Species and Serovars of the Genus *Listeria* Isolated from Different Sources in Brazil from 1971 to 1997

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Using phenotype techniques, characterization was made to species and serovar of 3,112 strains of Listeria, *isolated from different sources of infection such as human (247-7.9%) and animals (239-7.6%), as well as from various routes of infection, including food (2,330-74.8%) and environmental constituents (296-9.5%), all coming from different regions of the country and collected during the period 1971-1997. The following species were recovered in the cultures analysed:* L. monocytogenes *(774-24.8%),* L. innocua *(2,269-72.9%),* L. seeligeri *(37-1.1%),* L. welshimeri *(22-0.7%),* L. grayi *(9-0.2%), and* L. ivanovii *(1-0.03%).* L. monocytogenes *was represented by ten serovars, the most prevalent being 4b (352-11.3%), 1/2a (162-5.2%), and 1/2b (148-4.7%). The predominant serovar in* L. innocua *was 6a (2,093-67.2%). Considerations about laboratory methods for diagnosis and epidemiological aspects are presented on the basis of the results obtained.*

Key words: *Listeria* - species - serovars - human - animals - environment - Brazil

The constituents of the genus *Listeria* are widely distributed in nature, although with a limited number of species of medical and veterinary importance (Schuchat et al. 1991, Low & Donachie 1997, McLauchlin 1997). Currently, listeriosis is considered to be an emergent disease, with its agent, *L. monocytogenes,* being mainly transmitted by food (Farber & Peterkin 1991). In humans, the disease presents clinical forms of a certain severity with a high socioeconomic impact among the food borne diseases (Roberts & Pinner 1990).

Among the various surveillance systems used for monitoring the disease or its etiologic agent, laboratory analysis data predominates (Rocourt 1988, Rocourt et al. 1997). ln most cases, this information is limited to a sporadic event with no clinical or epidemiological notification (Rocourt et al. 1997).

The basic laboratory methods used are usually concentrated on the isolation and phenotypic identification of species of the genus *Listeria,* followed less frequently by antigenic characterization of serovars (Rocourt & Seeliger 1985, McLauchlin et al. 1986, Farber & Peterkin 1991).

Considering the lack of information available in Brazil concerning these aspects, the present study was undertaken to assess the distribution and frequency of *Listeria* species and serovars from different areas of Brazil, isolated from various sources of infection and transmission vehicles during the period from 1971 to 1997.

MATERIALS AND METHODS

We studied a total of 3,112 *Listeria* strains maintained for 26 consecutive years in the form of a collection in properly sealed tubes containing a thick layer of semisolid medium (Bacto Tryptose Broth plus 0.4% of Bacto Agar, Difco) and stored at 4-8°C.

The cultures were shipped from different parts of Brazil by public and private entities in order to establish a conclusive laboratory diagnosis, or were isolated by the laboratory itself in the various investigations performed (Table I).

Identification and characterization of Listeria strains - They were assigned to the genus *Listeria* and its species using morphological and staining characteristics (Gram method) and biochemical tests: fermentation of D-xylose, D-mannitol, L-rhamnose and a-methyl-D-mannoside, plus routine analysis of hemolytic activity by the CAMP tests with *Staphylococcus aureus* and *Rhodococcus equi* (Rocourt et al. 1983).

Serogroups and serovars determinations were performed with polyclonal crossed absorbed factor antisera raised against somatic and flagellar *Listeria* antigens in rabbits, according to the method

described by Seeliger and Höhne (1979). The antisera were obtained from the Department of Bacteriology, Instituto Oswaldo Cruz.

RESULTS

During this period 3,532 cultures with presumptive original diagnosis of *Listeria* were analysed. Of these, 3,112 were morphologically and biochemically compatible with the genus *Listeria,* with 88.2% positivity.

Table I shows a 96% predominance of pathological processes in human isolates, 78.5% of them involving patients from the States of São Paulo and Rio de Janeiro. Most animal strains (96%) were originated from apparently normal cattle slaughtered in an abattoir in the State of Rio de Janeiro (Hofer 1972, 1983).

Among the strains obtained from foods consisting of dairy products (9.7%), meat products submitted or not to industrial processing (89.9%) and vegetables (0.25%), particularly the samples from Goiás, with a predominance of isolations from chicken carcasses and meat (98.9%). In a similar way, among isolations from the environment, most strains analysed were obtained from Goiás (88.8%), isolated from the effluents of meat-processing plants, strains from Rio de Janeiro (11.1%) from studies of different soils (Hofer & Póvoa 1984) and also material entering from a sewage treatment plant (Hofer 1975).

In the characterization of species of the genus *Listeria* (Table II), there was a prevalence of *L. innocua,* which represented 72.9% of the strains analysed, obtained mostly from foods, sewage, meat-processing plants, and apparently healthy carrier animals (cattle).

Among the strains of human origin, there was a prevalence of *L. monocytogenes* over the other species, being the latter always associated with temporary carrier status and fecal excretion.

It is interesting to point out the fortuitous occurrence of *L. ivanovii* isolated from the feces of an apparently normal cow, and the detection of *L. seeligeri* and *L. welshimeri* only in foods: *L. seeligeri* was more frequent in milk and in poultry, while *L. welshimeri* was predominantly in sausages and poultry.

Analysis of serovar distribution (Table III) showed that *L. monocytogenes* was represented by ten serotypes, although it was concentrated in three antigenic types in 85.4% of the cases: 4b (45.4%), 1/2a (20.9%) and 1/2b (19.1%). No isolation source revealed all *L. monocytogenes* serovars or exhibited a significant difference in the number of serovars identified

Some data listed in Table III concerning *L. monocytogenes* serovars are worthy of note, such as the predominance of serotype 4b in specimens from humans and meat products; the higher frequency of serovar 4a in animals, particularly healthy carriers; of 1/2a in dairy products; and of 1/2b in the environment. In general, serovar 4b was the most frequently serovar isolated among the antigenic representatives of the five *Listeria* species identified.

For the other species (Table III), we emphasize the high prevalence of serovar 6a of *L. innocua* (67.2%), as well as the occurrence of serovar 4ab in *L. innocua* of animal origin and of serovar 1/2b in *L. welshimeri* from meat products.

DISCUSSION

An immediate effect of the intensification of studies concerning various aspects of *Listeria* over the past few decades has been an increased detection of *Listeria* from different sources, especially

TABLE I

Distribution of *Listeria* strains according to isolation, source and geographic origin, collected from 1991 to 1997

State		Isolation source				Total	
		Human	Animal	Food	Environment	No.	%
Pernambuco		15	4	—	—	19	0.6
Bahia		2	—	—	—	2	0.06
Goiás		10	—	1,386	262	1,658	53.3
Distrito Federal		4	—	—	—	4	0.1
Minas Gerais		3	—	404	—	407	13.1
Rio de Janeiro		79	231	157	34	501	16.1
São Paulo		115	—	304	—	419	13.5
Paraná		15	—	—	—	15	0.5
Santa Catarina		—	—	8	—	8	0.3
Rio Grande do Sul		4	4	71	—	79	2.5
Total	No.	247	239	2,330	296	3,112	
	%	7.9	7.8	74.9	9.5	99.9	

TABLE II

Frequency of species of the genus *Listeria* isolated from different sources, from 1971 to 1997

Isolation sources	Species												
	L. monocytogenes		*L. innocua*		*L. ivanovii*		*L. seeligeri*		*L. welshimeri*		*L. grayi*		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Human	237	95.9	6	2.4	—	—	—	—	—	—	4	1.6	247	7.9
Animal	96	40.2	141	58.9	1	0.41	—	—	—	—	1	0.4	239	7.7
Food	382	12.3	1,886	80.9	—	—	37	1.58	22	0.9	3	0.1	2,330	74.9
Environment	59	19.9	236	79.7	—	—	—	—	—	—	1	0.3	296	9.5
Total	774	24.9	2,269	72.9	1	0.03	37	1.18	22	0.7	9	0.3	3,112	100

foodstuffs. This also holds good for Brazil, as can be seen in Table I, representing 26 years of uninterrupted analyses.

This clearly implies a necessity for the bacteriologic support of prospective epidemiological studies by the phenotypic characterization of isolates, indicating the genus, the species and the respective serovars (Rocourt & Seeliger 1985). Considering these aspects, the first step is the unequivocal identification of *L. monocytogenes,* responsible for human and animal listeriosis, in order to differentiate it from the remaining species, particularly *L. innocua.* Secondly, more accurate processes are used for somatic and flagellar antigenic characterization in combination with phagetyping and various molecular techniques (McLauchlin et al. 1986, Boerlin & Piffaretti 1991, Brosch et al. 1994, Pereira et al. 1994).

On the basis of the present results, we emphasize certain aspects such as the relatively easy identification of isolates of the genus *Listeria,* with an 88.2% rate of accuracy, although in 59.7% of this sample (1,860 cultures) there had been no definition of species or recognition of serogroups and serovars, or even mention that this analysis had been done. lt should also be pointed out that of the 1,374 cultures with species definition, we were able to confirm the original identification for 1,210 (88%). These cultures consisted of strains mostly from human sources and, also from animal sources, as explained by the fact that in most cases the bacteria were isolated from clinical specimens with a defined pathology (Hofer 1971, 1972, 1983, Hofer et al. 1984, 1998).

The major source of error in the primary phase of identification has been the conflicting interpretation of the bacterioscopic examination, especially in the presence of Gram-positive cocci arranged in pairs often simulating cocco-baciliary forms or, to a lesser extent, irregularly arranged Gram-positive or labile rods, or, even more rarely, Gram-negative bacilli. Most cultures not confirmed as *Listeria* (416) were excluded by bacterioscopy. During this phase, a search for catalase was originally rarely performed, although it represents the basic differentiation between *Listeria* and the genera *Enterococcus* and *Streptococcus,* with *Enterococcus* predominating in food and environmental isolates. We also draw attention to the possibly incorrect execution and reading of the motility test, sometimes performed with inadequate media associated, or not, with inappropriate inoculation procedure, temperature and incubation time. lt is interesting to note that most of the 416 cultures not confirmed as *Listeria* (383 or 92%) were isolated from food, and 271 of them were non motile at room temperature.

TABLE III

Frequency of serovars in the *Listeria* species characterized from 1971 to 1997

| Species | Serovar | Origin of the isolations | | | | | | | |
		Human	Animal	Dairy products	Meat products/ fish	Vegeta-bles	Environ-ment	No.	Total %
L. monocytogenes	1/2a	72	5	41	38	—	6	162	5.2
	1/2b	6	12	10	102	—	18	148	4.7
	1/2c	1	—	1	28	—	—	30	0.9
	3a	1	1	—	—	—	1	3	0.09
	3b	—	—	—	1	—	—	1	0.03
	4a	2	37	—	—	—	1	40	1.3
	4ab	7	5	—	—	—	11	23	0.7
	4b	146	34	17	140	—	15	352	11.3
	4c	—	—	—	2	—	—	2	0.06
	4e	—	2	1	1	—	7	11	0.4
	Rough	2	—	—	—	—	—	2	0.06
L. ivanovii	5	—	1	—	—	—	—	1	0.03
L. innocua	4ab	—	45	—	—	—	—	45	1.5
	6a	5	96	130	1,636	6	220	2,093	67.3
	6b	—	—	4	3	—	7	14	0.4
	NT	1	—	4	103	—	9	117	3.7
L. seeligeri	4ab	—	—	—	2	—	—	2	0.06
	NT	—	—	19	16	—	—	35	1.1
L. welshimeri	1/2b	—	—	—	12	—	—	12	0.4
	NT	—	—	1	9	—	—	10	0.3
L. grayi	—	4	1	—	3	—	1	9	0.3
Total		247	239	228	2,096	6	296	3,112	99.9

NT: not typable

Another problem observed in this investigation was the past use of countless biochemical tests, some of them being of no differential or specific importance. We emphasize the routine use of the scheme proposed by Rocourt et al. (1983), which is simple and accessible to most laboratories. It clearly differentiates species of the genus *Listeria,* with the fundamental requirement of detection of hemolysis either by direct observation on blood agar or, better still, by the CAMP test. The exclusion of this detail or an inadequate reading may have been the most frequent cause of error in the differentiation between *L. monocytogenes and L. innocua* in the past. In the present this identification can be easily obtained by a miniaturized process containing ten substrates (Bille et al. 1992).

Analysis of Table II supports the views discussed above, considering that 1,648 (99.3%) cultures of the 1,658 strains isolated from clinical specimens, food and environmental material in the State of Goiás did not present previous species identification.

Table II shows that most *Listeria* species were detected in food, especially *L. innocua and L. monocytogenes* in a similar way, in the environment and, also in animal sources. This result was obtained by analysis of feces from cows acting as apparently healthy carriers, but passing the pathogenic species *L. monocytogenes* and *L. ivanovii* into the external environment (Hofer 1972, 1983).

L. monocytogenes predominated in human origin strains (95.9%), frequently involved in pathological processes in the central nervous system and/ or with systemic distribution, reaching defined age groups and more rarely detected in other conditions or in healthy carriers (Hofer 1974, Nojimoto et al. 1997).

The most prevalent serovars of *L. monocytogenes* were usually limited to 4b, 1/2a and 1/2b, and, less frequently, 4a, 1/2c and 4ab (Table III). This result demonstrates that the antigenic characterization, represented by 13 serotypes, has limitations for epidemiological analyses due to the prevalence of a discrete number of serovars in most iso-

lation sources, and due to the fact that this property is of cosmopolitan nature. This, of course, does not invalidate the process of epidemiological investigation, since it is a fundamental point for the determination of the phenotypic profile, favoring the adoption of other more discriminatory methods such as phagetyping. Even so, there are some obstacles such as the large proportion of non-typable *L. monocytogenes* strains of serogroup 1/2 (McLauchlin et al. 1986), despite the addition of new phage preparations to the scheme, as opposed to the relative success of phagetyping for serovar 4b (Estela & Sofos 1993). These problems were actually observed in a part of the present sampling (107 cultures), mostly from human source origin, 79 of which (73.8%) permitted the recognition of lysotypes, with serovar 4b and 1/2a making up 78.3% and 63.1% of the typable samples, respectively (Nunes 1990).

Epidemiologically, the distribution of *L. monocytogenes* serovars (Table III) demonstrates the prevalence of 4b serotypes from human origin and from food, differently from data reported by Nicolas et al. (1989), Farber and Peterkin (1991) and McLauchlin (1997). These investigators reported that most part of Europe the serogroup 1/2 predominates in foods of any kind, with the emergence of serovar 1/2c (Rocourt 1988). Paradoxically this event has no major effect on cases of human listeriosis, especially during outbreaks, in which serovar 4b prevails (Jacquet et al. 1995). ln contrast, in the United States of America and in Canada (Schuchat et al. 1991, Farber & Peterkin 1991) comparison of serotypes isolated from human and from food reveals a certain identity, with serovars 1/2b and 1/2c being most commonly characterized, although in four of the five outbreaks of toxinfection that occurred in these regions, involving 254 persons, serovar 4b was the responsible agent (Farber & Peterkin 1991).

On the other hand, serovar 1/2a played a more effective role in the so-called sporadic cases of toxinfection recorded in countries of the northern hemisphere, as well as in Australia and New Zealand (McLauchlin 1997). ln a world calculation involving 29 events of outbreaks and of sporadic cases of toxinfection, McLauchlin (1997) reported the presence of serovar 4b and serogroup 4 in 72.4% of the events, as in opposition to a 27.5% frequency of serotypes 1/2a and 1/2b.

No reference to the above problem has been made in Brazilian studies, but the wide dissemination of the more incident serovars in the various sources (Table III) permits us to assume that the transmission of listerias by food, including fish (Hofer & Ribeiro 1990), represents one of the most important mechanisms of transmission.

With respect to the environment, and especially the studies on soil (Hofer & Póvoa 1984), the most frequent serovars were quite similar to those reported by Weis (1975). The data also demonstrated the resistance of *Listeria* in soil and the occurrence of contamination from animal hosts, especially in effluents from abattoirs.

ln summary, the results obtained till now agree with those reported in several parts of the world (Rocourt & Seeliger 1985, Schuchat et al. 1991, Farber & Peterkin 1991, Low & Donachie 1997, McLauchlin 1997, Rocourt et al. 1997), demonstrating that members of the genus *Listeria* are widely disseminated in various ecological systems and epidemiological cycles, in which food represents a common vehicle of transmission to countless sources of infection. This agrees with Jacquet et al. (1995) who emphasized the importance of the information obtained rapidly and in a relativeiy easy manner by phenotyping, and showed that this method is indispensable and should precede more refined analyses of a genotypic nature.

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