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Plasmid analysis of *Salmonella enteritidis* isolated from human gastroenteritis cases and from epidemiologically associated poultry flocks

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SUMMARY

Plasmid analysis of *Salmonella enteritidis* isolates from human gastroenteritis cases and from two commercial egg-producing poultry flocks was performed to determine if the poultry flocks were the source of the human infections. The plasmid profile and restriction fragment pattern (fingerprint) of five *S. enteritidis* isolates from human cases matched those of nine isolates from internal organs of egg-laying hens in one flock which was the source of eggs consumed by the cases. Another commercial flock was epidemiologically associated as the source of eggs consumed by affected persons in four separate gastroenteritis outbreaks from which *S. enteritidis* isolates were available. Five *S. enteritidis* isolates from human cases in these four outbreaks had the same profile and fingerprint, and they all matched those of the 24 isolates from hens in this flock. These results provide further documentation of egg-borne transmission of *S. enteritidis* to humans.

INTRODUCTION

Since the late 1970s, a large increase in the number of human gastroenteritis cases due to *Salmonella enteritidis* infections in humans has been observed on both sides of the Atlantic Ocean [1–4]. Epidemiological investigations of food items consumed by human cases have identified intact shell eggs or food containing eggs as the source of infection. It has also been shown that *S. enteritidis* can be isolated from the yolk and albumen of eggs following experimental infection of hens with *S. enteritidis* [5–8].

Although an epidemiological association between eating contaminated eggs and human infection provides indirect evidence of a cause and effect relationship, it is important to compare the *S. enteritidis* isolates from human cases and from poultry flocks which were the source of the eggs consumed, to determine if they are genetically related. Due to considerable strain diversity within the serotype *S. enteritidis*, phage typing and plasmid analysis have been used for epidemiological purposes. Whereas in England and Wales *S. enteritidis* phage type 4 (PT4) was the

predominant type [2], in the United States PT8 was the most common isolate from sporadic human cases and from animals [9]. Phage type 13a was the most common among US human outbreak isolates, followed by PT8 and PT14b [9].

Threlfall and co-workers [10] reported that plasmid analysis further discriminated strains of *S. enteritidis* within a phage type. In a previously reported analysis of *S. enteritidis* isolates obtained from US layer flocks whose eggs were epidemiologically associated with human *S. enteritidis* infections, the diversity of plasmid profiles was greater than that of phage types; 12 unique plasmid profiles verses 4 phage types [11]. Another study of *S. enteritidis* strains isolated from residents of a rural community in Spain found that 96% of the isolates possessed plasmids and 15 different plasmid profiles were identified [12]. The Centers for Disease Control Laboratories recognized 16 different plasmid profiles among 108 human and 46 animal isolates of *S. enteritidis* [13]. The analysis of plasmids using restriction enzymes to obtain restriction fragment patterns (fingerprints) can provide further evidence of genetic relatedness of strains [9]. The purpose of this study was to examine plasmid profiles and fingerprints of *S. enteritidis* strains isolated from human cases and from two epidemiologically associated egg-laying flocks for genetic relatedness that would corraborate the hypothesis of egg-borne transmission.

METHODS

Sources of bacterial strains

Two commercial layer flocks (LO1 and LO6) were selected for study because they were the source of eggs associated with *S. enteritidis* gastroenteritis outbreaks. In February 1990, the US Department of Agricultural initiated a *Salmonella enteritidis* Task Force which systematically collected samples from flocks implicated in human outbreaks. These samples consisted of the following pooled internal organs: heart, liver, gall bladder and contents, ovary and oviduct, and spleen. The culturing of the samples, identification of *S. enteritidis* and phage typing were performed by the National Veterinary Services Laboratory (NVSL) according to specific protocols [14]. Descriptions of *S. enteritidis* isolates from these layer flocks have been reported [11]. *S. enteritidis* isolates from faeces of humans involved in index outbreaks were acquired from the Centers for Disease Control where they had been phage typed [15].

Plasmid profiles and fingerprints

Plasmid DNA was extracted and purified by a previously described procedure [11]. To obtain profiles, plasmid DNA was electrophoresed on 0·6% (wt/vol) agarose gels in Tris-borate buffer (89 mM Tris base, 89 mM boric acid and 2·5 mM EDTA). The *Escherichia coli* strain V517 [16] was used as a standard for determining molecular sizes of plasmids. The plasmid DNA of each isolate was electrophoresed at least twice and a mean molecular size (kb) was calculated for each plasmid.

To obtain the plasmid fingerprints for each isolate, plasmid DNA was digested with restriction enzymes *Pst* I, *Sma* I and *Ava* II (Bethesda Research Laboratories, Gaithersburg, MD) and used according to the manufacturer's instructions. The digested DNA was electrophoresed on 0·8% (wt/vol) agarose gels in Tris-

Table 1. *S. enteritidis plasmid profiles of human isolates and poultry isolates from two epidemiologically associated layer flocks (LO1 and LO6)*

	Flock LO1		Flock LO6	
Plasmids (kb)	Human	Poultry	Human	Poultry
56	0	0	5*	24†
56, 4·8, 4·0, ⩽ 3	5	5‡	0	0

* Five isolates from four different outbreaks; all were PT8.

† Eleven isolates were phage typed; 10 were PT8 and 1 was PT23.

‡ Four additional *S. enteritidis* isolates isolated previously, in March 1989, had the same plasmid profile (56, 4·8, 4·0, ⩽ 3 kb) and the same phage-type (PT14b).

borate buffer (45 mM Tris base, 45 mM boric acid and 1 mM EDTA). Lambda phage DNA digested with *Pst* I was used as a molecular weight standard. All gels were stained with ethidium bromide (0·7 μg/ml) and photographed under UV transillumination using Polaroid 57 and Ektapan (Kodak) films.

RESULTS

Eggs from flock LO1 were associated with a *S. enteritidis* outbreak which occurred in a college preparatory school in March 1990. Five isolates from this outbreak were available and they all had the same profile consisting of four plasmids: 56, 4·8, 4·0 and ⩽ 3 kb (Table 1). Five samples of internal organs collected in April 1990 from laying hens were positive for *S. enteritidis* and the five isolates had the same plasmid profile as the human isolates (Table 1). Four additional isolates, obtained previously in March 1989 from ovaries of hens from this same flock, also had the same plasmid profile. The restriction enzyme digests of plasmid DNA revealed fingerprints with the same pattern, as shown for *Ava* II and *Sma* I in Fig. 1. These human and poultry isolates were all PT14b.

Eggs from flock LO6 were associated with several restaurant outbreaks during June–July 1990. Five *S. enteritidis* isolates from human cases in four of these outbreaks were examined. All five isolates had a single 56 kb plasmid (Table 1) and they were the same phage type (PT8). Twenty-four samples of pooled organs collected in August 1990 from individual laying hens contained *S. enteritidis* and they all had a 56 kb plasmid. The 56 kb plasmids of the human and poultry strains had the same restriction fingerprints when using *Ava* II and *Sam* I (unpublished observations). Twelve of the 24 poultry isolates were phage typed; 11 were PT8 and 1 was PT23.

DISCUSSION

The results of this study provide further documentation of egg-borne transmission of *S. enteritidis* from infected poultry flocks to humans consuming eggs or foods containing eggs. The *S. enteritidis* isolated from human cases had plasmid profiles and fingerprints which matched those of the isolates from poultry flocks which were the source of the eggs.

The 56 kb plasmid has been found in most *S. enteritidis* strains and it can be the only plasmid present [10, 12, 13]. Evidence that the Flock FLO6 related human and poultry isolates are the same strain is based on epidemiological trace-back

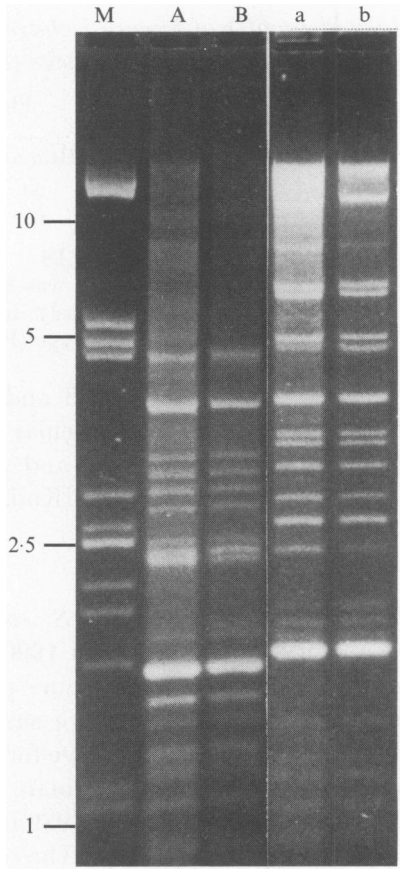

Fig. 1. Plasmid fingerprints of a *S. enteritidis* isolate (DO577, Lanes A and a) from a human outbreak case and a laying hen isolate (8501, Lanes B and b) from an epidemiologically associated *S. enteritidis* infected flock. Both the human and avian isolates had four plasmids (56, 4·8, 4·0 and $\leqslant$ 3 kb). Plasmid DNA was digested with *Ava* II (Lanes A and B) and *Sma* I (Lanes a and b). The molecular size measurements (kb) in the left margin were determined from lambda DNA digested with *Pst* I (Lane M).

information and the presence of a single plasmid of the same size and fingerprint. The fingerprint data alone, however, cannot be considered conclusive because epidemiologically unrelated strains bearing a single 56 kb plasmid may have the same fingerprint (unpublished observation). The presence of other plasmids in many 56 kb plasmid-bearing strains results in profiles that can be discriminated, as illustrated by the examination of flock LO1 related isolates.

The significance of the one PT23 isolate from a layer in flock LO6 is unclear. It had a 56 kb plasmid like the other isolates obtained from this flock in 1990. The phage typing of this isolate was confirmed by retesting; however, ribotyping of genomic DNA of the PT23 and PT8 isolates from flock LO6 revealed similar patterns, using *Pst* I and *Sma* I (unpublished observations). Furthermore, PT23 seems to be rare, as it was not found among *S. enteritidis* isolates from 46 animals between 1986 and 1987 in the US [13]. Therefore, the plasmid characteristics of this PT23 isolate seem to have more epidemiological meaning than its phage type.

Plasmid analysis was a useful tool in this epidemiological study of *S. enteritidis*. The methodology can be used to document transmission within and between animal species, to understand better the diversity of *S. enteritis* strains in populations and to provide data needed in the design, implementation and monitoring of infection control strategies.

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