Symposium Series on Food Microbiology

ABSTRACTS AND EXTENDED ABSTRACTS

Sponsored by the

ILSI North America Technical Committee on Food Microbiology

ILSI International Life Sciences INSTITUTE

International Association for Food Protection

in conjunction with the

International Association for Food Protection 88th Annual Meeting

August 5–8, 2001
Hilton Minneapolis
Minneapolis, Minnesota USA

Proceedings of the Symposium Series on Food Microbiology

in conjunction with the

International Association for Food Protection 88th Annual Meeting

August 5–8, 2001

Hilton Minneapolis

Minneapolis, Minnesota, USA

ABSTRACTS AND EXTENDED ABSTRACTS

The Symposium Series on Food Microbiology was sponsored by the ILSI North America Technical Committee on Food Microbiology in conjunction with the IAFP 88th Annual Meeting, held in Minneapolis, Minnesota, USA, August 5–8, 2001, and made possible in part by an education grant from the National Food Processors Association.

International Life Sciences Institute
One Thomas Circle, N.W., Ninth Floor
Washington, D. C. 20005-5802 USA
Tel: 202-659-0074
Fax: 202-659-3859

Printed in the United States of America

CONTENTS

***The speaker's name is capitalized, and only the speaker's affiliation is listed.**

Preface

The ***Symposium Series on Food Microbiology*** consisted of three international symposia sponsored by the North American Branch of the International Life Sciences Institute (ILSI N.A.) Technical Committee on Food Microbiology at the International Association for Food Protection (IAFP) 88th Annual Meeting, held August 5–8, 2001, in Minneapolis, Minnesota, USA.

The first of the three symposia, the day-long ***Symposium on Moving Beyond HACCP: Food Safety Objectives,*** was a collaborative effort between ILSI N.A. and the International Committee on Microbiological Specifications for Foods (ICMSF) to introduce the concept of food safety objectives, or FSOs, to a U.S. audience. FSOs, essentially statements of the maximum frequency or concentration of a microbiological hazard in a food considered consistent with an acceptable level of consumer protection, are realized through the use of GHP/GMP (Good Hygiene/Manufacturing Practice) and HACCP (Hazard Analysis Critical Control Point). FSOs are the brainchild of the ICMSF, an international standard-setting organization of world-renown scientists that provides guidance on appraising and controlling the microbiological safety of foods in international trade. The ICMSF recommended the concept to industry and worldwide control authorities as a way to translate "risk" into a definable goal for microbial food safety management. FSOs have the potential to link microbial standards, real-world public health requirements, and good science. FSOs could provide the scientific basis for industry to use in selecting and implementing measures to control the hazard(s) of concern in specific foods or food operations, and for control authorities in developing and implementing inspection procedures to assess the adequacy of control measures adopted by industry and to quantify the equivalence of inspection procedures in different countries. The ILSI N.A. Technical Committee on Food Microbiology invited scientists representing academia, government, and industry, as well as representatives of the international food microbiology community and the public sector, to discuss the application of FSOs to improve food safety and achieve public health goals.

Mycobacterium paratuberculosis is the etiologic agent of Johne's disease, a severe intestinal wasting disease in cattle. Surveys have shown that up to 41% of U.S. dairy herds carry the organism. This organism has a high tolerance to heat, and some studies have suggested that it may survive pasteurization conditions used for fluid milk. Similarities between Johne's disease in cattle and Crohn's disease in humans has led researchers to examine the association of this organism with human Crohn's disease, and some to postulate that it may be a causative agent of the disease. The second of the three symposia, the ***Symposium on*** Mycobacterium paratuberculosis***: Villain or Bystander?,*** brought together leading international experts to examine the evidence for and against the association of the organism with human Crohn's disease, implications for the future, and future research directions needed to definitively address the issue.

A primary objective of the ILSI N.A. Technical Committee on Food Microbiology is to promote improved understanding of microbial food safety hazards by sponsoring research. The results of research funded by the committee during its fifth grant cycle (1998–2001) were reported at the last of the three symposia. The research activities that were previewed at the symposium will be published in the peer-reviewed scientific literature by the individual investigators.

ILSI North America is a public, nonprofit foundation that advances the understanding of scientific issues related to the nutritional quality and safety of the food supply. By bringing together scientists from academia, government, industry, and the public sector, ILSI N.A. seeks a balanced approach to solving problems with broad implications for the well-being of the general public. The ILSI N.A. Technical Committee on Food Microbiology was formed in 1987 to address issues related to microbial food safety hazards. The committee has funded over two million dollars of research on several important foodborne pathogens and has sponsored numerous scientific meetings in the area of microbial food safety. Since 1993, the committee has collaborated with IAFP by sponsoring an annual international symposium series on food microbiology. ILSI N.A. and the Technical Committee on Food Microbiology hope that making the abstracts and extended abstracts of the presentations in these symposia available to the public will provide important information to a worldwide audience and will help stimulate initiatives to improve the safety of our global food supply.

MOVING BEYOND HACCP: FOOD SAFETY OBJECTIVES

The International Commission on Microbiological Specifications for Foods (ICMSF) Framework for Managing the Safety of Foods

TERRY A. ROBERTS, Food Safety Consultant, 59 Edenham Crescent, Reading RG1 6HU, United Kingdom (ICMSF member 1978–2000, Chairman 1991–2000)

History

The ICMSF was formed in 1962 through action by the International Committee on Food Microbiology and Hygiene, a committee of the International Union of Microbiological Societies (IUMS). Through the IUMS, the ICMSF is linked to the International Union of Biological Societies and the World Health Organization (WHO) of the United Nations.

In the 1960s the importance of foodborne disease was recognized, leading to greatly increased microbiological testing of foods. This created unforeseen problems in international trade in foods because different analytical methods, and sampling plans of doubtful statistical validity, were being used. Analytical results were used without agreed-upon concepts of biological significance and acceptance criteria, creating confusion and frustration.

The ICMSF was founded in this environment to (1) assemble, correlate, and evaluate evidence about the microbiological safety and quality of foods, (2) consider whether microbiological criteria would improve and assure the microbiological safety of particular foods, (3) propose, where appropriate, such criteria, and (4) recommend methods of sampling and examination.

Thirty years later the role of the ICMSF remains to give guidance on (1) appraising and controlling the microbiological safety of foods and (2) microbiological quality, since this influences consumer acceptance and losses from spoilage. Meeting these objectives assists international trade, national control agencies, the food industry, agencies concerned with humanitarian food distribution, and consumer interests.

Functions

The ICMSF provides scientific information through extensive study and makes recommendations based on that information without prejudice. Results of the studies are published as books, discussion documents, or refereed papers. Major publications are listed below.

The ICMSF functions as a working party - it is not a forum for the reading of papers. Meetings consist largely of discussions in subcommittees, debate to achieve consensus, the editing of draft materials, and planning activities. Most work is done between meetings by the editorial committee and members, sometimes with the help of nonmember consultants.

Since 1962, 33 meetings have been held in 17 countries. Commission members often participate in symposia organized by microbiologists or public health officials of the host country.

Currently, the membership consists of 15 food microbiologists from 11 countries, with combined professional interests in research, public health, official food control, education, product and process development, and quality control. The scientists come from government laboratories in public health, agriculture, and food technology, from universities, and from the food industry. The ICMSF is also assisted by consultants who are specialists in particular areas of microbiology that are critical to the success of the commission. New members and consultants are selected for their expertise, and are ***not*** national delegates. All work is voluntary without fees or honoraria.

Three subcommissions (Latin American, South-East Asian, and Balkan and Danubian) promote ICMSF activities among food microbiologists in their regions and facilitate communication worldwide.

The ICMSF raises its own funds to support its meetings. Support has been obtained from government agencies, WHO, and the food industry (more than 80 food companies and agencies in 13 countries). Some funds are received from the sale of its books.

Projects

The first book published by the ICMSF, initially published in 1968 and revised in 1978 (ICMSF 1968), fostered comparative and collaborative evaluation of methods with the objective of achiev-

ing agreed-upon methods appropriate for use in international trade. A worldwide program of comparative testing of methods resulted in 17 publications.

The second ICMSF book, published in 1974 and revised in 1986 (ICMSF 1986), recognized the need for scientifically based sampling plans for foods in international trade, and explained the statistical principles underlying attributes of sampling plans. It described two- and three-class sampling plans and introduced the concept of "choice of case" based on microbiological risk.

The ICMSF's third publication, a two-volume monograph on the ***Microbial Ecology of Foods*** (ICMSF 1980a, 1980b), summarized the factors affecting the growth, death, and survival of microbes on foods and provided a detailed overview of the microbiology of 14 commodity groups, identifying the most common types of spoilage, microbiological hazards, and their control.

The fourth book (ICMSF 1988) reflected the commission's conclusion that HACCP (Hazard Analysis Critical Control Point) systems, rather than extensive microbiological testing, offer the most effective approach to the control of microbiological hazards at all stages of the food chain.

Recognizing the need for a quick reference manual that summarizes individual foodborne diseases and provides a thorough but concise review of the literature on the growth, survival, and death responses of foodborne pathogens led to the development of the fifth book (ICMSF 1996). The information in that publication has many uses, including support of the control measures selected for use in food safety systems based on good hygiene practices (GHP) and HACCP.

A more recent ICMSF publication (ICMSF 1998) updates and extends an earlier publication (ICMSF 1980b) for 16 commodity areas, describing the initial microbial flora and the prevalence of pathogens, the microbiological consequences of processing, typical spoilage patterns, episodes implicating those commodities with foodborne illness, and measures to control pathogens.

An ICMSF book published in 2002 (ICMSF 2002) is based on a 1987 publication (ICMSF 1986), which put the microbiological testing of foods on a sounder statistical basis through sampling plans that remain useful at a port of entry when there is no information on the conditions under which a food has been produced or processed. No sampling plan can ensure the absence of a pathogen in food, and testing foods cannot guarantee food safety.

The 2002 publication (ICMSF 2002) illustrates how systems such as HACCP and GHP provide greater assurance of safety than does microbiological testing, but it also identifies circumstances where microbiological testing still plays a useful role in systems to manage food safety. The reader is introduced to a structured approach for managing food safety, including sampling and microbiological testing. The text outlines how to meet specific food safety goals for a food or process using GHP and HACCP.

The concept of a food safety objective (FSO) is recommended to industry and control authorities to use in translating "risk" into a definable goal for establishing food safety management systems that incorporate the principles of GHP and HACCP. FSOs provide the scientific basis for industry to use in selecting and implementing measures that control the hazard(s) of concern in specific foods or food operations, and for control authorities to use in developing and implementing inspection procedures to assess the adequacy of control measures adopted by industry and in quantifying the equivalence of inspection procedures in different countries.

Microbiological testing can be a useful tool in the management of food safety. However, microbiological tests should be selected and applied with awareness of their limitations in addition to knowledge of their benefits and the purposes for which they are used. In many instances other means of assessment are quicker and more effective.

The need for microbiological testing varies along the food chain. Points should be selected in the food chain where information about the microbiological status of a food will prove most useful for control purposes. Similarly, in a food operation, samples may be collected from different points of a process for control purposes.

Finally, a framework is provided by which importing countries can assess whether foods from other countries have been produced in a manner that provides a level of protection equivalent to that required for domestically produced foods. This book (ICMSF 2002) illustrates the insensitivity of even statistically based sampling plans, and encourages the use of a rational approach to microbiological testing in systems that manage food safety through GHP and HACCP.

Several new chapters are based on the experience of the food industry in controlling salmonellae, ***Listeria monocytogenes,*** and ***Escherichia coli*** O157:H7, on tightened or investigational sampling, on microbiological testing of the processing environment, and on the use of statistical process control to detect trends and work toward continuous improvement.

The book is intended to be useful for anyone engaged in setting microbiological criteria, be it for the purpose of governmental food inspection and control or for use by industry. For students of food science and technology, it offers a wealth of information on food safety management and many references for further study.

The commission believes that its original objectives are still relevant today. The creation of the European Union, the many other political changes occurring throughout the world, the growth of developing countries seeking export markets, and the growing trade in foods worldwide, as evidenced by the passage of the General Agreement on Tariffs and Trade and the North American Free Trade Agreement, all point to the continuing need for independent recommendations, such as those of the commission. It is essential that import/export policies be established as uniformly as possible and on a sound scientific basis.

The commission's overall goal will continue to be to enhance the safety of foods moving in international commerce. The commission will continue to strive to meet this goal through a combination of educational materials, the promotion of the adoption of food safety management systems using microbiological food safety objectives (i.e., this conference), encouragement of the use of HACCP and GHP, and the recommendation to use sampling plans and microbiological criteria where they have been developed according to Codex Alimentarius principles and where they offer increased assurance of microbiological safety. The future success of the ICMSF will continue to depend on the efforts of members, support from consultants who generously volunteer their time, and those who provide the financial support so essential to its activities.

Further information, including that on the ICMSF's membership, can be found at www.ICMSF.org

Acknowledgment

This extended abstract is based on information from an article that appeared in ***Food Control*** 1996;7:99–101.

References

International Commission on Microbiological Specifications for Foods (ICMSF). Application of the Hazard Analysis Critical Control Point (HACCP) system to ensure microbiological safety and quality. Microorganisms in Foods, book 4. London: Blackwell Scientific Publications, 1988 (also published in paperback as "HACCP in microbiological safety and quality")

ICMSF. Characteristics of microbial pathogens. Microorganisms in Foods, book 5. London: Blackie Academic & Professional, 1996 (also available from Aspen Publishers, Gaithersburg, MD)

ICMSF. Factors affecting the life and death of microorganisms. Microbial Ecology of Foods, vol. 1. New York: Academic Press, 1980 (a)

ICMSF. Food commodities. Microbial Ecology of Foods, vol. 2. New York: Academic Press, 1980 (b)

ICMSF. Microorganisms in foods: their significance and methods of enumeration, 2nd ed. Microorganisms in Foods, book 1. Toronto: University of Toronto Press, 1968, 2nd edn. 1978

ICMSF. Sampling for microbiological analysis: principles and specific applications, 2nd ed. Microorganisms in Foods, book 2. Toronto: University of Toronto Press, 1987 (originally published in 1974 and reprinted in 1978)

Additional Reading: Other ICMSF Technical Papers

ICMSF. Choice of sampling plan and criteria for *Listeria monocytogenes*. Int J Food Microbiol 1994;22:89–96

ICMSF. Establishment of microbiological safety criteria for foods in international trade. World Health Stat Q 1997;50:119–123

ICMSF. Microbial ecology of food commodities. Microorganisms in Foods, book 6. London: Blackie Academic & Professional, 1998 (also available from Aspen Publishers, Gaithersburg, MD)

ICMSF. Microbiological testing in food safety management. Microorganisms in Foods, book 7. Gaithersburg, MD: Aspen Publishers / Kluwers Academic, 2002

ICMSF. Potential application of risk assessment techniques to microbiological issues related to international trade in food and food products. J Food Protect 1998;61(8):1075–1086

ICMSF. Principles for the establishment of microbiological food safety objectives and related control measures. Food Control 1998;9(6):379–384

Simonsen B, Bryan FL, Christian JHB, et al. Report from the International Commission on Microbiological Specification for Foods (ICMSF): prevention and control of food-borne salmonellosis through application of Hazard Analysis Critical Control Point (HACCP). Int J Food Microbiol 1987;4:227–247

van Schothorst M. Sampling plans for *Listeria monocytogenes*, Food Control 1996;7(4/5):203–208

Assessing Microbial Food Safety Risks and Establishing Food Safety Objectives

ROBERT L. BUCHANAN, ICMSF and Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Room 3832, 200 C Street, SW, Washington, D.C. 20204, USA

Foods have a unique place in the minds of consumers worldwide. This reflects the fact that, in addition to nourishing our bodies, the act of eating plays a large role in defining our experiences as individuals, as families, and as part of communities and regions. Our memories are often tightly linked with events revolving around meals eaten at family gatherings or repasts during our travels or vacations. Foods even play a central role in literature. For example, the simple act of having tea and pastries has been the focus of literary endeavors that have ranged from the surreal meeting of Alice and the Mad Hatter to the philosophical pondering of Marcel Proust. Thus, it is not surprising that risks associated with foods are viewed in a unique manner and are typically considered separately from the risks associated with life's other activities. Foods are produced and consumed with a great deal of care, and most foods have enviable records in relation to safety. Although no food can be made completely free of all risks, the food safety systems and control measures that have been developed by industry and food control agencies effectively manage public health risks to a high degree.

One of the basic concepts underlying the development of food safety systems is that the degree of control required in the production, processing, distribution, marketing, preparation, and consumption of a food is proportional to the risk to public health that would be incurred if control were not exercised. An assumption underlying this basic concept is that we are capable of measuring food safety risks. In the case of microbiological food safety concerns, this was typically done in a qualitative manner, based generally on expert advice or hazard analyses. It was generally thought that the microbiological food safety concerns related to foodborne infectious agents were too complex to be amenable to the types of quantitative risk assessments that were increasingly used for chemical food safety concerns over the past three decades. However, beginning in the second half of the 1990s, the first "research" microbial food safety risk assessments began to be published, followed quickly by increasingly sophisticated risk assessments by regulatory bodies and international agencies. Examples of quantitative microbial food safety risk assessments that have been completed or are currently underway include ***Salmonella enteritidis*** in eggs and egg products, ***Salmonella*** spp. in broilers, ***Campylobacter jejuni*** in broilers, ***Vibrio parahaemolyticus*** in molluscan shellfish, and ***Escherichia coli*** O157:H7 in ground beef.

Different types of microbial risk assessments can be conducted depending on the specific risk management questions that are being posed. Probably the best known are pathogen/product pathway analyses where a specific pathogen/product pair is examined in detail. These are typically done to identify the parameters that contribute to the overall risk or to evaluate the likely impact of potential intervention strategies. Risk ranking is another type of risk assessment that is used to compare the relative risks among different foods for a single pathogen, multiple pathogens for a single food, or potentially for multiple pathogens and multiple foods. This type of risk assessment is generally conducted as a means of establishing priorities, such as determining which foods should receive the greatest attention to decrease the public health impact of a pathogen. A third type of microbial food safety risk assessment is the "geographical" assessment, which examines the likelihood of an agent such as Bovine Spongiform Encephalopathy (BSE) entering a new region. Another type of risk assessment that could be potentially used in addressing microbial food safety concerns is risk/risk trade-off analysis. This type of risk assessment is conducted when an intervention strategy that will be used to decrease a risk associated with one hazard has its own risks. Examples of this type of risk assessment are the evaluations that have been conducted to compare the reduction of risks from infectious diseases achieved by chlorination of drinking water versus the increased toxicological risks associated with the increased incidence of chlorinated organic compounds such as chlo-

roform. This type of risk assessment can be considered a "risk reduction optimization evaluation" wherein both risks can be reduced to the lowest possible extent.

While the rapid emergence of quantitative microbial food safety risk assessment is dramatically changing the manner in which food-associated microbial threats to public health are evaluated, it is not a paradigm shift. Instead, it can be viewed as the next step in the evolution of hazard evaluations in terms of sophistication and transparency. Through the risk assessment frameworks that have been recommended by bodies such as the Codex Alimentarius Committee on Food Hygiene, the U.S. National Advisory Committee on Microbiological Criteria for Foods, and the International Commission on Microbiological Specification for Foods, the scientific information pertinent to a microbial food safety concern can be laid out in a structured manner to determine what knowledge is available and the uncertainty associated with that knowledge. Although there are some differences in the details of the approaches recommended by these bodies, all of them divide the process into four segments: (1) hazard identification (definition of the problem), (2) exposure assessment (the population exposure to the hazard), (3) hazard characterization (the relationship between the levels of an agent and the frequency and severity of adverse public health effects), and (4) risk characterization (overall calculation of risk plus an evaluation of the associated uncertainty). Simplistically, a quantitative risk assessment can be viewed as a mathematical relationship:

$$R = f(e, dr),$$

which denotes that risk is a function of the exposure of a population to an agent and the relationship of the level of exposure and the extent of adverse effects. By conducting the exposure assessment and determining the hazard characterization (i.e., the dose-response relationship), it is possible to measure the risk (i.e., risk characterization).

As the degree of sophistication with which the measurement of food safety risks has advanced in the past 10 years, there is increasing interest in how these new capabilities can be harnessed to address food safety risk management at both the national and the international level—particularly at the international level. This has been fueled by the issuance of the World Trade Organization's "Sanitary and Phytosanitary" and "Technical Barriers to Trade" agreements, which have articulated a clear role for risk assessment and international standards-setting bodies such as the Codex Alimentarius Commission. One of the key concepts that has arisen from these international agreements is the articulation and establishment of a nation's "appropriate level of protection" (ALOP). In essence, this is the degree to which a country intends to manage a risk—a challenge that has both scientific and societal components.

If risk assessment is the process by which a food safety risk is measured and characterized, then an ALOP is the decision regarding the level to which the risk will be managed. Initially there were concerns raised related to this concept, particularly in relation to whether this is an articulation of the amount of disease a country is willing to tolerate in its citizens. However, this is not the case, because implicit in the concept is the idea of continual improvement: a country always strives to decrease the disease burden that is attributable to foods. Instead, the concept considers the reality that any societal endeavor produces a burden, or "costs," on that society. Such "costs" are not limited to economic considerations, but also include the human, medical, ethical, legal, and moral ramifications of the selection of a particular level of risk that will be tolerated by a society. Through a combination of scientific and societal considerations, societies ultimately reach decisions related to conflicting costs associated with the control of a hazard. For example, decreasing the stringency of a food control measure will typically decrease the economic burden in relation to product costs but at the same time will increase the burden of human and medical costs. Thus, the ALOP can be considered the "cost" that a society is willing to bear to achieve a specific level of control over a hazard.

ALOPs typically have been described in terms of a country's public health goals, generally stated in units related to the incidence of disease (e.g., cases per 100,000 population). For example, in the United States, the government publishes on a 10-year basis its Healthy People series, which states the public health goals against which its public health programs are evaluated. In the case of the most recent edition, ***Healthy***

People 2010, examples of goals for foodborne diseases are 12.3, 0.25, 1.0, and 6.8 cases per 100,000 population for ***Campylobacter***, ***Listeria monocytogenes***, enterohemorrhagic ***Escherichia coli***, and ***Salmonella***, respectively. This poses a problem in terms of implementing food control programs in that the goals are not in a form that are directly measurable either by the segment of the food industry that controls the hazard that contributes to the public health concern or by the food control agencies responsible for oversight of industry activities. Typically, they focus on such features as the frequency or extent of contamination of a food with a specific microbiological hazard, not on the incidence of a specific disease. Thus, the challenge is to convert an ALOP into a value that can be controlled by the food industry.

During the past several years the International Commission on Microbiological Specifications for Foods and other international and national bodies have been exploring the potential use of food safety objectives (FSOs) as a means of bridging the gap between a country's ALOP and the performance and process standards that a company would use in its HACCP program. The definition of a food safety objective currently used by the Codex Alimentarius Commission is: "the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides the appropriate level of protection." Thus, an FSO can be viewed as the maximum level of a hazard that can occur in a specific food that is considered to meet the level of stringency needed to achieve a country's stated public health goal. The key to developing this stringency measure is the ability to relate, via a risk assessment process, the relationship between the level of a population's exposure to a hazard and the incidence of disease in a population. The following are hypothetical examples of FSOs:

- The amount of staphylococcal enterotoxin in cheese must not exceed 1 µg/100g.
- The frequency of ***S. enteritidis*** in eggs cannot exceed 1 egg/100,000.
- The concentration of aflatoxin B_1 in peanuts is not to exceed 15 µg/kg.
- The concentration of salmonellae in powdered milk is less than 1 CFU/100 g.

From these examples, it is apparent that an FSO has three components: the hazard, the food, and the maximum level or frequency. Whenever possible, an FSO should be quantitative and verifiable. This does not mean, however, that FSOs must be verifiable by microbiological testing. For example, an FSO for low-acid canned foods might be that the "the probability of a viable spore of ***Clostridium botulinum*** is less than 0.000000000001 per can of low-acid canned food." This is clearly a goal that could not be verified by microbiological testing; however, it could be verified by physical means using well-established relationships between the extent of heating and the thermal destruction of the ***C. botulinum*** spores. Although FSOs appear to be microbiological criteria, it is important to recognize that they are distinctly different: an FSO is a measure on which a microbiological criterion should be based. Put simply, a microbiological criterion is highly dependent on the methodologies employed, the sampling plans selected, and the variability in results that have to be accounted for in a microbiological criterion, whereas an FSO is a value based on relating a measure of exposure to an ALOP. Thus, a microbiological criterion is a further translation of an FSO and would typically have to be more stringent than the FSO to ensure that the FSO is not exceeded.

The establishment of an FSO is not that dissimilar to current processes for establishing levels of stringency for a food control system, except that an FSO relies more extensively on a formal assessment of risk, particularly the hazard characterization phase of a risk assessment in which the relationship between the incidence of disease and the frequency and extent of contamination with the microbiological agent is derived. However, unlike acute chemical toxicants where a threshold value can deduced, the risks associated with foodborne pathogens seldom reach a notional zero value. Thus, a decision must be reached concerning an ALOP in order to establish the degree of stringency that will need to be adopted in the manufacture of a food. On a national basis, this is typically the responsibility of a food control agency, although procedures are often established to ensure that the facts and opinions of various stakeholders are considered in reaching the final decision. For example, in the United States the establishment of a new food safety regulation involves the development of a proposal followed by a formal public comment period. On an international basis, the development

of FSOs would typically fall to a standards-setting body such as the Codex Alimentarius Commission or would be handled through bilateral or multilateral agreements among nations.

A strong consideration in establishing an FSO is that it must be technologically feasible, because an infeasible FSO is not likely to be "enforced." This is not to say that every current manufacturer of a food must currently be capable of meeting an FSO. In fact, an FSO can be a highly effective tool for increasing overall food safety effectiveness in an industry by providing a food safety goal that the poorer performers in an industry, along with the better performers, are expected to achieve.

If a newly proposed FSO appears to be technologically feasible, the next step is its implementation by industry. However, if the FSO is found to be technologically infeasible during the implementation process, the food control agency is faced with the options of considering a less stringent FSO, a close alternative product substitution (e.g., pasteurized versus nonpasteurized milk), or a ban if the product affected by the FSO is inherently dangerous to health. Examples of the latter option are rare.

An important feature of FSOs is the fact that they are based on the frequency and/or extent of contamination at the time of consumption. This location in the food chain was selected because it allows the levels of a microbial hazard to be directly related to the ALOP. In many instances, however, the control of the agent occurs earlier in the food chain where the specific target may be different from the goal of the FSO. For example, the maximum extent of a contaminant at the time of manufacture of a ready-to-eat food might have to be substantially below the FSO if the food supports the growth of the pathogen of concern between the time of manufacture and the time of consumption. The goal that would have to be achieved at the time of manufacture to meet the FSO would be considered a performance criterion. It is anticipated that both FSOs and performance criteria would be needed in many instances to establish effective quantitative management of microbial food safety risks.

One of the underlying goals of an FSO is to make food safety risk management decisions in a manner that directly relates the level of control to the level of public health protection that will be achieved. Of necessity, this is an information-intensive process. However, it provides several distinct advantages, such as a high degree of transparency, the effective use of scientific information, and the articulation of clear public health/food safety goals. FSOs also have strong potential in resolving concerns regarding equivalence among food safety risk management options and systems, both nationally and internationally. The most obvious application would be in the area of evaluating and assuring the equivalence of food produced under different HACCP plans. FSOs would provide flexibility in approaches employed while providing a clear articulation of the "bottom line" performance that needs to be achieved by all manufacturers. While the fact that FSOs are based on a consideration of specific hazard/food combinations that would have to be integrated to consider the overall equivalence of entire food safety systems, the use of FSOs has clear advantages in that they are directly related to a nation's stated public health goals.

In summary, the concept of food safety objectives is increasingly viewed as a means of establishing a clear bridge between the articulation of food safety public health goals and the development of performance criteria that provide the level of control needed to achieve those goals. Furthermore, this risk assessment–based approach to establishing the stringency of food control systems provides a means of increasing the transparency of decision making and uses the best available science in the decision-making process.

On-the-Line: Performance, Product, and Process Criteria

MARTIN B. COLE, ICMSF and Food Science Australia, P.O. Box 52, North Ryde, New South Wales 1670, Australia

Establishing Control Measures to Meet a Food Safety Objective

From the information provided in a food safety objective (FSO), regulatory authorities and food operators can select appropriate control measures to achieve the intended safe level of pathogens. A control measure is ***any action and activity that can be used to prevent or eliminate a food safety hazard or reduce it to an acceptable level.*** One or more control measures may be necessary at each stage along the food chain to ensure that a food is safe when consumed. In the design of control measures it is necessary to establish what needs to be achieved (the ***performance criterion***) and how it will be achieved (the ***process and product criteria***).

Control measures should be established according to good hygiene practices (GHP) and Hazard Analysis Critical Control Point (HACCP). They generally fall into three categories:

Controlling Initial Levels

- Avoiding foods with a history of contamination or toxicity (e.g., raw milk, raw molluscan shellfish harvested under certain conditions).
- Selecting ingredients (e.g., pasteurized liquid eggs or milk).
- Using microbiological testing and criteria to reject unacceptable ingredients or products.

Preventing the Increase of Levels

- Preventing contamination (e.g., adopting GHP to minimize contamination during slaughter, separating raw from cooked ready-to-eat foods, implementing employee practices that minimize contamination, using aseptic filling techniques).
- Preventing the growth of pathogens (e.g., chilling and holding temperatures, pH, water activity [a_w], preservatives).

Reducing Levels

- Destroying pathogens (e.g., freezing to kill certain parasites, disinfectants, pasteurization, irradiation).
- Removing pathogens (e.g., washing, ultrafiltration, centrifugation).

Performance, Process, and Product Criteria

In the design and control of food operations, it is necessary to consider pathogen contamination, destruction, survival, growth, and possible recontamination. Consideration should also be given to subsequent conditions to which the food is likely to be exposed, including further processing and potential abuse (time, temperature, cross-contamination) during storage, distribution, and preparation for use. The ability of those who control foods at each stage in the food chain to prevent, eliminate, or reduce food safety hazards varies with the type of food and the effectiveness of available technology.

A ***performance criterion*** is the required outcome of one or more control measures at a step or combination of steps that contribute to assuring the safety of a food. When establishing performance criteria, one must take into account the initial levels of the hazard and changes of the hazard during production, processing, distribution, storage, preparation, and use. A performance criterion is preferably less than, but at least equal to, the FSO and can be expressed by the following equation:

$$H_0 - \Sigma R + \Sigma I \leq FSO, \qquad (1)$$

where FSO = the food safety objective, H_0 = the initial level of the hazard, ΣR = the total (cumulative) reduction of the hazard, and ΣI = the total (cumulative) increase of the hazard. (FSO, H_0, R, and I are expressed in $\log_{10}$ units.)

These criteria are usually not established for control measures that involve the avoidance of certain foods or ingredients, although they may be applied to ensure that the initial levels of hazards in ingredients are not excessive. Microbiological testing may thus be used to select ingredients or to obtain information on the initial level of a hazard.

This systematic approach can provide the framework for the validation of control measures

and the establishment of verification activities for use by food operators and control authorities. The application of processes validated to achieve specified performance criteria is more reliable for ensuring food safety than are attempts to rely on the microbiological testing of foods to separate "safe" from "unsafe" foods.

An example of a performance criterion is a 6D kill of salmonellae when cooking ground beef or <15% contamination of freshly slaughtered broilers with ***Salmonella***. It should be noted that a performance criterion specifying the frequency and/or concentration of a pathogen is identical to the "acceptable level" to be achieved at a Critical Control Point (CCP). A CCP is defined as "a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level."

Process criteria are the control parameters (e.g., time, temperature, pH, a_w) at a step, or combination of steps, that can be applied to achieve a performance criterion. For example, the control parameters for milk pasteurization in the United States are 71.7°C for 15 seconds (Food and Drug Administration 1997). This combination of temperature and time assures the destruction of ***Coxiella burnetii***, as well as other non-spore-forming pathogens that are known to occur in raw milk. Process criteria are identical to critical limits when the control point is a CCP in a HACCP plan.

Product criteria consist of parameters that are used to prevent unacceptable multiplication of microorganisms in foods. Microbial growth is dependent on the composition and "environment" in the food. Consequently, pH, a_w, temperature, gas atmosphere, etc., have an influence on the safety of particular foods where those factors are the main reasons for microbiological safety. For example, it may be necessary for a food to have a certain pH (e.g., pH 4.6 or below) or an a_w (e.g., 0.86 or below) to ensure that it will meet an FSO for a pathogen for which growth in the product must be limited (e.g., ***Clostridium botulinum, Staphylcoccus aureus,*** or ***Listeria monocytogenes***).

The Use of Microbiological Sampling and Performance Criteria

Increasingly, it is realized that food safety management systems based on preventing hazards through GHP and HACCP are much more effective in ensuring safe foods than is end-product testing.

Two uses for microbiological criteria can be identified: (1) to validate that control measures meet performance criteria and (2) to determine the acceptability of a food when more effective means of providing such assurance of safety are not available, i.e., in the absence of knowledge that GHP and HACCP have been properly applied.

Different control measures or options can be applied in manufacturing safe foods to meet an FSO. The equivalence of these measures, in comparison to an established performance criterion, needs, however, to be established. For a number of processes and products, this can be expressed in terms of the frequency or the concentration of a microbiological hazard in a food. Traditionally, the performance of sampling plans and microbiological criteria has been expressed in terms of "defect rate." The proportion of defective samples can be established by using the distribution of bacteria for relating the performance of attribute plans (i.e., plans based on a positive or negative result) to the concentration of a hazard (Foster 1971, Legan et al. 2000). Homogeneous distribution or random sampling has to be assumed.

For example, given below is the number of 25-g samples that would be needed and that tested negative to be able to conclude (with a probability of 95%) that the concentration of organisms per grams of food was at a given level or below:

13 × 25 g negative corresponds to <1 cell/125g,
29 × 25 g negative corresponds to <1 cell/250g,
and
60 × 25 g negative corresponds to <1 cell/500g.

With this approach, microbiological testing can be used to ensure that the concentration of the hazard (H_0) in an ingredient does not exceed a given concentration (assuming a homogeneous distribution or random sampling). In this way a microbiological criterion can be used as a control measure to help meet a performance criterion.

Validation of Control Measures

Validation in the context of this paper means the process of ensuring that a defined set of control measures achieves appropriate control over a specific hazard(s) in a specific food(s).

Validation of a defined set of control measures requires that their effectiveness be measured against an expected outcome in controlling a haz-

ard, normally expressed in terms of a performance criterion (e.g., a 5-log reduction in the level of a pathogen, i.e., a specific incidence reduction of a pathogen in a product). Thus, control measures should be validated to prove that they meet established performance criteria for controlling a specific hazard(s) in a food(s) in order to meet a given FSO.

Validation can include the use of laboratory data in the form of predictive microbial models and challenge tests, the use of data collected during normal processing in the food operation, comparison with similar processes/products, as well as the use of other expert knowledge.

Example

The following example is taken from an ICMSF publication (ICMSF 2002) and is concerned with the risk of ***Escherichia coli*** O157:H7 and similar enteric foodborne pathogens in fermented sausages.

In December 1994, an outbreak of foodborne illness caused by ***E. coli*** O157:H7 in a fermented sausage product occurred on the West Coast of the United States. In response, the U.S. Department of Agriculture established a requirement that all manufacturers use processes that control the risk of illness from ***E. coli*** O157:H7. In this case, the agency proposed a performance criterion (i.e., 5D kill of ***E. coli*** O157:H7) and left it to industry to decide how to satisfy the criterion and still produce products of acceptable quality. The agency's proposal of a 5D kill was based on very limited evidence suggesting that up to 1000 ***E. coli*** O157:H7/g could occur in the raw meat used for processing. Industry-sponsored research led to five options that were accepted by the agency (Nickelson et al. 1996):

1. Apply an existing approved heat treatment as specified in former USDA regulation 9 CFR 318.17 (i.e., heating to an internal temperature of 62.8°C for 4 minutes or to a lower temperature for such time as required to obtain an equivalent level of safety).
2. Apply a process that is validated by research to cause a 5D kill of ***E. coli*** O157:H7 before the product is released for distribution.
3. Combine raw material testing with a process that is validated by research to cause a 2D kill of ***E. coli*** O157:H7 before the product is released for shipment. The sample procedure must ensure that the level of ***E. coli*** O157:H7 in the raw sausage blend does not exceed 1/g. One such sampling procedure could consist of analyzing 15 samples (25 g each) collected at the time the meat blend is stuffed into the casings.
4. Apply a hold-and-test program for the finished product before distribution. Products intended to be heated before serving (e.g., pepperoni for pizza) would be sampled at a rate of 15 samples per lot. Products normally consumed without heating before serving (e.g., salami) would be sampled at a rate of 30 samples per lot. An analytical unit of 25 g for each sample tested would be used.
5. To allow for new technology or ideas, this option permits the use of alternative processes that would provide the equivalent of a 5D reduction

All of the options are intended to ensure that the level of ***E. coli*** O157:H7 is 1 cell/100 g or less when the products are released for distribution. The agency considered that this, at the time, provided an acceptable level of consumer protection for this class of product. The five options include process criteria, performance criteria, and microbiological criteria. Options 1 and 2 assume an initial level of 1000 ***E. coli*** O157:H7/g in the raw sausage blend. The process criterion for option 1 (heating to an internal temperature of 62.8°C and holding for 4 minutes) is derived from an existing regulation for roast beef and is based on research data demonstrating a 5D kill of salmonellae and ***E. coli*** O157:H7 in beef. The 4-minute hold time was an added requirement, since the roast beef regulation does not require a hold time at 62.8°C. Processors choosing this option would not take advantage of the faster rate of kill that would occur with the reduced pH of a fermented product. The performance criterion in option 2 specifies a 5D kill of ***E. coli*** O157:H7. To satisfy this option, the processing plant must have on file, and available for review, research data validating that the process being used will achieve a 5D kill. The validation research must have been developed with a protocol approved by the USDA.

Option 3 incorporates both a reduction step and an elimination step based on microbiological criteria. It involves a performance criterion of a 2D kill, in combination with obtaining a microbiological negative result (Foster 1971). Although

this may be a prudent sampling plan for some operations, this level of sampling exceeds by approximately 100-fold the detection level required in option 3.

Option 4 establishes microbiological criteria for finished product and assumes no prior knowledge of the level of ***E. coli*** O157:H7 in the raw sausage blend or the lethality of the process. Reliance is placed solely on the use of a sampling plan that may detect ***E. coli*** O157:H7 if it is present in the finished product. The sampling plans are based on an ICMSF recommendation (in book 2 [ICMSF 1986]) and an assignment of ***E. coli*** O157:H7 to cases 13 and 14 for pepperoni and salami, respectively. Case 13 involves $n = 15$ and $c = 0$. Case 14 involves $n = 30$ and $c = 0$. A negative result with 15 25-g samples (375 g total) provides a 95% probability of no more than 1 cell/125 g. A negative result with 30 25-g samples (750 g total) provides a 95% probability of no more than 1 cell/250 g (Foster 1971).

References

Foster EM. The control of salmonellae in processed foods: a classification system and sampling plan. JAOAC 1971;54:259–266

ICMSF. Microbiological testing in food safety management. Microorganisms in Foods, book 7. Gaithersburg, MD: Aspen Publishers / Kluwers Academic, 2002

Legan JD, Vandeven MH, Dahms S, Cole MB. Determining the concentration of microorganisms controlled by attributes sampling plans. Food Control 2000;12:137–147

Nickelson R, Luchansky J, Kaspar C, Johnson E. Update on dry fermented sausage *Escherichia coli* O157:H7 validation research. Research Report 11-316. Chicago: National Cattlemen's Beef Association, 1996.

U.S. Food and Drug Administration. Milk and cream, pasteurized, 21 CFR 131.3b (1997)

Use and Misuse of Microbiological Criteria for Foods

MICHIEL van SCHOTHORST, Secretary, ICMSF; Wageningen University, The Netherlands; and Nestlé, Avenue Nestlé 55, Vevey, CH-1800, Switzerland

Microbiological criteria are used in the management of food safety. They are used, for example, in the inspection of imported or locally produced foods, in HACCP verification, and in trade agreements between commercial partners. The terms used for the various criteria differ according to their purpose. For instance, in the examination of consignments of raw materials and end products, they are called "acceptance criteria." In production, microbiological limits that are used to express the outcome of a certain processing step are often called "performance criteria." In the context of Codex (Codex Alimentarius Commission 1997), "microbiological criterion" has a specific meaning that will be explained below. Food safety objectives (FSOs) differ from microbiological criteria in many respects, although they also may express the number of microorganisms per unit or mass of a food product.

Microbiological Criteria According to Codex

Codex defines a microbiological criterion as "the acceptability of a product or a food lot, based on the absence or presence or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot." In the establishment of microbiological criteria, consideration should be given to the following:

- ***Evidence of actual or potential hazards to health***. This could be, for instance, the outcome of a risk assessment or epidemiological evidence that a certain microorganism in the food for which the criterion has to be established was implicated in a foodborne disease outbreak.
- ***Microbiology of raw materials***. This is the H_0 value, which can be found in the FSO equation $H_0 + \Sigma R + \Sigma I \leq FSO$.
- ***Effect of processing***. The various effects of processing and food preparation are designated in the FSO equation as ΣR.
- ***Likelihood and consequences of contamination and growth during handling, storage, and use***. This reflects the ΣI from the FSO equation.
- ***The category(ies) of consumers at risk***. The very young, the very old, the diseased, and the immunocompromised are more sensitive to many foodborne pathogens than others are. This difference may also be reflected in the outcome of a risk assessment.
- ***The cost-benefit ratio associated with the application of the criterion***. Codex recognizes that resources are limited and should be used efficiently.
- ***The intended use of the food***. Food products for export may need to be treated differently (often because of a longer and more complicated transport and distribution chain) than foods for the local market.

According to Codex, a microbiological criterion should state the food and the point in the food chain at which it applies and any actions to be taken if the criterion is not met. Additionally, the following components of a microbiological criterion are listed:

- microorganisms and reasons for concern,
- analytical methods to be used,
- sampling plan and size of analytical units,
- microbiological limit, and
- numbers of units to be in conformity.

The sampling plans should take into account:

- risks to public health associated with the hazard,
- the susceptibility of the target group of consumers, and
- the acceptable quality level and the desired statistical probability of accepting a nonconforming lot,

Codex does not give further guidance on how sampling plans should be established, other than reference to the ICMSF book 2 (ICMSF 1986). The first part of that book, dealing with the subject of sampling, is being revised and will be published as book 7 in the series Microorganisms in Foods (ICMSF 2002).

Sampling Plans

The ICMSF describes three categories of hazards: moderate, serious, and severe. Three food-handling conditions are also distinguished: those that result in a decrease in the hazard level, those where no change will occur, and those that may

lead to an increase of the hazard level. Examples of moderate hazards are ***Bacillus cereus*** and ***Staphylococcus aureus*** enterotoxin; examples of serious hazards are ***Salmonella*** serovars and ***Listeria monocytogenes;*** and examples of severe hazards are ***Escherichia coli*** O157:H7 and ***Vibrio cholerae*** O1. When the three categories of hazards and the three handling conditions are combined, nine so-called cases, numbered 7 to 15, are obtained. The risk from eating certain foods obviously increases from "case 7" (moderate hazard/decrease in hazard level) to "case 15" (severe hazard/increase in hazard level). The ICMSF has proposed sampling plans that increase in stringency with increased risk.

Statistical calculations can be used to express the likelihood of accepting or rejecting a consignment of food based on various sampling schemes. For example, when 10 units of a milk powder lot containing 40,000 units, each of 250 g, are examined, and when 25-g samples from the 250-g units are tested without finding one ***Salmonella***-positive sample, there is a 35% probability that lots containing up to 4,000 contaminated units are accepted. Statistical interpretation of test results is valid only when samples are randomly taken or when there is a homogeneous distribution of the microorganism throughout the lot, which is often not the case. This example clearly demonstrates that microbiological testing alone is not an adequate tool in food safety management. More efficient tools are good manufacturing practices (GMP) and HACCP programs.

The Selection of Microbiological Criteria in Relation to FSOs

FSOs are concerned with the level of a hazard at the moment of consumption. Microbiological testing is not normally applied at this point in the food production chain. Microbiological criteria are often based on what can be achieved during food production/processing and are expressed as performance criteria.

A further distinction is that many FSOs cannot be measured microbiologically, particularly when production includes a step intended to kill (reduce, inactivate) the hazard. Examples include the level of ***Salmonella*** in milk powders and pasteurized meat products. In such circumstances, indicator microorganisms may be used as part of a microbiological criterion to check the likelihood that an FSO is met. An indicator should detect unacceptable survival, recontamination, or growth of the hazard. The indicator should have the same, or very similar, heat resistance, growth characteristics, etc., as the pathogen of concern. It must be emphasized that using an indicator can never be relied upon as a "proof" that the pathogen of concern is absent. Tests for "Enterobacteriaceae" in the examination of pasteurized egg products have proved reliable when the testing limit was based on a performance criterion. Useful indicators are not always available, for instance, in the case of ***Clostridium botulinum*** in canned meat products (where the level of the hazard is too low to be determined); in this case a microbiological criterion cannot be established.

Sometimes an FSO is measurable microbiologically; e.g., the FSO suggested for ***L. monocytogenes*** is <100/g at the moment of consumption. The ICMSF has published sampling plans (ICMSF 1994) based on this FSO, which are currently under discussion by the Codex Alimentarius Food Hygiene Committee. For example, when a product has been adequately heated before consumption, no testing is advised. When an increase in a hazard may be expected, analysis of 20 samples is proposed, none of which should contain ^{3}100/g or mL at the moment of consumption. Because such an examination will be made before the expiry date, a lower number of ***L. monocytogenes*** per gram needs to be established as a microbiological limit. That number can be based on product-specific growth data obtained from the manufacturer or on more general growth data obtained from microbiological models. When ***Listeria*** spp. cannot grow in a product, such as in ice cream, it is proposed that 10 samples be examined, none of which should contain ^{3}100/g. Because there is no change in numbers between manufacture and consumption, this microbiological criterion could also be the performance criterion.

In many instances, microbiological criteria will not have any mathematical relationship with FSOs. This is due partly to the differences in purpose. An FSO is an objective that should give sufficient consumer protection, but is not designed for testing. Microbiological criteria, on the other hand, are intended to give guidance for testing. They should confirm that GMP and HACCP are properly established and applied.

Examples of the Use and Misuse of Microbiological Criteria

FSOs are a new concept and, to our knowledge, have not yet been applied officially. Microbiological criteria have been used in commerce for many decades. The first Codex document on the subject of microbiological criteria was elaborated in 1977. However, the principles described have rarely been applied. Microbiological criteria are not always used correctly. For example, they are used to ensure food safety or to provide evidence of food safety (due diligence), they are used at the wrong point in the food chain, "zero tolerance" criteria have been established, quality criteria are used as "nontariff" barriers, etc.

The correct use of mandatory microbiological criteria are clearly described in a Codex document (CAC 1997): "They shall apply to those products and/or points of the food chain where no other more effective tools are available, and where they are expected to improve the degree of protection to the consumer."

References

Codex Alimentarius Commission. Principles for the establishment and application of microbiological criteria for foods. CAC/GL 21-1997. Rome: Food and Agriculture Organization of the United Nations, 1997

International Commission on Microbiological Specifications for Foods (ICMSF). Choice of sampling plan and criteria for *Listeria monocytogenes.* Int J Food Microbiol 1994;22:89–96

ICMSF. Microbiological testing in food safety management. Microorganisms in Foods, book 7. Gaithersburg, MD: Aspen Publishers / Kluwers Academic, 2002

ICMSF. Sampling for microbiological analysis: principles and specific applications, 2nd ed. Microorganisms in Foods, book 2. Toronto: University of Toronto Press, 1986

Applying ICMSF Processes for Foods

R. BRUCE TOMPKIN, Vice President of Food Safety, ConAgra Refrigerated Prepared Foods, 3131 Woodcreek Drive, Downers Grove, Illinois 60515, USA

For decades, microbiological criteria have been used as a means to assess food operations and the safety of foods. When the number of microbes of concern or the proportion of defective units is high, microbiological testing can be an effective means of detecting unacceptable product. However, in many modern food operations where the prevalence and concentration of pathogens are very low, microbiological testing is a weak, unreliable means to detect unacceptable lots of food and to assess food operations. For example, in operations that produce a contaminated food with a prevalence rate of 0.5%, there is a 61% probability of accepting a positive lot even though 100 samples are analyzed. In addition, testing product is of limited value for assessing food operations because this provides only a snapshot and little, or no, confidence for what may be produced over an extended period of time. These weaknesses pointed to the need for a new approach to assess control and ensure food safety.

The International Commission on Microbiological Specifications for Foods (ICMSF) concluded that a more effective approach would be to strengthen food safety systems through the application of good hygiene practices (GHP) and HACCP (Hazard Analysis Critical Control Point). To confirm the effectiveness of that approach, an objective is needed to define the necessary level of control. Ideally, this objective should be linked to specific public health goals (e.g., level of consumer protection). These considerations led to the food safety objective (FSO), a new concept that can offer significant advantages over the testing of individual lots of food.

FSOs are statements of the maximum frequency or concentration of a microbiological hazard in a food. In practice, FSOs are realized through the use of GHP and HACCP. This may lead to the development of performance criteria that specify the expected level of control at one or more steps in the food chain that may be necessary to meet an FSO. FSOs and performance criteria provide the basis for review of food operations by regulatory agencies and other auditors. They also can be used by food operators to validate that their operations will meet an FSO. This approach shifts the decision to accept or reject individual lots of food to an assessment of whether the control measures adopted are adequate to meet the FSO and, thus, provide the desired level of consumer protection.

What Is a Food Safety Objective, and How Do FSOs Relate to Public Health Objectives?

R. BRUCE TOMPKIN, Vice President of Food Safety, ConAgra Refrigerated Prepared Foods, 3131 Woodcreek Drive, Downers Grove, Illinois 60515, USA

Risk managers in government and industry share the common goal of providing consumers with safe, wholesome foods. National food safety goals, however, are a responsibility of governments and are established by risk managers in federal agencies following input from all affected stakeholders. Food safety goals are expressed in terms of the risk that a society regards as tolerable in the context of, and in comparison with, other relevant risks in everyday life (i.e., the tolerable level of risk, or TLR). Other factors that must be considered are the public health impact of the hazard, technological feasibility of controlling the hazard, consumer willingness give up their freedom of choice (e.g., rare hamburger, soft egg yolk), and economic implications (e.g., higher food prices).

The TLR can be expressed as the number of illnesses occurring because of a certain microbial hazard per 100,000 population per year. A hypothetical example could be 0.5 cases of listeriosis per 100,000 population per year. Although deciding on a TLR is a societal issue, sound scientific principles should underline the evaluation of risk. Thus, epidemiologic data to document the role of specific foods and the conditions leading to foodborne illness are essential. This information along with other scientific data can be used in qualitative or quantitative risk assessments to guide risk managers as they consider establishing an appropriate TLR.

An effective surveillance system is a key component of this process. Comprehensive surveillance systems may include different means to monitor and report the incidence of foodborne diseases, such as passive notification systems, active surveillance systems, case-control studies, outbreak investigations, and sentinel studies.

None of these systems alone can yield all of the data necessary for a risk assessment, and some (e.g., passive notification systems) often fail to identify food as a source. Passive notification systems follow trends in disease and can be useful for measuring the impact of changes in technology, preventive measures, and regulatory policies. Another approach is through active surveillance systems such as EnterNet or FoodNet. EnterNet is being used to more accurately determine the incidence of salmonellosis and infections caused by ***E. coli*** O157 in Europe and to identify outbreaks in Europe from a common food source. The U.S. surveillance program, FoodNet, is an active, sentinel site program that collects weekly updates on gastroenteritis and listeriosis from clinicians in certain regions of the country. Isolates of selected pathogens are compared for commonality to identify outbreaks stemming from a common food source.

Data on foodborne disease is also collected through case-control studies by interviewing patients to learn their food consumption history and to identify food sources. In parallel, a number of individuals are selected to serve as controls. This methodology has been used to identify not only the foods that may be involved, but also risk factors that the patients may share and that may explain increased susceptibility to the disease. Case-control studies are useful for identifying pathogen-food combinations where it has been difficult to isolate the causative organism from the food source or the role of foods in diseases with long incubation times before onset of symptoms (e.g., listeriosis).

Case-control studies can also be used to help identify the source(s) of sporadic cases of foodborne illness and the factors that contribute to their frequency. Different sources may be more important in sporadic cases than in outbreaks. The identity of the food source and conditions leading to foodborne illness may also be determined through epidemiologic investigations of outbreaks.

Sentinel studies monitor selected health events in a group of persons representative of the whole population. Laboratory testing may be limited or may include, for example, examination of all fecal samples for a range of pathogens.

In addition to epidemiologic data, information is necessary about the food(s), how they relate to the food chain, and why they may be a source of disease. Collectively, this information

is used to establish a TLR. In some cases, the TLR may be expressed simply as the number of cases/100,000 population per year, and the TLR also may specify a food or food group (e.g., ***Salmonella enteritidis*** from eggs).

Food operators cannot use a public health goal or a TLR to design and control processing conditions to prevent, eliminate, or reduce a microbiological hazard. For this and other reasons, the ICMSF has proposed the use of a food safety objective (FSO) to enable risk managers to communicate to industry and trade partners precise food safety goals. An FSO is the maximum frequency and/or concentration of a pathogen, toxin, or metabolite in a food considered tolerable for consumer protection. FSOs are typically expressions of concentrations of microorganisms or toxins at the moment of consumption. FSOs, along with performance criteria, define the level of control expected of a food establishment's food safety management system. In practice, FSOs and performance criteria are realized through the application of the establishment's prerequisite programs and the HACCP system.

Ideally, a TLR would be linked through the risk assessment process to an FSO so that if the FSO is met, the anticipated TLR also will be met. It is highly questionable, however, that the data available for risk assessments are adequate and accurate enough to permit such a relationship. In addition, the process of establishing an FSO also should be transparent, based on technological feasibility and other scientific criteria and with input from all affected stakeholders. This could influence the FSO that is adopted and its relationship to the TLR, thus requiring reconsideration of the TLR. The relationship may be more direct for certain pathogen-food combinations. For example, a TLR for foodborne botulism linked to an FSO for the control of ***Clostridium botulinum*** in low-acid canned foods would be more likely than a TLR for listeriosis linked to an FSO for the control of ***L. monocytogenes*** in either dairy or meat products. These issues must be resolved through experience and application of the concepts.

In the meantime, the FSO concept offers a new management tool to address new, emerging food safety concerns. Depending on the urgency of the situation, the hazard, and the available knowledge of the factors that lead to a foodborne illness, an interim FSO may be established based on advice from an expert panel or following a risk assessment. The FSO is intended to lead to the adoption of control measures to prevent, eliminate, or reduce the risk. As more information becomes available, the FSO can be adjusted to further enhance the safety of the implicated food(s).

What Role Should Food Safety Objectives Play in the U.S. Food Industry, and How Will They Affect the Way Industry Does HACCP?

DON L. ZINK, Future Beef Operations, LLC, Suite 325, 19751 East Mainstreet, Parker, Colorado 80138, USA

A food safety management system incorporating good manufacturing practices and HACCP (Hazard Analysis Critical Control Point) is now widely accepted as the preferred system for controlling food safety risks. However, a HACCP system cannot be credible or effective unless it is designed to meet specific food safety goals. A current weakness of many HACCP plans is that the level of control needed is not known with certainty.

Scientifically sound food safety goals, or food safety objectives (FSOs), provide a common basis for the food industry to use in developing and implementing control measures, for regulatory agencies to use in assessing the adequacy of these control measures, and for use in determining the equivalence of control measures applied in other countries. The safety of low-acid canned foods is an example of how a well-defined FSO combined with scientifically sound and effective control measures can virtually eliminate a food safety hazard.

The food industry urgently needs FSOs to drive the development of improved hazard control measures. FSOs will have the greatest impact on HACCP systems that do not have at least one control process that eliminates a hazard. In many cases, particularly for unprocessed foods, the industry must rely on a combination of partially effective control processes. FSOs will determine the required effectiveness of these control processes and allow industry to implement the most effective combination of controls. Finally, the food industry will be able to establish acceptance criteria based on a public health goal. This will provide an important rational basis for control and allows the consequences of failure to be seen in public health terms. The result will be better HACCP plans and more effective control measures.

An International Perspective on Food Safety Objectives

STEPHEN C. HATHAWAY, Food Assurance Authority, Ministry of Agriculture and Forestry, P.O. Box 646, Gisborne, New Zealand

International Trade

After many years of good hygienic practices by the food industry, the introduction of HACCP principles and risk assessment in the last decade has resulted in fundamental changes in the design of food control programs and the roles of the various stakeholders. For foods in international trade, the World Trade Organization Sanitary and Phytosanitary (WTO SPS) Agreement has arguably been the most important catalyst in shifting the regulatory focus from the prescription of process to risk-based measures that can be demonstrated as achieving specified levels of consumer protection. The application of a particular range of hygiene measures by a country provides the level of protection chosen for its consumer population, i.e., the "appropriate level of protection" (ALOP).

It is often the case that food control systems operating in an exporting country differ from those in an importing country. In this respect, the WTO SPS Agreement requires that if requested by an exporting country, claims of equivalence for identified hygiene measures should be considered by an importing country. Equivalence as described in the WTO SPS Agreement is the objective demonstration that different hygiene measures in an exporting country achieve the level of consumer protection delivered by the hygiene measures of the importing country.

Food Safety Objectives

The concept of a food safety objective (FSO) is founded on the need for an objective measure of the level of hazard control in food that is required to achieve a desired level of consumer protection (Hathaway and Cook 1997, Jouve 1998, van Schothorst 1998). A working definition of an FSO is "a statement which expresses the level of a hazard in food that is tolerable in relation to an ALOP." Thus, FSOs provide an important tool for

- controlling food safety problems (design, validation, and implementation of controls),
- elaborating food standards,
- judging the equivalence of different hygiene measures, and
- facilitating communication of risk management decisions to all stakeholders in food safety.

Reference to a risk management framework positions an FSO in the stepwise development of a HACCP system (Anonymous 2000). In the case of microbiological hazards in foods, the broad food safety goal will usually be to reduce risks to a level that is "as low as reasonably achievable." During the assessment of risk management options, an ALOP will be "arrived at" by considering desired levels of protection and the availability and effectiveness of different risk management options. Following the final risk management decision, establishment of an FSO will utilize data on hazards that are available from the risk assessment, together with knowledge of food technology.

In the case of chemical hazards in food, an ALOP is usually predetermined by regulatory food safety policy, e.g., "notional zero risk" in the case of residues of veterinary drugs. Here, assessment of risk management options usually has a different function. Risk assessment can be used to determine the level of control of the hazard in the food that is required to achieve the designated level of protection, and hygiene measures will be selected so as to achieve that level of protection. Establishment of an FSO flows from this process.

Application in Food Control Systems

In the ideal situation, a quantitative risk assessment (QRA) model will be available for a particular food/hazard combination. The QRA model will likely present estimates of risk resulting from different levels of hazards at the point of consumption. Once a decision on an ALOP is taken, the FSO will be a quantitative representation of that ALOP.

In the absence of a QRA model, no decision can be taken on an ALOP relative to different exposure scenarios for hazards in the food supply. Notwithstanding this, the current level of hazard control can be deemed to be "reflective" of an ALOP if it is not seen to be resulting in an unacceptable food safety situation. Objective measurement of the level of hazard control is then neces-

sary to establish an FSO on this basis.

Where there is no QRA model available and there are food safety concerns associated with a particular hazard/food combination, establishing an FSO has additional constraints. An FSO representing a more stringent level of hazard control can be set, but this can be considered to represent only stakeholder "expectations" in terms of reducing foodborne risks to human health. The FSO will be properly reflective of an ALOP only if ongoing monitoring of the consumer population demonstrates that the more stringent hygiene measures needed to achieve the FSO are actually delivering an acceptable reduction in risk.

If an FSO has been established, the detailed design of a HACCP system is likely to include performance and/or process parameters, either alone or in combination, that are validated as achieving or contributing to the achievement of the FSO. Once performance/process parameters have been validated as meeting the required level of hazard control at particular point(s) in the food process, ongoing verification will assure the required level of consumer protection.

FSOs can be set at different steps in the food chain, providing that there are established correlations between the required level of hazard control at a step and the level of hazard control at the point of consumption that delivers the ALOP. At the least, this requires detailed knowledge on the prevalence and concentration of the hazard in the food as it progresses through the food chain. Modeling of the whole food chain in this manner presents obvious advantages in providing food safety assurances for foods produced in one country and consumed in another. For example, demonstrating that the microbiological status of fresh meat at the point of packaging will achieve the importing county's ALOP should preempt any need for routine monitoring at the port of entry.

Conclusion

As international food standards increasingly specify outcomes rather than processes, i.e., as they become genuinely risk based, all stakeholders should welcome the increased transparency and objectivity that the concept of FSOs will bring to food safety risk management. For foods in international trade, FSOs facilitate verifiable "targets" for industry and regulators, and they are vital components in assuring consumers that their safety expectations for imported foods are being met. Furthermore, FSOs provide an objective basis for judging the equivalence of alternative hygiene measures in different countries, and they can be used to build precautionary buffers into the required level of hazard control when risk estimates are highly uncertain.

It is also incumbent on governments to promote the design of genuinely risk-based HACCP systems. In the absence of FSOs, HACCP systems in many ways become empirical experiments rather than being validated as achieving contemporary food safety goals.

References

Anonymous. Food administration in New Zealand: a risk management framework for food safety. Wellington: Ministry of Health and Ministry of Agriculture and Forestry Joint Food Harmonisation Project, 2000

Hathaway SC, Cook RL. A regulatory perspective on the potential uses of microbial risk assessment in international trade. Int J Food Microbiol 1997;36:127–133

Jouve JL. Establishment of food safety objectives: 2nd International Food Safety and HACCP Conference. Nordwijk, The Netherlands, June 8–10, 1998

van Schothorst M. Principles for the establishment of microbiological food safety objectives and related control measures. Food Control 1998;9:379–384

How Can We Educate the Public About Tolerable Level of Risk/ Acceptable Level of Protection?

KATIJA BLAINE and Douglas A. Powell, Department of Plant Agriculture, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

Public awareness and discussion of food-related risks have reached unprecedented levels in terms of media coverage and survey responses. In the wake of the ***Escherichia coli*** O157:H7 outbreak in fast-food restaurants in 1993, stories about microbial food safety began appearing more frequently and more prominently in the U.S. media. A two-fold increase in North American media coverage of microbial food safety was seen from late 1993 through mid 1994 and has remained front and center through December 31, 1999 (Powell 2000). Outbreaks of food- and waterborne illnesses from such pathogens as ***Listeria monocytogenes*** in hot dogs and ***Escherichia coli*** O157:H7 in a municipal water supply in Walkerton, Ontario, are increasingly associated with other food-related risks, such as mad cow disease and foot-and-mouth disease. Growing concern is also being expressed about farm animal welfare issues, genetically engineered crops, and the environmental impacts of farming practices. These and other issues are cumulatively leading to a rejection of science-based approaches to producing safe, abundant food.

FoodNet data show very little change in the incidence of foodborne illness over the last 5 years (Foodborne Diseases Active Surveillance Network 2001). It is therefore not surprising that bacteria and pesticides consistently top consumers' lists of their top food safety concerns (Canadian Food Inspection Agency 1998, CMF&Z 2000, International Food Information Council 2001, Food Marketing Institute 1995). Hormones, Bovine Spongiform Encephalopathy, food spoilage and food related technologies like irradiation and genetic engineering are also important consumer concerns. Foot-and-mouth disease was mentioned as a food safety concern for the first time in the 2001 Ipsos-Reid poll (Ipsos-Reid 2001), an annual North American survey on food. This is an indicator not only that animal health and welfare issues are adding to consumer concerns about food, but also that consumer perceptions of food-related risks may not accurately reflect the actual levels of risk to society.

Survey responses have indicated that the greatest perception of safety is often associated with products and organizations that are the least regulated and scientifically assessed. For example, in a recent CMF&Z survey (CMF&Z 2000), respondents cited "natural foods" as the safest. The 2001 Ipsos-Reid poll (Ipsos-Reid 2001) found that Canadian consumers had the greatest confidence in farmers' markets over retailers, government, and food manufacturers. These findings also point to a lack of trust in government regulation and industry.

The current state of risk management and communication research suggests that those responsible for food safety risk management must be seen to be reducing, mitigating, or minimizing a particular risk. Those responsible must be able to communicate their efforts effectively, and they must be able to prove that they are actually reducing levels of risk. The current climate of distrust in regulatory agencies and industry makes communicating about risks not only more challenging but also much more important. In the absence of credible, honest messages about the nature of a food-related risk, consumers will use stigma, a powerful shortcut, to evaluate a risk (Gregory et al.1995).

British beef and mad cow disease are prime examples of stigma. Poor risk communication efforts and insistence by the U.K. government that there was no risk to the public from Bovine Spongiform Encephalopathy (BSE) undermined the faith of the British in their food safety regulatory system, which has not been fully restored (Powell and Leiss 1997). The Canadian government still does not like to talk about BSE. In February 2001, Canada, like the United States, banned imports of Brazilian beef, an action that was widely criticized as being a political and trade issue rather than a food safety issue. Theories of government conspiracies abounded.

BSE, however, is a health-related risk. The ban was implemented because Brazil could not, or would not, provide the requested paperwork on the whereabouts of several thousand cattle

imported from European Union countries that have since been found to have cattle with BSE. After repeated requests for information were denied, the veterinarians at the Canadian Food Inspection Agency imposed the ban. Canada lifted the ban when authorities were convinced that Brazil was taking appropriate measures to control the risk (Powell 2001).

This is an example of good risk management but poor risk communication. Health Canada failed to communicate proactively to the public the underlying risks that led to the decision to implement the ban. Perhaps fearing that Canadians could not handle the message of a possible risk to health from Brazilian beef and the efforts taken to control the situation and reduce risk, Health Canada said nothing at all. As a result, in the absence of readily available, credible information from regulators, people began to make up their own stories, which included conspiracy theories and rumors of trade wars (Powell 2001). Subsequent media coverage focused more on the trade and political aspects of the ban than on potential human health aspects. What can be done to alleviate poorly supported food-related safety fears?

Focus on Building Trust

Current research on perceptions of food-related risks has identified trust in food safety regulators as the primary predictor of consumer support for food technologies and consumer confidence in the food supply (Frewer et al. 1994, Dittus and Hillers 1993, Covello 1992). If trust is a better predictor of consumer support, then which factors influence perceptions of trust? Frewer et al. (1996) conducted two sets of in-depth interviews with about 45 people each followed by a larger quantitative survey to better understand how trust is formed. Overall, there were many findings of relevance to the effective communication of food-related risks, including the following:

- The single most important determinant of gain or loss of trust in a source is whether the information is subsequently proven right or wrong and whether the source is subsequently demonstrated to be unbiased.
- Trust appears to be linked with perceptions of accuracy, knowledge, and concern with public welfare.
- If government sources and risk regulators are seen to be proactive in their interactions with the media and other trusted sources—including discussions of risks—this may positively influence the way in which risk information is reported as well as increase trust in government regulation.
- Admitting to uncertainty, or facilitating public understanding of science as a "process," could increase communicators' trustworthiness.

Focus on Public Health and the Consumer

The U.S. Department of Agriculture and U.S. Department of Health and Human Services (2001) conducted a risk assessment of foodborne listeriosis and concluded that there is a risk to susceptible individuals, especially from the following foods: pâté and meat spreads, fresh soft cheeses, smoked seafood, deli meats, and deli salads. Recommendations focus on risk reduction for these food categories, including identifying new approaches for protecting human health.

What is needed next is to be honest and open and to communicate the risks that these foods may pose to susceptible groups: pregnant women, the elderly, and immunocompromised individuals. This includes an understanding of consumer concerns by framing risk messages in a context to which consumers can relate. The public will want to know what the risks are and, more importantly, what this means to them personally and what they should do about it.

Proactive Measures Can Also Improve Consumer Confidence

Fresh fruits and vegetables are increasingly recognized as vectors for foodborne illness. An on-farm food safety program was developed, implemented, and analyzed for the Ontario Greenhouse Vegetable Growers, in Ontario, Canada, over a two-and-a-half-year period (Powell et al. 1999). This HACCP-based system was designed to reduce the potential for microbial contamination throughout the production and distribution process. Through the use of microbiological testing, on-site visits, and surveys, it was determined that the program has increased growers' knowledge, understanding, and awareness of the microbial risks associated with fresh produce, and has brought about improvement in practices used in the greenhouse and packing sheds. The Ontario Greenhouse Vegetable Growers are playing a proactive role in the

produce industry with its verifiable on-farm food safety program. Through press releases, its website, and newsletters, the group also has been proactive in communicating information about the program to its consumers and producers.

Consumers want honest information on the nature of risks. Producer-led risk management programs like that of the Ontario Greenhouse Vegetable Growers are an appropriate risk management strategy that demonstrates that producers are aware of consumer concerns about food safety. Cooperating with the media and communicating early and often about such programs and initiatives can also enhance the perception of trust. In addition, providing continuous updates on the findings and progress of such programs will help show that actions match words.

Agricultural biotechnology is another example of technology and products that have been stigmatized. A survey by the International Food Information Council (2001) found that the majority of consumers surveyed incorrectly cited tomatoes and fresh fruits and vegetables as examples of supermarket foods produced through genetic engineering. In the absence of credible messages on the nature of the risks of biotechnology and the efforts taken by regulators to reduce these risks, opponents of genetic engineering have filled the void with their own highly memorable propaganda, e.g., the fish gene in tomatoes (Greenpeace 2001).

In a trial of consumer acceptance of genetically modified foods that took place on a commercial fruit and vegetable farm near Hillsburgh, Ontario, Canada, genetically engineered Bt sweet corn and Bt potatoes were grown side by side with conventional varieties. Neither the Bt sweet corn nor the Bt potatoes required any insecticides. The corn and potatoes harvested through the trial were segregated and labeled, and direct consumer testing for purchasing preference was conducted. Overall, the Bt sweet corn outsold the regular sweet corn. The final sales numbers were 680 dozen Bt sweet corn sold compared with 452.5 dozen conventional sweet corn sold. Surveys of consumers who were given the opportunity to buy the Bt corn indicated that reduced pesticide use and improved taste and quality influenced purchasing decisions.

This trial clearly indicates that consumers can handle messages about risk. The corn was clearly labeled as genetically engineered, and background information was provided on what "genetically engineered" meant. The majority of consumers, after reading the information, chose to buy the genetically engineered corn.

Conclusions

Slovic (1997) noted: "We live in a world in which information, acting in concert with the vagaries of human perception and cognition, has reduced our vulnerability to pandemics of disease at the cost of increasing our vulnerability to social and economic catastrophes of unprecedented scale. The challenge before us is to learn how to manage stigma and reduce the vulnerability of important products, industries, and institutions to its effects, without suppressing the proper communication of risk information to the public."

Appropriate risk management strategies, such as on-farm food safety programs and honest communication with consumers about agricultural technologies, such as occurred in the demonstration farm and market study described above, can enhance consumer confidence in the safety of improved foods produced through biotechnology.

Producer-led risk management programs are active, appropriate risk management strategies that demonstrate to consumers that producers are cognizant of their newfound concerns about food safety, and that show that producers and others in the farm-to-fork continuum are working to reduce levels of risk. When the next outbreak or crisis of confidence comes—and microorganisms can adapt and evolve to any food production and distribution system that is created—producers need to demonstrate due diligence to minimize potential losses.

Surveys and media analyses have shown that levels of perceived trust in promoters and regulators of technologies are the most accurate gauges of consumer support. People who demonstrate low trust have the highest concern about possible risks regarding pesticides and agricultural biotechnology (Frewer and Shepherd 1994), whereas those with high trust perceive greater benefits from both. In conclusion, trust in government and industry is a more important factor in influencing risk perception than is perceived potential safety or danger from a particular food product or technology.

References

Canadian Food Inspection Agency. Safe Food Handling Survey. Ottawa: CFIA, 1998

CFM&Z. Food Safety Survey. Des Moines, IA: CMF&Z, 2000. Available at http://www.cmfz.com/foodsafety/. Accessed on October 4, 2001

Covello VT. Trust and credibility in risk communication. Health Environ Digest 1992;6:1–5

Dittus KL, Hillers VN. Consumer trust and behavior related to pesticides. Food Technol 1993;477:87–89

Food Marketing Institute (FMI). (1995). Trends in the United States: Consumer Attitudes and the Supermarket, Food Marketing Institute, Washington, DC.

Foodborne Diseases Active Surveillance Network. Preliminary FoodNet data on the incidence of foodborne illnesses: selected sites, United States, 2000. MMWR Morb Mortal Wkly Rep 2001;50(13):241–246

Frewer LJ, Howard C, Hedderley D, Shepherd R. What determines trust in information about food-related risks? underlying psychological constructs. Risk Anal 1996;16:473–486

Frewer LJ, Shepherd R. Attributing information to different sources: effects on the perceived qualities of information, on the perceived relevance of information, and on attitude formation. Public Understand Sci 1994;3:385–401

Frewer LJ, Shepherd R, Sparks P. The interrelationship between perceived knowledge, control and risk associated with a range of food-related hazards targeted at the individual, other people and society. J Food Safety 1994;14:19–40

Greenpeace. Fishtomato.com. Available at http://www.fishtomato.com/. Accessed on October 4, 2001

Gregory, R., Slovic, P. and Flynn, J. 1995. Risk perceptions, stigma, and health policy. Health and Place 2(4): 213–220.

International Food Information Council (IFIC) (Wirthlin Group Quorum Survey). 2001. U.S. Consumer Attitudes Toward Food Biotechnology. Available:

http://www.ificinfo.health.org/foodbiotech/survey.htm

Ipsos-Reid. 2001. New Thoughts for Food: Consumer perceptions and attitudes toward foods. Final Report. Wave 1, March 30. Winnipeg, Manitoba.

Powell DA. Food safety and the consumer: perils of poor risk communication. Can J Anim Sci 2000;80(3):393–404

Powell DA. How Canada's beef ban helped protect consumers. Natl Post, February 28, 2001, p. C19

Powell DA, Leiss W. Mad cows and mother's milk: the perils of poor risk communication. Montreal: McGill-Queen's University Press, 1997

Powell DA, Ruiz MB, Whitfield A. On-farm food safety guidelines for greenhouse vegetables: final report. Leamington, Ontario: Ontario Greenhouse Vegetable Growers Association, 1999. Available at http://www.plant.uoguelph.ca/safefood/onfarm/ogvga/report.htm. Accessed on October 4, 2001

Slovic P. Perceived risk, stigma, and the vulnerable society. Presented at risk conference, June 13, 1997, City University, London

U.S. Department of Agriculture and U.S Department of Health and Human Services. Draft Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods, January 2001. Available at http://www.foodsafety.gov/~dms/lmrisk.html. Accessed on October 4, 2001

MYCOBACTERIUM PARATUBERCULOSIS: VILLAIN OR BYSTANDER?

The Evidence for and Against the Association of *Mycobacterium paratuberculosis* with Human Crohn's Disease

R. BALFOUR SARTOR, Department of Medicine, University of North Carolina, CB 7038, Room 032, Glaxo Building, Chapel Hill, North Carolina 27599-7038, USA

Crohn's disease bears a clinical resemblance to intestinal tuberculosis and Johne's disease. However, a possible mycobacterial etiology of this chronic, spontaneously relapsing granulomatous inflammatory disorder, which preferentially affects the distal ileum and colon of humans, remains a matter of intense debate. Recovery of ***Mycobacterium paratuberculosis*** from Crohn's disease tissues is quite low (<15%), even in the most experienced hands, with slow-growing spheroplasts slightly more frequently isolated. Although initial polymerase chain reaction studies detected IS900, a multicopy genomic DNA insertion element, in 65% of Crohn's disease tissues versus 13% of controls, recent investigations using similar techniques have been either negative or nonspecific.

Several groups have recently reported highly specific serologic responses to defined ***M. paratuberculosis*** proteins (p14, p35, and p36) in Crohn's disease patients, but no consistent T-cell responses to this organism have been detected. Some antibiotic trials report improvement of active Crohn's disease or prevention of relapse after steroid therapy, but the most favorable study was uncontrolled, and all protocols used agents active against normal commensal enteric bacteria, making interpretation difficult. Current evidence does not support a mycobacterial cause of the majority of cases of Crohn's disease, but it does suggest either that a small subset of patients are infected with this organism or that the ulcerated tissues of Crohn's disease patients selectively harbor ***M. paratuberculosis*** acquired as an environmental contaminant. Definitive proof of an etiologic role for ***M. paratuberculosis*** in Crohn's disease will depend on long-term improvement after effective antibiotic therapy that clears the organism from tissues.

The Etiology of Bovine Paratuberculosis and On-Farm Management Strategies

SCOTT J. WELLS, College of Veterinary Medicine, University of Minnesota, 435F Animal Science/ Veterinary Medicine Building, 1988 Fitch Avenue, St. Paul, Minnesota 55108, USA

Johne's disease is increasingly recognized as an important cause of animal disease in dairy cattle throughout the world, with increasing recognition in beef cattle and other ruminant livestock species. The causative bacterium is ***Mycobacterium avium*** subspecies ***paratuberculosis*** (***M. paratuberculosis***), an intracellular bacterium closely related to ***M. avium*** subsp. ***avium*** (***M. avium;*** Sweeney 1996). After initial infection in young calves, the disease progresses slowly, with clinical signs of diarrhea and weight loss most frequently appearing in cows from 3 to 6 years of age. There is no effective approved treatment for Johne's disease; clinically affected cattle are usually sold for slaughter purposes.

The National Animal Health Monitoring System (NAHMS) has estimated that the annual cost to infected U.S. dairy operations is more than $100 per cow in inventory, with higher costs of more than $200 per cow in inventory in herds with high infection levels (Ott et al. 1999). With at least 22% of U.S. dairy cattle herds and 8% of U.S. beef cattle herds infected with ***M. paratuberculosis*** (U.S. Department of Agriculture 1997) and with the continuing expansion of dairy herds and movement of cattle, implementation of Johne's disease control strategies is critically needed.

Control of Johne's disease on the farm requires an understanding of the clinical course of the disease, routes of transmission, and current use of specific herd management practices. Control measures often recommended to cattle producers include the following: (1) screening of purchased cattle to prevent introduction of infection to the herd, (2) identification of infected cattle and removal from the herd to prevent further transmission, (3) prevention of calf ingestion of the organism from adult cow manure, milk or colostrum, and water, and (4) decreased environmental contamination to reduce overall exposure to the organism. Because diagnostic tests are unable to detect the disease incubating in young cattle and because the tests lack desired sensitivity and specificity in adult cows, test-and-cull control programs are often not currently economically feasible (Van Groenendaal and Galligan1999) and individual cattle intended as herd replacements cannot be effectively screened prior to introduction to a herd. Because of these reasons, pathogen eradication is not a realistic goal in most dairy cattle operations.

Instead, control strategies at infected farms should preferentially focus on reducing the potential of transmission of infection. Studies have shown that the time of highest risk of transmission is the calving period and early calfhood (Goodger et al.1996, Johnson-Ifearulundu and Kaneene 1998, Wells and Wagner 2000). Researchers at the University of Pennsylvania modeled Johne's disease control using herd management strategies focusing on reduction of infection to the newborn calf and growing heifer (Van Groenendaal and Galligan 1999) and showed that the disease can be controlled cost effectively over a period of several years. The development of herd control plans has been facilitated recently with the development of herd risk assessment protocols to identify the areas of highest risk of transmission specific to each farm. The Minnesota Board of Animal Health in collaboration with faculty from the University of Minnesota developed a risk assessment–based Johne's disease control program. Several infected dairy and beef cattle herds are currently participating in a demonstration Johne's disease herd control program that will model the changes in herd infection subsequent to management changes (University of Minnesota 2001). Although it is still too early in the project to see results, we believe that the control of Johne's disease using a herd management plan developed from a herd risk assessment is scientifically valid and economically feasible for dairy producers.

References

Goodger WJ, Collins MT, Nordlund KV, et al. Epidemiologic study of on-farm management practices associated with prevalence of *Mycobacterium paratuberculosis* infections in dairy cattle. J Am Vet Med Assoc 1996;208:1877–1881

Johnson-Ifearulundu YJ, Kaneene JB. Management-related risk factors for *M. paratuberculosis* infection in Michigan, USA, dairy herds. Prev Vet Med 1998;37:41–54

Ott SL, Wells SJ, Wagner BA. Herd-level economic losses associated with Johne's disease on U.S. dairy operations. Prev Vet Med 1999;40:179–192

Sweeney RW. Transmission of paratuberculosis. In:Veterinary Clinics of North America: food animal practice. Philadelphia: WB Saunders, July 1996:305–312

University of Minnesota, Center for Dairy Health, Management and Food Quality. Johne's disease information source, September 2001. Available at www.cvm.umn.edu/dairycenter/johnes. Accessed on October 4, 2001

U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Centers for Epidemiology and Animal Health. Johne's disease on U.S. dairy operations. N245.1097. Fort Collins, CO: National Animal Health Monitoring System, 1997

Van Groenendaal H, Galligan DT. Economical consequences of Johne's disease control programs. Kennett Square, PA: Center of Animal Health and Productivity, University of Pennsylvania, 1999

Wells SJ, Wagner BA. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in U.S. dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in the herd and use of preventive measures. J Am Vet Med Assoc 2000;216 (9):1450–1457

Ecological and Physical Characteristics of *Mycobacterium paratuberculosis*

MICHAEL T. COLLINS, School of Veterinary Medicine, University of Wisconsin, 2015 Linden Drive, Madison, Wisconsin 53706, USA

Mycobacterium paratuberculosis has emerged as a major infectious disease problem in multiple domestic animal species. This slow-growing mycobacterial pathogen infects the intestinal tract, eventually disseminates to other organs of the body, and is excreted from animals both in manure and in milk. The majority of infected animals are in the preclinical stage of infection (healthy appearing) but are excreting the organism. Clinical manifestations of this chronic insidious disease, known as Johne's (pronounced YO-nees) disease, are wasting (cachexia) and diarrhea (common in cattle, but uncommon in sheep and goats). Methods to control this infection on the farm involve changes in farm management systems, in particular those relating to the rearing of herd replacement animals to minimize the likelihood of infection transmission, and testing programs to identify for early removal from the herd those animals most likely to be infectious. For a review of on-farm control of paratuberculosis, see the abstract by Scott Wells herein. Comprehensive coverage of ***M. paratuberculosis*** and the diseases it causes in animals can be found on the Internet (University of Wisconsin 2001).

Crux of the Problem

Foods originating from infected animals can be contaminated with ***M. paratuberculosis*** both before harvest (antemortem) and at harvest by fecal contamination. ***M. paratuberculosis*** is unique among mycobacteria in its inability to produce a siderophore called mycobactin. This "handicap" means that in nature the organism can multiply only in infected animals. To grow ***M. paratuberculosis*** in vitro, mycobactin must be added to the culture medium; hence, ***M. paratuberculosis*** is considered mycobactin dependent. To compensate for this apparent biological disadvantage, ***M. paratuberculosis*** has evolved mechanisms for persisting in the environment as it waits to be consumed by its next host animal and resume multiplication. As a result, the organism is notoriously resistant to heat, cold, drying, and other physical or chemical factors.

Understanding the capacity of ***M. paratuberculosis*** to persist in the environment is important for animal disease control programs. If the agent is a foodborne pathogen of humans, it is also important to understand, and if possible to quantify, the ability of ***M. paratuberculosis*** to resist killing by thermal, chemical, physical, and mechanical processes used in the manufacture of foods of animal origin. Unfortunately, very little research on ***M. paratuberculosis*** has been done in this field, so that results and observations made here should be considered preliminary at best.

Frequently, estimations about the capacity of ***M. paratuberculosis*** to resist killing by chemical or physical factors must be made by extrapolation of information on closely related microorganisms such as ***Mycobacterium avium***. Bulletin 362/2001 of the International Dairy Federation (ordering information can be found at http://fil-idf.org) provides the most current and comprehensive review of ***M. paratuberculosis*** with emphasis on dairy products (Task Force on ***Mycobacterium paratuberculosis*** 2001).

Specific Data

Most research to date has focused on the ability of ***M. paratuberculosis*** to survive pasteurization. Measurement of thermal D values (time required to decrease the viable count by 1 log) under standard laboratory conditions indicates that this agent is more thermal resistant than most other microbial pathogens (z value = 7.43°C) (Collins et al. 2001). Results of studies using laboratory-scale pasteurizers, however, are conflicting; some studies report 100% efficacy at killing ***M. paratuberculosis*** whereas others do not (Grant et al. 1998, Stabel et al. 1997). A survey of retail pasteurized milk in the United Kingdom resulted in recovery of ***M. paratuberculosis,*** supporting the suggestion that ***M. paratuberculosis*** is capable of resisting pasteurization (Advisory Committee on the Microbiological Safety of Food 2000, Stabel et al. 1997).

Low pH seems to adversely affect survival of ***M. paratuberculosis*** in buffered solutions as well as in cheese (Sung and Collins 2000). This find-

ing, however, conflicts with epidemiologic data indicating that low soil pH is associated with higher ***M. paratuberculosis*** infection rates in dairy cattle, presumably because the organism can persist in low-pH soils better than in alkaline soils (Johnson-Ifearulundu and Kaneene 1997, 1999). The ability of almost all other food manufacturing processes to kill or remove ***M. paratuberculosis*** from foods is unknown.

Because it is resistant to chlorine, ***M. paratuberculosis*** could potentially contaminate domestic water supplies. What adaptations ***M. paratuberculosis*** makes to a harsh extracellular environment and how long it may persist there are not known. It is also not known whether there are environmental niches such as free-living amoebae that may provide places for replication outside warm-blooded animals.

Quick and Crude Risk Analysis

M. paratuberculosis infections are common in domestic animals, and the prevalence of infection is rising globally. To date there are no systematic efforts of significant magnitude in any country except Australia to bring this epidemic under control. The host range is expanding. Recent published reports have shown for the first time that the infection spreads from domestic animals to rabbits and then to predators of rabbits. In Australia, marsupials that share habitat with infected domestic animals have acquired the infection. In ecological terms, the bioburden of ***M. paratuberculosis*** in the environment is steadily rising.

M. paratuberculosis is pathogenic for many animals. The host range is broad and includes nonhuman primates. All other pathogenic mycobacteria are considered zoonotic, and the genetically closest relative of ***M. paratuberculosis***, namely, ***M. avium***, infects humans. It is thus plausible that ***M. paratuberculosis*** is also capable of infecting humans.

M. paratuberculosis contaminates foods of animal origin, both preharvest (it is a disseminated bacterial infection, it can be cultured from multiple internal organs, and excretion occurs via the udder), at harvest via fecal contamination (for both meat and milk), and potentially postharvest via contaminated water.

M. paratuberculosis appears to be more resistant to chemical and physical inactivation than most microbes (based on limited data). It thus has a higher probability of contaminating processed foods of animal origin than do other microbes. However, once it is outside the animal, it is unlikely that ***M. paratuberculosis*** can replicate. Amplification of ***M. paratuberculosis*** numbers in products with low-level contamination should not be the problem it is with microbes like ***E. coli*** and ***Listeria monocytogenes***.

The consequences of human exposure to ***M. paratuberculosis*** are not known. However, in people with diseases that have clinical signs and pathology similar to those of Johne's disease in animals—namely, Crohn's disease—microbiological, molecular, and serologic evidence indicates that such people are infected with ***M. paratuberculosis***. However, whether ***M. paratuberculosis*** is present in these human patients as a primary (causative) or a secondary agent remains to be determined.

The Future

It is important that more research be done to understand the microbial ecology of ***M. paratuberculosis*** and to characterize the ability of ***M. paratuberculosis*** to resist killing or removal by physical and chemical methods used in food manufacturing and water treatment. It is not prudent to wait until medical science decides whether ***M. paratuberculosis*** is a human pathogen.

References

Advisory Committee on the Microbiological Safety of Food. Preliminary results from the national study on the microbiological quality and heat processing of cows' milk: *Mycobacterium avium* subsp. *paratuberculosis*. January 9, 2001. ACM/485, 1–5. Available at http://www.foodstandards.gov.uk/pdf_files/papers/acm485.pdf. Accessed on October 30, 2001

Collins MT, Spahr U, Murphy PM. Ecological characteristics of *M. paratuberculosis.* In: IDF Task Force on *M. paratuberculosis,* ed. Mycobacterium paratuberculosis. Brussels: International Dairy Federation, 2001:32–40

Grant IR, Ball HJ, Rowe MT. Effect of high-temperature, short-time (HTST) pasteurization on milk containing low numbers of *Mycobacterium paratuberculosis*. Lett Appl Microbiol 1998;26:166–170

Johnson-Ifearulundu Y, Kaneene JB. Distribution and environmental risk factors for paratuberculosis in dairy cattle herds in Michigan. Am J Vet Res 1999;60:589–596

Johnson-Ifearulundu YJ, Kaneene JB. Relationship between soil type and *Mycobacterium paratuberculosis.* J Am Vet Med Assoc 1997;210:1735–1740

Stabel JR, Steadham EM, Bolin CA. Heat inactivation of *Mycobacterium paratuberculosis* in raw milk: are current pasteurization conditions effective? Appl Environ Microbiol 1997;63:4975–4977

Sung N, Collins MT. Effect of three factors in cheese production (pH, salt, and heat) on *Mycobacterium avium* subsp. *paratuberculosis* viability. Appl Environ Microbiol 2000;66:1334–1339

Task Force on *Mycobacterium paratuberculosis*. *Mycobacterium paratuberculosis*. IDF Bulletin 362/2001. Brussels: International Dairy Federation, 2001

University of Wisconsin School of Veterinary Medicine. Johne's Information Center. Available at http://johnes.org. Accessed on October 4, 2001.

Methods for Detecting *Mycobacterium paratuberculosis* in Food Products

JUDITH R. STABEL, U.S. Department of Agriculture, Agricultural Research Service, National Animal Disease Center, 2300 Dayton Road, Ames, Iowa 50010, USA

Paratuberculosis

The first significant recognition of paratuberculosis in cattle was by Johne and Frothingham (1895), who successfully isolated acid-fast bacilli from sections of diseased intestine from cattle with severe enteritis. Subsequently, it was demonstrated that the causative agent of paratuberculosis, a chronic granulomatous enteritis of ruminants, was the acid-fast bacterium ***Mycobacterium paratuberculosis*** (Twort and Ingram 1912). Clinical paratuberculosis is characterized by profuse, nontreatable diarrhea, emaciation, and eventual death. During the course of the disease, the intestine becomes thickened and corrugated, disallowing proper absorption of nutrients. Although cattle usually become infected as calves by ingestion of feces contaminated with ***M. paratuberculosis***, clinical signs of disease may not appear until the animals reach 3 to 5 years of age. Indeed, not all infected cattle will develop clinical disease (Larsen et al. 1975). Clinical disease may be precipitated by stressors such as parturition, lactation, dietary deficiencies, or parasitemia.

Paratuberculosis represents a significant economic loss to the dairy industry, because the disease has been reported in 22% of dairy herds in the United States (Wells and Wagner 2000). In addition to losses incurred by culling clinical animals from the herd, producers are faced with further economic losses from cows with subclinical paratuberculosis through an increased incidence of mastitis, decreased milk production, and increased calving intervals (Chiodini et al. 1984).

Crohn's Disease

Crohn's disease in humans manifests as severe inflammatory enteritis involving the terminal ileum. It thus shares some characteristics with paratuberculosis in ruminants. However, an etiologic agent for Crohn's disease has not yet been identified. Studies have been conducted in an attempt to identify a single agent that may be responsible for this. Despite many reports of successful isolations of viral and bacterial pathogens from affected intestinal biopsies of Crohn's patients, however, a singular causative agent has not been recognized. Although some studies have demonstrated the presence of ***M. paratuberculosis*** in biopsy tissue of patients, several other species of mycobacteria, including ***M. fortuitum***, ***M. avium-intracellulare***, ***M. cheloni***, and ***M. kansasii*** have been identified (Chiodini 1989). Because the clinical symptoms of Crohn's disease closely mimic those found in animals with paratuberculosis, a number of laboratories have proposed that ***M. paratuberculosis*** may be the causative agent of Crohn's disease (Sanderson et al. 1992, Mishina et al. 1996).

Modes of *M. paratuberculosis* Transmission

Several potential modes of transmission of the infective organism, ***M. paratuberculosis***, from the host animal to humans have been discussed. Early studies established that ***M. paratuberculosis*** is capable of surviving in water, soil, and manure for periods of up to 1 year (Lovell et al. 1944). There has thus been speculation that human beings may be exposed to the bacteria through contact with contaminated water sources. Closely related mycobacterial species such as ***M. avium*** and ***M. intracellulare*** have been found in water and biofilm samples from water distribution systems in the United States (Falkinham et al. 2001). ***M. paratuberculosis*** is also shed in the colostrum and milk of infected dams, albeit at low numbers. Although shedding of the bacterium in colostrum has not been adequately quantitated, it is highly correlated with heavy shedding in the feces of infected dams (Streeter et al. 1995). Shedding of ***M. paratuberculosis*** in the milk of infected dams is also associated with the degree of fecal shedding by the dam, and ***M. paratuberculosis*** has been found in viable numbers at low concentrations (5 to 8 cfu/50 mL milk) (Taylor et al. 1981, Sweeney et al. 1992). Exposure of humans to ***M. paratuberculosis*** has largely been thought to be through contaminated food products.

Culture Methods for *M. paratuberculosis*

The current concerns regarding a possible relationship between Crohn's disease and ***M. paratuberculosis*** were stimulated by a report in 1994 that ***M. paratuberculosis*** DNA could be detected in pasteurized retail milk samples purchased from markets in the United Kingdom (Millar et al. 1996). This report was followed by several studies that evaluated the effects of heat treatment on the survivability of ***M. paratuberculosis*** in milk (Grant et al. 1996, Hope et al. 1996, Stabel et al. 1997, Sung and Collins 1998, Keswani and Frank 1998).

At great issue in the interpretation of the results of these studies are the different methods used by the different laboratories to recover the organisms. All of the published studies performed either liquid- or solid-medium culture on treated milk samples to recover viable organisms. The most common form of culture for ***M. paratuberculosis*** uses Herrold's Egg Yolk Medium, but other solid mediums such as Lowenstein-Jensen or Middlebrook 7H10 agar have been used successfully to isolate ***M. paratuberculosis*** from milk. Alternatively, liquid-culture systems such as BACTEC radiometric medium have also been employed. Interestingly, a single study that reported recovery of viable ***M. paratuberculosis*** from the breast milk of Crohn's disease patients used two liquid-medium systems, BACTEC and the Mycobacterial Growth Indicator Tube (Naser et al. 2000).

The sensitivity of detection in solid compared with liquid mediums has been reported to be between 1 and 130 cfu/mL milk (Grant and Rowe 2001). Preenrichment of heat-treated bacteria in Dubo's broth medium prior to culture on conventional liquid or solid mediums may improve the recovery of sublethally injured organisms (Phillip Hammer, personal communication, 2001).

Decontamination Protocols

Decontamination of treated milk samples prior to culture in either solid or liquid medium is often necessary to circumvent overgrowth by faster-growing contaminants. Chemical decontamination introduces unknown variables into the efficient recovery of sublethally injured cells. Protocols that have been employed for decontamination include preincubation with either 0.75% or 0.9% hexadecylpyridinium chloride (HPC) for periods of time ranging from 1 hour to overnight; 4% NaOH plus N-acetyl L-cysteine and 2.9% sodium citrate for 30 minutes; and overnight decontamination in 0.9% HPC in 0.5X brain heart infusion broth followed by second overnight incubation in antibiotic solution (vancomycin, naladixic acid, fungizone). Another decontamination protocol that has been successful for recovery of ***M. bovis*** in milk samples used C_{18}-carboxybetaine (CB-18), a zwitterionic detergent (Cornejo et al. 1998).

PCR

Confirmation of the presence of ***M. paratuberculosis*** in the culture medium should be performed to rule out false-positive readings by other contaminating microorganisms. This can be done by polymerase chain reaction (PCR) using a known specific gene for ***M. paratuberculosis*** such as IS900. Although PCR cannot distinguish between live and dead organisms, it is frequently used as a detection tool. Methods for extraction of ***M. paratuberculosis*** DNA from milk samples are quite varied and range from a simple boil technique in Tris-EDTA buffer to release the DNA to more sophisticated, protracted methods employing enzymatic and chemical treatment with and without a bead beater to mechanically disrupt the cells (Grant and Rowe 2001).

Implementation of immunomagnetic separation of ***M. paratuberculosis*** organisms from the milk before extraction of DNA prior to PCR yielded a sensitivity of detection similar to that of culture (10 cfu/50 mL milk) (Grant et al. 2000). Yet this technique is expensive and can be problematic when used with raw bulk tank milk, since other microorganisms present in milk may bind nonspecifically to the antibody-coated beads. Application of this capture method to direct culture of the ***M. paratuberculosis*** bound to the beads has not been successful to date.

Fluorescent Staining

More novel methods for detecting viable ***M. paratuberculosis*** were recently evaluated (Herman et al. 1999). Direct epifluorescence filter technique microscopy using acridine orange staining and solid-phase cytometry (ChemScan) using a fluorogenic substrate for esterase were used successfully to detect viable ***M. paratuberculosis*** in milk. The BactoScan 8000, an instrument that separates bacteria from other milk components, stains them with acridine orange, and counts the

bacteria using a continuous-flow fluorescent microscope, was also evaluated for detection of viable ***M. paratuberculosis,*** but results were more variable than for other methodologies tested. These techniques were compared with PCR and culture for relative sensitivity of recovery of spiked organisms in milk, and the detection levels were found to be far superior.

In summary, detection thresholds for current techniques to recover viable ***M. paratuberculosis*** from milk and dairy products may be the limiting factor in studies that evaluate the survival of this bacterium after heat treatment. Owing to the low level of ***M. paratuberculosis*** shedding into milk by naturally infected cows, it is essential to improve the detection threshold to as high a degree of sensitivity as possible. The improvement of existing techniques is confounded by the need to decontaminate milk samples to reduce the load of other microorganisms that may overgrow ***M. paratuberculosis*** in culture.

References

Chiodini RJ, Van Kruiningen HJ, Merkal RS. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 1984;74:218–262

Chiodini RJ. Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. Clin Microbiol Rev 1989;2:90–117

Cornejo BJ, Shagun-Ruiz A, Suarez-Guemes F, et al. Comparison of C_{18}-carboxypropylbetaine and glass bead DNA extraction methods for detection of *Mycobacterium bovis* in bovine milk samples and analysis of samples by PCR. Appl Environ Microbiol 1998;64:3099–3101

Falkinham JO, Norton CD, LeChevallier MW. Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. Appl Environ Microbiol 2001;67:1225–1231

Grant IR, Ball HJ, Neill SD, Rowe MT. Inactivation of *Mycobacterium paratuberculosis* in cows' milk at pasteurization temperatures. Appl Environ Microbiol 1996;62:631–636

Grant IR, Pope CM, O'Riordan LM, Ball HJ, Rowe MT. Improved detection of *Mycobacterium avium* subsp. *paratuberculosis* in milk by immunomagnetic PCR. Vet Microbiol 2000; 77:369–378.

Grant IR, Rowe MT. Methods of detection and enumeration of viable *Mycobacterium paratuberculosis* from milk and milk products. Bull Intl Dairy Fed 2001; 362:53–60.

Herman L, Reybroeck W, D'Haese E, et al. Detection and enumeration of *Mycobacterium avium* subsp. *paratuberculosis* in milk. In: Proceedings of the 6th International Colloquium on Paratuberculosis, Melbourne, Australia, February 14–18, 1999: 543–552

Hope AF, Tulk PA, Condron RJ. Pasteurization of *Mycobacterium paratuberculosis* in whole milk. In: Proceedings of the 5th International Colloquium on Paratuberculosis, Madison, WI, September 29-October 4, 1996:377–382

Johne HA, Frothingham J. Ein eigenthuemlicher Fall von Tuberculose beim Rind. Deutsch Z Tiermed Pathol 1895;21:438–454

Keswani J, Frank JF. Thermal inactivation of *Mycobacterium paratuberculosis* in milk. J Food Protect 1998;61:974–978

Larsen AB, Merkal RS, Cutlip RC. Age of cattle as related to resistance to infection with *Mycobacterium paratuberculosis*. Am J Vet Res 1975;36:255–257

Lovell R, Levi M, Francis J. Studies on the survival of Johne's bacillus. J Comp Pathol Therapeut 1944;54:120–129

Millar D, Ford J, Sanderson J, et al. IS900 PCR to detect *Mycobacterium paratuberculosis* in retail supplies of whole pasteurized cows' milk in England and Wales. Appl Environ Microbiol 1996;62:3446–3452

Mishina D, Katsel P, Brown ST, et al. On the etiology of Crohn's disease. Proc Natl Acad Sci USA 1996;93:9816–9820

Naser SA, Schwartz D, Shafran I. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from breast milk of Crohn's disease patients. Am J Gastroenterol 2000;95:1094–1095

Sanderson JD, Moss MT, Tizard ML, Hermon-Taylor J. *Mycobacterium paratuberculosis* DNA in Crohn's disease tissue. Gut 1992;33:890–896

Stabel JR, Steadham EM, Bolin CA. Heat inactivation of *Mycobacterium paratuberculosis* in raw milk: are current pasteurization conditions effective? Appl Environ Microbiol 1997;63:4975–4977

Streeter RN, Hoffsis GF, Bech-Nielsen S, et al. Isolation of *Mycobacterium paratuberculosis* from colostrum and milk of subclinically infected cows. Am J Vet Res 1995;56:1322–1324

Sung N, Collins MT. Thermal tolerance of *Mycobacterium paratuberculosis*. Appl Environ Microbiol 1998;64:999–1005

Sung N, Collins MT. Effect of three factors in cheese production (pH, salt and heat) on *Mycobacterium avium* subsp. *paratuberculosis* viability. Appl Environ Microbiol 2000;66:1334–1339

Sweeney RW, Whitlock RH, Rosenberger AE. *Mycobacterium paratuberculosis* cultured from milk and supramammary lymph nodes of infected asymptomatic cows. J Clin Microbiol 1992;30:166–171

Taylor TK, Wilks CR, McQueen DS. Isolation of *Mycobacterium paratuberculosis* from the milk of a cow with Johne's disease. Vet Rec 1981;109:532–533.

Twort FW, Ingram GLY. A method for isolating and cultivating *Mycobacterium enteriditis chronicae pseudotuberculosis bovis,* Johne, and some experiments on the preparation of a diagnostic vaccine for pseudotuberculosis enteritis of bovines. Proc R Soc Lond B Biol Sci 1912;84:517–542

Wells SJ, Wagner BA. Herd-level risk factors for infection with *Mycobacterium paratuberculosis* in US dairies and association between familiarity of the herd manager with the disease or prior diagnosis of the disease in that herd and use of preventive measures. J Am Vet Med Assoc 2000;216:1450–1457

Detection of *Mycobacterium paratuberculosis* in Retail Milk in the United Kingdom: Analysis and Perspectives

NORMAN A. SIMMONS, Emeritus Consultant in Microbiology, Guy's and St. Thomas' Hospital Trust, 7–9 William Road, London NW1 3ER, United Kingdom

In the United Kingdom, the Ministry of Agriculture, Fisheries and Food (MAFF) has funded research on ***Mycobacterium avium*** subsp. ***paratuberculosis*** (MAP) since 1993. The organism is known to be the cause of Johne's disease in cattle, sheep, goats, wild ruminants, and deer. What is in dispute is whether or not it causes Crohn's disease in humans. Consideration of the answer to that question is beyond the scope of this presentation, but it is relevant that specialist groups and committees in the United Kingdom, United States, and European Union have failed to reach a consensus on whether MAP is the cause, and in the short term at least the debate seems set to continue.

If MAP does cause Crohn's disease, one potentially important vehicle of transmission is milk. Cows with Johne's disease may excrete MAP in their milk, which may also be contaminated with feces in which the organism may be present in large numbers. Even milk from animals in the asymptomatic phase of Johne's disease may contain MAP, albeit probably in smaller numbers.

Pasteurization was introduced into the U.K. dairy industry as a public health measure around 1920. It was designed to kill the most heat-resistant, non-spore-forming pathogenic organisms likely to be found in milk at that time, namely, ***Mycobacterium tuberculosis,*** the cause of tuberculosis, and ***Coxiella burnetii,*** the cause of Q fever. This was achieved by the application of one of the following time-temperature combinations: 63°C for 30 minutes (holder process) or 72°C for 15 seconds (high-temperature, short-time, or HTST, process). It was hoped and expected that HTST, the process used in most dairies, would be effective in killing MAP, but that was disputed; consequently, the effectiveness of pasteurization in killing MAP needed investigation.

Studies of all aspects MAP, including its pathogenicity in man and animals, the prevalence of Johne's disease in herds throughout the world, the detection of MAP excretion in asymptomatic animals, and the heat resistance of the organism, have all been severely hampered by technical difficulties. The organism is slow and difficult to grow, and cultures of milk in which pasteurization studies are carried out frequently become contaminated with other bacteria. Consequently, investigations that with other, more amenable organisms could be carried out rapidly take months or years to complete.

Preliminary results of laboratory tests indicated that at holder pasteurization temperature (63° C), the thermal inactivation temperature was not linear and exhibited tailing, perhaps owing to clumping; that with an inoculum of 10^6 cfu/mL spiked into raw milk, sporadic survival of MAP after HTST pasteurization was possible at temperatures up to 90° C; and that with the same inoculum, one of three laboratory strains survived 72°C for 15 seconds but not 25 seconds. (This information was presented to the Advisory Committee on the Microbiological Safety of Food. See the Food Standards Agency website at http://www.foodstandards.gov.uk/pdf_files/papers/acm486.pdf; accessed on October 31, 2001.)

In the same year, a preliminary survey of 62 milk samples from dairies in Northern Ireland using immunomagnetic separation and polymerase chain reaction (PCR) to identify acid-fast bacteria in cultures had found MAP in 10 of 31 samples of raw milk and 6 of 31 samples of pasteurized milk. Another survey of retail samples from south and central England demonstrated MAP in 22 of 312 samples of retail milk by PCR, but cultures were negative.

As a consequence of these findings, tests for MAP were included in a survey of samples of milk taken from 258 of the 755 approved U.K. dairies. The Food Standards Agency made the results of MAP tests on 679 of these samples available in 2000. (This information was presented to the Advisory Committee on the Microbiological Safety of Food and appears on the Food Standards Agency website at http://www.foodstandards.gov.uk/pdf_files/papers/acm485.pdf; accessed on October 31, 2001.)

Viable MAP was found in 4 (2%) of 201 samples of raw milk and 10 (2.1%) of 476 samples

of pasteurized milk. The organism was found in 3 (1.6%) of 191 samples of pasteurized whole milk, 5 (3.4%) of 145 samples of pasteurized semi-skimmed milk, and 2 (1.4%) of 140 samples of pasteurized skimmed milk. The 10 positive pasteurized milk samples came from 8 dairies, 3 of which had treated the milk at temperatures just above 72°C (72.2, 74, and 75°C) for at least 25 seconds.

In May 2001, the Food Standards Agency convened a conference of stakeholders to consider the ways in which the risks might be minimized. At the present time it is impossible to detect and eliminate all infected animals from herds, but the removal of symptomatic animals with Johne's disease and general measures to reduce bacterial contamination of milk should reduce the amount of MAP in the milk. This in turn should improve the effectiveness of pasteurization. Research is being conducted into the effectiveness of other measures, such as double pasteurization and high-pressure homogenization.

ILSI NORTH AMERICA–SPONSORED RESEARCH UPDATES

Engineering Vegetative Buffer Strips for the Removal of Amphixenotic *Cryptosporidium parvum* from Runoff of Dairies and Grazed Agricultural Land

EDWARD R. ATWILL, Lingling Hou, Betsy M. Karle, Thomas Harter, Kenneth W. Tate, and Randy A. Dahlgren, Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California–Davis, Tulare, California 93274, USA

Cryptosporidium parvum has emerged as a widespread and persistent waterborne microbial pathogen with specific genotypes able to be transmitted ambidirectionally between livestock and humans (i.e., amphixenotic) (Peng et al. 1997, Spano et al. 1997, DuPont et al. 1995). Although we still do not know the percentage of annual cases of human cryptosporidiosis that are attributable to livestock-derived waterborne ***C. parvum***, it may be prudent to reduce the likelihood that animal agricultural operations contaminate surface water with infective ***C. parvum*** oocysts as a proactive goal to support water quality and public health (Rosen et al. 2000).

There are several general strategies for minimizing the likelihood that an animal agricultural operation will contaminate surface water with infective ***C. parvum*** oocysts. One such strategy is to reduce the incidence or the intensity of fecal shedding of ***C. parvum*** oocysts by livestock populations, thereby reducing the rate of environmental loading of ***C. parvum*** per livestock unit (Hoar et al. 2000). These herd-health efforts remain hampered by our poor understanding of the medical ecology of ***C. parvum*** within livestock populations (Atwill et al. 1999, Mohammed et al. 1999, Garber et al. 1994) and how to interrupt transmission between the biological reservoir and susceptible animals (Atwill et al. 1998) and by the lack of an affordable vaccine proven to be efficacious under commercial agricultural settings (Harp et al. 1996, Perryman et al. 1999).

Another strategy is to manage the manure produced by livestock so that the survivability and off-site transport of infective ***C. parvum*** are substantially reduced (Sischo et al. 2000, Grazyck et al. 2000, Atwill et al. 1999, Jenkins et al. 1999). One method being advocated for minimizing the transport potential of ***C. parvum*** oocysts from animal manure to surface water is to place vegetated buffer strips between animal agricultural operations and vulnerable surface water supplies (Entry et al. 2000, Rosen et al. 2000, Younos et al. 1998, Mawdsley et al. 1996a, 1996b, Coyne et al. 1995, Larsen et al. 1994, Young et al. 1980). Optimal design criteria for on-farm vegetated buffer strips currently do not exist for waterborne microbial contaminants. Moreover, studies on the fate and transport of zoonotic protozoal pathogens such as ***Cryptosporidium parvum*** in overland and subsurface flow through such buffers are severely lacking (Mawdsley et al. 1996a, 1996b, Harter et al. 1999, Tate et al. 2000).

Our goal for this project was to assess the ability of a meter of vegetated buffer to remove waterborne ***C. parvum*** oocysts from overland and shallow subsurface flow as a function of soil and vegetative characteristics, slope of the buffer, and rainfall intensity, and to provide design criteria for vegetated buffers that remove = 99.9% of ***C. parvum*** oocysts from agricultural runoff.

Material and Methods

Our primary goal was to estimate the total log reduction of waterborne ***C. parvum*** oocysts attributable to a meter of vegetated buffer strip during conditions of rainfall and overland flow. The filtration efficiency (or log reduction) was estimated as a function of soil type, soil bulk density, vegetative height, percent slope (5%, 10%, or 20%), and two rainfall intensities, with three replicates per treatment combination. The three soils were a Hanford fine sandy loam (Typic Xerorthent), Argonaut loam (Mollic Haploxeralf), and a Capay silty clay (Typic Chromoxerert). The rainfall emitter was a drip-type box simulator (Chow and Hardaugh 1965) and operated to emit mean rainfall intensities of 1.5 and 4.0 mL/cm^2 per hour over 4 hours. These rainfall intensities functioned as idealized storm events occurring every 2 to 3 and 20 to 30 years for various agricultural regions in the United States (Hershfield 1961). Soil boxes that measured 15 cm wide × 100 cm long × 20 cm deep were designed to capture overland and subsurface flow. Perennial fescue seed was used to vegetate the soil boxes to a level of ≥ 80% vegeta-

tive cover. ***C. parvum*** oocysts were collected and purified from naturally infected calves. Two to four boxes were rained on simultaneously, and once steady-state conditions were established, a peristaltic pump located at the upper end of the soil boxes delivered to each box a suspension of $5X10^4$ oocysts/mL at a rate of 10 mL/minute ($5X10^5$ oocysts/minute) for 60 minutes. After 60 minutes, oocyst application was stopped but the rainfall was continued for an additional 180 minutes to quantify unbound and desorbed oocysts moving in overland or subsurface flow. Approximately $3X10^7$ oocysts were applied to each box. Adjusted for percent recovery (Pereira et al. 1999), ***C. parvum*** oocysts were enumerated by fluorescence microscopy using commercially prepared well-slides (Meridian Diagnostics, Inc., Cincinnati, OH) and a fluorescein isothiocyanate–labeled anti-***Cryptosporidium*** immunoglobulin M antibody (Waterborne, Inc., New Orleans, LA).

Our covariates (independent variables) for each treatment combination were soil type, mean grass height (cm) across the soil box, mean soil bulk density (g/cm^3), percent slope, rainfall intensity, and total volume of overland and subsurface flow during the 4-hour rainstorm. Mean 4-hour total subsurface flow was converted to the mean infiltration rate (mL/cm^2 per hour). The outcome (dependent) variable was the total number of nonfiltered and desorbed ***C. parvum*** oocysts discharging off the soil box in either overland or subsurface flow during the 4-hour rain event. A linear mixed-effects regression model (S-Plus 2000, MathSoft, Inc., Seattle, WA) was used to determine the association between the covariates (or fixed effects) and the total number of oocysts discharging off the vegetated buffer. Values for the total number of oocysts were transformed via the natural logarithm, and the experiment (15 randomized sets of two to four soil boxes per experiment) was modeled as a random effect. We used a forward-stepping algorithm to build the model, with significance for inclusion in the model set at a ***P*** value ≤ 0.10, using a restricted maximum-likelihood ratio test.

Results and Brief Discussion

Factors significantly associated with total oocysts in the effluent of overland flow (the inverse of filtration efficiency) were soil series and percent slope of the vegetated buffer, but these associations were strongly influenced by the underlying bulk density of the soil (i.e., strong interactions). Increasing the soil's bulk density from 0.5 to 1.7 g/cm^3 reduced the ability of the buffer to remove waterborne oocysts in overland flow. This reduction in oocyst filtration associated with higher bulk densities (i.e., more compacted and less porous soils) was greatest for Argonaut loam and buffers at 10% slope. In general, buffers constructed of Hanford fine sandy loam compared with the other two soils were least effective at removing oocysts in overland flow, particularly at high bulk densities whereby up to $3X10^6$ oocysts remained unfiltered or had desorbed. Given that soil series, percent slope, and interactions with bulk density were in the regression model, the infiltration rate, percent vegetative cover, vegetative height, rate of total overland flow, and precipitation rate (2–3 or 20–30 year return interval storm) were not associated with filtration efficiency for oocysts in overland flow.

Factors significantly associated with total oocysts in the effluent of subsurface flow were soil series and infiltration rate (mL/cm^2 per hour), but these associations were strongly influenced by the storm series (precipitation rate of ≈1.5 or ≈4.0 mL/cm^2 per hour for 4 hours) (i.e., strong interactions). At the lower precipitation rate (≈1.5 mL/cm^2 per hour), for every increase of 0.1 mL/cm^2 per hour in the infiltration rate, the total number of oocysts in the effluent of subsurface flow was increased by 86%. In contrast, at the higher precipitation rate (≈4.0 mL/cm^2 per hour), for every increase of 0.1 mL/cm^2 per hour in the infiltration rate, the total number of oocysts in the effluent of subsurface flow was increased by only 29%. The magnitude of these associations was the same for each soil series. Buffers constructed of Hanford fine sandy loam resulted in 81% more oocysts in subsurface effluent relative to buffers constructed of Capay silty clay. All other biotic and abiotic factors were not significant.

There was reasonable agreement between the observed number of total nonfiltered and desorbed oocysts in overland flow and the values predicted by the linear mixed-effects model. Therefore, coefficients from the linear mixed-effects models were used to calculate the mean filtration efficiency ($\log_{10}$ reduction) associated with the use of a meter of vegetated buffer. These predictions were calculated as a function of soil se-

ries, percent slope of buffer, and interactions with bulk density, given that these factors were significant in the regression model. Hence, the mean $\log_{10}$ reduction of waterborne ***C. parvum*** oocysts (bovine genotype A) associated with a vegetated buffer 15 cm wide × 100 cm long under these experimental conditions ranged from 1.0 to 3.1 (Table 1). Oocyst counts in subsurface flow were excluded from these calculations because in almost all cases total oocyst counts in subsurface flow did not exceed 10,000 oocysts (0.0003 of total possible oocysts), resulting in a negligible effect on the calculated $\log_{10}$ reduction of waterborne ***C. parvum*** oocysts. Based on the data presented above, vegetated buffer strips with a soil bulk density ≤ 1.7 g/cm^3, ≤ 20%slope, and ≥ 3 m long should function to remove 99.9% of ***C. parvum*** oocysts from overland flow generated during mild to moderate rainfall events. These minimal design criteria for a 3-$\log_{10}$ reduction are recommended under the assumption that vegetated buffers will be actively maintained such that overland and subsurface preferential flow paths will be kept to a minimum. Communities with high levels of water quality risk intolerance, substantial amounts of recreational contact with surface water, or watersheds with high rates of ***C. parvum*** loading may want to strengthen these standards to further minimize overland flow of ***C. parvum***.

References

Atwill ER, Johnson E, Pereira M, Das Graças C. Association of herd composition, stocking rate, and calving duration with fecal shedding of *Cryptosporidium parvum* oocysts in beef herds. J Am Vet Med Assoc 1999;215:1833–1838

Atwill ER, Harp JA, Jones T, et al. Evaluation of periparturient dairy cows and contact surfaces as a reservoir of *Cryptosporidium parvum* for calfhood infection. Am J Vet Res 1998; 59:1116–1121

Chow VT, Hardaugh TE. Raindrop production for laboratory watershed experimentation. J Geophys Res 1965;70:6111–6119

Coyne MS, Gilifillen RA, Rhodes RW, Blevins RL. Soil and fecal coliform trapping by grass filter during simulated rainfall. J Soil Water Conserv 1995;50:405–408

DuPont HL, Chappell CL, Sterling CR, et al. The infectivity of *Cryptosporidium parvum* in healthy volunteers. N Engl J Med 1995;332:855–859

Entry JA, Hubbard RK, Thies JE, Fuhrmann JJ. The influence of vegetation in riparian filterstrips on coliform bacteria, I: Movement and survival in water. J Environ Qual 2000;29:1206–1214

Garber LP, Salman MD, Hurd HS, et al. Potential risk factors for *Cryptosporidium* infection in dairy calves. J Am Vet Med Assoc 1994;205:86–91

Grazyck TK, Evans BM, Shiff CJ, et al. Environmental and geographical factors contributing to watershed contamination with *Cryptosporidium parvum* oocysts. Environ Res 2000;82:263–271

Harp JA, Jardon P, Atwill ER, et al. Field testing of prophylactic measures against *Cryptosporidium parvum* infection in calves in a California dairy herd. Am J Vet Res 1996;57:1586–1588

Harter T, Wagner S, Atwill ER. Colloid transport and filtration of *Cryptosporidium parvum* in sandy porous media (soils and groundwater). Environ Sci Technol 1999;34:62–70

Table 1. Predictions of $\log_{10}$ reduction of waterborne *Cryptosporidium parvum* oocysts in overland flow attributable to 1 m vegetative buffer strip filtration[a]

	Bulk density (g/cm^3)			
	0.66	1.0	1.33	1.66
Soil series and % slope of vegetative buffer strip	$\log_{10}$ reduction of *C. parvum* oocysts per meter of vegetative buffer strip[a]			
Capay silty clay, 5% slope	2.2	2.0	1.8	—
Capay silty clay, 10% slope	3.1	2.5	1.9	—
Capay silty clay, 20% slope	2.7	2.4	2.1	—
Argonaut loam, 5% slope	—	2.4	1.8	—
Argonaut loam, 10% slope	—	2.9	1.9	—
Argonaut loam, 20% slope	—	2.8	2.1	—
Hanford fine sandy loam, 5% slope	—	—	1.7	1.4
Hanford fine sandy loam, 10% slope	—	—	1.7	1.0
Hanford fine sandy loam, 20% slope	—	—	1.9	1.4

[a] Predictions calculated as the $\log_{10}$ [(mean number of total oocysts discharged from buffer in overland flow effluent, estimated from the linear mixed-effects regression model) / (3X10^7 spiked oocysts)].

Hershfield DM. Rainfall frequency atlas of the United States for duration from 30 minutes to 24 hours and return periods from 1 to 100 years. Technical Paper No. 40. Washington, DC: U.S. Department of Commerce, 1961

Hoar B, Atwill ER, Farver TB. Estimating maximum possible environmental loading amounts of *Cryptosporidium parvum* attributable to adult beef cattle. Quant Microbiol 2000;2:21–36

Jenkins MB, Walker MJ, Bowman DD, et al. Use of a sentinel system for field measurements of *Cryptosporidium parvum* oocyst inactivation in soil and animal waste. Appl Environ Microbiol 1999;65:1998–2005

Larsen RE, Miner JR, Buckhouse JC, Moore JA. Water quality benefits of having cattle manure deposited away from streams. Biores Tech 1994; 48:113–118

Mawdsley JL, Brooks AE, Merry RJ. Movement of the protozoan *Cryptosporidium parvum* through three contrasting soil types. Biol Fertil Soil 1996;21:30–36 (a)

Mawdsley JL, Brooks AE, Merry RJ, et al. Use of a novel titling table apparatus to demonstrate the horizontal and vertical movement of the protozoan *Cryptosporidium parvum* in soil. Biol Fertil Soil 1996;23:215–220 (b)

Mohammed HO, Wade SE, Schaaf S. Risk factors associated with *Cryptosporidium parvum* infection in dairy cattle in southeastern New York State. Vet Parasitol 1999;83:1–13

Peng MM, Xiao L, Freeman AR, et al. Genetic polymorphism among *Cryptosporidium* isolates: evidence of two distinct human transmission cycles. Emerg Infect Dis 1997;3:567–572

Pereira M, das Graças C, Atwill ER, Jones T. Comparison of sensitivity of immuno-fluorescent microscopy to that of a combination of immunomagnetic separation and immuno-fluorescent microscopy for detection of *Cryptosporidium parvum* oocysts in adult bovine feces. Appl Environ Microbiol 1999;65:3236–3239

Perryman LE, Kapil SJ, Jones ML, et al. Protection of calves against cryptosporidiosis with immune bovine colostrum induced by a *Cryptosporidium parvum* recombinant protein. Vaccine 1999;17:2142–2149

Rosen BH, Croft R, Atwill ER, et al. Waterborne pathogens in agricultural watersheds. Technical Note 2. Burlington, VT: Watershed Science Institute, University of Vermont, Natural Resource Conservation Service, USDA, 2000:1–64

Sischo WM, Atwill ER, George J, Lanyon LE. Cryptosporidia on dairy farms and the role these farms may have in contaminating surface water supplies in the northeastern United States. Prev Vet Med 2000;43:253–267

Spano F, Putignani L, McLauchling L, et al. PCR-RFLP analysis of the *Cryptosporidium* oocyst wall protein (COWP) gene discriminates between *C. wrari* and *C. parvum*, and between *C. parvum* isolates of human and animal origin. FEMS Microbiol Lett 1997;150:209–217

Tate KW, Atwill ER, George MR, et al. *Cryptosporidium parvum* mobilization and transport from livestock fecal deposits on California rangeland watersheds. J Range Manage 2000;53:295–299

Young RA, Hundtrods T, Anderson W. Effectiveness of vegetated buffer strips in controlling pollution from feedlot runoff. J Environ Qual 1980;9:483–487

Younos TM, Mendez A, Collins ER, Ross BB. Effects of a dairy loafing lot-buffer strip on stream water quality. J Am Water Resources Assoc 1998;34:1061–1069

Optimization of Conditions to Kill *Escherichia coli* O157:H7 in Manure

Xiuping Jiang, Jennie Morgan, and MICHAEL P. DOYLE, Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223-1797, USA

Raw animal manure, which carries human pathogens, is routinely used by farmers to fertilize crops. Contamination of food either directly or indirectly by animal manure is a contributing factor in many outbreaks of foodborne illness. The objectives of our studies were to identify conditions affecting the survival and inactivation of ***Escherichia coli*** O157:H7 in manure when applied to soil or used in compost. We used a five-strain mixture of green fluorescent protein-labeled ***E. coli*** O157:H7 to conduct studies in which the pathogen in manure was added to autoclaved or unautoclaved soil in ratios of 1:10, 1:25, 1:50, and 1:100 and held at 5, 15, or 21°C and monitored for rates of inactivation. In addition, we conducted studies of the pathogen in cow manure compost of 38% moisture content to determine rates of inactivation at constant temperatures of 50, 55, 60, 65, and 70°C. We also conducted studies in a bioreactor at a controlled external temperature of 21 or 50°C to develop a model system to use in identifying conditions that influence the inactivation of ***E. coli*** O157:H7 in compost during composting.

Our results revealed that ***E. coli*** O157:H7 survived for up to 91 days at 5°C in manure-amended autoclaved soil. The pathogen survived well in autoclaved soil when manure was added to soil at a ratio of 1:25, 1:50, and 1:100, with a reduction of only 1 to 2 $\log_{10}$ cfu/g over 60 days. In autoclaved soil amended with manure at a ratio of 1 part manure to 10 parts soil, ***E. coli*** O157:H7 was inactivated more rapidly, with reductions of 3 and 5 $\log_{10}$ cfu/g over 60 days at 15 and 21°C, respectively. However, pathogen populations declined most rapidly in unautoclaved manure-amended soil, owing probably to antagonistic interactions with indigenous soil microorganisms. The moisture content of soil appeared to be a contributory factor to the pathogen's survival in manure-amended soil.

Studies on the inactivation of ***E. coli*** O157:H7 in commercial cow manure compost at constant temperatures of 50, 55, 60, 65, and 70°C revealed that a reduction of at least 4 $\log_{10}$ cfu/g in autoclaved compost occurred within 10 hours, 3 hours, 20 minutes, 15 minutes, and 5 minutes, respectively. Less time was required to kill the pathogen in unautoclaved compost.

Inactivation profiles of ***E. coli*** O157:H7 in inoculated cow manure–based compost were determined in a 15-L bioreactor held at an external temperature of 21 or 50°C. At 21°C, self-heating of compost resulting in stratified temperatures within the bioreactor occurred for 0 to 3 days. ***E. coli*** O157:H7 populations increased by 1 to 2 $\log_{10}$ cfu/g during the initial 24 hours of composting, and decreased by 3.5 $\log_{10}$ cfu/g in the bottom portion and by 2 $\log_{10}$ cfu/g in the middle and top portions of the bioreactor during 36 days of composting. At 50°C, ***E. coli*** O157:H7 was inactivated rapidly, 4.9 $\log_{10}$ cfu/g in the top portion, 4.0 $\log_{10}$ cfu/g in the middle portion, and 5.9 $\log_{10}$ cfu/g in the bottom portion of the bioreactor within 24 hours of composting. When inoculated at an initial population of $\approx 10^7$ cfu/g, ***E. coli*** O157:H7 survived for 7 days but not for 14 days in all locations of the bioreactor. Compost at the top portion retained a relatively constant moisture content of 60%, whereas the moisture content of the bottom portion decreased steadily to 37–45% during 14 days of composting. The pH of the compost decreased to pH ≈6 within 1 to 3 days, and subsequently increased to pH 8–9. Results indicate that large populations (10^4 to 10^7 cfu/g) of ***E. coli*** O157:H7 survived at 36 days during composting in a bioreactor at an external temperature of 21°C, but was inactivated to undetectable populations between 7 and 14 days when the external temperature of the bioreactor was 50°C. This bioreactor model will be useful to determine the effects of different compost parameters such as pH, moisture content, ingredient formulations, aeration, and microbial competitors on the inactivation of pathogens during composting.

Effect of Organic Acid Content of Silages on the Growth of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 on Total Mixed Rations

DALE D. HANCOCK, College of Veterinary Medicine, Washington State University, Pullman, Washington 99164, USA

The prevalence of ***Escherichia coli*** O157:H7 increases several-fold during warm months in cattle operations. One hypothesis for this increase is that environmental replication of ***E. coli*** O157:H7 occurs in wet feeds and that the rate of growth increases in warm weather. Wet feed is commonly fed to dairy cattle in the form of silage-based total mixed rations (TMRs).

Preliminary studies have indicated that some silage-based TMRs support the growth of generic ***E. coli*** (Lynn et al. 1998). To further examine this phenomenon, 57 silages (corn, alfalfa, or grass) were collected from dairy farms in the northwestern United States, mixed with standardized ingredients to make experimental TMRs, divided into three aliquots, and inoculated with ***E. coli*** O157:H7, ***Salmonella enterica*** serotype Typhimurium mr-dt104, and fecal ***E. coli***. Levels of organic acids (lactic, propionic, butyric, acetic, isovaleric, and valeric) were measured in each of the silages. Inoculated TMRs were allowed to stand in open containers at room temperature. Although a wide variety of organic acid levels were observed among silages, none of the silage-based TMRs supported substantial growth of the test organisms. In most cases, declines of 0.5 to 2 log from the initial concentration occurred. This was so even though the nonsilage ingredients supported a 3- to 4-log growth of test organisms when moisture equivalent to that in the silage-based TMR was added.

A further experiment using 23 additional silages examined TMRs made with wet-weight concentrations of silage of 0%, 10%, 20%, 30%, 40%, and 50%. Moisture was raised to a minimum of 40% for each of the silage dilutions, and test silages were inoculated with ***E. coli*** O157:H7. Even 10% silage was inhibitory compared with the 0% silage mix, although greater declines from initial concentrations occurred at the higher concentrations of silage.

Based on these results with experimental TMRs, we concluded that silage-based TMRs in the northwestern United States should be uniformly inhibitory to the growth of ***E. coli*** O157:H7 and ***Salmonella*** Typhimurium. This was corroborated in a parallel field study in which we sampled TMRs from 30 farms soon after mixing (fresh) and again after they were in the feed bunks for 8 to 24 hours (stale). The total ***E. coli*** counts of fresh and stale TMRs were found to be highly correlated, with the counts in stale TMRs being somewhat lower on average. Inoculation of fresh TMRs collected from the 30 farms with ***E. coli*** O157:H7, ***Salmonella*** Typhimurium DT104, and fecal ***E. coli*** corroborated results from experimental TMRs in that growth was inhibited.

Although these results do not rule out environmental replication as playing an important role in the epidemiology of ***E. coli*** O157:H7 and ***Salmonella*** Typhimurium mr-dt104, they do seem to rule out a major role of replication in silage-based TMRs.

Reference

Lynn TV, Hancock DD, Harrison JH, Besser TE, et al. 1998. The occurrence and replication of *Escherichia coli* in cattle feeds. J Dairy Sci 1998;81:1102.

Molecular Tools for the Identification of *Listeria monocytogenes* Serotype 4b Strains

SOPHIA KATHARIOU, Department of Food Science, North Carolina State University, 339 Schaub Hall, Raleigh, North Carolina 27695, USA

Three serotypes of ***Listeria monocytogenes***, 1/2a, 1/2b, and 4b, account for the majority (95% or more) of human cases of listeriosis. Of these, serotype 4b is of special relevance because it has frequently been involved in major foodborne outbreaks of the disease. The bacteriologic and possibly virulence-associated attributes of this serotype that may be responsible for its relatively high incidence in foodborne listeriosis remain largely uncharacterized.

We have pursued the characterization of genomic regions of ***L. monocytogenes*** that are present in serotype 4b but that are either lacking, or extensively diverged, from the genome of strains of other major serotypes. Such regions have been designated as serogroup specific. We now know accurately the boundaries of the serogroup 4–specific region, which includes the gene ***gtcA***, determined in our laboratory to be essential for the addition of galactose and glucose substituents onto the antigenic cell wall polymer, teichoic acid. In serotype 4b, teichoic acid is unique in bearing both galactose and glucose substituents. Mutants deficient in ***gtcA*** were constructed in both a sporadic and an epidemic strain. In both cases, ***gtcA*** mutants were found to lack galactose and to have only traces of glucose in the teichoic acid (Promadej et al. 1999).

Our current data have revealed two other serogroup-specific genes upstream of the ***gtcA*** gene on the genome of ***L. monocytogenes*** serotype 4b. One of these genes (termed ***rho***) is involved in termination of transcription, whereas the other (termed ***murZ***) mediates the first step in the biosynthesis of cell wall (peptidoglycan). Southern blot data suggested that the sequences of both of these genes (***rho*** and ***murZ***) were specific to serogroup 4, because under high-stringency conditions hybridizing bands were seen with DNA from all screened serogroup 4 strains but with no other serotypes. Southern blots under low stringency, however, suggested that ***rho*** and ***murZ*** had counterparts in other serotypes in the same genomic locus.

To accurately compare the nucleotide sequence in this region between serotype 4b and the other major serotypes of ***L. monocytogenes***, we examined the corresponding locus in serotypes 1/2a and 1/2b. The strains chosen were G2228 (serotype 1/2a, implicated in a human case of listeriosis caused by contaminated turkey franks) and F4242 (human clinical isolate). Surprisingly, our analysis revealed that the genome of G2228 (serotype 1/2a) harbored homologues not only of ***rho*** and ***murZ*** but also of ***gtcA***. The ***gtcA*** gene in serotype 1/2a had only 80% identity with its counterpart in serotype 4b. This is in agreement with the finding that probes derived from ***gtcA*** of serotype 4b produced hybridizing bands only with serogroup 4 strains but not with any of the other serotypes (Promadej et al. 1999). The reverse was observed with probes derived from ***gtcA*** of serotype 1/2a, which yielded hybridizing bands with DNA from bacteria of serogroups 1 /2, 3, and 7 but not with DNA from bacteria of serogroup 4. The ***gtcA*** gene sequences were found to have 99% identity between serotypes 1/2a and 1/2b. These data suggest that in ***L. monocytogenes, gtcA*** exists in one allelic form in serogroup 1/2 (and likely serogroups 3 and 7 as well) and in another, significantly divergent allelic form in serogroup 4.

Another unexpected finding from the genetic characterization of the ***gtcA*** locus in serotypes 1/2a and 1/2b was the discovery of a novel gene, located between ***rho*** and ***gtcA***. The new gene has been tentatively termed ***mtrA*** (mannosyl transferase). Probes derived from ***mtrA*** were used in Southern blots under both high- and low-stringency conditions, and the results indicated that ***mtrA*** is absent from the genome of serotype 4b (and other serogroup 4) strains, but is present in all other screened serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, and 7). Thus, ***mtrA*** appears to represent a gene that is present exclusively in one serogroup cluster in ***L. monocytogenes*** (this cluster includes serogroups 1 /2, 3 and 7). This genetic situation is similar to two previously described genes (***gltA*** and ***gltB***) that we found were present in serotypes 4b, 4d, and 4e but that lacked detectable homologues in any other screened

serotypes (Lei et al. 2001).

We have constructed both insertion and deletion mutants in the ***mtrA*** of serotype 1/2a. The mutants were viable, but certain important aspects of their cell surface composition were altered. Specifically, the teichoic acid of the mutants lacked ***N***-acetylglucosamine, one of the sugars present on the teichoic acid of serogroup 1/2 strains. The mutants also were resistant to the ***Listeria*** genus-specific bacteriophage A511, which was surprising because the receptor for A511 is considered to be peptidoglycan and the mutants were normal in peptidoglycan composition. Teichoic acid is covalently linked to peptidoglycan, and we can speculate that the addition of ***N***-acetylglucosamine substituents on the teichoic acid is essential for presentation of the receptor on the peptidoglycan. A similar finding was obtained with ***gtcA*** mutants of serotype 4b, which also were found to be resistant to A511.

In conclusion, each of the serogroup-specific genes identified in this work (***gtcA***, ***rho***, ***murZ***, and ***mtrA***) can readily serve for detection of the corresponding strains of ***L. monocytogenes*** using either hybridizations (Southern or colony hybridization) with probes derived from the genes or polymerase chain reaction–derived approaches. The ***rho***, ***murZ,*** and ***gtcA*** genes have diverged significantly between serogroup 4 and the other serogroups. Thus, probes and PCR primers derived from these genes can be used to detect different serogroups, depending on whether the probes and primers were derived from serotype 4b or from serotype 1/2a sequences. In the case of ***mtrA***, the gene lacks homologues in serogroup 4 and therefore can be readily used to determine the preliminary serogroup status (serogroup 1 /2, 3, or 7 versus serogroup 4) of a strain. The mechanisms that drive the observed genetic diversity in this genomic region of ***L. monocytogenes*** remain to be identified, and much will be learned about the genetics and evolution of serotype-specific surface antigens from the continued characterization of these genes. Current data suggest that DNA-based tools derived from these genes will be valuable in the implementation of "molecular serotyping" for ***L. monocytogenes***.

References

Lei X-H, Fiedler F, Lan Z, Kathariou S. A novel serotype-specific gene cassette (*gltA-gltB*) is required for expression of teichoic acid–associated surface antigens in *Listeria monocytogenes* of serotype 4b. J Bacteriol 2001;183:1133–1139

Promadej N F, Fiedler P, Cossart S, et al. Cell wall teichoic acid glycosylation in *Listeria monocytogenes* serotype 4b requires *gtcA,* a novel, serogroup-specific gene. J Bacteriol 1999;181:418–

425

Effects of Environmental Conditions and Management on Antibiotic Resistance in Bacteria Associated with Swine

ALAN G. MATHEW and Patricia Cullen, Department of Animal Science, P.O. Box 1071, 2505 River Drive, University of Tennessee, Knoxville, Tennessee 37996, USA

Over the past five decades antibiotic use in the livestock industry has become widespread. These products can increase production and lower costs (Stahly et al. 1980), reduce the environmental impact of livestock operations (Roth and Kirchgessner 1993), and reduce the prevalence of pathogens (Kyriakis et al. 1996). However, these benefits have recently been overshadowed by the threat of emerging antibiotic-resistant microorganisms. Previous studies have linked the development of resistant enteric bacteria to exposure of subtherapeutic levels of antibiotics (Langlois et al. 1984, Ebner and Mathew 2000). The transfer of resistance factors from one bacterial species to another has compounded the problem because of the risk that foodborne pathogens may acquire resistance, thus lowering the efficacy of human antimicrobial therapies should they be needed. Some studies have suggested that stress, such as that resulting from transportation, can influence the prevalence of resistant bacteria in livestock (Langlois and Dawson 1999, Corrier et al. 1990). These reports suggest that factors other than exposure to antibiotics may play a significant role in the development and/or prevalence of resistant organisms.

Methods

To determine whether various animal stressors affect the prevalence of antibiotic-resistant bacteria in pigs, 58 18-day-old weaned pigs with no history of antibiotic exposure were challenged intranasally with 10^{11} cfu ***Salmonella enterica*** serovar Typhimurium (***Salmonella*** Typhimurium) containing a nalidixic acid resistance marker. Pigs were randomly assigned to one of eight treatments and separated accordingly in identical isolation rooms equipped with swine pens and associated husbandry equipment similar to that of modern commercial swine operations. All treatment groups, with the exception of control group 1 (see below), were exposed to the antibiotic apramycin at a concentration of 150 g/ton feed for a 14-day period. Treatments included (1) optimal production conditions without exposure to apramycin (control group 1), (2) optimal production conditions with exposure to apramycin (control group 2), (3) crowding via a 30% increase in animal density, (4) cold stress via maintenance of room temperature 6°C below recommended housing temperature, (5) heat stress via maintenance of room temperature 6°C above recommended housing temperature, (6) intermingling via placement of nonmedicated pigs (but previously challenged with ***S.*** Typhimurium) in contact with treatment pigs, (7) intervention with an unrelated antibiotic, oxytetracycline (100 g/ton feed), and (8) low sanitation via decreased cleaning regimen, allowing manure accumulation beneath and surrounding the pen. Optimal conditions were based on those described in the ***Pork Industry Handbook*** (National Pork Producers Council 1996). Strict biosecurity was maintained between rooms to avoid transfer of bacteria between treatment groups.

Fecal samples were obtained from pigs prior to inoculation (day 0), on days 2, 7, 14, 28, 64, and 148 (prior to shipping), and on day 149 (postshipping) for the recovery of the test isolates. For the isolation of ***Enterococcus faecalis,*** samples were cultured in Stomacher bags containing enterococcosel broth (Becton Dickenson, Sparks, MD), followed by inoculation and culture on Steptosel agar plates (Beckton Dickinson, Cockeysville, MD) containing 0.04% potassium tellurite. APIStrep strips (Vitek bioMerieux, Syosett, NY) were used to confirm to species. For ***E. coli*** isolation, samples were cultured on lactose MacConkey agar, and suspected ***E. coli*** colonies were further cultured on trypticase soy agar plates containing 5% defibrinated sheep blood for selection of nonhemolytic species before confirmation using API20E test strips (Vitek bioMerieux, Syosett, NY). The salmonella challenge organism was recovered by initially culturing samples in Mueller-Hinton II cation-adjusted broth, followed by enrichment in tetrathionate. Aliquots of the enrichment culture were then

transferred to XLT4 agar containing 50 µg/mL nalidixic acid. Presumptive salmonella colonies were confirmed using triple sugar iron and lysine iron agar. Isolates were tested for sensitivity to apramycin sulfate, ceftiofur sodium, oxytetracycline, and sodium sulfamethazine via a minimum inhibitory concentration (MIC) broth dilution method according to the National Committee for Clinical Laboratory Standards (1997).

Data were analyzed using analysis-of-variance mixed-model procedures to determine the effects of treatments and interactions of treatment by time (SAS Institute Inc. 1990). The percentages of resistant organisms and multiple resistance were compared using the SAS PROC FREQ procedure (SAS Institute Inc. 1990).

Results

Among test isolates, ***E. coli*** demonstrated the most pronounced effects of stressors on antibiotic resistance as measured by MIC (Table 1). Isolates from control group 1 exhibited the lowest resistance throughout the study. Peak resistance was observed in isolates from pigs exposed to apramycin by day 14. Upon withdrawal of apramycin, MICs for isolates from control group 2 returned to pretreatment levels, although some stressed groups maintained greater MICs through day 28 ($P < 0.05$). Cold stress, overcrowding, and oxytetracycline treatments demonstrated greater MICs up to day 64 before returning to pretreatment levels. Although there was a slight increase in MIC values in all groups following transport, with the exception of control group1 and the poor sanitation groups, posttransportation MICs were not significantly different from pretransportation levels. Treatment X time interactions, as well as treatment effects, were noted for ceftiofur, oxytetracycline, and sulfamethazine; however, no isolates demonstrated clinical resistance to ceftiofur (breakpoint = 8 µg/mL) at any time during the course of the study. High levels of resistance to oxytetracycline were exhibited by the majority of isolates throughout the study. There was a general increase in MICs for sulfamethazine as the study progressed.

Salmonella Typhimurium was recovered from most pigs between days 0 and 64, with concentrations generally declining after day 28. Throughout the study, salmonella isolates remained susceptible to apramycin (Table 2) and ceftiofur, with no significant treatment effects being noted, whereas effects were detected for oxytetracycline and sulfamethazine. Treatment X time ($P < 0.05$) interactions were noted for apramycin, ceftiofur, oxytetracycline, and sulfamethazine. MICs for oxytetracycline in salmonella isolated from pigs administered that antibiotic demonstrated increased resistance following tetracycline application and continuing through day 28 of the trial. Inability to detect salmonella at later sampling dates hindered further evaluation of this trend.

Despite numerous attempts, few ***E. faecalis*** could be detected immediately following antibiotic administration (days 7 and 14), when salmo-

Table 1. Effects of various treatments on sensitivity to apramycin by *E. coli* isolated from pigs over time

Day	C-1	C-2*	Cold*	Heat*	Crowd*	Sanit*	Oxy*	Int*
0	2.3	3.0	4.8	2.4	2.0	3.6	3.2	2.2
2	2.8	1.5	1.7	2.1	2.5	4.0	4.4	1.8
7	1.3	1.3	17.3	1.5	1.2	5.7	1.9	1.8
14	1.2	245.1	224.8	101.6	25.4	194.0	90.5	138.2
28	1.0	1.3	76.8	4.1	10.0	4.9	5.0	11.3
64	1.2	1.2	15.5	3.5	11.6	2.2	8.5	2.6
148	1.0	1.8	1.1	1.1	1.2	1.5	1.1	1.3
149	1.3	1.6	1.4	1.9	1.6	1.4	1.4	1.4

Data are presented as least-squares means of minimum inhibitory concentrations (MIC) in micrograms per milliliter. Day = days postchallenge, C-1 = control group 1 (optimal conditions, no apramycin), C-2 = control group 2 (optimal conditions and apramycin exposure), cold = room maintained at 6 °C below optimal temperature, heat = room maintained at 6 °C above optimal temperature, crowd = overcrowding, sanit = poor sanitation, oxy = treatment with oxytetracycline, and int = intermingled pigs.

*Treatments differ from control group 1 ($P < 0.05$), overall effect.

Table 2. Effects of various treatments on sensitivity to apramycin by *Salmonella* Typhimurium isolated from pigs over time

Day	C-1	C-2	Cold	Heat	Crowd	Sanit	Oxy	Int
0	1.3	3.8	1.8	—	2.4	2.7	4.6	1.8
2	1.9	2.2	2.2	2.1	2.0	2.0	2.5	1.9
7	1.4	1.2	1.9	1.9	1.7	1.3	1.4	1.3
14	1.5	1.7	1.5	1.4	1.2	1.5	1.8	1.6
28	1.6	1.8	1.6	1.3	1.8	1.4	1.1	1.5
64	2.0	2.6	—	—	2.0	—	—	—
148	—	—	—	—	—	—	—	—
149	—	—	—	—	—	—	—	—

Data are presented as least-squares means of minimum inhibitory concentrations (MIC) in micrograms per milliliter. See note to Table 1 for explanation of column headings. Dash = no isolate recovered.

nella demonstrated their greatest numbers. However, ***E. faecalis*** numbers returned to pretreatment levels by the end of the study. In general, recovered ***E. faecalis*** exhibited resistance to all test antibiotics throughout the study regardless of treatment.

Discussion and Implications

These data clearly demonstrate the effect of antibiotic exposure on resistance patterns in swine-derived bacteria, particularly ***E. coli***. Additionally, we were able to show that a number of animal stressors, including cold, heat, and crowding, can lengthen the duration that animals shed resistant ***E. coli.*** The salmonella challenge organism was less affected by treatment of animals with apramycin and/or stressors. This may reflect the invasive nature of this organism, which may allow the bacterium to escape exposure to the antibiotic by internalization into host cells. Additionally, our recovery of salmonella was strictly selective for the challenge strain, which was known to be initially sensitive to the test antibiotics. This is in contrast to ***E. coli***, for which a variety of isotypes were likely present, including some strains that may have been resistant initially and then proliferated or disseminated resistance factors within the species upon exposure to the antibiotic. The low recovery of ***E. faecalis*** during the primary stages of the treatment period may have been the result of competition with the challenge organism, because recovery of these two species appeared to demonstrate an inverse relationship over the course of the study. Analysis of recovered ***E. faecalis*** demonstrated little effect of stressors on resistance patterns, with resistance to most tested antibiotics considerably greater than that demonstrated by ***E. coli*** or salmonella.

In summary, these data indicate that poor husbandry practices can exacerbate problems associated with antibiotic resistance in bacteria from livestock. In particular, ***E. coli*** appears to be most affected by stress imposed on its animal hosts. Because this bacterium is thought to be a key enteric species involved in the transfer of genetic resistance elements to other bacteria, including foodborne pathogens, husbandry practices that reduce the prevalence of antibiotic-resistant strains should be employed. Such practices should also be consistent with optimal production, and thus will provide a monetary incentive while limiting the presence of antibiotic-resistant organisms.

References

Corrier DE, Purdy CW, DeLoach JR. Effects of marketing stress on fecal excretion of *Salmonella* spp in feeder calves. Am J Vet Res 1990;51:866–869

Ebner PD, Mathew AG. Effects of antibiotic regimens on fecal shedding patterns of pigs infected with *Salmonella typhimurium*. J Food Protect 2000;63:709–714

Kyriakis SC, Sarris K, Kritas SK, et al. Effect of salinomycin in the control of *Clostridium perfringens* type C infections in suckling pigs. Vet Rec 1996;138:281–283

Langlois BE, Dawson KA. Antimicrobial resistance of gram-negative enteric bacteria from pigs in a nonantimicrobial-exposed herd before and after transportation. J Food Protect 1999;62:797–799

Langlois BE, Dawson KA, Staly TS, Cromwell GL. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. J Anim Sci 1984;58:666–667

National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Wayne, PA: NCCLS, 1997:1–63

National Pork Producers Council. Pork industry handbook. Lafayette, IN: Purdue Extension Publications Media Distribution Center, 1996

Roth FX, Kirchgessner M. Influence of avilamycin and tylosin on retention and excretion of nitrogen in finishing pigs. J Anim Physiol Anim Nutr 1993;69:245–250

SAS Institute Inc. SAS/STAT users guide, version 6. Cary, NC: SAS Institute Inc., 1990

Stahly TS, Cromwell GL, Monegue HJ. Effects of dietary inclusion of copper and(or) antibiotics on the performance of weanling pigs. J Anim Sci 1980;51:1347–1351

Factors Affecting the Transfer of Genes Encoding Multiple Antimicrobial Resistance to *Salmonella* Typhimurium DT104

CORNELIUS POPPE, Laura Martin, John Prescott, Scott McEwen, Anne Muckle, David Alves, and Kim Ziebell, Health Canada, Laboratory for Foodborne Zoonoses, Office International des Épizooties Reference Laboratory for Salmonellosis, 110 Stone Road West, Guelph, Ontario, Canada N1G 3W4

In recent years, ***Salmonella*** Typhimurium phage type or definitive type 104 (DT104) has been isolated increasingly from animals and humans in many countries (Poppe et al. 1998). A high percentage of the isolates are resistant to ampicillin (Amp), chloramphenicol (Chl), streptomycin (Str), sulfonamides (Sul), and tetracycline (Tet). ***Salmonella*** spp. are foodborne pathogens that can infect humans through consumption of contaminated food products; thus, the increased occurrence of DT104 isolates in animals and in food of animal origin poses a threat to public health (Davies et al. 1996). Until recently, ***S.*** Typhimurium DT104 was primarily a pathogen of cattle and humans, but more recently increasing numbers have been isolated from other animal species including pigs and chickens.

In DT104 strains, genes encoding antimicrobial resistance to Amp, Chl, Str, Sul, and Tet are commonly found on a chromosomal sequence of about 13 kilobases (kb) containing two class I integrons (Briggs and Fratamico 1999). Gene cassettes encoding resistance to spectinomycin (Spc) and Str (the ***ant***(3")-***Ia*** gene) and Amp (the $\boldsymbol{bla}_{PSE1}$ gene) inserted into the integrons have been mapped to the sequence. The ***floR*** and the ***tetR*** and ***tetA*** genes, encoding resistance to florfenicol (Flo) and Chl, and to Tet, respectively, have been mapped between the two integrons. The intestinal tract may constitute an ideal milieu for the exchange of antimicrobial resistance genes among bacteria and bacteriophages.

The objectives of the study were to examine whether antimicrobials administered to animals previously infected with an antimicrobial-sensitive ***S***. Typhimurium DT104 strain would cause the strain to become resistant, to determine whether bacteriophages in the intestinal tract transfer antimicrobial resistance genes to DT104 strains, and to examine whether ***E. coli*** in the intestinal tract possess bacteriophages or self-transmissible plasmids able to transfer antimicrobial resistance genes to DT104 strains. Other objectives were to examine whether ***S***. Typhimurium DT104 bacteria isolated from the feces of animals contain bacteriophages that transfer antimicrobial resistance to ***E. coli*** and to determine whether ***Enterococcus*** spp. bacteria present in the intestinal tract of animals possess conjugative plasmids that are able to transfer antimicrobial resistance genes to ***E. coli*** and ***S***. Typhimurium.

Methods

A pilot study was conducted to determine the dose of a bovine antimicrobial-sensitive ***S***. Typhimurium DT104 isolate in calves that would cause bacterial shedding but not serious illness. We then carried out a trial to examine the putative acquisition of antimicrobial resistance genes by the antimicrobial-sensitive DT104 strain and the transfer of drug resistance genes among ***E. coli***, ***Enterococcus*** spp., and DT104 bacteria in the intestinal tract. Two calves were placed in each of four isolation rooms. After 2 weeks, all eight calves were challenged with 10^6 to 10^7 bacteria of the drug-sensitive DT104 strain. Two calves in one pen were given 55 mg/kg oxytetracycline hydrochloride (Tet) in the feed for 6 weeks and then placed on a diet without Tet for another 6 weeks. The two calves in the second pen were given 350 mg sulfamethazine (Sul) per day for a 6-week period and were thereafter on a diet without sulfamethazine for another 6 weeks. The calves in the third pen were dosed every 2 weeks with a bovine ***E. coli*** isolate resistant to Amp, Chl, Spc, Sul and Tet (resistances commonly observed in ***S***. Typhimurium DT104 isolates) for 6 weeks and then kept for a 6-week period without receiving the resistant ***E. coli*** strain. The calves in the fourth pen were challenged with the antimicrobial-sensitive ***S***. Typhimurium DT104 strain but were neither given antimicrobials nor challenged with the antimicrobial-resistant ***E. coli***.

Fecal samples were collected every 2 weeks from all of the calves and cultured to obtain 10 ***Salmonella*** spp., 10 ***E. coli***, and 2 ***Enterococcus*** spp. isolates per sample. The ***S***. Typhimurium iso-

lates were phage-typed, randomly selected ***E. coli*** isolates were serotyped, and the ***Enterococcus*** spp. isolates were speciated. The ***Salmonella*** and ***E. coli*** isolates were examined for resistance to amikacin (Amk), Amp, apramycin (Apr), carbadox (Car), ceftiofur (Ctf), ceftriaxone (Cef), cephalothin (Cep), Chl, ciprofloxacin (Cip), Flo, gentamicin (Gen), kanamycin (Kan), nalidixic acid (Nal), neomycin (Neo), nitrofurantoin (Nit), Spc, Str, Sul, Tet, cotrimoxazole (trimethoprim-sulfamethoxazole) (Cot), and tobramycin (Tob). The ***Enterococcus*** spp. isolates were examined for resistance to Amp, bacitracin (Bac), Car, Chl, clindamycin (Cli), Cip, Cot, erythromycin (Ery), Gen, lincomycin (Lin), Nit, rifampicin (Rif), Str, Tet, tylosin (Tyl), vancomycin (Van), and virginiamycin (Vir). Filtrates to isolate bacteriophages were made from all fecal samples. Transfer of resistance genes by conjugation and transduction was examined by methods described previously (Provence and Curtiss 1994, Cutting and Youngman 1994, Schmieger and Schicklmaier 1999). The presence or absence of type I integrons in the DT104 and the ***E. coli*** challenge strains was determined by polymerase chain reaction (PCR) using the primers described by Sandvang et al. (1997).

Results

The antimicrobial-sensitive DT104 challenge strain did not produce an integron-associated PCR product, whereas the ***E. coli*** challenge strain possessed the class I integron containing the ***ant***(3")-***1a*** gene. During the pilot study we obtained 4 fecal samples before and 14 after challenge, 15 tissue samples at necropsy on day 14 post-infection and 3 samples of shavings used as bedding for the calves. We obtained 8 ***S.*** Typhimurium isolates from 4 fecal and 4 tissue samples, 34 ***E. coli*** isolates (18 from fecal samples, 14 from tissues and 2 from shavings) and 16 ***Enterococcus*** spp. isolates (14 from 7 of 18 fecal samples and 2 from 1 of the samples of shavings). The ***Enterococcus*** spp. isolates consisted of 10 ***E. faecalis*** and six other ***Enterococcus*** spp. The DT104 challenge strain was reisolated from fecal and tissue samples from the two calves infected at 2 weeks of age but not from the two calves challenged at 3 to 4 weeks of age. None of the 18 fecal samples contained bacteriophages that formed plaques on the antimicrobial-sensitive DT104 challenge strain.

During the main trial we obtained 32 fecal samples from the eight calves during the 2-week period before challenge of the calves and isolated 128 ***E. coli*** (4 per fecal sample), 12 ***S.*** Typhimurium DT208 (from 3 fecal samples of one calf), and 60 ***Enterococcus*** spp. (from 30 of the 32 fecal samples; 2 per sample) that consisted of 39 ***E. faecalis***, 10 ***E. faecium***, 9 ***E. gallinarum*** and 2 ***E. casseliflavus***. We then infected the calves with 10^6 to 10^7 of the DT104 challenge strain. During the 6-week treatment period and the 6 weeks of no treatment, we obtained 96 fecal samples from the calves and isolated 550 ***S.*** Typhimurium DT104 strains from 55 of 96 fecal samples), 960 ***E. coli*** strains (10 from each of the 96 samples), and 86 ***Enterococcus*** spp. (2 isolates from 43 of the 96 fecal samples). The ***Enterococcus*** spp. consisted of 41 ***E. casseliflavus***, 14 ***E. faecalis***, 13 ***E. faecium***, and 18 other ***Enterococcus*** spp.

Resistance of the reisolated challenge ***S.*** Typhimurium DT104 strain was minimal and infrequent. Fifty of the 56 ***S.*** Typhimurium strains that became resistant showed reduced sensitivity to Cip at 0.125 µg/mL only. Although these DT104 isolates showed low-level resistance to Cip at 0.125 µg/mL at the initial isolation and drug resistance testing, subsequent examination of the same isolates and of strains isolated from the same fecal samples at a later date showed that they were sensitive to the antimicrobials in the test panel. The remaining strains were resistant to Nit (1 strain), Str (2), Kan, Neo, Str, Sul, Tet and Cot (1), Amp, Spc, Str, and Sul (1) and to Amp Spc and Sul (1). Nine of the fecal samples contained lysogenic bacteriophages that formed plaques on the antimicrobial-sensitive DT104 challenge strain. Six of the nine phages were obtained from fecal samples that contained multiresistant ***E. coli***. Two phages did not transfer antimicrobial resistance, although they plaqued on the recipient strain. Transduction of the antimicrobial-sensitive DT104 strain with each of the seven other phages resulted in the isolation of 10 transduced strains with varying antimicrobial resistance patterns. The strains became resistant to Str and Tob (2 strains), to Car (2), to Tob (2), to Spc (2) and to Str (2). The administration of Tet significantly increased the number of resistant ***E. coli*** strains isolated during and after the administration of Tet in comparison to the number of resistant ***E. coli***

strains isolated during the same periods from the control calves that did not receive Tet (P<0.01). The ***E. coli*** isolates were often multiresistant to antimicrobials including Chl, Kan, Neo, Spc, Str, Sul, Tet, and/or trimethoprim (Tmp). Similarly, the administration of Sul significantly increased the number of antimicrobial-resistant ***E. coli*** strains isolated during and after administration of Sul in comparison to the number of resistant ***E. coli*** strains isolated from the control calves during the same periods (P-value <0.01). The administration of the multiresistant ***E. coli*** also significantly increased the number of strains that were resistant among the ***E. coli*** strains isolated from the calves during and after the administration of the multiresistant ***E. coli*** in comparison to the number of resistant ***E. coli*** strains isolated from the calves in the control groups during the same periods (P<0.01). It appeared that calves receiving Tet or Sul shed ***Salmonella*** more frequently than calves receiving multiresistant ***E. coli*** or no treatment. However, we could not perform an animal level analysis since there were too few animals (only 2 calves) per treatment group.

Thirty-six ***E. coli*** fecal isolates representing 14 of 31 observed different antimicrobial resistance patterns contained large (>30 kb) plasmids and were conjugated with recipient strains. Three of the strains transferred resistance to Spc, Sul, Tet, and Cot (2 strains) and to Sul, Tet and Cot (1 strain) by conjugation to ***S***. Typhimurium LB5000 but not to the antimicrobial-sensitive DT104 challenge strain. We induced 24 ***E. coli*** isolates that had different antimicrobial resistance patterns with mitomycin C to obtain bacteriophages. Ten ***E. coli*** strains contained bacteriophages that plaqued on the antimicrobial-sensitive challenge DT104 strain resulting in the isolation of 16 transduced strains with various antimicrobial resistance patterns. The transduced strains became resistant to Str (7 strains), Spc (8 strains), or Cep (1 strain). However, development of resistance may not have been caused solely by transduction of the antimicrobial-sensitive DT104 strain since the strain also became resistant to Str or Spc on five occasions when plated on agar containing either of these antimicrobials without prior exposure to the ***E. coli*** phages; some may therefore have developed resistance through spontaneous mutation. Conjugation of ***Enterococcus*** spp. with ***E. coli*** and ***S***. Typhimurium did not result in transfer of antimicrobial resistance.

Discussion

The main findings of this study were that during and after treatment of the calves with Tet, Sul, or antimicrobial resistant ***E. coli***, a significantly higher number of ***E. coli*** were resistant to antimicrobials in comparison to the number of resistant ***E. coli*** strains isolated from the calves in the control group during the same period. Also, under laboratory conditions, bacteriophages occasionally transferred antimicrobial resistance to the challenge antimicrobial-sensitive ***S.*** Typhimurium DT104 strain. The same class of integrons found in DT104 strains has been identified in other ***S.*** Typhimurium phage types, other ***Salmonella*** serovars (Cloeckaert et al. 2000), and other genera of the family of the Enterobacteriaceae including ***E. coli.*** They have been located on conjugative plasmids and transposons. Clonal spread of DT104 strains has been suggested as the common manner of dissemination of the DT104 strains (Frana et al. 2001). As observed in the present study, transfer of resistance genes integrated into or associated with type I integrons by conjugation or transduction to antimicrobial-sensitive DT104 strains may be an uncommon event. More work is needed to understand the complex dynamics of transfer of antimicrobial resistance genes in the intestine in the presence and absence of antimicrobial drugs.

References

Briggs CE, Fratamico PM. Molecular characterization of an antibiotic resistance gene cluster of *Salmonella typhimurium* DT104. Antimicrob Agents Chemother 1999;43:846–849

Cloeckaert A, Boumedine KS, Flaujac G, et al. Occurrence of a *Salmonella enterica* serovar Typhimurium DT104-like antibiotic resistance gene cluster including the *floR* gene in *S. enterica* serovar Agona. Antimicrob Agents Chemother 2000;44:1359–1361

Cutting SM, Youngman P. Gene transfer in Gram-positive bacteria. In: Gerhardt P, Murray RGE, Wood WA, et al, eds. Methods for general and molecular bacteriology. Washington, DC: American Society for Microbiology, 1994:348–364

Davies A, O'Neill P, Towers L, Cooke M. An outbreak of *Salmonella typhimurium* DT104 food poisoning associated with eating beef. Commun Dis Rep 1996;6:R159–R162

Frana TS, Carlson SA, Griffith RW. Relative distribution and conservation of genes encoding animoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. Appl Environ Microbiol 2001;67:445–448

Poppe C, Smart N, Khakhria R, et al. *Salmonella* Typhimurium DT104: a virulent and drug-resistant pathogen. Can Vet J 1998;39:559–565

Provence DL, Curtiss R III. Gene transfer in Gram-negative bacteria. In: Gerhardt P, Murray RGE, Wood WA, et al, eds. Methods for general and molecular bacteriology. Washington, DC: American Society for Microbiology, 1994:317–347

Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. FEMS Microbiol Lett 1997;157:177–181

Schmieger H, Schicklmaier P. Transduction of multiple drug resistance of *Salmonella enterica* serovar *typhimurium* DT104. FEMS Microbiol Lett 1999;170:251–256