

DETERMINATION OF DOMINANT SEROVARS OF *LISTERIA MONOCYTOGENES*

SAEED MIRDAMADI, MSc, NASRIN MOAZAMI, PhD,
AND SHAHNAZ RAFIEE TEHRANI, PhD*

From the Biotechnology Department, Iranian Research Organization for Science & Technology (IROST),
P.O. Box: 15815-3538 Tehran, 15819, and the *Immunology Department, Tehran University of Medical
Sciences, Tehran, Islamic Republic of Iran.

ABSTRACT

Serovars of *Listeria monocytogenes* were determined. Sera of aborted samples (200) were collected from different hospitals in Tehran and were tested serologically by immunofluorescent antibody methods (IF tests). 137 positive sera were identified. Positive sera were tested against 12 serovars of *Listeria monocytogenes* separately. Titers of antibody in patients' sera for all serovars were determined. The results showed that the dominant serovars of *L. monocytogenes* which caused listeriosis in the samples were 4b, 1a, 2 and 3. None of the sera had antibodies against serovars 4a, 6a or 6b. Some of the sera which had high titers of antibody against dominant serovars (4b, 1a, 2 and 3), showed a faint result with serovars 4d, 4e, 5 and 7.

MJIRI, Vol. 8, No. 3, 173-175, 1994.

INTRODUCTION

Listeria monocytogenes, a small gram positive rod which shows beta hemolysis in sheep blood agar, was first isolated by Murray et al. in 1926,¹ as a result of their investigation of an epidemic of perinatal infection among a colony of rabbits. Since then the organism has been isolated with increasing frequency from man, particularly during the newborn period.^{2,5}

This microorganism is pathogenic for many animals, such as mammals and birds.³ Handling of these animals and drinking infected milk cause infection in man.^{3,7}

In 1981, 34 cases of perinatal listeriosis, including 16 deaths, occurred in the maritime provinces of Canada during a seven month period.⁸

Gray and Seeliger in 1963 first reported human infections by *Listeria monocytogenes*. Lashkari et al. found three positive cultures of *Listeria monocytogenes* among 100 abortion cases in 1974 in Iran.⁹ In 1979 she and her colleague reported oculoglandular infection in Iranian patients.¹⁰ In this study, we bacteriologically tested 200 abortion samples,

among which five were positive.²¹

This investigation was designed to find the serovars of *L. monocytogenes* which cause listeriosis in Iran.

The genus *Listeria* is divided into four main serologic types and 11 subtypes (7 main types according to Seeliger).¹²

The detailed antigenic structure of *Listeria* species was studied by L. Gray and L. Murray.¹²

MATERIALS & METHODS

Samples obtained from 200 aborted cases were tested serologically to determine whether the sera have antibody against *Listeria monocytogenes*. Positive sera were stored at -70°C.

Plasma protein-antisera (gamma globulin-fraction) fluorescein conjugate was obtained from Behring. All the reagents were prepared in PBS (Phosphate Buffered Saline, pH 7.6).

Serogroups of 1a (PTCC 1294), 2 (PTCC 1295), 3 (PTCC 1296) 4a (PTCC 1297), 4b (PTCC 1298), 4d (PTCC 1301),

Serovars of *L. monocytogenes*

4e (PTCC 1302), 5 (PTCC 1303), 6a (PTCC 1304), 6b (PTCC 1305) and 7 (PTCC 1306) of *Listeria monocytogenes* were kindly donated by Persian Type Culture Collection (PTCC).^{11,12} Serogroups of *L. monocytogenes* (antigens) were cultured and fixed on slides for immunofluorescent antibody (IF test). Sera were added on the slides of antigens, and excess sera were washed off with PBS.

Fluorescein conjugated antibody was added to the slides to check the positive sera reaction. Excess antibody was washed and the preparation was examined by the fluorescence microscope.^{11,12}

RESULTS

From August 1989 to 1990, 137 sera of patients who had antibody against *L. monocytogenes* by IF tests were collected. 200 mothers who had aborted their fetuses were selected. Some of these patients had had more than one abortion.

The sera were tested against 12 serovars of *L. monocytogenes* separately. Titers of antibody against serogroups of *L. monocytogenes* in blood of patients were determined (Table I).

DISCUSSION

The present investigation was designed to determine the serovars of *Listeria monocytogenes* which cause serious infection in Iran. We tested 200 sera of mothers who had aborted. Out of the tested samples, sera were separated. These samples had positive IF tests against *L. monocytogenes*. The positive sera were then tested against 12 serovars of *L. monocytogenes*.

All the sera showed negative IF reaction for serovars 4a, 6a and 6b. A faint (weak) result was obtained with serovars 4d, 4e, 5 and 7, with titres of 400 and 800.

Table I: Results of antibody titers in relation to different listeria serogroups.

SEROGROUP OF LISTERIA	NUMBER OF PATIENTS				TOTAL No. POSITIVE	PERCENT POSITIVE
	TITER 400	TITER 800	TITER 1600	TITER 3200		
1a	15	54	28	10	107	78.1
2	9	45	26	9	89	64.9
3	2	68	18	2	90	65.6
4a	0	0	0	0	0	0
4b	8	66	31	12	117	85.4
4d	1	6	0	0	7	5
4e	1	4	0	0	5	3.65
5	2	4	0	0	6	4.37
6a	0	0	0	0	0	0
6b	0	0	0	0	0	0
7	2	7	0	0	9	6.56

Serovars 1a, 2, 3 and 4b showed strong reactions with titres of 1600 and 3200 in some cases (Table I).

Numerous sera showed positive reactions with several serogroups of *L. monocytogenes*, but only one of the serogroups had higher titers than the others.

All the sera with faint positive reaction (4d, 4e, 5 and 7) showed a strong positive reaction with one of dominant serovars of 1a, 2, 3 and 4b. Similar reactions between these serogroups come not only from similarity between some antigens of serovars of *L. monocytogenes*,¹² but also from cross antigenicity between *L. monocytogenes* and some species of bacteria such as micrococcus, *Staphylococcus aureus*, hemolytic streptococci, *Escherichia coli* K8, *Staphylococcus epidermidis* and corynebacterium.^{11,12} Many people carry these organisms throughout their lives (especially *Staphylococcus aureus*).¹³⁻¹⁵

The results showed that the most dominant serovars of *L. monocytogenes* which cause listeriosis in Iran are 4b (85.4% of positive tests), 1a (78.1%), 3 (65.6%) and 2 (64.9%).

The obtained results from this investigation compare with Seeliger and Hohne (1979)¹⁶ who determined serogroups of 1, 3 and 4 of *L. monocytogenes* in the samples of human infections in U.K. Similar results were obtained by J. McLaughlin, et al.¹⁷ who confirmed the existence of serogroups 1, 3 and 4 in the 153 samples by phage typing method.

Gray and Killinger¹⁸ reported that the listeria serotypes identified in infants and children were types 1 and 4b which are types commonly encountered in the United States,^{18,19} but types 2 and 3 are rare. Type 4b comprises approximately two-thirds of all cultures.¹¹ Ahlfors et al.²⁰ isolated serotypes 1b, 4b and 1a in five patients.

So far type 1 is the predominant type in Europe, whereas in the mid-1960s, the dominant type was 4b.¹²

ACKNOWLEDGEMENTS

We wish to thank Dr. R.Z. Mirdamadi for collecting the clinical samples; Dr. M. Mazaheri, Biotechnology Department of IROST, for help in preparing this manuscript; Dr. Babaii, Tarbiat Modarres University, for statistical studies and Miss M. Hooshdaran, Medical University of Tehran, for her advice on the IF tests. Thanks are also due to Mr. M. Azin, Mrs P. Samadi, Mrs. M. Tabatabai and other colleagues at the Persian Type Culture Collection (PTCC) and Tarbiat Modarres University for economical and equipmental support.

REFERENCES

1. Murray EGD, Webb RA, Swan MBR: A disease of rabbits characterized by large mononuclear leukocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.).

- J Pathol Bacteriol 29: 407-439, 1929.
2. Seeliger HPR, Hohne K: Serotyping of *Listeria monocytogenes* and related species. In: Bergan T, Norris JR, (eds): Methods in Microbiology 13. London, Academic Press, 31-49, 1979.
3. Wilper M, Sword CP: Mechanism of pathogenesis in *Listeria monocytogenes*. Infection Bacteriol 93: 331-538, 1967.
4. Hoop M: Listeriosis as an infection of pregnancy manifested in the newborn. Pediatrics 27: 390-396, 1961.
5. Fleming DW, Cochi SL, Macdonald KL: Pasteurized milk as a vehicle of infection in outbreak of listeriosis. New Engl J Med 312: 404-407, 1985.
6. Willett HP: Listeria and Erysipelothrix. In: Joklik WK, Willett HP, Amos DR, Wilfert CMM (eds): Zinsser Microbiology. Nineteenth edition, USA, Appleton & Lange, 409-414, 1988.
7. Bortolussi R: Neonatal listeriosis: Where do we go from here?. Pediatrics Infectious Disease 4(3): 228-229, 1985.
8. Schlech WF, Lavigne PM, Bortolussi RA: Epidemic listeriosis: Evidence for transmission by food. N Engl J Med 308: 203-206, 1983.
9. Lashkari KB, Etemadi H, Abdi H: Three Cases of Listeria abortion. Med J Tehran University 5,6: 92-94, 1974.
10. Lashkari KB, Miremadi T: First case of oculo-glandular listeriosis in Iran. Medical J Tehran University 7: 46-48, 1979.
11. Sonnenwirth AO, Leonard J: *Listeria monocytogenes*. In: Sonnenwirth AO, Leonard J, (eds): Gralwohl's Clinical Laboratory Methods and Diagnosis, Vol 2. St. Louis, C.V. Mosby Company, 1666-1679, 1980.
12. Seeliger HPR, Dorothy J: Listeria genus. In: Sneath PHA (ed.): Bergey's Manual of Systematic Bacteriology. Vol 2, Baltimore, Williams & Wilkins, 1986.
13. Kampelbacher EH, Huysingaw TH, Van Jansen LM: The presence of *Listeria monocytogenes* in feces of pregnant women and neonates. Zentralbl Bakteriologie Mikrobiol Hyg(A), 222: 258-267, 1972.
14. Kwantes W, Isaac M: Listeriosis. Br Med J 4: 296-97, 1971.
15. Ralovich B: Listeriosis research: Present situation and perspective. Budapest, Akademiai, Kiado, 1984.
16. Seeliger HPR, Hohne K: Serotyping of *Listeria monocytogenes* and related species. In: Bergan T, Norris JR, (eds): Methods in Microbiology. 13, Academic Press, New York, 31-49, 1979.
17. McLaughlin J, Audurier A, Taylor AG: The evaluation of a phage-typing system for *Listeria monocytogenes* for use in epidemiological studies. J Med Microbiol 22: 357-365, 1986.
18. Gray ML, Killinger AH: *Listeria monocytogenes*, and listeric infections. Bacteriol Rev 30: 309-382, 1966.
19. Visintine A, Oleske JM, Nahmias AJ: *Listeria monocytogenes* infection in infants and children. Am J Dis Child 131: 393-397, 1977.
20. Ahlfors CE, Goetzman BW, Halsted CC, Sherman MP, Wennberg RP: Neonatal listeriosis. Am J Dis Child 131: 405-408, 1977.
21. Mirdamadi S, Moazami N, Rafiei Tehrani S: Rate of listeria abortion in Tehran. Med J Islam Repub Iran. In Press.