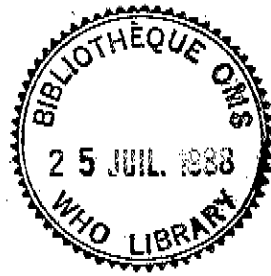




WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE

WHO/EHE/FOS/88.5

Distr.: GENERAL



FOODBORNE LISTERIOSIS

Report of a WHO Informal Working Group
Geneva, 15-19 February 1988



REPORT OF THE WHO INFORMAL WORKING GROUP ON FOODBORNE LISTERIOSIS
Geneva, 15-19 February 1988

CONTENTS

INTRODUCTION	1
1. THE NATURE AND EXTENT OF THE PROBLEM	2
1.1 Epidemiology of foodborne listeriosis - current state	2
1.2 The presence (qualitative and quantitative) of	6
<u>Listeria monocytogenes</u> in foods	
1.3 The heat resistance of <u>L. monocytogenes</u> in food with	8
particular reference to the pasteurization of milk	
1.4 Methods for the detection of <u>L. monocytogenes</u> in foods	9
2. FACTORS RESPONSIBLE FOR THE CONTAMINATION OF FOOD WITH <u>L. MONOCYTOGENES</u>	10
2.1 Presence of the organism in foods	10
2.2 Survival, Growth and Transmission of <u>L.monocytogenes</u>	11
2.3 Control measures	11
3. RESEARCH NEEDS	12
3.1 Epidemiology	12
3.2 Virulence/Pathogenicity	12
3.3 Methodology	13
3.4 Contamination of raw and processed food	13
3.5 Effects of processing	13
3.6 Effects of extrinsic and intrinsic factors	13
3.7 Other Listeria species	13
4. CONCLUSIONS AND RECOMMENDATIONS	14
4.1 Conclusions	14
4.2 Recommendations to national public health authorities on how	15
to ensure safeguarding the consumer	
4.3 Recommendations to the food industry	16

Annex 1 List of participants

R 688

This document is not a formal publication of the World Health Organization (WHO), and all rights are reserved by the Organization. The document may, however, be freely reviewed, abstracted, reproduced or translated, in part or in whole, but not for sale or use in conjunction with commercial purposes.

The views expressed in documents by named authors are solely the responsibility of those authors.

Ce document n'est pas une publication officielle de l'Organisation mondiale de la Santé (OMS) et tous les droits y afférents sont réservés par l'Organisation. S'il peut être commenté, résumé ou cité sans aucune restriction, il ne saurait cependant être reproduit ni traduit, partiellement ou en totalité, pour la vente ou à des fins commerciales.

Les opinions exprimées dans les documents par des auteurs cités nommément n'engagent que lesdits auteurs.

Introduction

Dr W. Kreisel, Director, Division of Environmental Health, World Health Organization welcomed the participants to this meeting on foodborne listeriosis on behalf of the Director-General, WHO. He emphasized the fact that although this urgent meeting appeared timely in relation to the recent decisions made by several European public health authorities to protect consumers against Listeria in various samples of cheeses and the reactions of the food industry and consumer organizations, it had been planned by WHO long before foodborne listeriosis reached the media as a new threat to public health.

He reminded the participants that the threats to public health stemming from contaminated food are manifold. The toxic oil syndrome in Spain, the adulterated wine problems in several European countries, radionuclides in food following a major nuclear accident and now contamination of various foods with Listeria are only a few of the better known of these problems. Consumers, particularly in industrialized countries are concerned about the potential risk associated with food additives, environmental chemicals and pesticide residues in food and with new processes such as food irradiation. The public health sector is attempting to assess the nature and the magnitude of these risks and to communicate this assessment to the public. The role of WHO is to facilitate a consensus among scientists and to inform senior public health officials in Member States, consumers, and consumer organizations, where the real dangers in the food supply lie. The message from WHO should be clear enough to inform individuals, families and communities as to what they can and must do, in addition to measures taken by governments and the food industry, to protect themselves from foodborne disease.

This meeting provides an opportunity to review and to evaluate available data on the occurrence of Listeria in foodstuffs and to assess the risk to individual and public health associated with such contamination. Experience has taught that a no-risk approach is probably not feasible in matters of this sort, particularly in view of the widespread occurrence of Listeria in the environment. Host response should be taken into consideration in order to identify risk groups in the population and to develop specific protective measures. Not all the facts about the epidemiology of human listeriosis are known and additional research is needed. However, this lack of knowledge should not justify unwarranted and hasty conclusions which may cause unnecessary upheavals.

Dr Kreisel anticipated that by the end of the meeting the Working Group would be able to make recommendations to public health authorities, the food industry and to consumers, which address in perspective, the problem of foodborne listeriosis.

On Dr Käferstein's proposal (secretary of the Working Group), the Working Group agreed that Professor E.H. Kampelmacher should act as chairman and Dr D. Roberts as rapporteur. Professor Kampelmacher, in turn, invited Dr C. Broome to act as vice-chairperson. This was also accepted by the Working Group.

1. THE NATURE AND EXTENT OF THE PROBLEM

1.1 Epidemiology of foodborne listeriosis - current state

1.1.1 Introduction

Recent advances in understanding of the epidemiology of human listerial infection, particularly of the potential for common source foodborne outbreaks, and an increased and increasing awareness of the human toll exacted by this organism, have created an atmosphere of uncertainty among the health professions, governments, the food industries and the general population. Though an uncommon cause of human disease, the case fatality rate is high: approximately one third of outbreak cases have resulted in deaths or stillbirths.

This anxiety is heightened, and a sense of urgency created by the knowledge that some part at least of this human toll is preventable by presently available methods. It remains to refine our knowledge so that preventive strategies can be implemented both efficiently and with maximum benefit.

1.1.2 Background

Of all *Listeria* species, only *Listeria monocytogenes* (L.m.) has been regularly implicated as being pathogenic to humans and animals. Most other recognised species are harmless, though it is possible that *L. ivanovii* is responsible for occasional human disease.

Listeriosis is recognized and studied to any extent mainly in industrialized countries. While sporadic cases and occasional outbreaks of human listeriosis and examples of food contamination are detected in other countries, reported prevalences in Africa, Asia and South America are non-existent or low. Whether this is a result of different consumption patterns and dietary habits or represents a lack of available reference facilities (whether because of their absence or because of their dedication to other public health priorities) is not known. Listeriosis remains a world wide problem and with increasing urbanization, social evolution and changes in dietary habit, may assume greater significance in developing countries.

The Working Group reviewed recent data on human listeriosis and concluded that foodborne listeriosis is predominantly transmitted by non-zoonotic means. Nor can it be categorically stated to be a soilborne disease, though soil may often be the origin of the organism. Transmission of foodborne listeriosis to man is primarily the result of environmental contamination and the ease of transmission from the environment to animals and food contact surfaces.

Listeria monocytogenes is perhaps best considered as an environmental contaminant whose primary means of transmission to humans is through foodstuffs contaminated during production and processing. However, this should by no means be considered a complete or exclusive definition.

The basic epidemiological pattern of human listeriosis as seen in industrialized countries would seem to be that of an endemic "background" of sporadic disease, on which may be superimposed outbreaks of disease. This is the picture seen in those countries which practise surveillance for listerial infection; these are on the whole laboratory based and passive (e.g. United Kingdom) or "semi-active" (e.g. France). A special active surveillance project in the United States has given much useful information. The more sensitive the surveillance system, the more effectively it can distinguish outbreak phenomena from background sporadic cases; however, since outbreaks result in overall disease rates of 10 to 50 cases per million, they may still be difficult to detect even with good surveillance.

Four major outbreaks of listeriosis have been well described in the literature (Nova Scotia, 1981¹; Boston 1983²; Los Angeles, 1985³; Canton of Vaud, Switzerland, 1983-87⁴). In Nova Scotia, Los Angeles and Vaud an epidemiological link with supporting microbiological evidence was established with a particular foodstuff (coleslaw, Mexican-style soft cheese, Vacherin soft cheese). In Boston epidemiological evidence suggested whole milk, but there was no microbiological confirmation. These outbreaks have demonstrated clearly the association of human listeriosis with a number of foodstuffs and a variety of mechanisms of contamination. It is clear that not all possible causative foodstuffs, nor all possible mechanisms whereby these foodstuffs may become contaminated, have yet been satisfactorily elucidated.

As well as these few documented outbreaks, other outbreak phenomena have been observed in many countries in which epidemiology and/or microbiology could not establish common sources (e.g. Denmark 1986⁵, Philadelphia, USA 1987⁶). These remain, however, highly probable examples of foodborne outbreaks.

An implication that can be drawn from all these observed outbreaks is that if listeriosis is caused by foodborne transmission in epidemic situations, it may also be caused by such transmission in part (even the larger part) in sporadic cases. This conclusion is supported by anecdotal case reports in the literature associating listeriosis with isolation of the same phage type from an ingested foodstuff.

No single strain has been repeatedly found to be associated with different outbreaks. Outbreaks observed to date have been associated with a range of serovars and lysovars.

Apparent increases in the incidence of listeriosis have also been noted, occurring particularly after publicity about the disease. These are, perhaps, due more to increased awareness, with subsequent increased diagnosis and compliance with reporting systems, than to real increases in incidence. The establishment of stable monitoring systems is crucial to the ability to assess such changes over time.

There has been evidence from some countries (e.g., United Kingdom) of an increase in the incidence of human listeriosis over recent years. Even when the factors mentioned in the previous paragraph are taken into account, it would seem that some if not all of this increase reflects the real situation.

¹ Schlech, W.F., Lavigne, P.M., Bortolussi, R.A. *et al.* (1983) Epidemic listeriosis - evidence for transmission by food. New England Journal of Medicine 308, 203-206

² Fleming, D.W. Cochi, S.L., MacDonald, K.L. *et al.* (1985) Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. New England Journal of Medicine 312, 404-407.

³ James, S.M., Fannin, S.L., Agee, B.A. *et al.* (1985) Listeriosis outbreak associated with Mexican style cheese - California. Morbidity and Mortality Weekly Report Report 34, 357-359.

⁴ Bille, J. and Glauser, M.-P. (1988) Listériose en Suisse. Bulletin des Bundesamtes für Gesundheitswesen. No. 3 28-29.

⁵ Broome, C.V. personal communication.

⁶ Frederiksen, W. personal communication

Again, even allowing for differences in reporting systems and effectiveness of surveillance, the range of reported incidences in countries with surveillance systems is worthy of note. For instance, reported incidences from Scandinavian countries, France, the United Kingdom and the United States range from less than 2 to 11.3 per million. Whether this reflects different dietary habits, consumption patterns, diagnostic routines or surveillance methods remains speculative.

Available evidence from France and the United Kingdom has led to an impression that the distribution of serovars and lysovars isolated from foodstuffs and from humans are different. Whether this is the result of a sampling bias or reflects a real phenomenon is as yet undetermined and warrants further investigation.

1.1.3. Natural History

Risk Groups. Pregnant women and their foetuses or newborn children are at particularly high risk; in the United States active surveillance project, 120 sporadic cases occurred per million births. Other broad and inclusive groups at increased risk of listerial infection are well characterized; they include those whose immune system is compromised or incompetent by virtue of a wide variety of reasons, and narcotic addicts whose resistance to infection is diminished. The role of gastric acid defences against infection or colonization remains unclear.

There is as well an ever present proportion of cases amongst those who have been previously healthy; in whom, at least, no predisposing cause could be found. An intriguing question exists about the role of intercurrent infection in changing the carrier state to one of clinical illness, by decreasing resistance or through some other mechanism. This needs further exploration, as does also the question of differential dietary factors and therefore different exposures in different risk groups.

It must be recognized that the proportions of immunocompromised and elderly persons in many populations are rising, increasing the numbers of those at risk from listerial disease.

Infectious Dose. Virtually nothing is known about the infectious dose of *L. m.* in man, nor is there good quantitative information relating to the amount of contaminated foodstuff ingested with the risk of acquiring disease. It is likely that infectious dose may be related to host susceptibility. Another possible influence worthy of investigation may be related to the food substrate.

Incubation Period. Evidence from both Switzerland and the United States, in those instances where both data on ingestion of the contaminated foodstuff and on onset of illness could be reliably determined, suggests an incubation period in adult disease of one to several weeks. This again raises the possibility of clinical illness being triggered in a carrier by some factor such as intercurrent viral infection. From this it is obvious that much has still to be learned concerning the incubation period.

Clinical Spectrum. Septic abortion, newborn and adult septicaemia, meningitis or meningo-encephalitis are the major clinical manifestations of listerial infection. There is conflicting and inconclusive evidence of the differing expression of clinical disease among the different risk groups. Clinical expression on the whole does not seem to be different in outbreak situations from that in sporadic cases; this, perhaps, further lends support to the speculation that many sporadic cases are associated with foodborne transmission. No association has yet been demonstrated between particular sero- or lysovars and particular clinical illnesses.

Carriage. The existence of a carrier state, and its relationship with disease, has long been the subject of speculation.

The question of immunity to listerial infection is poorly understood; a major reason for this is the continuing lack of definitive serology for Listeria and the possibility that cell-mediated immunity may be more important. The ratio of clinical to subclinical cases is not known also because of the lack of availability of specific serological techniques.

There has been speculation that carriage of the organism may be related to foodborne transmission, and that carriage may perhaps be triggered into illness.

1.1.4. Surveillance

In order to monitor the occurrence of human listeriosis, and to detect outbreaks, it is crucial that countries establish a surveillance system. The two major factors which define an outbreak are an increase in the number of cases over that expected and the detection of a common strain amongst isolates from the majority of such cases. Detection of the former requires a sensitive and stable system for monitoring listerial infection; and the latter efficient access to effective laboratory facilities for subtyping by some method - phagetyping, isoenzyme typing or gene restriction methods.

For both these requirements, there is a fundamental need for strong networks of reference laboratories at local, national and international levels.

The method of identification of a specific strain chosen will vary with the purposes of typing. If the purpose is the detection of a common (epidemic) strain different from other strains in the area, any method capable of effectively performing such discrimination is adequate. If the purpose is that of testing associations of virulence of particular strains, then the method must be amenable to international standardization. Similarly, evaluations of efficacy of different methods require international standards.

Effective surveillance exists to detect and identify outbreaks and changes in background pattern of disease; surveillance is of no use if the information it generates is not acted upon. Resources must therefore be made available for the rapid investigation particularly of outbreak phenomena but also of other changes in disease pattern.

The strategy plans of the United States, Switzerland, France and the United Kingdom include establishing and improving surveillance systems, and providing resources for investigation of outbreaks which include the collection and finer identification of isolates.

1.1.5. Conclusion

The most potent epidemiological tool available is the effective surveillance system; to be effective, however, the ability to investigate outbreaks rapidly is necessary. Here, the most helpful epidemiological tool is the case-control study. More than one such study may be necessary in a particular investigation. In conjunction with this, reference laboratories with the ability to rapidly and effectively sero- and phagetype isolates are indispensable to the control of listerial infection.

Sufficient evidence exists to be able to state that many cases of epidemic human listeriosis are due to foodborne transmission of L.m., and that this may also be the case with many sporadic ("non-epidemic") cases. However, the full range of foodstuffs thus implicated, and of the mechanisms of contamination of these foodstuffs, has by no means been totally described.

Cheeses, especially certain types of soft cheeses, have received much attention as vehicles of transmission; and rightly so, as they are foodstuffs which can have long shelf or refrigerator storage and which undergo no further listericidal treatment* before ingestion. In such a situation, in which the organism may have the conditions and time favourable for multiplication, there can be no such thing as a level of contamination which can be said with any confidence not to lead to a risk of infection.

Many other foodstuffs meet the above criteria as well as cheeses. Available evidence strongly suggests that other food products may well be vehicles for transmission (e.g. coleslaw has been proven to be a vehicle of infection). This Working Group urges that due attention in outbreak investigation be paid to the potential role of a wide variety of foodstuffs in the transmission of L.m. to humans.

1.2 The presence (qualitative and quantitative) of L. monocytogenes in foods

1.2.1 Preamble

Although much information has accumulated in the past three years on the qualitative and quantitative presence of L.m. in foods, the data are difficult to interpret and compare. Some of the difficulties involved in assessing the degree of contamination of the food supply are not unique to L.m., but some are. The areas of difficulty can be divided into 1) sampling/analysis and 2) other variables.

In the area of sampling/analysis, specific contributors to confusion include 1) lack of information on sampling design or inadequate sample size, 2) lack of information on where in the food chain the sample was collected (e.g. at time of processing, at retail, when during product shelf life), 3) lack of information on how the sample was collected (e.g. aseptically or not, conditions of storage prior to analysis), 4) lack of standardized analytical methodology and effect of method on outcome (e.g. method contraselective to specific serotype) and 5) lack of uniform reporting of data (e.g. whether quantitation was done, serotype(s) isolated). Some of these shortcomings were unavoidable, given the relative newness of the Listeria problem. While qualitative data on the presence of L.m. in foods is useful, reliable, quantitative data using valid sampling/analysis procedures are urgently needed.

In the area of "other variables", comparisons between countries is made difficult by 1) possible differences in the geographic distribution of L.m., 2) variation in animal husbandry practices and food processing, 3) variations in sanitary standards and practices between nations and between industries, 4) the only too recent awareness of the problem and 5) the inadequacy of refrigeration as a control mechanism.

All data on the qualitative and quantitative presence of L.m. must be interpreted cautiously and with the above provisos in mind.

1.2.2 Isolations from Dairy products

L.m. has been isolated from raw milk. In some surveys up to 5% of samples contained the organism, at levels of ≤ 10 cells per ml. The origin of L.m. in milk is mainly from faecal contamination. Several studies have confirmed a link between faecal excretion of L.m. and the condition of silage fed to the cows.

*Describes any process which will kill Listeria

Infected cows, suffering from an L.m. mastitis, have been reported as shedding L.m. in numbers of approximately 10^3 per ml in their milk. However, reports of such cows are infrequent. L.m. may occur intracellularly and this may be difficult to detect.

Raw goat and ewe milk are frequently used for cheese production but there are only limited data on the occurrence of L.m. in these milks.

The reported incidence of L.m. contamination of cheeses varies greatly between different surveys. Of all foods, cheeses have been found to be frequently contaminated with Listeria and associated with human disease. Soft-ripened cheeses (especially white mould and red smeared surface ripened) appear to be most suitable both to contamination and growth of L.m. This may be due to the higher pH of these cheeses in the later stages of ripening. Incidence figures from surveys vary from 1% of product being contaminated to 5-10%. When contaminated, certain cheeses are capable of supporting outgrowth of L.m. to populations of $10^4 - 10^7$ L.m. per gram. Knowledge of sampling time is critical in interpreting these numbers. Variations in manufacturing practices result in opportunities for post-process contamination. In theory, cheeses manufactured from L.m. contaminated raw milk would be more likely to be ultimately contaminated, but only a low percentage of contaminated raw milk has been reported. Surveys in Germany, Switzerland and France strongly suggest that cheeses made from pasteurized milk are as frequently contaminated with L.m. as cheeses made from unpasteurized milk due to contamination during manufacturing processes and handling.

Other dairy products vary in their risk of contamination with L.m. due to many factors. Acidified dairy products (e.g. cottage cheese) are, in principle, free of L.m. Some instances of ice cream contamination have been attributed to post-process contamination. Quantitative data are limited, but suggest contamination levels of from less than 1 to 15 L.m. per gram. The incidence data from surveys varies from zero to approximately 5.5% of products tested.

1.2.3 Meat and meat products

To interpret the data on incidence and numbers of L.m. in meats and meat products, the previously mentioned variables must be reemphasized.

In raw, ready to eat meat products, up to 30% have been reported to contain L.m. In sausages, receiving technologically a listericidal heat treatment, post processing manipulations, such as slicing, appear to be processing steps which are responsible for L.m. contamination of the product.

Quantitative studies on these products are lacking although in prospective studies on cooked poultry, an inoculum of 50 L.m. yielded populations of 10^7 L.m. within 2 weeks at 4.4°C storage temperature.

Not surprisingly, given the faecal carriage of L.m. by many mammals and birds and opportunity for contamination in the abattoir environment, numerous isolations from raw meats and poultry have been reported. L.m. has been isolated from raw beef and pork, lamb, ground and/or minced meat, and various poultry. Up to 30% (15-20% is the norm) of minced meat has yielded L.m. in some surveys with reported numbers of L.m. ranging from < 20 to 10^3 L.m. per gram. From 15-80% of retail poultry has been reported to be contaminated, depending on the sampling method (i.e. surface, whole carcass wash, swab). The numbers of L.m. in refrigerated, retail poultry have been observed to increase during product storage by up to 2 logs₁₀ in 10 days. Freezing appears to have no detrimental effect on L.m.

Fermented sausage products have also been surveyed and the contamination incidence varies greatly and may be up to 20%. When quantitative studies have been conducted on these products, numbers of L.m. have generally been lower than numbers found in non-fermented ready-to-eat cooked meats. When L.m. is present in cooked, ready to eat meats, surveys conducted thus far strongly suggest recontamination after cooking.

1.2.4 Other foods of animal origin

Although the data are limited, recent surveys suggest that cooked fish and other seafoods may also be contaminated with L.m. From 4-8% of cooked crabmeat and 3-4% of shrimp may yield L.m. on analysis. One enumeration study on frozen, butterfly shrimp conducted using a genetic probe suggested 200 L.m. per gram may be present. It is likely that game animals may be contaminated, but survey data are lacking. Neither internal nor external contamination of eggs has been reported.

1.2.5 Non-animal foods

Salad vegetables have been surveyed and found to be contaminated with L.m. Pre-cut, packaged salad vegetables have been reported to be contaminated. Certain vegetables, once cut, support the growth of L.m. Numbers of samples, at present, are too small to determine the incidence of contamination. Fruits have thus far been free of L.m. contamination.

1.2.6 Conclusions

Other foods may be added to the list of L.m. positives in the near future. Any food subject to contamination from bird or mammalian excreta, decomposing vegetation, or contaminated soil or water may ultimately be reported as contaminated with L.m. This underscores the importance of gathering information about the ability of various foods to support the growth of L.m., as well as obtaining quantitative data. It is important to note that of proven cases of foodborne listeriosis, the foods responsible, chopped cabbage (as a component of coleslaw) and cheeses, are foods capable of supporting the growth of L.m.

Although not necessarily indicative of a potential health problem, data on the presence of other Listeria species, particularly L. innocua, may be useful nonetheless. L. innocua has frequently been found in foods contaminated with L.m. and sometimes in greater numbers. In processed foods, the presence of L. innocua or other Listeria species may be a useful indicator of post-process contamination and requires further investigation.

1.3 The heat resistance of L. monocytogenes in food with particular reference to the pasteurization of milk

Many studies have been carried out to evaluate the effect of heat treatment on inactivation of L.m. in milk and conflicting results have often been reported. Differences in results generally can be explained by differences in experimental procedures used to assess thermal resistance. Early studies made with L.m. in milk contained in test tubes which were only partially submerged in a water bath during the heat treatment indicated that L.m. could survive a pasteurization treatment of 61.7°C for 35 min. Studies by others comparing this experimental approach with heat treatment of L.m. in milk held in sealed containers which were totally submerged in a water bath revealed that the organism was readily inactivated at 62°C with the sealed container method (D_{62°C}=0.1 to 0.4 min) but not with the partially submerged container method in which excessive tailing of survivor curves occurred. It was concluded that the partially submerged container method is an inaccurate means of measuring rates of thermal inactivation.

Later studies with L.m. added to milk and heated in sealed tubes at 71.7°C for 15 seconds revealed D-values of 0.9 sec. These results indicate High Temperature Short Time (HTST) pasteurization (71.7°C, 15 sec) would be sufficient to kill 10^{15} L.m. per ml of milk. However, this study did not address the intracellular nature of L.m., i.e., the organism may be present within leucocytes of milk from cows with a Listeria infection. This point was addressed by a study in which milk from cows inoculated with L.m. was heated in a commercial-type HTST pasteurizer at 72°C (minimum) for 15 sec. Surviving L.m. was occasionally detected by extensive testing of heat-treated milk using enrichment procedures. However, consideration must be given to the fact that an unusually large number of intracellular L.m. (10^3 to 10^4 cells/ml) was present in the milk. This is an extreme condition since recent studies of raw milk supplies revealed that only a small percentage (< 5% of raw milk) is contaminated with L.m. and the number of listeriae present is ≤ 10 cells per ml.

Recent studies using sealed tubes or slug-flow heat exchange methods in conjunction with more sensitive recovery procedures for detecting heat-injured Listeria revealed a $D_{71.7^\circ\text{C}}$ of 2.75 to 3.1 sec for L.m. added to milk and 4.1 sec for L.m. within bovine leucocytes. Other studies with milk from Listeria-infected cows did not detect surviving L.m. Furthermore, milk before pasteurization is often homogenized which disrupts leucocytes and puts L.m. in a freely suspended state.

Based on this information, the Working Group concluded that pasteurization* is a safe process which reduces the number of L.m. occurring in raw milk to levels that do not pose an appreciable risk to human health. It was also the consensus of the group that further research on the pasteurization of milk is not necessary, but additional studies are needed to determine the heat resistance of L.m. in other foods such as meat products. It is generally felt that dairy products may be recontaminated by L.m. after pasteurization through environmental sources. This not only applies to dairy products but is true for most food products.

1.4 Methods for the detection of L. monocytogenes in foods

A careful review of the many existing methodologies for detecting L.m. in foods readily convinced the Working Group that while many were inadequate, some held promise and several appeared to be quite effective. Direct plating procedures that require selective formulations to recover low numbers have generally proved quite unsuccessful. Single step enrichments that employ low temperatures with or without inhibitory constituents are now inordinately time-consuming for routine work and may fail to resuscitate sub-lethally injured cells. The working group believes that serial enrichment procedures employing a less selective primary medium followed by a selective secondary enrichment and a differential isolation agar are currently more promising than either of the above methodologies for isolating foodborne L.m..

A primary less selective enrichment medium is advantageous for the recovery of thermally stressed cells present in heat-processed foods and also to support the growth of very low numbers of Listeria admixed with a dense population of competitive indigenous flora. The primary medium is also essential to overcome adverse fluctuations in pH resulting from the nature and size of the food inoculum and the growth of indigenous competitive flora. The second selective enrichment medium is crucial to the success of serial enrichment. Its constituents must be determined by the growth potential of L.m. and competitive strains comprising the primary mixed culture. The working group felt that efforts to improve enrichment methods should focus upon optimizing the performance of the selective enrichment medium taking into consideration the need to recover as many of the serotypes in the sample as possible. Furthermore, it is desirable that improved methods be applicable to a broad range of food groups.

*As defined in Codex Code of Hygienic Practice for Dried Milk (CAC/RCP31-1983).

The Working Group strongly recommends that investigators who have developed improved detection procedures submit them to evaluation by rigorous collaborative studies conducted according to the guidelines promulgated by such international validating bodies as the International Dairy Federation (IDF), International Standardization Organization (ISO), Association of Official Analytical Chemists (AOAC) and the International Commission on Microbiological Specifications for Foods (ICMSF).

Sampling procedures, size and microbiological limits can only be given when a satisfactory method has been approved. To this end the guidelines laid down in the general principles for the establishment and application of microbiological criteria for foods should be strictly followed*.

To provide screening techniques appropriate to the monitoring of *Listeria* spp. in processed products and in-line samples the development of rapid yet sensitive techniques is envisaged. Promising developments include an ELISA using monoclonal antibody to flagellar antigens and gene probes for haemolysins, delayed hypersensitivity factor or other markers. Confirmation of isolates from rapid screening procedures must be efficiently abridged. Examination of the exaggerated number of verification tests now proposed or in use for L.m. has convinced the Working Group that many are unnecessary. Only the minimum number of reactions consistent with species identification should be used.

There is an urgent need for more *Listeria* Reference Laboratories to provide mainly sero- and phage typing for confirmatory and epidemiological useages. The World Health Organization should encourage the establishment of new laboratories and the continuation of existing ones.

2. FACTORS RESPONSIBLE FOR THE CONTAMINATION OF FOOD WITH LISTERIA MONOCYTOGENES

2.1 Presence of the organism in foods

Due to its widespread occurrence in nature L.m. has become part of the microbial ecosystem of food production and processing environments and is established on surfaces that come into contact with food and/or man.

The presence of L.m. in raw and transformed raw foods may be unavoidable.* The use of raw fertilizers in vegetable production is a contributing factor for contamination. The presence of L.m. in processed foods and packaged processed foods which have had a listericidal process indicates post-processing contamination from the environment. Control of L.m. within the food processing and preparation environments is essential.

*Microbiological criteria for foods - summary of recommendations of FAO/WHO Expert Consultations and Working Groups 1975-1981. WHO/VPH/83.54.

2.2. Survival, Growth and Transmission of L.m.

The critical issues for L.m. are to control its survival and growth, and to minimize the recontamination of processed foods from the environment. Survival and growth of L.m. are determined by the food substrate (including pH, water activity and salt concentration), the time temperature relationship of a heating process and effectiveness of other listericidal processes. Cut vegetables and soft cheese are suitable substrates for L.m., due to their favourable combination of conditions including pH, moisture, salt concentration, and nutrients. The surfaces of meats also are suitable substrates to support growth of L.m. after contamination from the environment. Contrary to most foodborne pathogens, growth of L.m. is not completely inhibited at refrigeration temperatures (4-6°C). Hence extended storage time should be discouraged.

Suitable sources and vectors for contamination within the process environment include drains, conveyor belts and other equipment, water supplies, condensates, aerosols, humans, insects and rodents. Suitable sources for contamination within food distribution channels, retail food establishments, and home environments include cutting surfaces and knives, water supplies, humans, insects, rodents and drip from contaminated exposed products onto other foods.

2.3 Control measures

2.3.1 Process plant environment

Appropriate control measures include (a) separating non-contaminated foods from contaminated foods, (b) limiting the potential for growth by elimination of unnecessary use of water and by application of adequate sanitation principles and, (c) limiting suitable vectors for L.m. transmission. General guidance on Good Manufacturing Practices (GMPs) and hygienic principles can be found in: Recommended International Code of Practice - General Principles of Food Hygiene, Codex Alimentarius Commission, 1979. Codes of practice for some specific commodities are also available as part of the Codex Alimentarius. A systematic approach to the assessment and control of hazards within the processing environment is termed the "Hazard Analysis Critical Control Point" system (HACCP). General guidance on GMPs and HACCP are contained within a number of documents produced by ICMSF and WHO¹⁻³.

¹ ICMSF (1986) Microorganisms in Foods, 2. Sampling for microbiological analysis: Principles and specific applications. 2nd edition. pp 92-107. Toronto: University of Toronto Press.

(A further book on HACCP by the ICMSF is expected to be published in 1988)

² WHO (1983) Guidelines on Prevention and Control of Salmonellosis. WHO/VPH/83.42.

³ WHO (1986) Prevention and Control of Foodborne Salmonellosis through the application of the Hazard Analysis Critical Control Point System, Report of an International Commission on Microbiological Specifications for Foods (ICMSF) ad hoc Committee, WHO/CDS/VPH/86.65.

*for definition of food categories please refer to para. 4.2.2

2.3.2 Retail food establishments and home environments

Similar control principles apply for retail food establishments and home environments as for food processing environments. Cross contamination should be eliminated from the environment through suitable design of equipment and appropriate sanitation procedures, and contamination from contact surfaces should be minimized. Raw and cooked products must be kept separate to avoid cross contamination by food handlers, contact surfaces and other transmission vectors.

Listericidal processes (e.g. pasteurization, cooking procedures), applied correctly, rid food of L.m., and prevention of recontamination is achievable if adherence to good hygienic practices is maintained. This applies to food manufacturing establishments, retail establishments and the home setting.

3. RESEARCH NEEDS

Although considerable research has been done to assess the association of L.m. with cases of illness and the role of food in the transmission of the organism, several unresolved questions requiring future research remain. These include:

3.1 Epidemiology

(i) Determination of the true incidence of human disease which will require increased efforts to diagnose listeriosis, especially in cases of stillbirth or miscarriage.

(ii) Improved surveillance systems for listeriosis, including reporting systems, to determine the accurate incidence of sporadic cases of listeriosis and to increase the recognition of outbreaks. This will include collection of information about the cases, particularly risk factors and demographic data, possible sources of infection, details of illness and sero- and phage type of the isolates.

(iii) Development of better serological procedures for use in surveillance studies. More specific serological methods may be useful in assessing the immune status and susceptibility of women to L.m. before pregnancy, and also to more specifically characterize case-related isolates.

(iv) Further investigations on the role of intercurrent infections and other factors in changing the carrier state to one of clinical illness by decreasing resistance or through other mechanisms.

(v) Development of alternatives to phage typing of L.m. such as isoenzyme typing and gene restriction methods.

3.2 Virulence/Pathogenicity

(i) Further definition of the mechanism of pathogenesis.

(ii) Determination of minimum infectious doses especially in specific cases of listeriosis when the actual number of L.m. in the food incriminated can be detected.

(iii) Study of genetics of virulence factors, including the expression of virulence under different storage and growth conditions.

(iv) Development of alternative (non-animal) tests for assessment of virulence, e.g. egg testing.

3.3 Methodology

Development of:

- (i) improved isolation procedures including serial enrichment procedures and differential isolation media;
- (ii) quantitative methods to enumerate L.m. in foods;

The improved methods should be applicable to a broad range of food groups and subsequently submitted to evaluation by rigorous collaborative studies to become officially acceptable as reference methods. More simple and rapid methods should be derived, for example from the reference methods, to enable food processors to monitor their process and food processing environment.

3.4 Contamination of raw and processed foods

Establishment of:

- (i) contamination cycles of L.m. including the role of human and animal excreta, the environmental spread of L.m. in sewage and surface water and the role of vermin in the dissemination of L.m. to slaughter animals, food processing plants and finally food products;
- (ii) sources of L.m. and point(s) of entry into the food chain;
- (iii) measures for the prevention of contamination of food processing plants and post-processing recontamination.

3.5 Effects of processing

Apart from the pasteurization of cows milk there is no systematic knowledge of the effects of different processes on the survival and growth of L.m. Research is needed on:

- (i) the effects of heat treatment on L.m. in foods other than cows milk such as meat products.
- (ii) the application of food irradiation to eliminate L.m. from certain types of soft cheeses without adverse effects on the organoleptic quality.
- (iii) the development of procedures which will eliminate L.m. from 'ready-to-eat' foods using the latest available findings.
- (iv) the development of processing equipment to further the advance of more hygienic procedures in food production.

3.6 Effects of extrinsic and intrinsic factors

Growth of L.m. in food is affected by factors such as pH, a_w , microbial flora and food additives. Studies should be made of the effects of these factors (singly or in combination) in foods, especially those which are subjected to long distribution periods.

3.7 Other Listeria species

The presence of L. innocua or other Listeria spp in processed foods may be a useful indicator of post process contamination. A study is required of the role of these organisms.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Listeria monocytogenes is a widely distributed environmental contaminant, whose primary means of transmission to humans is through contamination of foodstuffs at any point in the food chain, from source to kitchen. The total elimination of L.m. from all food is impractical and may be impossible.

Several major food commodities are implicated: milk and dairy products, meat, especially raw meat products, poultry and its products, vegetables and salads, seafoods. Contrary to most other foodborne pathogens, L.m. is able to multiply at refrigeration temperature (4-6°C). The critical issue, therefore, is not how to prevent the presence of L.m. in food, but how to control its survival in order to minimize the levels in food.

Data available at present suggest that foodborne listeriosis has a relatively low morbidity but is significant for its relatively high case fatality. The occurrence of listeriosis can be expected to vary greatly throughout the world due to the tremendous variation in dietary practices and food consumption (e.g., consumption of raw meat in some countries).

Several groups of the population have been identified as at risk: pregnant women and the unborn child, patients receiving treatment which alters the natural immunity, alcoholics, drug abusers, diabetics, AIDS patients and the elderly. However, cases of listeriosis have been reported in those who have been previously healthy.

It is crucial that countries establish surveillance systems to monitor the occurrence of human listeriosis and to detect outbreaks. The ability to investigate outbreaks rapidly is necessary. Surveillance systems require strong supporting networks of reference laboratories for sero-, phage and other forms of typing at local, national and international levels.

It should be remembered that a wide variety of foodstuffs can be involved in the transmission of L.m. to humans thus it is important to gather information on the ability of various foods to support growth of L.m. as well as to obtain quantitative data. Information on the presence of other Listeria spp., e.g., L. innocua, may be useful as an indicator of post-processing contamination and requires further investigation.

Pasteurization is a safe process which reduces the number of L.m. occurring in raw milk to levels that do not pose an appreciable risk to human health. Further research on the pasteurization of milk is not necessary but additional studies are needed to determine the heat resistance of L.m. in other foods such as meat products.

Listericidal processes (e.g., pasteurization, irradiation, cooking) applied correctly will rid foods of L.m., but recontamination can occur during further manipulation of the products, particularly with those foods which are not aseptically packaged immediately after the listericidal treatment. The risk of recontamination can be reduced by adherence to good hygiene practices in food manufacturing establishments, retail establishments and the home environment.

There has been considerable research on methods for the detection of L.m. in foods which indicates that serial enrichment procedures employing a less selective primary medium followed by a selective secondary enrichment and differential isolation media are the most promising. Improved isolation methods should be applicable to a broad range of food groups, should be able to recover as many serotypes in the sample as possible and should be evaluated by rigorous collaborative studies. There is a need for simpler, rapid methods to be developed from reference methods to enable food processors to monitor their processes and food processing environments.

4.2 Recommendations to national public health authorities on how to ensure safeguarding the consumer

4.2.1 Statement of purpose.

1. To reduce the incidence of foodborne listeriosis.
2. To limit, or eliminate, where technologically feasible, the burden of L.m. in the food supply.
3. To enhance consumer confidence in the safety of the food supply.

4.2.2 Definitions of food categories

1. Raw foods (e.g. raw vegetables, raw meats).
2. Transformed raw foods (e.g. raw foods mixed with other ingredients (cole slaw), fermented sausage, raw milk cheeses).
3. Processed foods (listericidal* process applied) with subsequent handling (e.g. certain types of cheeses, commercially processed meats sliced or altered in retail establishments).
4. Processed foods (listericidal process applied) in intact packages (e.g. pasteurized milk, dairy products, cooked meats in sealed containers). Such foods receive the listericidal process in their packages or they are aseptically packaged immediately after the listericidal process is applied.

4.2.3 Specific recommendations to Public Health Authorities

The Working Group made the following recommendations that Public Health Authorities should:

1. Actively promote research to determine ways in which (a) L.m. can be reduced or eliminated from the raw food supply and, (b) the contamination of processed food with L.m. in areas of greatest public health impact (e.g. delicatessens, caterers, restaurants) can be lessened.
2. Commence or continue public education programmes to help consumers to protect themselves from L.m. in food categories 1 to 3 (raw foods, transformed raw foods and processed foods which are subsequently handled).
3. Ensure that consumers are not given a false sense of security about the safety of food categories 1 to 3.
4. Ensure that foods in intact packages which have received a listericidal process at any point in their production (food category 4) are free of L.m. during the product's normal shelf life and as long as the packaging integrity remains.
5. Encourage the use of ionizing radiation for the elimination of L.m., particularly for foods which are highly susceptible to L.m. contamination and growth and for any packaged food, processed or raw.

*Describes any process which will kill Listeria.

6. Consider the removal from the market of foods of category 4 found to be contaminated with L.m.
7. Withdraw from the market foods in any category which are demonstrated to be causally associated with human cases of listeriosis.
8. Fully consider, when withdrawal of food products from the market is indicated, all the ramifications and possible consequences prior to withdrawal. Such decisions should be based on the best available scientific information and made only after careful risk analysis, with the goal of maintaining consumer confidence in the food supply which cannot be made totally Listeria-free.
9. Work co-operatively with affected, or possibly affected, segments of the food industry to prevent, limit and, where possible, eliminate the presence of L.m. in foods.
10. Cooperate with the food industry, universities and research institutes to coordinate essential research on L.m.
11. Implement and maintain surveillance systems for all forms of human listeriosis to detect outbreaks, and to monitor progress towards its reduction and provide epidemiological and microbiological resources for energetic investigation of outbreaks.
12. When contributing to the WHO Surveillance Programme for Foodborne Infections and Intoxications in Europe, exchange data concerning foodborne listeriosis through the WHO Collaborating Centre coordinating the programme.
13. Educate all health professionals about the relatively new problem of foodborne listeriosis so that they can make appropriate recommendations to patients at high risk for the disease on the relative risks of foodborne listeriosis versus the benefit of consuming foods of categories 1 to 3.

WHO should act as a point of information exchange regarding foodborne listeriosis research and should facilitate the establishment of reference laboratories for L.m.

4.3 Recommendations to the Food Industry

The general recommendations made by the WHO Consultation on Prevention and Control of Listeriosis, Berlin (West), 1986* are still valid in principle. The Group considers, however, that certain points should be re-emphasized in the light of experience.

The measures to be taken to reduce levels or limit growth of L.m. on food contact surfaces in the environment of food factories have been shown from experience to be exactly the same as those for other pathogens. The fact that L.m. can grow at chill temperatures makes the reduction of their numbers or their elimination all the more important. It has been shown that schemes applied in the past for Salmonella spp in areas such as meat processing are also effective against L.m. if they are applied with great attention to detail (adequate washing, rinsing, sanitizer concentrations and contact time). It should be emphasized that L.m. is particularly common in wet environments in food factories and that maintenance of a dry environment wherever feasible is one of the best ways of limiting growth of this organism.

The HACCP approach has been recommended as the best way to assure safety and quality of foods, however, this approach is not uniform in all sectors of industry. These recommendations are made taking these factors into account.

*WHO (1986) Report of the WHO Consultation on Prevention and Control of Listeriosis
WHO/CDS/VPH/87.69.

4.3.1 Statement of objectives

1. Short-term:
 - (a) To assure food safety.
 - (b) To reduce or eliminate where technologically feasible the burden of L.m. in foods of category 3.
 - (c) To promptly enhance education, training and awareness in the food industry.
2. Medium-term:
 - (a) To continuously re-evaluate or verify HACCP procedures to assure the safety of processed foods.
3. Long-term:
 - (a) To overcome the problem of L.m. in foodstuffs which do not undergo listericidal treatments (e.g., in category 2) but which previously have been accepted as safe.
 - (b) To expand education and training to more segments of the food industry.

4.3.2 Specific recommendations to the Food Industry

The Working Group made the following recommendations to the Food Industry and/or Commodity Organizations that they should:

1. Promote the HACCP approach and assure the safety of food products, by education and motivation of all those working in the food industry.
2. Apply the HACCP approach in order to:
 - (i) identify pathogens associated with production environments and raw materials;
 - (ii) identify critical sources of contamination and eliminate them where possible;
 - (iii) identify vehicles of contamination and eliminate them;
 - (iv) identify opportunities for survival and growth of undesirable microorganisms in the factory, environment and product.
3. Concerning 2(iv), assure:
 - (i) that bactericidal treatments (heat, irradiation, etc.), are adequate and result in the killing of L.m.;
 - (ii) that sanitizer concentrations and sanitization regimes are adequate for killing L.m..
4. Carry out or promote research to seek new ways of eliminating or limiting the growth of L.m. in foods using natural or synthetic inhibitors.
5. Cooperate with regulatory agencies regarding the presence of L.m. in manufactured products and industry's efforts to eliminate the organism.

6. Collaborate closely with food processing equipment manufacturers to improve hygienic design.
7. Collaborate closely with regulatory/public health authorities to elaborate codes of hygienic practice for different sectors of food production.
8. Collaborate with international (e.g. WHO) and national organizations and universities to devise model food microbiology curricula which include the HACCP approach.
9. Research into new technological solutions to the problem of L.m. in products which do not undergo listericidal treatments before consumption, but which have traditionally been regarded as safe (e.g. foods of category 2).

WHO INFORMAL WORKING GROUP ON FOODBORNE LISTERIOSIS,

Geneva, 15-19 February 1988

LIST OF PARTICIPANTS

- ¹ADAMS, C.E. Food Safety and Inspection Service, US Department of Agriculture, Office of the Administrator, Rm 335-E Administration Building, Washington, D.C. 20250, USA
- ARCHER, D. Division of Microbiology, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, D.C., 20204, USA
- ²BILLE, J. Laboratoire de bacteriologie medicale, Hopital Cantonal Universitaire de Vaud, Lausanne, Switzerland
- ²BILLO, N. Medical Epidemiology, Federal Office of Public Health, Bollwerk 27, CH-3001 Bern, Switzerland
- BIND, J.L. Laboratoire Vétérinaire Départemental, 46 Avenue Gustave Eiffel, 37023 Tours Cedex, France
- ³BREER, C. Institute for Clinical Microbiology and Immunology, Frobergstrasse 3, CH-9000 St. Gallen, Switzerland
- BROOME, C.V. (Vice-chairperson) Meningitis and Special Pathogen Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga 30333, USA
- ⁴COX, L.J. Microbiology, Central Quality Assurance Laboratory, Nestec Ltd., CH-1800 Vevey, Switzerland
- ⁵CROFTS, N. Central Public Health Laboratory Service, Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ, UK
- ⁶DOYLE, M.P. Food Research Institute, University of Wisconsin, Madison, Wisconsin 53706, USA
- ¹ENGEL, R.E. Food Safety and Inspection Service, US Department of Agriculture, 300 12th Street, S.W., Washington, D.C. 20250, USA
- ⁷FRANCIOLI, P. Division of Hospital Preventive Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
- ⁸FREDERIKSEN, W. Department of Diagnostic Bacteriology, State Serum Institute, Amager Boulevard 80, Copenhagen S, Denmark
- ⁶HOPPER, P.F. General Foods, White Plains, N.Y., U.S.A. (Committee of Food Microbiology) International Life Science Institute
- KAMPELMACHER, E.H. (Chairman) National Institute of Public Health and Environmental Hygiene, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.
- ROBERTS, D. (Rapporteur) Food Hygiene Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

- ⁹SEELIGER, H. Institute for Hygiene and Microbiology, University
Würzburg, Josef Schneider Str. 2, Bau 17, D-8700
Würzburg, Federal Republic of Germany
- TEUFEL, P. Bundesgesundheitsamt, Thielallee 88-92, Postfach 330013,
D-1000 Berlin 33
- TERPLAN, G. Chair for Hygiene and Technology of Milk, University of
Munich, D-8000 München 40, Schellingstrasse 10/III,
Federal Republic of Germany
- ¹⁰TWEDT, R.M. Division of Microbiology, Center for Food Safety and
Applied Nutrition, Food and Drug Administration,
Washington, D.C., 20204, USA
- VUJICIC, I.F. Faculty of Agriculture, Livestock Research Institute,
21000 Novi Sad, Veljka Vlahovica 2, Yugoslavia
- ¹¹YNDESTAD, M. Dept. of Food Hygiene, Norwegian College of Veterinary
Medicine, Post Box 8146, DEP, Oslo 1, Norway

OBSERVERS

- DAELMAN, W. Direction Générale de l'Agriculture, Commission des
Communautes Européennes, 200 rue de la Loi, B-1049
Bruxelles, Belgium
- RALOVICH, B.S. University Medical School of Pécs, Szigeti st. 12. H-7643
Pécs, Hungary

SECRETARIAT

- BECKERS H.J.
(WHO Temporary Adviser) National Institute of Public Health and Environmental
Hygiene, PO Box 1, 3720 BA Bilthoven, The Netherlands
- FUJIKURA, T. Veterinary and Public Health Unit, Division of
Communicable Diseases
- KAFERSTEIN, F.K.
(Secretary) Manager, Food Safety Unit, Division of Environmental
Health
- PROST, A. Division of Environmental Health
- REY, M. Division of Communicable Diseases

-
- ¹ Sponsored by US Department of Agriculture
- ² Sponsored by Swiss Public Health Service
- ³ Sponsored by Institute of Clinical Microbiology and Immunology,
St. Gallen, Switzerland
- ⁴ Sponsored by Nestec Ltd., Switzerland
- ⁵ Sponsored by Public Health Laboratory Service, London, UK
- ⁶ Sponsored by International Life Science Institute
- ⁷ Sponsored by the Département de l'Intérieur et de la Santé Publique,
Canton de Vaud, Switzerland
- ⁸ Sponsored by the Danish Ministry of Public Health
- ⁹ Sponsored by University Würzburg, Federal Republic of Germany
- ¹⁰ Sponsored by US Food and Drug Administration
- ¹¹ Sponsored by Government of Norway.