutiny of nursing notes in two representative wards for two monthly periods before and after deliveries of raw chicken ceased, did not show any significant differences in recording of diarrhoeal disease. It may be that constant exposure had induced sufficient immunity to prevent clinical disease, or that the degree of contamination did not constitute an 'infectious dose'. Even in the absence of reported illness, it is apparent that salmonella infection and excretion or transient carriage took place.

The epidemiological relationship between the chicken carcases prepared in the hospital kitchen and presumed salmonella excretion by the patients is based on the comparability of the salmonellae isolated from the poultry meat and the sewers, and the changes following removal of raw chicken from the kitchen.

Eleven of the 16 (69%) different salmonella types detected in the sewer swabs were also recovered from the carcases. Seven of the 11 salmonella types were isolated from chicken and sewer on matching weeks. The isolation of common types, e.g. *S. enteritidis*, *S. typhimurium* and *S. virchow* from both sources during corresponding weeks could be expected to occur by chance, considering the high frequency of the three types in chicken and in the sewers. However, the isolation of *S. minnesota*, *S. eimsbuettel* and *S. montevideo* in matching weeks is of greater significance. These three serotypes were recovered infrequently from the chicken during the 38-week period. Altogether, the same salmonella type was detected in both chicken carcases and sewer drains in 13 of 35 matching weeks with the detection in the sewers preceded by the recovery of the same salmonella type in the carcases. The presumed risk factor (consumption of contaminated chicken or

other foods cross-contaminated by the raw carcases) predated the observed outcome (salmonella infection and excretion) and the association was statistically significant.

Towards the latter period of the bacteriological survey, pre-cooked whole chicken was introduced into the hospital kitchen as a substitute for the raw chicken carcases. For a period of 5 weeks specimens were taken from a 10% sample of the pre-cooked chicken. While none of the 102 packs examined was positive for salmonella the use of pre-cooked meat was not without risk, as 29 (28·4%) were contaminated with *Staphylococcus aureus*.

The change in kitchen policy from raw to pre-cooked chicken, coincided with or resulted in a marked change in the recovery of salmonella from the sewer. The removal of the presumed or associated risk factor (contaminated raw chicken) was followed by a statistically significant reduction in the unwanted outcome (salmonella excretion by the patients). This again suggests that raw chicken was an important source of salmonella to the hospital patients.

While the production of salmonella-free poultry and poultry products must be a longterm objective, some immediate control measures must be undertaken. Much may be achieved by recent changes in legislation but it is unlikely that salmonella can be eliminated with present methods of production and processing [26]. Every effort must continue to be exerted to reduce or remove salmonella from the final product entering the retail outlets and kitchen environments. In addition to controlling salmonella infection within the poultry industry some form of treatment of the final product such as irradiation would be a helpful adjunct.

During this study there was a change in kitchen policy – from raw chicken carcases to pre-cooked chicken. Elsewhere in Scotland other hospitals followed similar procedures or abandoned chicken in the patients' diet. Certainly, total rejection of chicken cannot be the best solution to the problem, not the least in terms of national economy, good nutrition and public health. Measures to ensure the elimination or reduction of salmonella contamination of fresh or frozen carcases would seem a more productive approach. While processes such as irradiation are not yet acceptable in the UK, consideration should be given to requiring the use of pre-cooked chicken in catering establishments such as hospital kitchens.

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