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Histopathology and Pathogenesis of Listeriosis Caused by *Listeria monocytogenes* Isolated from Raw Milk in Mice

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Abstract: Pathogenicity and histopathology of *listeria monocytogene* isolates (11) from raw milk in mice was done. Swiss albino mice (18-22 g) were used as experimental animals. All the isolates were pathogenic and caused mortality within 2 to 6 days after intraperitoneal injection. Reisolation of the microorganism from different organs was done. Pathological changes in the liver, spleen, brain, kidneys and intestines were recorded.

Key words: Histopathology, listeria, listeriosis

INTRODUCTION

Listeriosis is one of the most important foodborne diseases that different regulatory agencies throughout the world have been dealing with for the last few years. The causal agent of this illness is *Listeria monocytogenes*, a pathogen widely distributed in the natural environment (Fenlon *et al*., 1996; Moshtaghi *et al*., 2004) and consequently present in many animal and plant food products (Billiard, 2000). It is well established that the ingestion of contaminated food products causes a range of symptomatic manifestations (including septicaemia and meningitis), with an approximate 20% case-fatality rate (Rouquete and Berche, 1996), that increases up to 75% in high-risk groups, such as pregnant women, neonates and immunocompromised adults. In the last few years, several outbreaks of listeriosis due to consumption of contaminated food products have been reported (Anonymous, 1999). The extended distribution of *L. monocytogenes* in the environment, combined with the specific growth conditions of the pathogen, appear to be the main cause of its high prevalence in different kinds of food products. On the other hand, when outbreaks of human listeriosis occur, it is important for clinicians to be able to verify the pathogenicity of associated isolates, particularly isolates from the suspected vehicle of infection. Hemolytic activity correlates with the pathogenicity of *L. monocytogenes* (Groves and Welshimer, 1977; Skala *et al*., 1982). However, hemolysis is frequently weak and hemolysis tests often yield ambiguous results (Dominguez Rodriguez *et al*., 1986).

The main objective of this study was to understand the pathogenicity and histopathology of milk isolates of *Listeria monocytogenes*.

MATERIALS AND METHODS

Eleven isolates of *Listeria monocytogenes* from raw milk that collected from private dairy farms in Shahrekord city (Iran) from May 2004 to December 2005, were examined for their pathogenicity and histopathological changes in mice.

Initially, pathogenicity was determined by a modification of the method of Ralovich (1984). For each isolate, five 18-22 g Swiss albino mice were given 0.1 mL of the suspension, containing 10^8 cells, intraperitoneally (i.p.). A group of 5 mice was inoculated with 0.1 mL peptone water to act as control. Strains that killed three or more mice were considered to be pathogenic. The mice were provided with food and water *ad lib* and were observed daily for clinical signs and mortality up to 12 days and deaths were recorded. Strains that killed three or more mice were considered to be pathogenic. The mice, which survived, were sacrificed after 12 days.

The mice were examined by necropsy for gross changes. Portions of the liver, spleen, intestine and brain were collected aseptically in sterile vials and reisolation of the test organism from these was done by direct plating on PALCAM agar. Sections were stained by eosine and haematoxyline staining method and Taylors method (Taylor, 1966).

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RESULTS AND DISCUSSION

All the isolates of *L. monocytogenes* were serotype 4b which is associated with the majority of cases of human listeriosis and were pathogenic to mice and led to mortality. The mortality started two days post- inoculation and continued up to 6 days, after which the mice survived the infection. Grey and Killinger (1966) and Pine *et al.* (1990) have also reported up to 6 days after inoculation, but our results were not in agreement with the results of Low and Donachie (1997) that had been reported serotype- 4b strains are not pathogenic in mice. However, of the 35 mice inoculated with serotype-4b strains, 51% (18 mice) 2 days, 5.7% (2 mice) 3 days, 2.9% (1 mouse) 4 days after inoculation had died and mortality was recorded 5.7% (2 mice) on day 6.

Microbiological examination of all the dead mice led to recovery of *L. monocytogenes* in large numbers and in pure cultures. Presence of the organisms in small numbers was also recorded in the organs of the mice, which survived the infection. Between days 5 and 7 postinfection, *L. monocytogenes* bacteria start to disappear from mouse organs until their complete clearance as a result of gamma interferon (IFN-g)-mediated macrophage activation and the induction of an acquired immune response primarily mediated by CD8 lymphocytes, which together destroy *Listeria*-infected cells (Mielke *et al.*, 1988; Kaufmann, 1993; Jose *et al.*, 2001). The above course of events is accelerated in immune animals, resulting in rapid elimination of *L. monocytogenes* from the liver. This is probably the most common outcome of *L. monocytogenes* infection in humans and animals in normal conditions, given the potentially high frequency of exposure to the pathogen via contaminated food and the relatively rare occurrence of clinical disease.

Different organs of the mice in control group did not show any gross and histopathological changes. In the infected group, grossly there were a number of small, white necrotic foci in the liver and spleen. At places, the liver was congested. The number of necrotic foci was more in mice that died 2 or 3 days post-inoculation. Similar findings have been reported by Mandel and Cheers (1980) and Mainou-Flower *et al.* (1988).

Histopathological examination of the liver revealed congestion and pyo granulomas consisting of necrosed hepatic cells, macrophages and neutrophiles (Fig. 1). There was lymphocyte depletion and necrosis of lymphocytes in white pulp of spleen of the infected mice (Fig. 2). Brain tissue showed microabcesses and necrosis of neurons, which was characterized by satellosis and

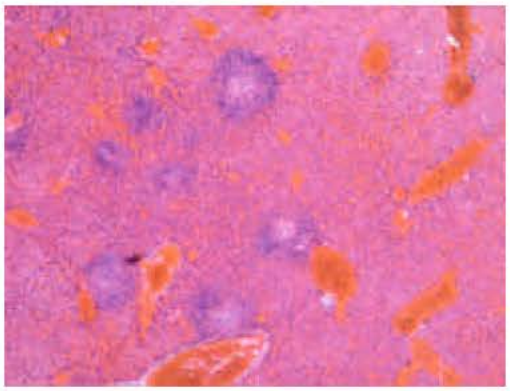

Fig. 1: Section of liver of *Listeria monocytogenes* infected mouse showing congestion and necrotic foci (H and E X4)

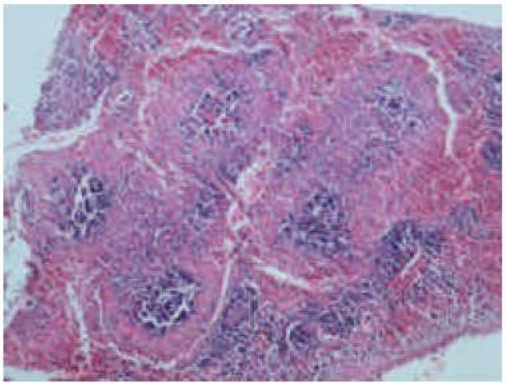

Fig. 2: Section of spleen of *Listeria monocytogenes* infected mouse showing necrosis of lymphocytes in white pulp and congestion (H and E X4)

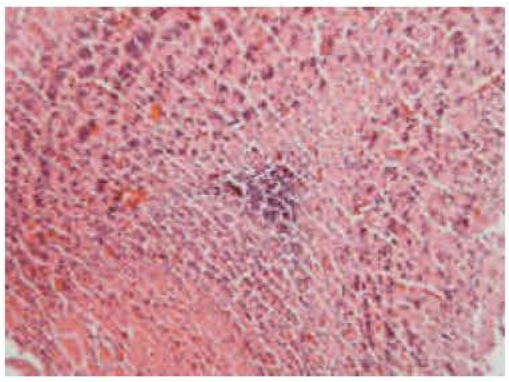

Fig. 3: Section of brain of *Listeria monocytogenes* infected mouse showing gliosis and congestion (H and E X10)

neurophagia (Fig. 3). In kidneys, histopathological changes were characterized by the presence of focal interestitial nephritis, focal areas of neutrophiles and lymphocytes in interestitial tissue and atrophy of tubules. Besides these, hyaline casts in tubules and cloudy swelling were recorded. Sections of the intestine showed characteristics of enteritis. The lesions in the liver, kidney, spleen and intestine showed the presence of the microorganisms, which stained blue to blue-black by Taylors stain. The lesions recorded in mice were similar to those reported by earlier workers (Grey and Killinger, 1966; Mandel and Cheers, 1980; Mainou-Flower *et al*., 1988).

CONCLUSIONS

The findings suggested that *L. monocytogenes* isolates, which are commonly known to saprophytic existence in the environment, are potential pathogens. These may pose serious threat to animal and human health.

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