Report

Isolation of listerias from farm milk and abortion cases in women

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Introduction

Listeria has attracted the wide attention of dairy microbiologists in recent years. *Listeria* germs are Gram-positive rods occurring singly, in pairs or in short chains and are catalase-positive, oxidase-negative, non-spore forming, facultative anaerobe bacteria. The genus *Listeria* is composed of seven species now that *L. denitrificans* has been moved from the *Listeria* group to a new genus known as *Jonesia* [*1*]. *Listeria* germs are intracellular bacteria that grow at refrigeration temperature and may be thermoduric at large doses [*2*]. Although *L. monocytogenes* is believed to be the most common listerial causative agent of fetal meningitis, abortion and food poisoning, a big change in the general attitudes of both public health workers and dairy technologists has occurred since the presence of other *Listeria* species in milk and dairy products was reported [*3,4*].

During one summer outbreak in Massachusetts, 49 patients were hospitalized with listeriosis; 7 cases occurred in infant–mother pairs and 42 in adults; 14 patients died. The illness was strongly associated with drinking pasteurized milk [*2*]. McLauchlin [*5*] reported that a cutaneous form of listeriosis affected a veterinarian at a dairy farm. *L. monocytogenes* was the causative agent. The seriousness and epidemic nature of *Listeria* necessitates the inspection of milk and humans on farms and in hospitals.

The objective of the study was to obtain information on the occurence of listerias in dairy farm milk and abortion cases in women.

Materials and methods

This work was conducted at Zagazig University, Faculty of Veterinary Medicine, Egypt, in cooperation with the Department of Medical Microbiology and Immunology, Faculty of Medicine, El-Fateh University for Medical Sciences, Tripoli, Libyan Arab Jamahiriya.

A total of 163 samples were subjected to listerial examination. Of these 138 were raw milk samples from 138 apparently normal cows and 25 were vaginal swabs taken from women who had spontaneously aborted.

Milk samples

A total of 138 individual cow milk samples (each approximately 100 ml) were collected aseptically in sterile sampling bottles from the Sharkia government dairy farm, Egypt in September 1995. The samples were sent in an ice-box without delay to a laboratory for listerial examination.

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From each sample 25 ml of milk were diluted with 225 ml of US Food and Drug Administration (FDA) broth. The level of acriflavin HCl was reduced to 10 mg/l instead of 15 mg/l to avoid damage to the bacterial cell wall. This was then incubated at 30 °C for 24 hours. 0.1 ml of primary enrichment broth was inoculated into 10 ml of modified FDA broth, which contained a high concentration of acriflavin (25 mg/l). The broth was incubated at 30 °C for 24 hours. Loopfuls were streaked onto two selective media, Oxford and Palcam agar. The media were used to increase the possibility for isolation of listeria species. The two media were incubated at 30 °C for 48 hours and 37 °C for 48 hours. Suspected listerial colonies appeared to be dark greenish in colour with a dark brown halo (Palcam agar) and greyblack with a black halo (Oxford agar).

Vaginal swab samples

Two vaginal swabs each were taken from women who had experienced spontaneous abortions in the Department of Obstetrics and Gynaecology, Zagazig Central Hospital, Egypt. One sample from each woman was immersed into lithium chloride enrichment broth in the hospital, then placed in an ice-box and sent to the laboratory. The incubation in lithium chloride was at 30 °C for 48 hours and was followed by streaking onto selective media. The second swab was directly streaked onto plating media (Oxford-Palcam). Two sets of plates were incubated for 48 hours at 30 °C and 37 °C. Incubation at 30 °C is very necessary for *L. ivanovii*.

Suspected listerial colonies were selected and streaked onto Tryptone Soya Agar (TSA) plates and incubated at 30 °C for 24 hours. The plates were examined for typical blue colonies by the oblique lighting technique and identified according to Lovett [*6*] and Bille et al. [*7*].

Antibiogram test

This test was done in cooperation with the Department of Medical Microbiology and Immunology, Faculty of Medicine, El-Fatih University of Medical Sciences. Clinical isolates of *L. monocytogenes* were subjected to antibiotic sensitivity assay using the Kirby Bayer method with ampicillin, gentamicin, erythromycin, chloramphenicol and tetracycline.

Results and discussion

Farm milk

Five samples (3.6%) were contaminated with *Listeria*. Both *L. ivanovii* and *L. seeligeri* were detected in two samples each (1.4%), while *L. welshimeri* was found in one sample (0.7%).

Raafat [8] isolated *L. welshimeri*, *L. ivanovii* and *L. seeligeri* from raw milk samples in Assiut, Egypt at 0.7%, 0.5% and 0.2% respectively. Other investigators observed *Listeria* species in 4.56% of raw milk samples. *L. ivanovii, L. seeligeri* and *L. welshimeri* were found with low percentages (0.2%–0.4%) by Rodriguez et al. [*9*]. A higher incidence of *Listeria* in raw milk was obtained by other researchers [*10,11*] at 15.4% and 15.6 % respectively.

Food-borne transmission through milk appeared to be the major means of zoonotic transmission of listeriosis. Cow's milk proved to be a possible source of listerial infection indicating that milk might be the first blamable animal source to infect man with food poisoning [*12*]. Farber et al. [*13*] discussed the danger of shedding *Listeria* in apparently normal milk, thus constituting a potential public health hazard.

Cases of abortion

A total of 50 vaginal swabs taken from 25 cases of spontaneous abortions were examined bacteriologically for *Listeria*. Only one case, a woman aged 21 years, proved to be infected with *L. monocytogenes*. The history of the case indicated that abortion occurred in the second half of the pregnancy. Shortly before abortion, the woman suffered from fever (38.5 °C), chills and dizziness. There was no evidence for other causes of abortion and the aborted fetus was not subjected to microbiological studies for bacterial isolation.

Raafat [*8*] found that the occurrence of human listeriosis was 4.5%. The species were identified as *L. monocytogenes* [*3*], *L. ivanovii* [*2*] and *L. innocua* [*2*]. Farid observed that 3 out of 200 pregnant women (1.5%) were infected with *L. monocytogenes* in Banha and Ain Shams University hospitals [*14*]. The observation of a close association between late abortion and listeriosis was reported by Acha and Szyfres [*15*].

Zoonotic significance of listerias other than *L. monocytogenes*

Elischerova et al. [*16*] isolated *L. ivanovii* from a woman who had aborted. Others announced the pathogenicity of *L. welshimeri* and *L. seeligeri* in men and animals [*17,18*]. Despite the lack of associations found between cases of abortion and milk consumption, the epidemiological link between food, especially cow's milk and human listeriosis, can be explained by the isolation of *L. monocytogenes* from humans, highlighting the possible contamination of foods by direct or indirect methods. Cross-contamination between foods, including milk and dairy products, may be via hand contact or by kitchen implements. Beumer et al. [*19*] reported that *L. monocytogenes* was isolated from dishcloths, drains and washing brushes taken from more than two hundred domestic kitchens in the absence of *Listeria*. The occurrence of *L. ivanovii* in raw cow's milk may indicate the possible transmission of the pathogen to humans through consumption of inefficiently heat-treated milk and dairy products such as soft cheese and home-produced frozen desserts.

Treatment

The clinical isolates of *L. monocytogenes* were sensitive to erythromycin, chloramphenicol and ampicillin. These findings substantiate those reported by Carter and Chengappa [*3*] and Colonna et al. [*20*].

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References

1. Rocourt J, Wehmeyer U and Stackebrandt E. Transfer of *Listeria denitrificans* to a new genus, *Jonesia* gen. nov, as *Jonesia denitrificans* comb. nov. *International journal of systematic bacteriology*, 1987, 37:266–70.
2. Fleming DW et al. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New England journal of medicine*, 1985, 312:404–7.
3. Carter GR, Chengappa MM. *Essentials of veterinary bacteriology and mycology.*

4th ed. Philadelphia/London, Lea and Febiger, 1991.

4. Cahen P et al. Infected dissecting aneurysm of the thoracic aorta due to *Listeria monocytogenes*. In: Glauser MP, Francioli P and Bille J, eds. Abstracts of the 8th European Congress of Clinical Microbiology and Infectious Diseases, Lausanne, Switzerland, 25–28 May 1997. *Clinical microbiology and infection*, 1997, 3(suppl.2):339.

5. McLauchlin J. *Listeria* and listeriosis. *Clinical microbiology and infection*, 1997, 3(4):484–92.

6. Lovett J. Isolation and identification of *Listeria monocytogenes* in dairy products. *Journal—Association of official analytical chemists*. 1988. 71(3):658–60.

7. Bille J, Doyle MP. *Listeria* and *Erysipelothrix*. In: Balows A et al., eds. *Manual of clinical microbiology*, 5th ed. Washington DC, American Society for Microbiology. 1991:287–95.

8. Raafat AH. *Epidemiological studies on the occurrence of* Listeria *infection in animal and man* [Thesis]. Assiut, Egypt, Assiut University, 1994.

9. Rodriguez JL et al. Incidence of *Listeria monocytogenes* and other *Listeria* species in ewes raw milk. *Journal of food protection*, 1994, 57(7):571–5.

10. Donnell E. The incidence of *Salmonella* and *Listeria* in raw milk from bulk tanks in England and Wales. *Journal of the society of dairy technology*, 1995, 48(1):25–9.

11. Fenlon DR et al. The incidence, numbers and types of *Listeria monocytogenes* isolated from farm bulk tank milks. *Letters in applied microbiology*, 1995, 20:57–60.

12. Skovgaard N. Pathogenic microorganisms: does the dairy sector have problems? *Maelkeritidende*, 1996, 109:11, 257–60.

13. Farber JM et al. Growth of *Listeria monocytogenes* in naturally contaminated raw milk. *Lebensmittel-Wissenschaft and Technology*. 1990. 23(3):252–4.

14. Farid IA. Listeria monocytogenes *during pregnancy and neonatal period* [Thesis]. Banha, Egypt, Zagazig University, 1989.

15. Acha PN, Szyfres B. *Zoonoses and communicable diseases common to man and animals*, 2nd ed. Washington DC, Pan American Health Organization, 1991:105–11 (Scientific Publication No. 354).

16. Elischerova K et al. Isolation of *Listeria ivanovii* in Slovakia. *Ceskoslovenska Epidmiologie, Microbiologie and Immunologie*, 1990, 39(4):228–36.

17. Seeliger HPR, Jones D. Genus *Listeria*. In: Sneath PHA et al., eds. *Bergey's manual of systemic bacteriology*, 2nd ed. Baltimore, MD, Williams and Wilkins, 1986:1235–45.

18. Chakraborty T, Goebel W. Recent developments in the study of virulence in *Listeria monocytogenes*. In: Goebel W, ed. *Current topics in microbiology and immunology*. Heidelberg, Springer-Verlag Berlin, 1988:41–58.

19. Beumer RR et al. Listeria species in domestic environments. *Epidemiology and infection*, 1996, 117:437–42.

20. Colonna V et al. Observations on the presence of *Listeria monocytogenes* in milk products. *Atti XI Congresso Nazionale*. Perugia. Italy. 1–4 June 1994:439–42.