Epidem. Inf. (1987) 98, 271–276
Printed in Great Britain

A comparative study of strains of salmonella isolated from irrigation waters, vegetables and human infections

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(*Accepted 13 October 1986*)

SUMMARY

A total of 181 samples of irrigation water from the farmlands of Granada were examined for the presence of *Salmonella* spp. At the same time 849 samples of the crops from these farmlands and of vegetables sold in city market-places were studied. Sampling was done regularly over the period of study which ran from March 1981 to February 1983. Isolates from these sources were compared with 93 salmonellas isolated from human pathological material at various hospitals of the city of Granada from 1979–80, and again from 1981–3.

The most commonly isolated serotypes of human origin were *S. typhimurium* and *S. enteritidis*. In irrigation waters and in crops, *S. typhimurium*, *S. kapemba*, *S. london* and *S. blockley* were found to be the most common. The results indicate a close relationship between the isolates from the irrigation waters and those from the vegetables, but their relationship to prevalent human infections is less clear.

INTRODUCTION

The number of outbreaks and sporadic cases of human salmonellosis is steadily increasing in many countries and in Spain *Salmonella* sp. are responsible for the majority of cases of food poisoning.

Salmonellas may be found in residual waters from rivers and lakes and can contaminate crops, unprotected environments and processing plants (Cherry *et al.* 1972; Parvery *et al.* 1974; Dondero *et al.* 1977; Goyal, Gerba & Melnick, 1977; Nardy & Tanzi, 1977; Sanchez-Buenaventura & Cortina Creus, 1977; Smith, Jones & Watson, 1978; Teltsch *et al.* 1980). Contaminated irrigation water, the use of animal waste as fertilizer and the lack of hygiene on the part of foodhandlers or in the care of processing machinery make vegetables potential carriers of *Salmonella* sp.

The presence of *Salmonella sp.* has been investigated in the processing of salads (Christiansen & King, 1971), in frozen vegetables (Splittstoesser & Segen, 1970; Conejo *et al.* 1978) and in a wide variety of dishes prepared with cooked vegetables (Velaudapillai *et al.* 1969; Ercolani, 1976; Tamminga *et al.* 1978).

The purpose of this investigation was to define the serotypes of salmonella found in the waters that irrigate the farmland of Granada, in the vegetables irrigated by these waters and in the vegetables at the city's centre of distribution, and then to compare these serotypes with those others isolated from human sources in the same catchment area. The study might demonstrate an avenue in the epidemiology of salmonellosis amenable to appropriate measures.

MATERIALS AND METHODS

Water and vegetable samples

The irrigation water of the farmland of Granada comes from the Darro River in its confluence with the Genil River, and from the Gorda canal, a branch of the Genil. Water samples were collected every 15 days from two places on each river, the period of study extending from 1981 to the end of February 1983. A total of 181 samples were collected in sterile 1-litre glass containers with hermetically sealing lids and were transferred to the laboratory nearby unrefrigerated.

Over the same time period, 849 samples of vegetables were collected from the farmland of Granada irrigated by the aforementioned waters, and from local commercial establishments and centres of distribution. Of the samples gathered, 204 were stems and roots, 346 were vegetables consisting mainly of leaves and 299 were fruits. They were transferred to the laboratory in sterile plastic bags and were stored refrigerated for a maximum period of 3 h before examination.

A hundred grammes of vegetable matter were diced and washed in 250 ml of peptone water, agitated for 1 h and kept at room temperature for a further 2 h.

Bacteriological procedures

Ten times concentrated tetrathionate–Kauffman broth containing 40 μg/ml novobiovin was used for enrichment. The plating medium was SS differential agar and biochemical characterization was by standard techniques. Irrigation water, 750 ml, was enriched with 250 ml of tetrathionate–Kauffmann broth and the solution incubated at 37 °C for 24–72 h before plating out. This final concentration of broth ($\times$ 2·5) was found by experiment to be the most satisfactory for salmonella isolation. Five suspect salmonella colonies were picked from positive plates for biochemical tests and serological identification.

A similar procedure was followed for the vegetables after 150 ml of the sample were mixed with 75 ml of enrichment broth.

The serotypes were identified at the Centro Nacional de Virologia e Inmunologia Sanitarias (Majadahonda, Madrid) using the microtechnique of Shipp & Rowe (1980).

Statistical method

Results were analysed by the χ^2 test.

https://doi.org/10.1017/S0950268800062026 Published online by Cambridge University Press

Table 1. *Salmonellas isolated from different kinds of vegetables*

Vegetables	No. of samples	No. of. salmonellas	% of salmonellas
Stem and roots*	204	10	4·9
Leaves†	346	26	7·5
Fruits‡	299	10	3·3
Totals	849	46	5·4

* Garlic, sweet potato, onion, chive, mushroom, turnip, potato, leek, radish, beet and carrot.
† Beet leaves, artichoke, celery, cardoon, cabbage, brussel sprout, cauliflower, endive, escarole, asparragus, spinach, lettuce and parsley.
‡ Eggplant, marrow, pumpkin, broadbean, bean, cucumber, pepper and tomato.

RESULTS

Salmonellas were isolated from 53·6% of the water samples and from 6·5% of the vegetables sampled on the farmland but from only 1·7% at the wholesale market. The proportion rose again at the retail outlets, 2·9% of samples from supermarkets and 8·3% from small establishments being positive, presumably due to cross-contamination. Table 1 shows the type of vegetable involved and that fruit is perhaps marginally less likely to carry salmonellas than stem, root or leaf vegetables.

The serotypes of salmonella isolated in the various samples are shown in Table 2.

Isolates of human origin over the whole period of study belonged to 26 different serotypes. The predominant serotypes for both time periods were *S. typhimurium* and *S. enteritidis*. In irrigation waters the most frequent serotypes amongst the 25 isolated were *S. kapemba*, *S. london* and *S. blockley*. In vegetables only 10 serotypes were isolated, *S. typhimurium*, *S. kapemba* and *S. london* being the most frequent. It should be noted that 11 strains of *S. typhimurium* came from a single place on the same day from 20 samples submitted.

Table 3 summarizes the numbers and proportions of the five main serotypes isolated from the three sources. The relationship between the serotypes found in the irrigation water and the vegetables is significant ($P < 0.001$). Conversely, there is a significant difference between the human strains and those from the environment ($P < 0.001$).

DISCUSSION

When *Salmonella* sp. isolated from human samples in 1979–80 were compared with those isolated in 1981–83, a marked increase in the numbers of *S. enteritidis* was apparent and this organism now causes most of the outbreaks of food poisoning in Spain. It was responsible for 10 of the 12 outbreaks recorded in Granada in 1985. The next most common human isolate in this study was *S. typhimurium*, the strains of which could unfortunately not be phage-typed owing to the lack of facility to do so. Both of these were fairly commonly found in the irrigation waters, but rarely in vegetables (bearing in mind that 11 of the 12 samples containing *S. typhimurium* were obtained from the same source on the same day).

Table 2. *Serotypes of* Salmonella *isolated from samples of human origin during the periods 1979–80 and 1981–83, and those isolated in irrigation waters and vegetables during the latter period*

Serotypes	Human origin, 1979–80	Human origin, 1981–3	Irrigation waters, 1981–3	Vegetables, 1981–3
S. typhymurium	26	26	7	12
S. enteritidis	16	34	12	1
S. kapemba	—	1	28	7
S. london	—	—	18	6
S. blockley	1	8	15	—
S. ohio	—	—	14	2
S. heidelberg	2	1	8	—
S. panama	4	—	8	—
S. lagos	8	1	—	—
S. agona	7	2	5	1
S. bredeney	—	1	5	1
S. hadar	4	—	5	—
S. infantis	2	3	6	1
S. fyris	4	1	—	—
S. java	2	—	—	—
S. manhattan	2	—	1	—
S. montevideo	1	—	1	—
S. frintrop	1	—	—	—
S. brandenburg	1	—	—	—
S. bareilly	1	—	—	—
S. paratyphi B	1	1	—	—
S. limete	1	—	1	—
S. nagoya	1	—	—	—
S. tennessee	1	—	1	—
S. oranienburg	1	—	1	—
S. remo	—	3	—	—
S. Saint-paul	—	2	—	—
S. inganda	—	2	—	—
S. concord	—	1	—	—
S. cleveland	—	—	2	1
S. jamaica	—	—	2	—
S. newport	—	—	2	—
S. takoradi	—	—	2	—
S. anatum	—	—	1	—
S. lindenburg	—	—	1	—
S. manchester	—	—	1	—
S. malmoe	—	—	1	—
S. bovis-morbificans	—	—	—	1
Unidentified	6	6	33	13
Totals	93	93	181	46

Of the salmonellas most frequently found in water irrigation samples, only *S. blockley* was responsible for more than one or two human cases. Increasing numbers of infections due to this serotype are now reported in Granada and in one incident 49 persons were affected in an outbreak thought to have originated in unhygienically prepared chicken. The large number of other serotypes in these waters presumably reflects a fairly high level of contamination by animal excreta. The origin of the other dominant strains, *S. kapemba* and *S. london* are not known,

Table 3. *The predominant serotypes of* Salmonella *isolated in the various types of samples*

Serotypes	Human origin, 1979–80	Human origin, 1981–3	Irrigation water, 1981–3	Vegetables
S. typhimurium	26 (27·9)*	26 (27·9)	7 (3·9)	12 (26·1)
S. enteritidis	16 (17·2)	34 (36·6)	12 (6·6)	1 (2·2)
S. blockley	1 (1·1)	8 (8·6)	15 (8·3)	0
S. kapemba	0	1 (1·1)	28 (15·5)	7 (15·2)
S. london	0	0	18 (9·9)	6 (13·0)
Others	50 (53·8)	24 (25·8)	101 (55·8)	20 (43·5)
Totals	93	93	181	46

* Percentage of serotypes.

and no data on animal isolations is available for this area. They are occasionally reported in Spain as responsible for food poisoning, though not recently in this region. Other studies on residual waters have realized similar results (O'Shanahan *et al.* 1985; Navarro *et al.* 1985).

The incidence of contamination in vegetables was very much lower than in water and even then was inflated by the *S. typhimurium* cluster. There was, however, a correlation between the strains most commonly found in waters with those most commonly found in vegetables. No such relationship was demonstrable with human case isolates. In a similar study where a high proportion of lettuces and fennel were found to be contaminated with salmonella, no related human cases were identified (Ercolani, 1976).

Although our results indicate the presence of salmonella in vegetables, these foods do not appear to constitute a major source of human salmonellosis. In a previous study we showed that washing fresh vegetables reduced the level of contamination by micro-organisms to a considerable extent (Garcia-villanova *et al.* 1985). Nevertheless, the potential hazard of pathogenic bacteria in vegetables should not be underestimated, particularly in those eaten raw or only lightly cooked. It is possible that some of the recent rise in the numbers of cases of sporadic salmonellosis may be from such sources which remain relatively free from suspicion.

REFERENCES

Cherry W. B., Hanks, J. B., Thomason, B. M., Murlin, A. M., Biddle, J. W. & Croom, J. M. (1972). Salmonellae as an index of pollution of surface waters. *Applied Microbiology* **24**, 334–340.

Christiansen, L. M. & King, N. S. (1971). The microbial content of some salads and sandwiches at retail outlets. *Journal of Milk and Food Technology* **34**, 289–293.

https://doi.org/10.1017/S0950268800062026 Published online by Cambridge University Press

CONEJO, J. A., UBEDA, M., JORDANO, R., RODRIGUEZ, R. D., HERRERA, A. & POLO, L. M. (1978). Estudio sobre la calidad microbiológica de ensaladilla y menestra de verduras congeladas. *Anales de Bromatologia* **30**, 55–62.

DONDERO, N. C., THOMAS, C. T., KHARE, M., TIMOTEY, J. F. & FUKUI, G. M. (1977). Salmonella in surface waters of central New York State. *Applied and Environmental Microbiology* **33**, 791–801.

ERCOLANI, G. L. (1976). Bacteriological quality assessment of fresh marketed lettuce and fennel. *Applied and Environmental Microbiology* **31**, 847–852.

GARCIA-VILLANOVA RUIZ, B., JURADO CHACON, M. D. & BOLAÑOS CARMONA, M. J. (1984). Estudio microbiológico de lechuga y tomate (*Lactuca sativa y Solanum lycopersicum*. II. Influencia del aliñado. *Anales de Bromatologia*, **36**, 317–326.

GOYAL, S. M., GERBA, CH. P. & MELNICK, J. L. (1977). Occurrence and distribution of bacterial indicators and pathogens in canal communities along the texas coast. *Applied and Environmental Microbiology* **34**, 139–149.

JONES, P. W., RENNISON, L. M., LEWIN, V. H. & REDHEAD, D. L. (1980). The occurrence and significance to animal health of salmonellas in sewage and sewage sludges. *Journal of Hygiene* **84**, 47–62.

NARDI, G. & TANZI, M. L. (1977). La ricerca delle salmonelle nelle acque superficali. *Annali Sclavo* **19**, 251–272.

NAVARRO, M., CASTILLO, J., LARRAZ, Ma V. & GOMEZ LUS, R. (1985). Estudio de Salmonella en aguas residuales, comparación con aislamientos clinico *X Congreso Nacional de Microbiología, Valencia, España.*

O'SHANAHAN, L., MEDINA, A. & SANTANA, C. (1985). Estudio sobre Salmonella y *Vibrio Cholerae* en aguas de mar y residuales. *X Congreso Nacional de Microbiologia, Valencia, España.*

PARVERY, F., CHAMBREUIL, G., BECAUD, J. P. & CAYEUX, P. (1974). Etude ecologique des Salmonella dans une rivière en zone urbaine: La Maine a Angers. *Revue d'Epidémiologie et Sante Publique* **22**, 125–136.

SANCHEZ-BUENAVENTURA, J. & CORTINA GREUS, P. (1977). Estudio ecológico y epidemiológico del género Salmonella en Valencia. *Revista de Sanidad e Higiene Pública* **51**, 1093–1108.

SHIPP, CH. R. & ROWE, B. (1980). A mechanised microtechnique for Salmonella serotyping. *Journal of Clinical Pathology* **33**, 595–597.

SMITH, P. J., JONES, F. & WATSON, D. C. (1978). Salmonella pollution of surface waters. *Journal of Hygiene* **81**, 353–360.

SPLITTSTOESSER, D. F. & SEGEN, B. (1970). Examination of frozen vegetables for Salmonella. *Journal of Milk and Food Technology* **32**, 111–113.

TAMMINGA, S. K., BEUMER, R. R. & KAMPELMACHER, E. H. (1978). The hygienic quality of vegetables grown in or imported into the Netherlands: a tentative survey. *Journal of Hygiene* **80**, 143–154.

TELTSCH, B., KEDMI, S., BONNET, L., BORENZATAN-ROTEM, & KATZENELSON, E. (1980). Isolation and identification of pathogenic micro-organisms at waste water irrigated fields: ratios in air and waste water. *Applied and Environmental Microbiology* **39**, 1183–1190.

VELAUDAPILLAI, R., NILES, G. R. & NAGARATNAM, W. (1969). Salmonellas, shigellas and enteropathogenic *Escherichia coli* in uncooked food. *Journal of Hygiene* **67**, 186–191.