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RATE OF LISTERIA ABORTION IN TEHRAN

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ABSTRACT

The rate of listeria abortion in Tehran was investigated. Abortion samples (200) were cold enriched at 4°C and subcultured on selective culture media containing acriflavin, nalidixic acid and potassium thiocyanate. Sera of patients were tested serologically (IF method) for screening, and results were confirmed by culturing the positive samples. Antibody against *L. monocytogenes* was obtained in 70.7% of sera but the bacteria was isolated from five samples only.
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INTRODUCTION

Listeria are small gram-positive coccobacilli that have a tendency to occur in short chains of three to five organisms. In stained preparations they often assume a typical diphtheroid palisade arrangement, a property that was responsible for their previous incorrect classification with the corynebacteria. *Listeria monocytogenes* is 0.4-0.5 μm x 0.5-2.0 μm in size.[6,23,25]

Listeriosis is regarded as a zoonosis. The host range is wide, including some 40 mammals, 20 birds, crustaceans, ticks and fish. The disease in animals is characterized by septicemia, monocytosis, and multiple focal abscesses.[7,23] Transmission of listeria from animals to man occurs by handling of newborn calves, infected dogs, and drinking infected milk.[7,9,19]

A wide variety of clinical syndromes are caused by *Listeria monocytogenes*, ranging from a mild influenza-like illness to fulminant neonatal listeriosis associated with mortality rates of 54-90 percent. In the adult, the major infections are meningitis (55 percent), primary bacteremia (25 percent), endocarditis (7 percent), and nonmeningitic central nervous system infection (6 percent). More than half of these patients have underlying disorders such as malignancy, alcoholism, cirrhosis, diabetes, and vasculitis or are receiving immunosuppressive drugs.[6,22]

In any event, the source of infection may be the environment or maternal carriage. Possible sites of carriage include the gastrointestinal tract, the lower genital tract and the pharynx.[5,10] Fetal infection may arise from haematogenous transplacental spread of the organism or by direct acquisition (antepartum or intrapartum) from the mother's lower genital tract.[6,14,27]

MATERIALS & METHODS

Under sterile conditions, 200 abortion samples and sera from patients were collected

Listeria-selective media and cold enrichment broth was obtained from Difco which contained 0.01% nalidixic acid, 3.75% KCNS and 0.0025% acriflavin.

Blood agar base and brain heart infusion (BHI) agar was obtained from Difco for isolating other bacteria such as staphylococci and streptococci.

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).

Serotypes of 1a (PTCC 1294), 3 (PTCC 1296) and 4b (PTCC 1298) of *Listeria monocytogenes* were obtained from Persian Type Culture Collection (PTCC) and fixed on slides for immunofluorescent antibody (IF) testing.

Collected samples were homogenized in 5-10 ml of selective broth and inoculated in two flasks. One flask was incubated at 35°C for 24 hours. A drop of this broth was added to a blood agar plate and a nalidixic acid selective medium agar plate and incubation was carried out in a candle jar for 48 hours, along with the original broth flask. The second flask was stored in a refrigerator at 4°C and subcultured at weekly intervals for one month. Later to check listeria growth, the process was repeated by keeping the culture at 4°C and subculturing at weekly intervals for 3 months. The isolated strains were studied bacteriologically and species determined.

After 24 hours at 35-37°C, colonies of *L. monocytogenes* were small (0.5-1.5 mm in diameter), smooth, and translucent. By reflected light they had a blue-green color on horse blood agar,[13] and were surrounded by a narrow zone of beta-hemolysis.[21,22,26]

All sera were tested by indirect immunofluorescence. In this test, sera of patients were added to the slides of antigens (listeria). The excess sera was washed off with PBS.

Fluorescein-conjugated antibody was added to the slides to determine whether the sera have antibodies against *L. monocytogenes*. Excess antibody was washed off and the preparation was examined by the fluorescence microscope.[23,25]

RESULTS

From August 1989 to Aug. 1990, 200 placenta samples of abortions were tested bacteriologically and the sera of these pregnant women tested by immunofluorescence method (IF).

The age of these women ranged from 16 to 48 years. Samples were cold enriched at 4°C and subcultured in common media (blood agar, BHI agar). The isolated listeria were obtained using the selective media containing acriflavin, nalidixic acid and potassium thiocyanate.

About 66% of samples showed bacterial infection. A total number of 174 bacteria were isolated (Table I). The results further showed that 72 strains were pathogens and 5 were *Listeria monocytogenes* which had caused abortion, while the others had caused infection but not abortion.

Table I: Isolated bacteria from the samples

Microorganisms	Number of bacteria	Percent of bacteria	Percent of Infected Samples*
Coagulase-positive staphylococci	32	18.4	16
Coagulase-negative staphylococci	46	26.4	23
Beta-hemolytic streptococci	19	10.9	9.5
Candida albicans	8	4.6	4
Bacillus spp.	54	31	27
Enterobacteriaceae	8	4.6	4
Lactobacilli	2	1.2	1
Listeria monocytogenes	5	2.9	2.5
TOTAL	174	100	66

(*From some samples more than one type of bacteria was isolated)

We tested the sera against 3 serovars of *L. monocytogenes* (1a, 3, 4b) through immunofluorescence test (IF), where 70.7% of the sera showed existence of antibody against *L. monocytogenes* and were regarded positive.

Listeria monocytogenes were isolated from pregnant women of 22,25,30, and 36 years old (Table II).

Results showed that the rate of listeria recognized by culture was 2.5%, whereas by IF tests it was 70.7%.

Table II: Results of IF tests and relationship between age and positivity.

AGE	Number	Positive	Percent positive
16-19	40	30	15.7
20-25	58	43	22.5
26-31	53	40	20.9
32-37	33	18	9.5
38-43	4	3	1.6
44-49	3	1	0.5
TOTAL	191	137	70.7

In addition, sera of all five positive cultures showed an antibody titer of 3200 or more against listeria.

DISCUSSION

The present investigation was designed to determine the rate of listeria abortion in Iran and compare two different tests for the diagnosis of listeriosis.

From August 1989 to Aug. 1990, a total of 200 abortion samples were tested bacteriologically and serologically. Among the tested cultures, only five (2.5%) samples were positive, but IF tests showed the existence of antibody against *L. monocytogenes* in 70.7% of sera.

According to Biegeleisen,[1] Cherry & Moody,[2] Eveland,[3] Nelson and Shelton[16] and Smith, et al[24] immunofluorescent

antibody methods can be used for detecting microorganisms in tissue (direct immunofluorescent method) and the presence of antibody against one strain of microorganisms in sera is also determined (indirect immunofluorescent method). In this investigation, we used the indirect immunofluorescent method for detecting listeria antibody in the sera of patients, and false-positive reactions in IF methods were also studied. Antibody against *L. monocytogenes* was detected in 70.7% of samples. On the contrary, *L. monocytogenes* was isolated from only 5 samples.

From these samples, strains of bacteria such as staphylococci, streptococci, enterobacteriaceae and bacillus (Table I) were also isolated. False-positive reactions are caused by cross reactions between *L. monocytogenes* and these bacteria. This point has also been mentioned by Seeliger and Dorothy,[22] who demonstrated cross reactions between *L. monocytogenes* and various groups of streptococci, including enterococci, *Staphylococcus aureus*, *Escherichia coli* K8, *Staphylococcus epidermidis* and corynebacteria.

Neter, et al [25] by means of the hemagglutination test, suggested that *L. monocytogenes* contains the so-called Rantz antigen, an antigen of undetermined chemical composition common to many gram-positive bacteria. Aside from this, it is possible that many of these patients were contaminated by *L. monocytogenes* for a short period of their life before aborting, and as a result they had positive IF tests without true infection. Studies of Kampelmacher[8] and colleagues showed that up to 70% of the population may carry *L. monocytogenes* for a short period with or without any apparent symptoms. Also, Kwantes et al[11] and Ralovich[17] reported that fecal carriage of *L. monocytogenes* is seen in about 0.6%-16% of the population at any time. Kampelmacher and colleagues found *L. monocytogenes* in the feces of 44% of 134 pregnant women, all of whom had normal pregnancies. Lamont & Postlethwaite[12] isolated *L. monocytogenes* from the feces of 2% of pregnant and 3.4% of non-pregnant women, which is similar to that reported in the population by Ralovich.[17]

These investigations showed that many women have antibody against *L. monocytogenes* but their abortion is not related to infection with *Listeria monocytogenes* and as we said, without any doubt, many positive IF tests are falsely positive.

In a logical way, to determine the rate of listeria abortion, we used IF methods as a screening test and the results were confirmed by culturing the samples. Similarly, Gray, Killinger and Morel, et al[15] showed that IF testing is only suitable for screening and must be complemented by culturing and isolation of *L. monocytogenes*, although the initial isolation attempts may not always be successful because *L. monocytogenes* is an intracellular pathogen[13,18] and resembles the other corynebacteria in many respects. To avoid these problems, the cold enrichment technique was performed using Seeliger[20] and Cherry's[21] method.

From 2.5% of abortion samples, *L. monocytogenes* was isolated; therefore, we may say that 2.5% of abortions are caused by *L. monocytogenes*. The same results were reported by Rost, et al[25] who suggested that *L. monocytogenes* may be responsible for some cases of abortion in women, and by Alex, et al[25] who described perinatal septicemia, and reported that it is a common form of listerial infection in Europe but is rare in the United States where it constitutes approximately 2% of all cases.

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