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Since 1993, the reporting of listeriosis has been mandatory in Italy. The surveillance system based on case notifications from physicians is managed by the Ministry of Health. The information collected includes only gender, age and case distribution by region. To gather more information, an active surveillance was conducted for 12 months (2002-2003). All hospital microbiological laboratories in Italy (n=103) were given clinical and food questionnaires and were requested to report positive cases and send strains for testing. A higher number of cases of listeriosis were reported by this active surveillance compared to the mandatory notifications. In addition, information on risk factors, clinical symptoms and outcomes of 77 reported cases were analysed. In one case it was possible to trace the source of infection.

Of the 77 cases of listeriosis, 41 *Listeria monocytogenes* isolates were characterised by serotype and pulsotype. More than 95% of the strains belonged to serotypes 1/2a, 4b and 1/2b; molecular analysis revealed 23 different *AscI* pulsotypes. The information collected is very important for understanding the real situation of listeriosis in Italy. It can be used to take effective actions in improving food safety and to provide dietary advice to individuals at greater risk of infection.

Introduction

Listeria monocytogenes (*L. monocytogenes*) is a ubiquitous Gram-positive facultative intracellular food-borne pathogen that causes listeriosis. In pregnant women the disease primarily causes preterm delivery, miscarriage and stillbirth, whereas in newborns it leads to sepsis, pneumonia and meningitis. In elderly and in immunocompromised individuals listeriosis causes sepsis, meningitis and focal infections. Foodborne transmission of *L. monocytogenes* can also cause a self-limiting acute febrile gastroenteritis in healthy adults [1,2].

Listeriosis is an infection of great concern to public health due its clinical severity and high case fatality.

Since 1993, the reporting of listeriosis has been mandatory in Italy. Physicians notify listeriosis cases on a notification form that is sent to the Ministry of Health and archived in a database. The notification form includes information only on gender, age and region. The mandatory surveillance does not comprise sending clinical and food strains to the National Centre for Food Quality and

Risk Assessment (Centro Nazionale per la Qualità degli Alimenti e per i Rischi Alimentari – C.N.Q.A.R.A.) for characterisation. To gather more information on the listeriosis situation in Italy, an enhanced surveillance was conducted between February 2002 and January 2003 in all 20 Italian regions. All hospital microbiological laboratories in Italy (n=103) were given clinical and food questionnaires and were requested to report positive cases and send strains for testing.

The objectives of this enhanced surveillance were: to estimate the incidence of listeriosis and compare it to the incidence calculated on the basis of mandatory surveillance, and to describe risk factors, clinical symptoms and outcomes associated with cases of listeriosis. Furthermore *L. monocytogenes* isolates were characterised by serotyping and PFGE, and epidemiological investigations were performed in order to trace source of infection.

Methods

Case definition

The case definition of invasive *Listeria* infection was based on the Commission Decision 2002/253/CE [3]; the case definition of *Listeria* gastro-enteritis was based on the "Proposed case definitions for a European surveillance network of listeriosis" [4]. A case is considered maternal/neonatal (MN) when diagnosed in a pregnant woman, foetus or a newborn below one month of age. When *L. monocytogenes* is isolated from both the pregnant woman and her newborn child or foetus, it is considered a single case. If a case does not apply to any of these, it is considered as non-maternal/neonatal (non-MN)

Enhanced surveillance

An enhanced surveillance was conducted for a 12-month period (from February 2002 to January 2003), targeting the whole Italian population. A clinical and food questionnaire was prepared with the collaboration of the National Centre for Food Quality and Risk Assessment (C.N.Q.A.R.A.) and the National Centre for Epidemiology Surveillance and Health Prevention (C.N.E.S.P.S.) of the Istituto Superiore di Sanità (ISS). The standardised questionnaires were sent to 103 hospital microbiological laboratories in Italy. The clinical questionnaire included information on gender, age, region, risk factors (underlying disease or condition), clinical symptoms, outcome of patients. The food questionnaire asked to provide a list of food items consumed within two months before the onset of illness. The laboratories were asked to report on all positive cases of listeriosis, with the information requested in the questionnaires, and to submit *L. monocytogenes* isolates to the C.N.Q.A.R.A. All data were obtained through the standardised questionnaires, and not via face-to-face or telephone interviews.

Serotyping

The isolates were serotyped using commercial *Listeria* antisera (Denka Seiken, Japan), in accordance with the manufacturer's instructions, with a few modifications [5].

PFGE

DNA isolation and pulsed-field gel electrophoresis (PFGE) were performed following the PulseNet Protocol [6], with *AscI* as restriction endonuclease. The gel was digitally photographed with Gel Doc 2000TM (Bio-Rad, USA). The TIFF images were compared using the Applied Maths BioNumerics software package (Version 4.0, Applied Maths, Saint-Martins-Latem, Belgium), and normalization was carried out by aligning the bands with database global standard *Salmonella* Braenderup strain H9812, loaded in 4 lanes in each gel. Pattern clustering was performed using algorithms within BioNumerics application: the unweighted pair group method using arithmetic averages (UPGMA) and the Dice correlation coefficient with a position tolerance of 1.0%. Strains

with the same number and band position were considered indistinguishable.

Results

During our enhanced surveillance 40 hospital microbiological laboratories of 10 regions reported 77 sporadic cases of listeriosis, corresponding to an incidence of 1.3 cases per 1,000,000 inhabitants/year, which was higher than the incidence reported by the Ministry of Health (0.8 cases per 1,000,000 inhabitants/year) in the same 12-month period. The most common underlying conditions among cases were cancer (24 cases, 31%), solid organ transplantation (24 cases, 31%), dialysis (7 cases, 9%), and pregnancy (6 cases, 8%). 16 cases (21%) were over the age of 65 years. The most common clinical manifestations were septicaemia (29 cases, 38%), meningitis (19 cases, 25%) and meningo-encephalitis (16 cases, 21%); less frequent were newborn with septicaemia (7 cases, 9%), miscarriage (3 cases, 4%) and febrile gastroenteritis (3 cases, 4%). The number of deaths reported was 15 (20%). Only in one listeriosis case, the source of infection was identified [7].

Of the 77 cases of listeriosis, 41 isolates were sent to C.N.Q.A.R.A and were characterised by serotyping and PFGE. More than 95% of the strains (Table) belonged to serotypes 1/2a (18 isolates, 44%), 4b (13 isolates, 32%) and 1/2b (8 isolates, 20%). Molecular analysis revealed 23 different *AscI* pulsotypes among the 41 strains. Isolates possessing identical restriction patterns were also recovered from different geographical areas, indicating that strains were not correlated (Table). Only in two regions (Lombardia and Puglia) and in a six-month period we found clusters of isolates with indistinguishable *AscI* profile in the same hospital. In Lombardia it was one MN case and one non-MN case with *AscI* profile 2 and serotype 4b, and in Puglia there were three non-MN cases with *AscI* profile 7 and serotype 1/2b, and two non-MN cases with profile 7 and serotype 4b.

TABLE

Listeria monocytogenes strains isolated during enhanced surveillance, Italy 2002-2003

Region	Serotype	AscI
Trentino Alto Adige	4b	2
	1/2b	3
	1/2b	4
	1/2a*	5*
	4b	7
	1/2a	12
Piemonte	1/2b	1
	1/2a	6
	1/2a	15
	1/2c	4
	1/2b	21
Friuli Venezia Giulia	1/2a	17
Lombardia	1/2a	4
	4b#	2#
	4b*#	2*#
	1/2a*	13*
	1/2a	14
	1/2a*	15*
	1/2b	19
Veneto	4b*	2*
	1/2a	18
	1/2a	22
	1/2a	20
	1/2a	23
Toscana	4b	8
	4b*	9*
	4b	7
Lazio	4b	16
Puglia	1/2b#	7#
	4b#	7#
	Not typable	10
	1/2a	11
	1/2b#	7#
	4b#	7#
	1/2b#	7#

* MN cases (the remaining ones, i.e. without*, are non-MN)

Cases occurred in the same hospital, in the same six-month period

Discussion and conclusion

Discussion and conclusion

The epidemiological data based on mandatory notifications show the incidence of listeriosis in recent years (2004-2006) to be about 0.8 cases per 1,000,000 inhabitants per year.

The enhanced surveillance described in this paper allowed the collection of more precise and complete information about this infection thus responding to the challenges underlined in the "Annual Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Antimicrobial Resistance in the European Union" with regard to a mortality rate of 8.3% for listeriosis. The Report pointed out that "the lower than expected reported mortality rate might be due to a lack of data on patient outcomes after the initial notification", and therefore "to assess the burden of listeriosis in the EU community better harmonisation of data collection systems is required" [8].

With the exception of one case related to the consumption of gorgonzola cheese [7], no clear source of infection was identified during the enhanced surveillance. There are a number of problems in collecting food items consumed by patients, including the potentially long incubation period (up to 91 days) [9], and the difficulty to collect food samples from homes or from where they were purchased.

Our results show that most of the strains isolated from listeriosis cases belong to serotype 1/2a. In comparison with the results of our previous work [5] in which we studied the distribution of *L. monocytogenes* serotypes in food, environment and human isolates collected in Italy during 1990 to 1999, we have currently observed that the distribution of serotypes among the food isolates has remained the same, while among human isolates, the frequency of serotype 4b has decreased and the frequency of serotype 1/2a has increased. This variation of serotype of clinical *L. monocytogenes* isolates has also been observed in a study conducted in Finland on *L. monocytogenes* isolates from invasive infections during 11-year period [10]. These results support findings in the United Kingdom [11], Denmark [12], Switzerland [13] and Sweden [14] suggesting that serotype 1/2a is replacing serotype 4b in human infections.

Molecular analysis revealed 23 different *AscI* pulsotypes among 41 strains and this supports the fact that isolates were from sporadic cases. There were only some exceptions of indistinguishable strains for serotype and PFGE profile collected in the same hospital and in a six-month period. In these few cases, epidemiological and microbiological investigations were unable to identify a probable common source of infection.

The following were considered to constitute the possible limitations of this study: participation of only half of the regions and no communication of zero reporting; difficult-to-perform epidemiological investigations; incomplete questionnaires. Notwithstanding these limitations, the information collected during the study is important in understanding the real situation of listeriosis in Italy. It can be used to take effective actions in improving food safety and to provide dietary advice to high-risk individuals in avoiding specific foods (like the consumer information made available by the Food Safety and Inspection Service in the United States – <http://www.fsis.usda.gov/>).

Serotypes and pulsotypes of clinical and food strains collected during our study will be added to the Italian database and to the PulseNet European Network, contributing information useful in detecting compatible cases and tracing probable sources of infection.

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