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Salmonella enterica serotype *Infantis* in southern Italy from 2000-2005: molecular typing of isolates from human and non human sources

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Abstract

Serotype Infantis ranks within the top-five *Salmonella* serotypes in many European countries. An association between this serotype and the poultry ecosystem is apparent in some geographic areas and could account for its persistent or emergent role as causative agent of human salmonellosis. Recently, in southern Italy isolation from eggs and poultry has become increasingly frequent, and in one case the egg yolk also cultured positive. Molecular typing was performed to assess the possible relationship among isolates from human, food and animal sources. Isolates of serotype Infantis identified in southern Italy between 2000 and 2005 from various sources were submitted to pulsed field gel electrophoresis (PFGE) by a CHEF-Mapper apparatus after endonuclease digestion of DNA by *Xba*I.

PFGE patterns of isolates from swine and poultry reservoirs showed distinctive PFGE profiles and human isolates shared pulsotypes with isolates from both sources. Moreover, egg isolates from different farms appeared very similar to each other.

Epidemiological investigation and risk assessment can obtain reliable and useful pointers from the application of molecular epidemiology techniques.

Key words: Salmonella enterica serotype Infantis, molecular epidemiology, foodborne disease, PFGE

Introduction

Salmonellosis is one of the most widespread foodborne zoonoses in industrial as well as developing countries even though the attributable risk fraction seems to vary between countries [1,2]. *Salmonella* infection in humans is mainly foodborne, but can also be acquired through such behaviour as pet ownership, farm visits, or international travel [3].

Foods from animal origins, especially from poultry, play a prominent role in causing human salmonellosis. Surveillance of *Salmonella* serovars from human, animal and food/feed sources is crucial for determining the dynamics of disease transmission and implementing effective prevention and control measures [4-6]. Many epidemiological problems, including detection and interpretation of outbreaks, by tracing transmission routes and identifying infection sources, can be addressed by molecular strain typing methods [7]. These methods are also useful to identify high-risk foods, high-risk practices and high-risk populations for specific pathogens. Microbial source tracing (MST) by strain typing has been in recent years formally enrolled among the epidemiological approaches aimed at the multidisciplinary task of attributing illnesses to food [8].

Serotype Infantis steadily ranks within the top-five *Salmonella* serotypes in Italy from human and non human sources [9]. In 2002, such a serotype was reported among the 5 most common human serotypes from all 6 regions of the world along with: *S.* Enteritidis, *S.* Typhimurium, *S.* Montevideo, *and S.* Typhi [10]. Moreover, it was the most common non human serotype in Europe [10]. In recent years, isolation from eggs and poultry has became more frequent in southern Italy, and in one case a positive culture was also obtained from an egg yolk pool collected in the context of a sampling plan performed in western Sicily, according to the objectives of the national *Salmonella* monitoring programs for laying hens.

Molecular typing by pulsed field gel electrophoresis (PFGE) was performed on *S.* Infantis isolates identified during the years 2000-2005 at the southern Italy Centre for Enteric Pathogens (CEPIM), Palermo, Italy, to assess possible relationships among isolates from

human, food and animal sources. An added aim of this study was to evaluate the ability of PFGE to discriminate among serotype Infantis isolates based upon their food animal sources.

Methods

A total of 55 strains were available. Their distribution by source is illustrated in Table 1. The human strains were isolated in southern Italy from a hospital as well as from the public health laboratories and sent to the Southern Italy Centre of Enteric Pathogens for serological identification or confirmation and typing. The food and animal strains were isolated at the Istituto Zooprofilattico Sperimentale of Sicily and then serotyped at the CEPIM. In particular, within the food isolates two were from poultry meat, one from pork meat, while ten strains were from hen eggs. These last isolates were from a sampling plan conducted in Sicily in the year 2005 on egg-laying hen farms and egg shells. Three isolates from poultry feed were also included.

The *Salmonella* cultures were identified biochemically, using API 20E system (Bio-Merieux, Marcy l'Etoile, France) and by agglutination using specific O and H antisera (Staten Serum Institut, Copenhagen, Denmark).

PFGE was performed at the CEPIM, according to previously described procedures [11]. Chromosomal DNA was digested with 50 U of *Xba*I (Promega, Madison, Wisconsin, USA). Electrophoresis was performed on a CHEF-Mapper system (Bio-Rad, Hercules, CA) in 0.5X Tris-Borate-EDTA (TBE) with recirculation at 14 °C. DNA macrorestriction fragments were resolved on 1.2% SeaKem Gold Agarose (Cambrex) in 0.5X TBE buffer. DNA from *Salmonella* Braenderup H9812 restricted with *Xba*I was used as a size marker. Pulse times rose from 2.2 to 56.2 s during a 21-h run at 6.0 V/cm. Macrorestriction profiles different by at least one band were identified as unique pulsotypes and designated consecutively by letters.

Results

PFGE of serotype Infantis isolates yielded well distinguishable profiles with a number of bands ranging from 11 to 16. Among the 55 isolates submitted to PFGE analysis, 12 different patterns were identified (Fig. 1a and b).

Some association between source and *Xba*I pulsotype was apparent (Table 1). Pulsotype J, the most frequently identified PFGE profile from human sources, was also found in one isolate from a sample of minced pork meat. Furthermore, a correlation between the poultry ecosystem and humans was suggested by the identification of the same pulsotypes from human sources and hen eggs – pulsotypes A and L, poultry meat – pulsotypes E and G, poultry feed – pulsotypes D and E, respectively.

Isolates from hen eggs sampled at different farms shared the same pulsotype A.

Figure 1a . *Xba*I- pulsotypes of *S.* Infantis, southern Italy 2000 – 2005 (MW is Braenderup H9812).

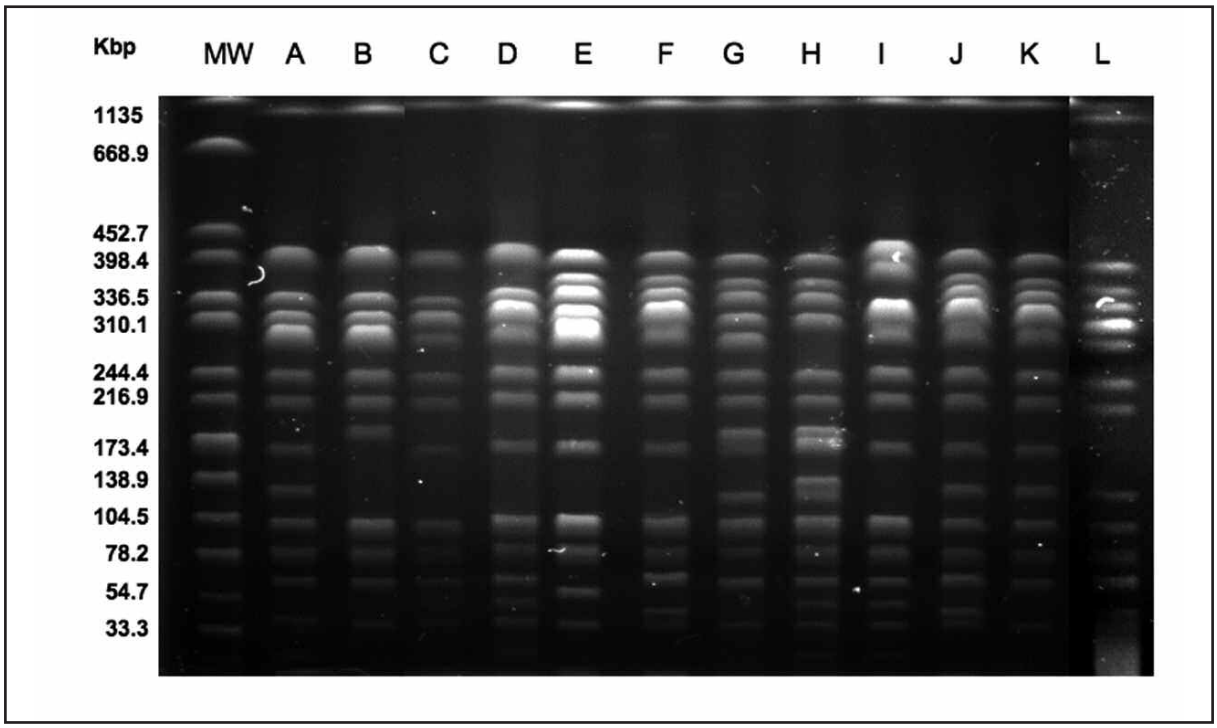

Figure 1b. Schematic diagram of the pulsotypes *Xba*I- pulsotypes of *S.* Infantis (MW is Braenderup H9812).

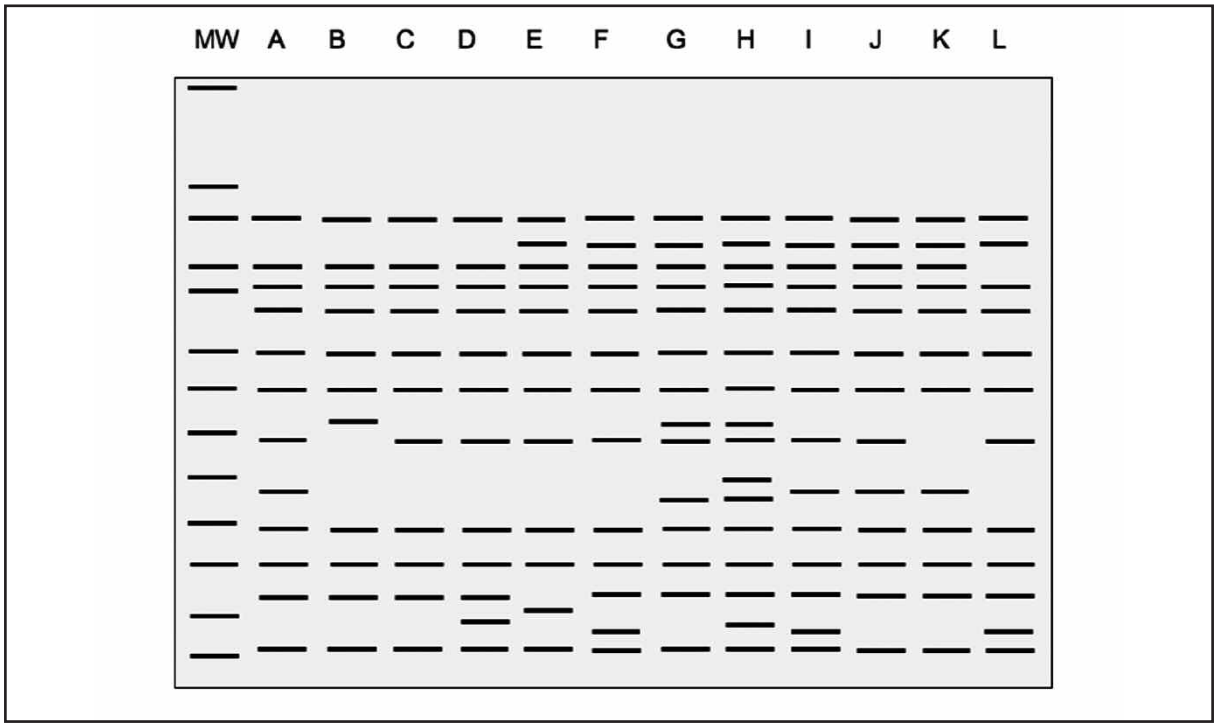

Table 1. Distribution of *S.* Infantis isolates by *Xba*I pulsotype and source of isolation

	Number of isolates by source					
XbaI pulsotype	human		food/feed	environmental		animal
A	1	8	hen eggs	1	-	
B	-	-		-	1	wild bird
C	1	-		-	-	
D	3	2	poultry feed	-	-	
		1	poultry meat			
E	1	1	poultry feed	1	-	
F	2	-		-	-	
G	1	1	poultry meat	-	-	
H	2	-		-	-	
I	6	-		-	-	
J	13	1	pork meat	3	-	
K	1	-		-	-	
L	2	2	hen eggs	-	-	

Discussion

Epidemiological and microbiological data are needed to inform public health authorities about the nature and magnitude of foodborne illness and to monitor trends over time. These data are also needed to identify outbreaks and implement targeted preventive and control measures.

Although in general, passive surveillance does not produce data timely enough to help in identifying outbreaks and put in place preventive and control measures, this information does lead to the generation of hypotheses which can then be assessed in analytical studies and contribute to the planning of scientifically sound interventions in the long term. Molecular typing techniques substantially add to a better understanding of the epidemiology of *Salmonella*, by contributing to extract cryptic outbreaks and animal-environment-food-human connections that otherwise could go undetected, drowned by the overflowing, apparently endemic background of the most successful *Salmonella* serotypes [4].

Our results have obvious limitations in terms of representativeness and comparability, but provide interesting insights into the transmission routes, within our geographic area, of a widely circulating serotype with a wide-range animal host spectrum. Indeed, two parallel and different food sources proved to be involved in the causation of human cases, both the swine and the poultry chains. Of

special significance is the seemingly emergent association serotype Infantis-hen eggs, because of the previous experience with serotype Enteritidis, which found in this extensively employed food/ingredient, a dramatically effective tool for dissemination. The presence of a single pulsotype in egg shells from different farms in western Sicily is a matter of further concern.

Although our findings should be confirmed by the analysis of a wider selection of isolates from different geographic areas, they do confirm the possible role of PFGE typing in tracing routes of human infection, especially when the causative agent is not host-specific and distinctive profiles could be detected across animal reservoirs.

Acknowledgments

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