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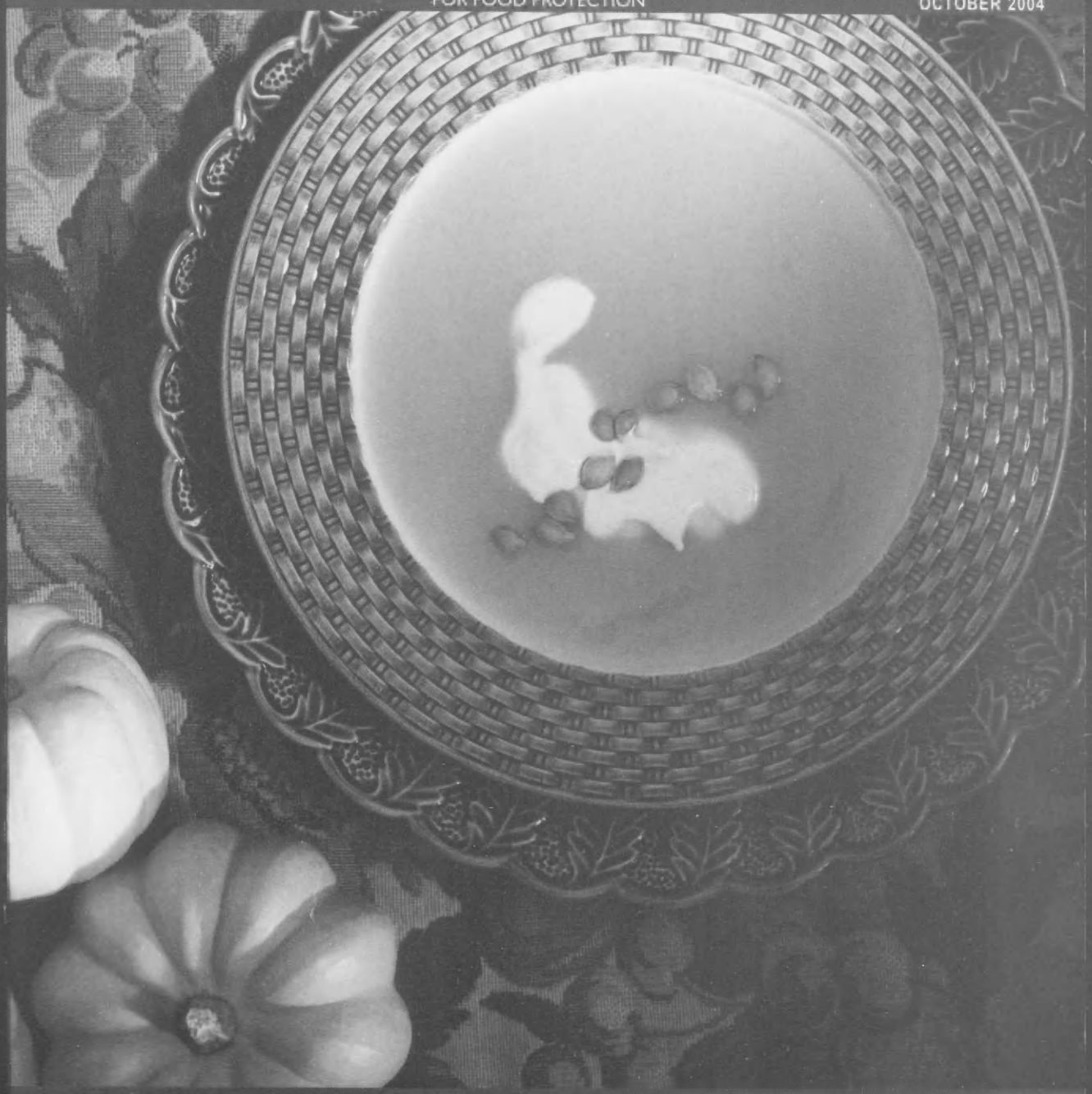
FOOD PROTECTION TRENDS

SCIENCE AND NEWS FROM THE INTERNATIONAL ASSOCIATION FOR FOOD PROTECTION

OCTOBER 2004



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ARTICLES

ASSOCIATION NEWS

DEPARTMENTS

EXTRAS

International Food Safety Icons

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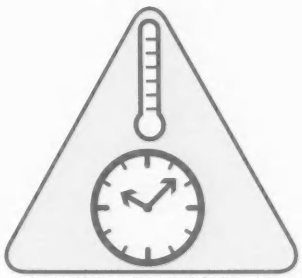
Handwashing

Potentially Hazardous Food



Cooking

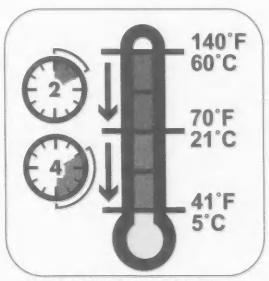
Do Not Work If Ill

Cross Contamination

Wash, Rinse, and Sanitize

No bare hand contact

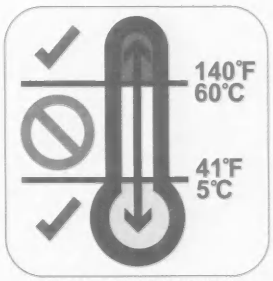
Cooling



Refrigeration/Cold holding

Hot Holding

Temperature Danger Zone



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FOOD PROTECTION TRENDS
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IAFP 2005

AUGUST 14-17

Baltimore Marriott
Waterfront Hotel
Baltimore, Maryland

IAFP 2006

AUGUST 13-16

Telus Convention Centre
Calgary, Alberta, Canada

IAFP 2007

JULY 8-11

Disney's Contemporary Resort
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International Association for Food Protection

FOOD PROTECTION TRENDS

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"A VIEW FROM WISCONSIN"

By **KATHLEEN A. GLASS**
PRESIDENT

"In order to realize our goals, we will need you, our members, to become part of the team working on accomplishing our objectives."

My family and co-workers will attest that I am a listmaker. I am a goal-orientated person who is intrinsically motivated by being able to check something off my "to-do list." Crossing out the daily chores on my list are great for instant gratification, but progress on lifelong goals such as professional development, seeing my sons safe, successful, and enjoying the educational and cultural abundance around us, and spending time with my husband, family, and friends, are all items that are continuously on my list of priorities.

IAFP similarly develops short and long term goals and measures our successes by our progress against those objectives. Our association developed a Strategic Plan in 1993 and reviewed progress in 1997. The primary objectives of the plan were to: Expand the Membership; Develop an Enhanced Education Program; Enhance Product and Services Offerings; and Develop a Formal Financial Plan. Certainly, many items from our original plan have been accomplished and can be checked off our collective task list, but the essence of these long term goals provides our vision for the future of IAFP in 2010.

Undoubtedly, the development of our educational program extends across all the target issues, is vital to our growing success, and must be maintained. As part of the education program, members of the Executive Board are regularly invited to conferences hosted by affiliates to deliver lectures in their area of expertise. The Speakers Bureau not only brings food safety experts to the local level, but also allows the leadership of your association to learn first hand the needs of the professionals who serve on the front line. Consequently, the Association can direct efforts to develop products and services that provide value to our international and affiliate members. Our Professional Development Groups (PDGs) and our partnership with the ILSI North America Technical Committee on Food Microbiology have flourished, developing the symposia and workshops which serve as the foundation of our Annual Meeting. The strong scientific program has allowed our Annual Meeting to enjoy phenomenal growth, increasing from an annual attendance of less than 1,000 only seven years ago to a record attendance of 1,584 attendees witnessed at this year's meeting in Phoenix. The growth in attendance, numbers of exhibitors, and financial sponsors, in turn, are partially responsible for a movement toward financial stability.

Certainly, while we are pleased with our past and current successes, we know that we cannot depend on reputation alone to sustain us. This brings us to our April 2004 planning session, during which the Board and staff collaborated to identify specific issues for growth such as: International Commitment; Publications; Affiliates; Outreach and Education; and Foundation Fund.

Daniel Burnham, a 19th century US architect said, "Make no little plans; they have no magic to stir men's blood...Make big plans, aim high in hope and work." We have no illusions that budget may be a hurdle to realizing some of our goals. Nevertheless, we aimed high and are excited about the potential we are about to see unfold. These are the initiatives for IAFP 2010.

International Commitment:

1. Demonstrate commitment to international members by hosting regional international meetings outside of North America; target inaugural meeting in Europe during fall 2005.
2. Increase international Affiliates to a minimum of 15.
3. Translate pertinent booklets or journal articles in response to multilingual needs.

4. Establish offices in Europe and Asia to be able to better serve needs of members in those regions.

Publications:

1. Increase accessibility to publications by adding back volumes to *JFP* Online and archiving *FPT* articles online after one year.
2. Develop applied food safety booklets; select topics and assign responsibility by 2005.
3. Encourage development of "white papers" on important food safety issues.
4. IAFP Press: investigate publishing books related to food science and safety issues.

Affiliates:

1. Increase communication with Affiliate organizations. Promote IAFP at all Affiliate meetings. Offer Affiliate Newsletter to all Affiliate members. Affiliate Presidents and Delegates teleconference to receive IAFP updates from Affiliate Council Chairperson and Secretary.
2. Increase percentage of Affiliate members who are IAFP Members.
3. Increase international Affiliates to a minimum of 15.
4. Restructure IAFP dues to make IAFP Membership more affordable and to attract Affiliate members to become IAFP Members.

Outreach and Education:

1. Form Special Committee by 2005 to address critical food safety issues as they arise; include procedure to quickly respond to issues through mid-year briefings, convene first meeting in 2006, if needed.
2. Establish travel grants to attend IAFP Annual Meeting; start with 2 in 2005 increasing to 25 by 2010.

Foundation Fund:

1. Set goal of $1 million by 2010. Develop a vision for the fund.
2. Make donating to the Foundation Fund easier for members. Develop a tiered recognition program.
3. Hold regular teleconferences throughout the year to keep the fundraising effort alive.
4. Consider hiring a fund raising professional.

Our strategic plan is more than just a to-do list, it is the Vision for IAFP 2010. In order to realize our goals, we will need you, our members, to become part of the team working on accomplishing our objectives. Review the list and think about how you can help. I welcome your ideas and look forward to working with you. Please feel free to email me at kglass@wisc.edu and let me know your view.

"COMMENTARY"
FROM THE EXECUTIVE DIRECTOR

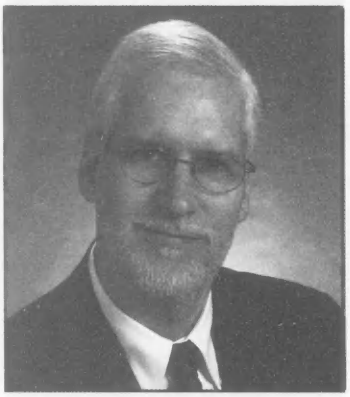
A meeting was held early on Tuesday morning during IAFP 2004 to encourage development of Affiliate organizations in Pacific Rim countries. There were over 40 people in the room as we talked about what it takes to become an IAFP Affiliate. Interest was strong from Members in Japan and New Zealand and we hope to be able to report positively on the progress of these two groups at IAFP 2005. There were also Members present from Thailand, Australia, and Korea (which already has an Affiliate).

For those of you who do not know, the requirements to become an Affiliate of IAFF are very simple. First off, our Affiliate organizations operate independently from IAFP. They are freestanding entities without financial obligations either to or from the parent organization, IAFP. They do, of course, hold the same interests and values that IAFP does in "Advancing Food Safety Worldwide." The IAFP Affiliate structure provides a base for many different types and styles of Affiliate organizations, both large and small.

To be considered for Affiliate status within IAFP, a group of at least five IAFP Members must write a letter of interest containing the following information:

- Names and contact information for the IAFP Members forming the Affiliate;
- A list of the proposed officers for the new organization;
- The name of the proposed Affiliate and a description of the geographical area to be covered by the new Affiliate; and
- A copy of the proposed Constitution and Bylaws for the new Affiliate.

The IAFP Executive Board reviews the information submitted and votes to approve issuance of Affiliate Charters. Once a group is officially recognized as an Affiliate, there are only three ongoing requirements to be an Affiliate.



By DAVID W. THARP, CAE
EXECUTIVE DIRECTOR

"We encourage development of new Affiliates and like to see this growth"

(1) The Affiliate group needs to have at least five of their members who are IAFP Members;

(2) They must maintain their President and Delegate as IAFP Members; and

(3) They must file an Annual Report.

Very simple really! We encourage development of new Affiliates and like to see this growth. Since 1997, we have seen a nice resurgence of Affiliate groups. Here is a recap of new Affiliate organizations:

1997 Korea Association of Milk, Food and Environmental Specialists

1999 British Columbia Association for Food Protection

2000 Quebec Food Protection Association

Mexico Association for Food Protection

Capital Area Food Protection Association

2002 Southern California Association for Food Protection

Brazil Association for Food Protection

2003 Portugal Association for Food Protection

United Kingdom Association for Food Protection

2004 Arizona Environmental Health Association

Ten new Affiliate organizations in eight years – not too bad of a record! As I said earlier, we have

two groups that are working hard on gathering support to establish a new Affiliate. Hopefully, we will see the results of their work at IAFP 2005 when we may be able to present their new Affiliate Charters like we did this year with the Arizona group.

I want to include a short note about our newest Affiliate, the Arizona Environmental Health Association. This group was an existing organization but saw affiliation with IAFP as a positive advantage when discussions began about forming an Arizona IAFP Affiliate. What I want to point out is that not all new Affiliates need to be a totally new group. We are able to accept new Affiliates who may already be an established group or even may be affiliated with other organizations.

If you have any questions on forming an Affiliate organization, please contact me to discuss this topic in greater detail. As Kathy Glass mentioned in her President's Column, we want to increase our number of active, international Affiliates!

Now, as a follow up to last month's column, yes, it was the best Annual Meeting ever! Attendance at IAFP 2004 was 1,584 people (a 7% increase over 2003), exhibits were up by 20% and sponsorship increased by 22%! Next month's *FPT* will include the Annual Meeting wrap up with pictures, reports and session summaries. Be sure to watch for the IAFP 2004 report in November's *Food Protection Trends*.

ARTICLES

Food Protection Trends, Vol. 24, No. 10, Pages 730-739

6200 Aurora Ave., Suite 200W, Des Moines, IA 50322-2864

International Association for Food Protection.

Risk Profile for Strawberries

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SUMMARY

This document describes a risk profile for strawberries intended for fresh consumption. Attention is paid to the current production methods for strawberries, consumption-related issues, including positive and negative health effects, and contamination with micro-organisms and pesticide residues. In addition, pre-harvest and harvesting requirements are described, as well as post-harvest measures, including decontamination and suitable storage conditions. Finally, there is a brief overview of existing regulations.

The study reveals that strawberries intended for fresh consumption have a relatively good safety record. To keep this status and maintain consumer confidence, it is recommended that codes of good agricultural practice be used, for which land and water use, application of organic and inorganic fertilizers, animal exclusion and pest control are described, together with recommended harvesting and cooling practices and measures for ensuring worker health and safety.

INTRODUCTION

The consumption of fresh fruits and vegetables is increasing because consumers strive to eat healthful and tasty foods. This applies particularly to strawberries; however, exact consumption data on strawberries are scarce. Data from the United States demonstrate an increase in production of strawberries from 316 million kg in 1970 to 494 million kg in 1993 *(5)*. In that period the consumption of fresh strawberries increased from 850 gram per capita per year in 1970 to 1,750 grams in 1992 *(5)*. In 2000 the consumption increased to 2,700 grams per capita *(13)*.

The consumption of frozen, sliced strawberries in the United States amounts to 600 – 700 grams per capita per year and has not changed significantly over the past 30 years *(5)*. In The Netherlands, data are collected by interviewing 100 families, selected at random, once every three years. In 1999, domestic consumption amounted to 965 grams per person. In 2002 it was 870 grams per person (personal communication, Commodity Board for Fruit and Vegetables, The Netherlands).

Although global trade in strawberries is increasing, the availability of the fruit is still largely seasonal. The price and consumption level depend largely on availability.

PRODUCTION METHODS FOR STRAWBERRIES

Strawberries are adapted to growing in many different regions. They require well-drained soil with a high concentration of organic matter; a pH between 5.0 and 7.0 is optimum. The majority of strawberries are produced outdoors, for which purpose the use of small protective polythene tunnels is increasing. Indoor production is mostly carried out in greenhouses made of glass. The use of large polythene tunnels is a cheap alternative to the greenhouse.

Outdoor production

Matted row strawberries. The 'matted row' method used by home gardeners and by some commercial growers has been used for many years. Plants are set out in spring or early summer on bare ground and allowed to send out runners. These give rise to daughter plants that also take root and form a wider 'matted' row. The field is allowed to produce fruit for 2–4 years and is then replanted. The production level varies from 6,000 to 8,000 kg per hectare (10,000 m^2).

Strawberries grown on plastic. Strawberries grow well (and weed-free) through a mulch of polythene. The great majority of strawberries are produced by setting plants out into black plastic in the fall. Irrigation and some fertilizer are supplied through a drip tape laid under the plastic at the time of planting. In late winter and early spring, the plants start to grow in earnest. Growers protect the early flowers from late (night) frosts during March and April by overhead irrigation at night. Although strawberry plants are perennial, those cultured in plastic are grown as

A peer-reviewed article

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annuals; they are harvested only 7–8 months after planting, and new plants are set out every year. When flowering is finished, the plastic is covered with straw, which protects the berries against contact with sun and soil and avoids mud splashes during periods of rain. Plant density is high (100,000 plants per hectare) and the investment of farmers in the crop is substantial. Production levels vary from 42,000 to 74,000 kg/hectare.

Plastic culture, first developed in the mid-1980s, is now widely used and, in particular, is being adapted for use in colder areas. Also, the use of small, protective polythene tunnels is increasing in the production of plastic-culture strawberries.

Indoor production

Hydroponic indoor production. The use of methyl bromide as a soil fumigant for greenhouses has been phased out in The Netherlands and many other countries in Europe, including Germany, Sweden, Switzerland and Denmark, because hydroponics (soil-free culturing) has been developed. This allows strawberries to be grown successfully in greenhouses on hanging shelves. Planting densities in greenhouses have been doubled by the intelligent use of this system. The hydroponic solution (nutrient-rich water) is pumped to the plants by means of a trickle/drip irrigation system. The solution is re-used after being sterilized.

CONSUMPTION RELATED MATTERS

The majority of fresh strawberries are consumed without further processing. At most, they will be washed gently in tap water before being eaten. They may be consumed directly, sometimes in combination with dairy products, such as yogurt and whipped cream, and they may also be used as toppings for pies and desserts.

Quality aspects

An important part of international trade in strawberries is the quality requirements of bodies such as the United Nations Economic Commission for Europe (UNECE) and the European Commission. In, for example, the 'Commission Regulation (EC) No 843/2002 of 21 May 2002,' laying down the marketing standards for strawberries, and the amending Regulation (EEC) No 899/87, the minimum requirements for strawberries are that they should be intact, sound (produce not affected by rotting or deterioration), clean (practically free from any visible foreign matter), fresh in appearance (but not washed), practically free from pests and from damage caused by pests, and with the calyx present (except in the case of wild strawberries). The calyx and the stalk (if present) must be fresh and green, free from any abnormal amount of external moisture, with no foreign smell and/or taste. The strawberries must have been picked carefully. They must be sufficiently developed and display satisfactory ripeness. The development and condition of the fruit must be such that they can withstand transport and handling, and arrive in a satisfactory condition at their final destination.

In the above-mentioned regulation, strawberries are divided into three classes, differing with respect to brightness, color and shape that are characteristic of the variety in question, and aspects related to quality, shelf life and presentation, which are degree of bruising, defects in shape, presence of white patches and amount of any attached soil.

Consumer data on quality

Consumer data on the identification of quality attributes and acceptance of defects are rarely published. The few studies that have been carried out include investigations of items dealing with, for example, flavor and sweetness; the price, quality relationship; and appearance and color.

Flavor and sweetness are attributes that are becoming increasingly important to the consumer *(38)*. In an Australian study *(45)*, the mean price/quality relationship for strawberries was low, suggesting that consumers do not rely on price as an indication of quality and price does not predict the level of consumption. Quality criteria mentioned by consumers in this study were odor and bruising. For a better understanding of the quality:price relationship, further study is required. In the design of these studies, it is recommended that the quality criteria used be clearly defined. Using quality attributes given by the UM FDA and the Joint Institute for Food Safety and Applied Nutrition in Food, quality criteria could be separated into external features (appearance, color), other sensory attributes (odor, taste) and a third category, including wholesomeness, nutritive value and safety *(28)*.

Positive health aspects

Because the health advantages of horticultural products have been proven scientifically, authorities of many countries recommend the consumption of at least 5 portions of a variety of fruits (such as strawberries) and vegetables each day to reduce the risks of cancer and coronary heart disease and many other chronic diseases. For the program introduced in the UK, visit the internet page http://www.doh.gov.uk/fiveaday/index.htm.

Strawberries are low in calories, are a good source of many bioactive phytochemicals in the human diet, and provide nutrients that a healthy body needs. In addition, they have a good flavor. Strawberries are high in iron and vitamin C and are a good source of folic acid, fiber, potassium and cancer-fighting antioxidants. Eight medium-sized strawberries provide 20% of the recommended daily amount of folic acid *(44)*, which works with vitamins B_6 and B_{12} in the body to metabolize homocysteine and bring blood levels down to a safe range. Homocysteine contributes to atherosclerotic plaque formation, which can ultimately lead to a heart attack. In this way, strawberries are a recommended part of a heart-healthy diet *(53)*.

Antioxidants reduce the oxidation of low-density lipoprotein which links to cholesterol to form LDL-cholesterol. It has been demonstrated that decreased oxidation of LDL-cholesterol greatly diminishes the development of atherosclerosis *(55)*. The antioxidant property depends on the food matrix, as has been shown recently in studies demonstrating that strawberries have more antioxidant activity than apple, apricot, peach or kiwi fruit *(48)*.

It has been claimed that antioxidants present in strawberries, including flavanoids, anthocyanidin, ellagic acid and other phenolic acids that may have also anti-inflammantory properties, reduce the risk of developing several forms of cancer (http//www.labspec.co.za/l_fruit2.htm# Strawberry).

Negative health aspects

Hazards associated with fresh produce include biological, chemical and physical agents. A general overview of these hazards that can also be associated with strawberries is presented in Table 1.

Microbiological pathogens such as bacteria, parasites and viruses are part of the environment. Many of them reside in the intestinal tract of animals and humans. They can contaminate strawberries

TABLE 1. A general overview of the hazards that can be associated with fruits and vegetables, including strawberries (6, 7, 20, 22, 29, 41, 43)

I Microbiological hazards	
Bacteria	*Salmonella*
	Shigella
	Escherichia coli (pathogenic)
	Campylobacter
	Listeria monocytogenes
	Vibrio spp.
Parasites	*Cryptosporidium*
	Cyclospora
	Giardia
	Entamoeba
	Toxoplasma
	Nematodes
	Plathelminthes
Viruses	Hepatitis
	Norovirus
	Rotavirus
	Enterovirus
II Chemical hazards	
Natural chemical hazards	allergens
	mycotoxins
Extraneous chemical hazards	Polychlorinated biphenyls
	Agricultural chemicals
	- pesticides
	- fertilizers
	Toxic elements and compounds
	- lead
	- zinc
	- cadmium
	- mercury
	- arsenic
	- cyanide
	Other contaminants
	- lubricants
	- cleaners
	- disinfectants
	- coatings
	- refrigerants
	- pest control chemicals
	From packaging materials
	- plasticizers
	- venyl chloride
	- adhesives
III Physical hazards	
Foreign bodies	Filth, foreign matter like soil

through infiltration of sewage waters into fields, irrigation with contaminated water, presence of animals in the field or use of inappropriately composted organic fertilizers. Especially during the growing period, many of these microorganisms can come into contact with strawberries. This can also occur during harvest, storage and handling. Microorganisms with infectious properties are of special concern because they are able to cause disease.

Strawberries may also contain hazardous chemical agents. They may be present naturally (allergens) or be added, deliberately or inadvertently, during agricultural production, post harvest handling and other operations. An important source of contamination is the use of plant protection agents (pesticides) and fertilizers.

In addition to microbiological and chemical hazards, strawberries may be contaminated with filth and other foreign matter.

A literature survey on the association between strawberries and adverse health effects has demonstrated that during the last 10 years, strawberries have only incidentally been involved in acute disease outbreaks. In addition to microbial diseases, strawberries might be involved sporadically in allergic reactions, such as rashes. This information is presented below.

Disease outbreaks. In the period 1998 – 2000, the US Centers for Disease Control and Prevention (CDC) reported a total of 1,135 foodborne disease outbreaks. In 43% of the outbreaks, a suspected vehicle was indicated. For this same period, a total of 4 outbreaks implicated strawberries as the possible vehicle (Table 2). In 3 cases the agent was hepatitis A virus and in 1 case a Norwalk-like virus. Water is a very common vector of these viruses. In one case the outbreak was caused by frozen sliced strawberries.

In 1997 two large outbreaks of hepatitis A associated with consumption of frozen strawberries occurred in the United States *(2, 27)*.

In an outbreak that occurred in Canada, the disease agent was the parasite *Cyclospora cayetanensis*. The incriminated food was a mixture of different types of berries, and uncertainty exists regarding which of the berries were contaminated. *C. cayetanensis* is a human parasite that can be transmitted to other people via water contaminated with human feces. Both food and water may act as transmission vectors *(42, 50)*.

In the period 1993–1998, the WHO Surveillance Program for Control of Foodborne Infections and Intoxications in

TABLE 2. Strawberryborne outbreaks reported to the Centers for Disease Control and Prevention with strawberries as the suspected vehicle (period 1998–2000) (http://www.cdc.gov/foodborneoutbreaks). Figures for Canada, Australia and in Europe (period 1993–1998) are also included

Year	Etiological agent	Nr ill	Suspected vehicle	Ref.
US Centers for Disease Control and Prevention (1998–2000)				
1998	*Hepatitis* A	29	Frozen strawberries	*
1998	*Hepatitis* A	41	Strawberries, honeydew melon	*
1999	Norwalk-like virus	63	Pasta salad, strawberries	**
2000	*Hepatitis* A	8	Strawberries	***
Canada (1998–2000)				
1999	*Cyclospora cayatenensis*	94	Blackberries, raspberries, strawberries	*3*
Europe (1993–1998)				
1997	*Hepatitis* A	> 8000	Frozen creams with strawberries	*46*
Australia, 2001				
2001	*S.* Typhimurium	5	Pastry-filled custard tart topped with strawberries in jelly	*37*

* Internet page: http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo1998/viral98.htm

** Internet page: http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo1999/viral99.htm

*** Internet page: http://www.cdc.gov/foodborneoutbreaks/us_outb/fbo2000/viral00.htm

Europe *(59)* registered a total 22,386 outbreaks in which the food vehicle was identified. Fruit, vegetables and spices were involved in 261 (1.2%) of these outbreaks. In none of these were strawberries involved. In 1997 there was a large outbreak of hepatitis A both in the Czech Republic and in Slovakia *(46)*. This outbreak could be traced back to the consumption of frozen-moderated strawberries. In Slovakia, more than 8,000 of the cases were hospitalized. It was suspected that the strawberry field was irrigated with contaminated water a few days before the fruit was picked, washed and frozen.

In Australia, an outbreak of *Salmonella* Typhimurium was linked to a pastry-filled custard tart topped with fresh strawberries in jelly *(37)* and five cases were reported. However, it has not been established if the strawberries were the source of the outbreak.

Sporadic cases. In most countries, it is mandatory to report outbreaks of foodborne disease only. As a consequence, there is not much data concerned with sporadic cases. Some countries (USA, UK and The Netherlands), however, collect foodborne infection data based on sentinel studies of the general population. A comparison of data from The Netherlands reported to the authorities and data collected by sentinel studies reveals that the large majority (> 90%) of foodborne diseases are, by definition, sporadic cases *(23)*. Because of the way strawberries become contaminated (for example, through fecal droppings of birds), it might be expected that many of the disease incidents will be sporadic.

Recalls. Another source of information on strawberry-borne diseases is recall action. Examination of the 'recall' internet sites of the US Food and Drug Administration (FDA) and Health Canada for the period 2000–2002 revealed only two such events.

Recall 1: On April 28, 2000, New West Foods, in conjunction with the FDA, initiated a nationwide recall of frozen strawberries (http://www.fda.gov/oc/po/firmrecalls/strawberries.html). The recall was in response to an outbreak of hepatitis A in early February among seven laboratory workers at Boston's Brigham and Women's Hospital. Following an investigation by the FDA, ice cream on which frozen strawberries were served as one of the toppings, was the most suspected food. After this recall, many others followed, involving frozen strawberry products that were associated with the same outbreak.

Recall 2: On May 23, 2000, the FDA announced that Expo Fresh LLC had recalled fresh strawberries, in bulk cardboard cartons, because the product was contaminated with *Salmonella* (http:www.safetyalerts.com/recall/f/00/460.htm).

Allergies. In allergic reactions, which are immunologically mediated, IgE plays an important role. Allergic reactions caused by strawberries are very rarely observed and when they are observed, the symptoms are relatively mild. The allergic reactions from eating strawberries are usually caused by the small hairs on the surface of the fruit.

Strawberries are a common cause of skin rashes, which are common symptoms in individuals that react to strawberries. These reactions are not immunologically mediated and are induced by aromatic and colored substances found in strawberries (http://www.labspec.co.za/l_fruit2.htm#Strawberry).

CONTAMINANTS

Microbiological contamination

A survey of the scientific literature for microbiological analyses of strawberries revealed that only a small number of studies have been carried out. In recent years, there were only two such studies.

In 2000, the FDA surveyed domestic fresh produce, including strawberries, for microbiological quality (http://vm.cfsan.fda.gov/~dms/prodsu10.html). A total of 136 samples of strawberries were investigated for the presence of *Salmonella*, *Shigella* and *Escherichia coli* O157. Each

sample consisted of 454 grams which was rinsed with a buffer solution. The rinse was tested for the presence of pathogens. No *Salmonella*, *Shigella* or *E. coli* O157:H7 were detected in any of the samples.

In a follow-up study, 143 samples of imported strawberries were tested for the presence of *Salmonella*, *Shigella* and *E. coli* O157:H7. Only in one sample were pathogenic organisms detected (http://vm.cfsan.fda.gov/~dms/prodsu6.html).

Johannessen et al. *(28)* tested the microbiological status of 120 samples of strawberries obtained from Norwegian markets. Samples were analyzed for thermotolerant coliform bacteria (an indicator of fecal contamination) and for the pathogens *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Yersinia enterocolica*. Thermotolerant coliform bacteria were found in only a small proportion of the samples; *Salmonella*, *Yersinia enterocolica* and *E. coli* O157 were not found in any. *L. monocytogenes* was found in one sample, while *S. aureus* was found more frequently.

Based on the above investigations, it may be concluded that strawberries are not often contaminated with human pathogens. However, the limited numbers of samples tested do not provide a guarantee of safety.

Pesticide residues

In The Netherlands, data are obtained from the pesticide residue monitoring program 'Programme for the quality of agricultural products'. These data are available only for the years before 1998. The results are presented in Table 3. Detailed information is available in the internet site http://library.wur.nl/cgi-bin/WebQuery/kapgf.

Also presented in Table 3 are data from the European monitoring program on pesticide residues in products of plant (source: http://europa.eu.int/comm/food/fs/inspections/fnaoi/reports/annual_eu/monrep_2001_en.pdf).

In 2001, five commodities (strawberries, apples, tomatoes, lettuces and table grapes) were tested for 36 different pesticides. Residues of pesticides at or below the Maximal Residue Levels (MRL) were found most often in table grapes (60%), followed by strawberries (51%), lettuce (49%), apples (47%) and tomatoes (33%). Residues exceeding the MRL were found most often in lettuce (3.9%) followed by strawberries (3.3%), table grapes (1.8%), tomatoes (1.5%) and apples (1.1%).

Chronic exposure assessments demonstrated that Acceptable Daily Intake (ADI) values were not exceeded for the pesticide/ strawberry combination. The finding indicates that there is no acute risk in this case.

PRE-HARVEST AND HARVEST

Pre-harvest

Land and water use. Soil is a rich environment for a variety of microorganisms. The non-pathogenic flora is important for the mineralization of plants and animals after their death, but the tissue-degrading properties of the microorganisms that contaminate fruits and vegetables may cause damage to the produce during transport and storage. Subsequently, the products are exposed to further microbial attack. In addition, soil is a reservoir of foodborne pathogens, such as *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens (35)*. *Listeria monocytogenes* has been isolated from non-cultivated soil. Pathogenic microorganisms from human/animal reservoirs can be found in the soil because of irrigation with contaminated water, fertilization with manure and sewage sludge, or droppings of animals in the farming area.

Water is often used for irrigation of plants. Its quality will vary depending on whether it is surface water or potable water. Water may be a source of contaminating microorganisms Surface water from streams and lakes may be contaminated with pathogenic protozoa, bacteria and viruses. The occurrence of *L. monocytogenes*, *Salmonella* and viruses in water has been reported *(10, 41, 42)*. The transfer of foodborne pathogens from irrigation water to fruits and vegetables will depend on the irrigation technique and the nature of the produce being grown *(39)*. Spray irrigation would be expected to increase the risk of contamination, in comparison with drip irrigation or flooding. Leafy vegetables provide large surfaces for contact with water and for the attachment of microorganisms.

In hydroponic systems, water is used for the transport of nutrients into the plant. Water from sewage plants can be used for this purpose. However, in the absence of prior treatment, it may represent a risk to crop contamination. There is a similar concern over the use of recycled water. Recycling of water for agricultural purposes is carried out in several countries, such as Australia, Germany, Israel, Spain, The Netherlands and USA *(10)*. The safety of treated sewage water depends on the efficacy and reliability of the treatments used to inactivate pathogens.

Organic fertilizers. Sewage, manure, slurry, sludge and compost of human and animal origin are commonly used as organic fertilizers for fruit and vegetable production, particularly for organic produce. The fecal origin of these fertilizers, however, represents a potential risk of contamination by viruses, bacteria and parasites pathogenic to humans.

Members of the family *Enterobacteriaceae*, such as *Salmonella*, *Shigella*, *Yersinia*, and *E. coli*, as well as *Campylobacter* spp. can be found in the intestinal tracts of a wide range of domestic, wild and companion animals. In Belgium and Finland, *L. monocytogenes* was found in 6.7 to 20% of the fecal samples analyzed *(26, 56)*, and also in sewage sludge *(52)*. De Luca et al. *(15)* found *Listeria* in sewage sludge and concluded that fertilizing land with this material for vegetable farming could present potential health risks. In Italy and The Netherlands, *L. monocytogenes* has been detected in sewage treatment-plant effluents *(4, 16)*. In the UK, in 1992, 1,029,555 tons (dry solids) of sewage sludge were generated, and over 460,000 tons of it were applied to agricultural land *(36)*. Even greater amounts of farm animal waste are applied to land. In the UK, some 21 million tons (dry solids) of farm animal waste are spread annually on the land *(39, 40)*.

In some foodborne outbreaks linked to the consumption of raw fruits and vegetables, epidemiological investigations have identified manure as the source of contamination, as in the case of *L. monocytogenes* on cabbage in Canada, and *Salmonella* and *E. coli* O157:H7 on apples used to make apple juice in the USA *(42, 54)*. The occurrence of *E. coli* O157:H7 on fresh produce may also result from field contamination, because of water run-off from nearby cow pastures or exposure to droppings from wild animals *(24, 47)*.

The microbiological processes during composting or aeration of manure are not well understood. Important factors are the increase in temperature to 50–60°C and the treatment time. If the composting process is managed carefully, it will kill those foodborne pathogens that do not form spores *(52)*. However, the adequacy of existing methods of composting and the relevant regulations need to be reviewed *(53)*. In general, increasing the delay between the application of organic fertiliz-

TABLE 3. Pesticide residue levels for (1) Dutch strawberries (domestic and imported) and (2) strawberries monitored in the European monitoring program for pesticide residues in products of plant origin (see for source under pesticide residues)

(1) Dutch strawberries (domestic and imported)

Year	The Netherlands							
	Domestic				Imported			
	Nr. of samples	No residue	Residue < MRL[1]	Residue > MRL	Nr. of samples	No Residue	Residue < MRL[1]	Residue >MRL
1993	381	30%	67%	2.9%	51	28%	55%	17.7%
1994	493	38%	53%	8.1%	87	17%	72%	10.3%
1995	547	48%	48%	4.0%	97	19%	71%	10.3%
1996	697	33%	64%	2.6%	60	30%	47%	23.3%
1997	900	47%	51%	3.3%	89	23%	63%	14.6%

(2) European strawberries

Year	Commodities	European Union			
		Nr. of samples	No residue	Residue < MRL	Residue > MRL
2001	Strawberries	1652	46%	51%	3.3%
2001	Strawberries, apples, tomatoes, lettuces, table grapes	9868	51%	47%	2.2%
2000	Strawberries, apples, tomatoes, lettuces, table grapes	3737	80%	17%	2.7%
1999	Strawberries, apples, tomatoes, lettuces, table grapes	4707	69%	22%	8.7%
1998	Strawberries, apples, tomatoes, lettuces, table grapes	3836	66%	32%	2.0%
1997	Strawberries, apples, tomatoes, lettuces, table grapes	6021	65%	34%	1.1%

ers and harvest could reduce the occurrence of foodborne pathogens on fruits and vegetables. More evidence is needed to establish the minimum delay necessary for pathogens to be completely eliminated.

Usually, vegetative pathogenic bacteria and viruses decline in numbers within a few days of their introduction into the soil *(17, 22, 51, 56, 58)* or presence on plant surfaces *(32, 43)* although they may survive (in low numbers) for several weeks or months *(1, 9, 17, 58)*. Survival in the soil is influenced by several factors, e.g. soil type, humidity, temperature and competing microflora *(7, 17, 51)*. *E. coli* O157:H7 has been found to survive in bovine and ovine manure for periods from several weeks up to 12 months, depending on environmental conditions *(21, 33)*.

Plant protection products. Chemical biocides are generally used to protect plants against pests and disease agents. Even though substances authorized for this purpose have undergone extensive safety evaluations, there is consumer concern about their need and safety. These substances are not authorized for use in organic production of fruits and vegetables and this has stimulated the development of alternative control methods based on microorganisms or their metabolites. A wide range of microorganisms are used in biological control, including members of *Bacillaceae, Micrococcaceae, Streptomyces, Trichoderma,* fungi, viruses, *Lactobacillaceae,* and the *Pseudomonas* group *(31)*. Strains of *Bacillus thuringiensis,* or its bioactive crystalline protein, have been used for the control of insects *(57)*. *B. thuringiensis* is also permitted in organic production of fruits and vegetables, and the gene for the active protein has been inserted into GM-plants for insect control. The genomic structure of *B. thuringiensis* is similar to that of *Bacillus cereus,* and discrimination between these organisms is largely based on the possession by *B. thuringiensis* of the crystalline protein. *B. thuringiensis* strains used for pest control have been found to express an enterotoxin similar to that of *B.cereus (14)*.

Viruses also have a long tradition for controlling pests and mites. A well-known example is the use of Baculo viruses against arthropods *(25)*.

Antibiotic substances such as kasugamycin, octhilinone, oxytetracycline, validamycin, polyoxin, and streptomycin are used for plant protection in some countries. The emerging risk related to

this practice was discussed in an opinion of the EU Scientific Steering Committee on antibiotic resistance *(49)*. Their advice was not to use antibiotics as plant production agents.

Harvest

Strawberries are mainly harvested by manual picking. However, mechanical picking has been considered. For example, a mechanical harvester for strawberries was developed in Sweden between 1986 and 1990 (http://www.actahort.org/books/348/348_35.htm). The harvester was based on experience of similar harvesters in the United States and Canada. The entire plant is cut at ground level and the leaves are removed inside the machine with the help of cross-flow fans. In a second step, the clusters of fruit become separated when the stems are raised in an air flow and hedgers cut them off. The harvester is designed to work in solid-bed plantations. In commercial trials, ten tons of fruit per hectare have been harvested. In some years, it has been possible to harvest 80% of the ripe fruit. The development of gray-mold fruit rots affects the quality and quantity of fruit harvested. In dry years, it has been possible to start harvest more than ten days after the primary berries are ripe. The harvested product can then be used for juice with no further sorting. Processors of quality products prefer the unripe berries to be removed. For jam or similar products, it is necessary to decap the berries. At this time, mechanical decapping is not practiced.

Fruits and vegetables can become contaminated with pathogenic microorganisms during harvesting through fecal material, human handling, harvesting equipment, transport containers, wild and domestic animals, air, transport vehicles, ice or water *(6)*. In an investigation of several foodborne illnesses associated with fresh produce (NACMCF, 1999a), agricultural workers were often the most likely source of contamination. Lack of suitable sanitary hand-washing facilities in the production area can create a potential hygienic problem. This appears to be particularly important in the transmission of enteric viruses, such as hepatitis virus. Beuchat *(6, 7)* referred to outbreaks of illness due to *Shigella flexneri* and hepatitis A, which could be traced to infected people working in the fields or the packaging facility.

Harvesting at the appropriate time and keeping the harvested product under controlled environmental conditions will help retard growth of post-harvest spoilage organisms and pathogens.

POST-HARVEST MEASURES

Post-harvest treatment of fruits and vegetables includes handling, storage, transportation and cleaning. During these practices, conditions may arise that lead to cross contamination of the produce from other agricultural materials or from the workers. Environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption.

Poor handling can damage fresh produce, rendering the product susceptible to the growth and/or survival of spoilage and pathogenic microorganisms. This damage can also occur during packaging and transport. The presence of cut and damaged surfaces provides an opportunity for microbial contamination and growth, as well as ingress of microbes into plant tissues *(20)*.

Decontamination

Strawberries are characterized by a very short post-harvest life because of fungal decay and deterioration in appearance and texture. In order to prolong post-harvest shelf life and the quality of these fruits, several decontamination techniques have been tested. They include washing with a solution containing disinfectants, gamma irradiation and modified atmosphere packaging.

Natural survival. Survival of *Escherichia coli* O157:H7 was studied on strawberries, together with the effect of washing with disinfectants *(30)*. Strains inoculated onto the surfaces of strawberries did not multiply during subsequent storage at ambient temperatures. Actually, a small decrease in numbers was observed.

To ascertain the potential for pathogenic enteric viruses to survive on strawberries, the fruit was inoculated with poliovirus and tested for survival *(34)*. It took a storage period of up 8.4 days before a one-$\log_{10}$ reduction was observed.

Disinfectants. Dipping of inoculated strawberries in water alone reduced the levels of pathogens by approximately 0.8 $\log_{10}$ units. None of the disinfectant compounds used (NaOCl, acetic acid, Na_3PO_4 and H_2O_2) reduced the numbers of *E. coli* O157:H7 by more than 2 $\log_{10}$ units *(30)*.

El-Ghaouth et al. *(18)* studied the effect of chitosan coating. Strawberries were inoculated with a mixture of spores of *Botrytis cinerea* and *Rhizopus stolonifer* and then coated with chitosan solution (10 and 15 mg/ml respectively). After storage for 14 days at 13°C, the coating markedly reduced decay by both mold species. It was concluded that the reduction in decay was related to the fungistatic properties of chitosan.

Irradiation. Brecht et al. *(8)* investigated the effects of gamma irradiation (100 and 200 krad). Irradiation did not influence the color of strawberries. Treatment delayed decay by *Rhizopus* and *Botrytis* molds, but resulted in a clear softening of the fruit.

Modified atmosphere packaging. Modified atmosphere packaging (MAP) (7% O_2 and 20% CO_2) did not influence the color of strawberries. However, there was little delay in spoilage by several strawberry pathogens *(8)*. The effects of different atmospheres (low O_2 and high CO_2) on biological changes and growth of fungal pathogens were also studied by Chambroy and colleagues *(11)*. At 20°C, control of fungal development was impossible, regardless of the composition of the surrounding atmosphere. At 10°C and with CO_2 concentrations of >10%, a reduction in the development of mold decay was observed. Under these conditions, the strawberries had a better appearance and firmer texture.

Despite all the attempts made to decontaminate strawberries, no practical control measure has been devised. One of the main reasons is that strawberries are so sensitive to damage during treatments such as washing and irradiation. There is also a consumer preference for 'natural' produce that has received minimal treatment. This means that Good Agricultural Practice (GAP) must be the main factor in controlling contamination. Cooling of the fruit is actually the only acceptable means of increasing shelf life.

Cooling

Immediately after harvest, strawberries must be chilled by forced-air cooling to a temperature of 7°C or less. Hydrocooling (flooding them with chilled water) is not recommended because wet berries are much more susceptible to decay. Cooling with crushed or 'liquid' ice would be even worse because, in this case, the berries are likely to sustain physical damage.

It is never sufficient simply to place the packaged strawberries in a chill room and allow them to cool gradually. For palletized loads, the cooling process would take too long, so that fruits at the center of the pallet would not be adequately cooled and would start to de-

cay. Without forced movement of the cooling air, the heat from plant respiration would destroy the fruit.

LAWS AND REGULATIONS

Codex Alimentarius. Good Hygienic Practice (GHP) as defined in the Codex document on "General Principles of Food Hygiene," in combination with HACCP, as the basis for safe food production *(12)*. A code of "Hygienic Practices for Fresh Fruits and Vegetables" including an Annex on "Ready-to-Eat Fresh Pre-cut Fruits and Vegetables" has been elaborated by the Codex Alimentarius Committee on Food Hygiene.

The Codes were initiated in response to the growing concern about fresh fruits and vegetables being a possible source of foodborne pathogens. They address Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) that will help to control microbial, chemical and physical hazards at all stages of the production of fresh fruits and vegetables, from primary production to packing. The following areas are acknowledged to be important in connection with microbial safety: environmental hygiene, hygienic production (water, manure, soil, agricultural chemicals, biological control, indoor facilities and personal hygiene), handling, storage, transport, cleaning, maintenance and sanitation.

The draft code follows the format of the Codex code on General Principles of Food Hygiene. It addresses hazards to be managed by the producer according to GAP and GMP. It does not generally define measures to be taken or criteria to be observed.

Food and Drug Administration (FDA) and United States Department of Agriculture (USDA). As guidance for the US industry *(19)*, the FDA and USDA define procedures to be followed with respect to microbial food safety hazards and good agricultural and management practices common to growing, packaging and transport of fruits and vegetables. The guide focuses on the quality of water used for different purposes, manure and municipal biosolids, worker health and hygiene, field sanitation, packaging-facility sanitation, transportation and traceability.

European Union (EU). The European Commission has produced several directives, regulations and recommendations related to the production and consumption of fresh strawberries. They include:

- **Pesticide residues in foodstuffs of animal origin:** Council Directive 86/363/EEC, Official Journal No L 221, 07/08/1986 p. 0043 – 0047
- **Fixed maximum levels for pesticide residues in and on products of plant origin:** Council Directives 86/362/EEC, Official Journal No. L 221, 07/08/1986 p. 0037 – 0042 Amended Council Directives 90/642/EEC, Official Journal No. L 350, 14/12/1990 p. 0071 – 0079
- **Inspections and monitoring:** Council Directive 89/397/EEC, Official Journal No. L 186, 30/06/1989 p. 0023 – 0026
- **Additional measures concerning the official control of foodstuffs:** Council Directive 93/99/EC, Official Journal No. L 290, 24/11/1993 p. 0014 – 0017
- **Sampling:** Commission Directive 79/700/EEC, Official Journal No. L 207, 15/08/1979 p. 0026 – 0028
- **Specific EU coordinated monitoring programme:** Commission Recommendation 2001/42/EC, Official Journal No. L 11, 16/01/2001 p. 0040 – 0045
- **Requirement of Member States to report to the Commission the results of the monitoring programme for pesticide residues carried out:** Article 7 of Council Directive 86/362/EEC and Article 4 of Council Directive 90/642/EEC, as amended by Council Directive 97/41/EC, Official Journal No. L 184, 12/07/1997 p. 0033 – 0049
- **Detailed implementing rules for the monitoring provisions:** Commission Regulation (EC) No. 645/2000, of 28 March 2000, Official Journal No. L 78, 29/03/2000, p. 0007 – 0009
- **Laying down the marketing standard for strawberries and amending Regulation (EEC) No 899/87:** Commission Regulation (EC) No. 834/2002 of May 21, 2002, Official Journal No. L 134,24 22/05/2000, p. 0024 – 0028.

CONCLUSIONS

The results of this risk profile reveals that strawberries have a healthful image and are appreciated by consumers. The consumption rate of strawberries continues to rise. The results also indicate that strawberries intended for fresh consumption have a relatively good safety record. Only a few strawberry-related outbreaks have been reported, and most of them are associated with consumption of frozen strawberries. Nevertheless, the quality and consumer aspects need attention and further studies are required.

Monitoring data for pesticide residues show that the majority of strawberries tested do not contain detectable residues. Only a small percentage exceed the Maximum legally permissible Residue Level (MRL). Acceptable Daily Intakes (ADI) values were not exceeded, indicating that there is no acute risk.

Microbiological examination of fresh strawberries reveals that pathogenic infectious organisms are rarely present. Nevertheless, in various stages of the production process of strawberries, contamination with pathogenic microorganisms may occur. Because no practical decontamination methodologies are available, attention needs to be paid to preventing contamination. Therefore, it is recommended that well-developed codes of good agricultural practices be used, in which land and water use, application of organic and inorganic fertilizers, and animal and pest control are described, together with recommended harvesting and cooling practices and measures for ensuring worker health and safety.

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Consumers' Perceptions of Irradiated Ground Beef After Education and Product Exposure

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SUMMARY

A consumer study of irradiated ground beef was conducted in Manhattan, Kansas to test the effects of education and product exposure on consumers' perceptions of food irradiation. Sensory evaluation was performed on irradiated and non-irradiated ground beef at the time of the consumer study and again following three months of frozen storage.

Sensory evaluation indicated that consumers could not differentiate between the two types of ground beef and had no preference for either ($P > 0.05$). For both the initial and follow-up sensory tests, irradiated and non-irradiated cooked ground beef were perceived the same ($P > 0.05$).

Educating consumers on irradiation had the most significant impact on their views of food irradiation ($P < 0.05$). Groups that received irradiation education were more accepting of the technology ($P < 0.05$) and more consumers positively changed their perceptions of irradiation ($P < 0.05$). Consumers not receiving education were skeptical, uninformed and had more negative perceptions. Some were unaware of irradiation technology. Product exposure had no effect on perception of irradiation.

INTRODUCTION

Food irradiation has been studied in the United States since the 1950s and has been determined to be a safe and effective means of preventing foodborne illness *(8, 9)*. The irradiation of food provides many consumer advantages, including decreased microbial levels, increased food safety and longer shelf life *(3)*. Though foodborne illness continues to affect millions of people each year, mainstream availability of irradiated products and consumer acceptance has been slow to develop *(6)*.

The Centers for Disease Control and Prevention (CDC) estimates that each year there are 76 million cases of illness, 325,000 hospitalizations and 5,000 deaths, resulting in nearly $6.7 billion in patient-related costs for treatment of bacterial infections *(6)*. Contaminated raw food or ingredients accounts for an estimated 42% of foodborne illness *(4)*. Food irradiation could reduce the levels of bacterial pathogens in raw meat and poultry, thus reducing the risk of illness *(9)*.

Irradiation technology is unfamiliar to consumers, and radiation provokes feelings of fear *(5)*. Most consumers are not aware of the benefits of irradiation, and positive aspects have not been enough to eliminate consumer concern. To increase public acceptance, emphasis must be placed on using scientific data to educate consumers about the effectiveness

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TABLE 1. Organization of sample groups

Group	Product Exposure	Education	Focus Group
1	Yes	Yes	Yes
2	Yes	No	Yes
3	No	Yes	Yes
4 (control)	No	No	Yes

and safety of the process *(3)*. Invariably, consumers who are well informed about irradiation are more accepting of the technology, and thus more willing to purchase irradiated food items *(1)*.

Irradiation at certain levels may incur undesirable sensory changes in food products, though research results are variable, depending on packaging, product and received irradiation dose. At increased doses for some foods, consumers' perceptions of quality both increased and decreased *(10)*. Progress in irradiation technology has led to lowered doses and improved handling. In addition, research has indicated that during prolonged storage, sensory changes may occur in irradiated foods *(7)*.

The objectives of this study were to determine: (1) the effects of education and product exposure on consumer acceptance of irradiation; (2) if consumers could determine differences between irradiated and non-irradiated ground beef; and (3) if consumers could detect sensory changes in a ground beef product irradiated and then stored frozen for three months.

MATERIALS AND METHODS

Sample selection and recruitment

Random consumer names, addresses and phone numbers were purchased from Survey Sampling Inc., Fairfield, CT, a national market survey company. One hundred thirteen consumers participated in the study. Those who ate ground beef no less than once per month and those over the age of 18 were eligible for the study. Participants were randomly assigned to one of four sessions. A small random sample of participants was asked to take part in a focus group following each session. All data collection sessions took place on the campus of Kansas State University.

Test design

The experiment was a 2 × 2 factorial design (Table 1). Treatments were:

1. Irradiation education (Ed-group)
2. No irradiation education (No-Ed group)
3. Exposure to irradiated ground beef
4. No exposure to irradiated ground beef

Data collection and questionnaires

Participants provided demographic information and completed a pretest-questionnaire prior to the beginning of each session. Those who partook in the sensory test completed a sensory questionnaire. At the end of each session, all participants completed a general questionnaire on food irradiation. The questionnaire had been developed and pre-tested at the University of Arkansas by Molly Longstreth, Ph.D. Minor modifications were made to it.

Sensory evaluation

Fifty-six people participated in an informal sensory test of unidentified irradiated and non-irradiated hamburger patties. An identical follow-up sensory test was conducted on hamburger patties that had been stored for three months at 0°C. Consumers from the initial sensory test also participated in the follow-up test. Frozen 4 oz. ground beef patties of the same manufacturing lot were received from Huisken Meats, Chandler, MN. The ground beef was 75% lean, and the irradiated patties were irradiated at a level of 1.5 kGy. Ground beef patties were prepared prior to tasting (160°F), using George Foreman grills. Each 4 oz. patty was divided into fourths and held in warming containers until use (approximately 15 minutes). Irradiated and non-irradiated products were kept separate from each other during the storage, cooking and holding processes.

Each participant completed a 10-item sensory questionnaire consisting of graphic, 8-point intensity and liking-scales on color, appearance, aroma, flavor, juiciness, texture, and overall liking.

Irradiation education

Participants in sessions one and three received education in the form of a 15-minute Microsoft Power-Point® presentation and an 8-minute video. The video, "Behind the Headlines: Food Irradiation", had been produced by Purdue University as part of a 10-state irradiation education project. The researchers prepared the Power-Point® presentation, which included general information about food irradiation.

Focus groups

A focus group (approximately 30 min) was conducted immediately following each large group session. Six to eight participants stayed for the discussion, by prearrangement. Focus group sessions were audio-recorded and a moderator's guide was followed.

Statistical analyses

Data were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, NC). The SAS univariate procedure was used to analyze sensory data. In addition, regression analysis was performed on selected main questionnaire data. Contingency table analysis was used to analyze remaining information. Statistical significance was defined as $P < 0.05$.

RESULTS

Of the 113 participants who completed the study, males represented 52% of the sample. More respondents were born in the 1950s (20%) or 1970s (21%) than in other decades. Most (96%) were Caucasian, and 61% were married.

When asked if they or any family member had ever experienced symptoms of foodborne illness, 80% responded "yes", 18% said no and 2% stated that they did not know. Ground beef was consumed by 99% of the participants at least once per month, while 38% responded

TABLE 2. Frequency and percentages of responses by consumers who were educated (Ed) and not educated (NoEd) on food irradiation

	Response Frequency (%)				
Question/Group	Definitely	Probably	Undecided	Probably Not	Definitely Not
Safe to eat?					
Ed (Groups 1 & 3)	45 (85)	7 (13)	1 (2)	0 (0)	0 (0)
NoEd (Groups 2 & 4)	18 (30)	28 (46)	11 (18)	2 (1)	0 (0)
Question/Group	Very Likely	Likely	Neither	Unlikely	Very Unlikely
Likely to purchase?					
Ed (Groups 1 & 3)	32 (60)	12 (23)	6 (11)	3 (6)	0 (0)
NoEd (Groups 2 & 4)	9 (15)	23 (38)	24 (40)	1 (1)	1 (1)

TABLE 3. Mean scores and *P*-value comparisons to the reference group

		Values			
Question	Variable	Group 1^	Group 2	Group 3^	Group 4*
1. Is irradiated meat safe	*P*-value	0.0001"	0.4896	0.0004"	*
for consumption?	Mean score	36.92	73.37	44.34	68.87
2. How likely are you to					
purchase irradiated vs.	*P*-value	0.0004"	0.783	0.0052"	*
non-irradiated ground beef?	Mean score	40.36	67.46	47.34	69.55

* Indicates reference group. Means (responses) of other groups are compared to the reference

"Low *P*-values < 0.05 indicate a difference in association to the reference, group 4

In this case, groups 1 and 3 are statistically different from group 4, the reference. Mean scores are based on 5-point scales. Higher means indicate responses closer to Question1. definitely not, Question2. very unlikely

^Indicates received irradiation education

they consumed ground beef more than once per week. Within that response, 80% stated they prepared and consumed ground beef one to three times per week at home.

Focus groups concluded each general session. It is important to note that groups one and three received education on food irradiation (Ed-groups) during the main session prior to the focus group, whereas groups two and four did not (NoEd-Groups). Participants were asked: What does irradiation mean to you? What is your perception? Ed-groups expressed a more positive attitude about the irradiation process. NoEd-groups stated concerns and often said they did not know enough about the topic to fully discuss it. Group one provided the most positive responses, though most participants from Ed-groups expressed initial skepticism before learning about irradiation.

After a Food and Drug Administration (FDA) definition of food irradiation was presented to groups, groups were asked if their perceptions had changed. Groups receiving irradiation education responded positively to the definition and it did not change their view on irradiation. NoEd-groups stated that it made sense and made irradiation sound more positive. Yet, the definition raised many questions about the energy source, shelf life, and nutritional changes that may occur in the product.

TABLE 4. Regression analysis of responses to "safety" and "purchase" questions

	Purchase Question*		Safety Question**	
Variable	Coefficient	*P*-value	Coefficient	*P*-value
Intercept	3.424732	2.082E-06	2.8433631	7.177E-08
Received irrad. education	0.666699	0.0002208	0.7666761	1.732E-08
Received product	0.119514	0.4841051	0.0029223	0.9809973
Had illness	0.212951	0.3467198	-0.020414	0.8999728
Consumption	-0.035893	0.6931275	0.0723257	0.2701352
Children under age 6	0.167171	0.5052778	0.0547932	0.761209
Over age 65 in household	-0.04414	0.8893281	-0.132006	0.5631214
Education level	-0.040396	0.5408283	0.0887757	0.0636583
Age	0.005562	0.4611078	0.0062694	0.2489205
Male gender	0.209019	0.2270132	0.3393839	0.007179

TABLE 5. Frequency and percentage of correct responses to "true" "false" and "don't know" category questions

	Group			
Question	1*	2	3*	4
	Frequency (Percentage)			
1. Recontamination	24 (96)	12 (39)	19 (70)	13 (48)
2. Spoilage	17 (71)	14 (45)	19 (68)	17 (63)
3. Retained Quality	25 (100)	19 (61)	24 (85)	19 (70)
4. FDA Approved	25 (100)	18 (58)	27 (96)	15 (55)
5. Radura	24 (96)	5 (17)	28 (100)	11 (40)
6. Radioactive	25 (100)	19 (61)	27 (96)	16 (59)

* Indicates an Ed-Group

All groups were generally unaware that items in everyday-use are irradiated for safety. Irradiation of mail was new to the consumers, although it was a widespread news story after the 2001 anthrax events.

Responses varied as to how participants felt about whether irradiated ground beef was available in their local supermarkets and fast food restaurants. No one in the groups had knowingly purchased irradiated products. Most did not know it was available in the Manhattan community via home delivery. Groups stated they would feel safer buying irradiated hamburgers from a fast food chain because of added safety, referring to the Jack-in-the-Box *E. coli* outbreak. One member from one of the NoEd-groups stated that irradiated beef could be offered at the grocery store, as long as consumers had a choice.

It appeared from the discussion that participants from Ed-groups had greater understanding of the benefits of food irradiation. From all groups, concerns tended to be preconceived ideas and initial stereotypes about the irradiation process. NoEd-group participants were not as aware of irradiation's benefits; they knew only what they had been told previously from the FDA definition. Participants from each group stated that they felt more comfortable purchasing irradiated hamburgers from restaurants because the cooking was not in their direct control.

Sensory responses of participants (groups 1 and 2) from the taste test were analyzed as one group. A graphic, 8-point intensity and liking scale was used for each of 10 attributes: color, overall appearance, aroma, flavor, juiciness, tenderness, overall flavor of each sample and overall liking of each sample.

The initial sensory test indicated that respondents had no preference for either unidentified sample. When specific attributes such as juiciness and texture were considered, irradiated ground beef was reported to be juicier (80%) and more tender (89%). The majority of respondents perceived color, aroma, appearance, flavor, juiciness and texture to be "as acceptable" or "better than" in the irradiated than in the non-irradiated sample, although these differences were not statistically significant. Follow-up sensory results were similar to those of the initial test. Statistically significant differences were not found between responses for irradiated and non-irradiated samples.

Consumers were asked if they "believe irradiated meat, processed using the latest scientific guidelines and according to the latest regulations, is safe to eat". Choices for response were on a 5-point

TABLE 6. *P*-values and means for responses to question: "How do you perceive food irradiation?" as compared to responses of reference Group 4

Test		Group 1^	Group 2	Group 3^	Group 4*
Pre-Test	*P*-value	0.4813	0.9915	0.5771	*
	Mean Score	60.16	54.00	58.75	53.91
Post-Test	*P*-value	0.0001	0.5996	0.0001	*
	Mean Score	75.48	41.11	74.22	37.51
Difference in	*P*-value	0.0002	0.1386	0.0001	*
Pre and Post-tests	Mean Score	69.10	50.09	70.48	39.08

^ Indicates received irradiation education

* Indicates reference group to which all other values were compared

The question was in the form of an 8-point scale (1-8 or negative-positive)

scale ranging from "definitely" to "definitely not". Ed-groups believed that irradiated foods were safe to eat, answering "definitely" 85% of the time and "probably" 13% of the time. NoEd-groups were more skeptical of the food's safety, responding "definitely" 30% of the time, "probably" 46% and "undecided" 18% (Table 2).

In a similar question, participants were asked, "Knowing that technology has potential benefits and risks, how likely are you to buy irradiated rather than non-irradiated ground beef?" A 5-point scale ranging from "very likely" to "very unlikely" was used. Ed-groups most often responded that they would be "very likely" (60%) and "likely" (23%) to purchase irradiated ground beef. NoEd-groups responded that they would be "likely" (38%) and "neither likely nor unlikely" (42%) to purchase irradiated ground beef (Table 2). In this hypothetical purchase question, Ed-groups were more willing to purchase the irradiated ground beef.

Data were compared across groups for both questions by the least significant difference (LSD) procedure of SAS. Data from groups were compared to the reference (group 4); significant differences were indicated ($P < 0.05$).

For both questions, groups 1 and 3, who received irradiation education, were not statistically associated to the reference group 4, as indicated by low *P*-values ($P < 0.05$) (Table 3). The close association among Ed-groups may infer they answered questions similarly, as indicated by mean scores. Group two was more closely associated to the reference, as indicated by an elevated *P*-value and mean score closer to the reference (Table 3).

Regression analysis was performed to investigate the effect of demographic variables on the responses to the "safe for consumption" and "likely to purchase" questions. Explanatory variables in the regression models included the level of ground beef consumption, the presence of younger children or elderly individuals in the household, the respondent's gender, age and level of education, and whether or not any individuals close to the respondent had suffered from foodborne illness. Also included as explanatory variables were the experimental treatments – i.e., whether or not the respondent received education about irradiation, and whether or not they sampled the products.

The results (Table 4) suggest that exposure to the educational program was the dominant factor explaining variation in the subjects' responses. The coefficient on the 'Received education' variable is relatively large, positive and statistically significant (Table 4). Of the demographic variables, only gender was significant in explaining responses to the safety question; males tended to evaluate the product as safer than did females.

Coefficient signs, however, indicate that those who tasted the product tended to give more favorable evaluations. Coefficient signs also indicate that those who had experienced or whose family member had experienced a foodborne illness, those with young children and those who were older gave slightly more favorable evaluations.

Data indicated statistical associations between groups within true/false/don't know questions. Statistical analysis indicated an association among Ed-groups and NoEd-groups (Table 5). Ed-groups more often answered the questions appropriately, thus indicating that they had retained information from the irradiation presentation. It can be assumed that, in general, the population in Manhattan, Kansas did not possess a large amount of prior food irradiation knowledge. When provided information about irradiation, people retained the information, and thus answered questions correctly. Education had a positive influence on participants' ability to correctly respond to food irradiation questions.

At the beginning and end of each session, participants rated their perception of food irradiation on an 8-point scale that ranged from negative to positive. For the pre-test scores, there was no statistical difference among groups' perceptions of food irradiation (Table 6). At the conclusion of each session, significant differences were noted in perception among groups. Groups that received irradiation education differed from the reference, group 4, in their perception of food irradiation. Ed-groups had similar mean scores (Table 6).

Group 4, which functioned as the control and reference group, did not receive irradiation education or product exposure and its perception of food irradiation was unchanged and uninfluenced. Overall, education had the strongest influence on perception of food irradiation. Groups that received education had more positive perceptions.

DISCUSSION

Consumers did not have a strong liking preference when given unidentified samples of irradiated and non-irradiated ground beef, either initially or after three months of frozen storage. These results are a positive sign for irradiated meat, considering that early industry research found irradiated products to be unpalatable. And, if irradiated and non-irradiated products are perceived the same, sensory changes cannot be a reason for avoiding irradiated ground beef.

In the focus group sessions, it was apparent that groups who had received irradiation education were more open and informed in discussing irradiation. Given their knowledge, they were more accepting and less fearful of the process, and more willing to accept irradiated foods. Yet, they recognized the need for public education on the topic. It was evident that the general public is in need of education on food irradiation. Mainstream irradiated foods were not available in local grocery stores, and until the public is informed on the topic of food irradiation, negative perceptions will remain.

Education had a strong influence on the perception of food irradiation ($P < 0.05$). Groups who received irradiation education were more positive about the technology. This is consistent with other research that found that education had a positive impact on the image perception of food irradiation *(2, 3, 5)*. Irradiation is not a mainstream food process and consumers are not familiar with irradiated food. Once consumers were informed about food irradiation, perceptions changed.

The effect of product exposure on the perception of irradiation was slight. Most people do not have personal experience with irradiated food products. One can assume that, as irradiated products become mainstream and consumers become familiar with them, product exposure may have a positive impact. When local grocery retailers carry these products, acceptability of the products may follow.

Though irradiation is not a new process to the scientific and academic communities, it remains unknown to most consumers. Education is the key to acceptance. Product exposure may also help to relieve fears. Our data indicate that consumers are accepting of the overall taste and flavor of irradiated ground beef and that consumers who are informed about the benefits of irradiation are more accepting of the process. Education, followed by market introduction, would be positive steps towards increasing food safety in the United States.

ACKNOWLEDGMENTS

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Manchester Grand Hyatt San Diego
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PREFACE

The *Symposium Series on Food Microbiology* consisted of three international symposia sponsored by the ILSI North America Technical Committee on Food Microbiology at the International Association for Food Protection (IAFP) 89th Annual Meeting, held June 30–July 3, 2002, in San Diego California, USA. Sessions addressed antibiotic resistance in humans and feed animals, *Listeria* research, and chronic wasting disease and other transmissible spongiform encephalopathies.

The North America branch of the International Life Sciences Institute (ILSI North America or ILSI N.A.) is a public, non-profit scientific foundation that advances the understanding and application of scientific issues related to the nutritional quality and safety of the food supply as well as health issues related to consumer self-care products. The organization carries out its mission by sponsoring relevant research programs, professional education programs and workshops, seminars, and publications, as well as by providing a neutral forum for government, academic, and industry scientists to discuss and resolve scientific issues of common concern for the well-being of the general public. ILSI N.A. also strives to foster the career development of outstanding new scientists. Its programs are supported primarily by its industry membership.

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 worth 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.

ABSTRACTS AND EXTENDED ABSTRACTS

ANTIBIOTIC RESISTANCE IN HUMANS AND IN FEED ANIMALS

Historical Perspective on Antimicrobial Resistance

THOMAS F. O'BRIEN, Brigham and Women's Hospital, Boston, Massachusetts, USA

Resistance in *Salmonella* Newport

AMITA GUPTA, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Multiple Drug Resistance: Trends and Implications

PAULA J. FEDORKA-CRAY, Marcia L. Hedrick, Mark E. Englen, Jeffrey T. Gray, Charlene R. Hudson, Jeanetta Tankson, Neena Anandaraman, Ben Salamone, Bonnie Rose, David A. Dargatz, and Linda Tollefson, Agricultural Research Service, U.S. Department of Agriculture, Athens, Georgia, USA

Antibiotic Resistance Trends in Europe

E. JOHN THRELFALL and Ian S.T. Fisher on behalf of the Enternet participants, Central Public Health Laboratory, London, United Kingdom

Perspectives in Addressing the Safety of Cephalosporin Use in Food Animal Medicine

SUSAN F. KOTARSKI, Scott A. Brown, Edward J. Robb, and Rex Hornish, Pharmacia Animal Health, Kalamazoo, Michigan, USA

Consequences of the Removal of Subtherapeutic Antibiotics from Danish Farms

HANNE-DORTHE EMBORG and Ole E. Heuer, Danish Veterinary Institute, Copenhagen, Denmark

LISTERIA RESEARCH UPDATE

This symposium was made possible in part by a grant from the National Food Processors Association.

Use of Sequence Typing for Characterization of Virulence Factors and for the Development of a Novel Molecular Typing Scheme for *Listeria monocytogenes*

Franco J. Pagotto, Sandra C. Smole, and JEFFREY M. FARBER, Health Canada, Ottawa, Ontario, Canada

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

Identification of Potentially Unique Genetic Markers and Virulence Attributes of Epidemic-associated Strains of *Listeria monocytogenes*

Matt R. Evans, BALA SWAMINATHAN, Lewis M. Graves, Steven Bowen, and Sophia Kathariou, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Molecular and Phenotypic Characterization of *Listeria monocytogenes* Isolates from Humans and Foods to Define Human Pathogenic Strains

MARTIN WIEDMANN, Mario Roma, Katy Windham, Mike Gray, and Esther Fortes, Cornell University, Ithaca, New York, USA

Rapid Nucleic Acid–based Detection and Enumeration of *Listeria* spp. Flow Cytometry

BYRON F. BREHM-STECHER and Eric A. Johnson, Department of Food Science and Human Nutrition, 2581 Food Sciences Building, Iowa State University, Ames, IA 50011, USA; University of Wisconsin, Madison, Wisconsin, USA

CHRONIC WASTING DISEASE AND OTHER TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Overview of Transmissible Spongiform Encephalopathies

DEAN O. CLIVER, University of California, Davis, California, USA

Diagnostic Tests for Chronic Wasting Disease: What Is Needed? What Will Be Available?

KATHERINE I. O'ROURKE, Aru Balachandran, Elizabeth S. Williams, and Terry R. Spraker, Agricultural Research Service, U.S. Department of Agriculture, Pullman, Washington, USA

In Vitro and In Vivo Models for the Biology, Pathogenesis, and Transmission of Chronic Wasting Disease

SUZETTE A. PRIOLA, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA

Epidemiology of Chronic Wasting Disease in Wildlife

ELIZABETH S. WILLIAMS and Michael W. Miller, University of Wyoming, Laramie, Wyoming, USA

Control Measures for Chronic Wasting Disease

LYNN H. CREEKMORE, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Fort Collins, Colorado, USA

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

ANTIBIOTIC RESISTANCE IN HUMANS AND IN FEED ANIMALS

HISTORICAL PERSPECTIVE ON ANTIMICROBIAL RESISTANCE

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The World's Bacterial Populations

Bacteria have been evolving and diversifying on this planet for more than 3 billion years, penetrating into and adapting to earth's countless environmental niches. There are reasons to believe that there are many times more bacterial species than the 4,500 characterized to date, most of which are perhaps unable to grow with the culture methods on which we have largely depended *(1)*. Those that live on the surfaces and within the guts of animals and humans have presumably increased in proportion to the expansion of their host populations with the development of agriculture over the past 5,000 to 10,000 years. Most of these live harmlessly in or on their hosts, but a bacterial strain of a small number of pathogenic species occasionally invades and infects the tissues of a normal host, or one of a larger number of opportunistic species infects the tissues of an immunocompromised host.

Bacteria isolated from patients before antimicrobial agents were first manufactured, 60 or more years ago, were rarely resistant to the agents that were to come *(2)*. As each new agent became widely used thereafter, years or even decades often elapsed before a strain of bacteria resistant to that agent was found within a species that had been entirely susceptible. When a resistant strain finally did emerge, it was usually found to carry a gene that expressed the resistance by encoding a product that inactivated the antimicrobial or otherwise circumvented its effect on its lethal target site within the bacterial cell.

Emergence of A New Antimicrobial Resistance Gene

Study of resistance genes sometimes provides insight into their ultimate origins. Some, such as genes expressing resistance to fluoroquinolones and those expressing resistance in mycobacteria, can be seen to have arisen as single or multiple mutations in chromosomal genes carried by the resistant strain's susceptible ancestors. Most, however, are encoded not on the strain's chromosome but on extrachromosomal genetic elements called plasmids, many of which also express products that transfer the plasmid from one strain to another. These plasmid-encoded resistance genes are often unlike any other genes in the strain.

The processes by which new resistance genes emerge are largely unobserved, but can in a general way be imagined. A widely used new antimicrobial agent must eventually encounter somewhere in the world's diverse microbial populations a strain of bacteria that can withstand slightly higher concentrations of the agent than can the strains with which it competes in its niche *(3)*. For the periods of time in which that niche is exposed to such concentrations, that strain will overgrow to replace its inhibited competitors. As the strain's numbers are thus amplified, so also are the chances of a mutational event allowing one of them to tolerate still higher concentrations of the agent to evolve a more effective resistance mechanism.

Spread of an Emerged Resistance Gene

If the new resistance emerged on the chromosome of its strain of origin, it could spread in the clone of its descendants but would tend to be confined to the niche or niches for which that strain was fit. If it emerged on a mobile genetic element such as a plasmid, however, or if it had become mobilized onto one, it could be transferred to other strains and species within that plasmid's host range and so come to occupy additionally the niches for which those strains and species were fit. If the resistance gene were encoded within a functioning transposon inserted in the plasmid, it could be transposed to a different plasmid and so gain access to the new plasmid's host range of strains and species. Yet another level of mobility would be available if the resistance gene were, as they often are, in a cassette of an integron and so able to be excised singly from that integron and inserted into another of another strain or species *(4)*.

These multiple mechanisms of genetic mobility have led to the evolution of complex genetic constructs encoding multiple closely linked resistance genes. Once they begin to acquire resistance genes, moreover, their further evolution is driven by selection owing to the wide use of any of the antimicrobials, because such selection amplifies not just the resistance genes selected for but also, of course, the genetic constructs that carry them *(5)*. In time, under selection by antimicrobials, such evolving genetic constructs come to compete with one another. From such competition under selection, the genetic constructs and strains that evolve to be most efficient, carry the most useful complement of resistance genes, cost the least to the hosts that carry them, and so on, would be expected to prevail *(6)*, and the history of the spread of different types of resistance seems to provide examples of this.

Historical Examples of the Spread of Resistance

Within the first decade of the use of penicillin, penicillinase-producing strains of *Staphylococcus aureus* resistant to penicillin emerged and spread widely, at first through hospitals and later through the broader community. Studies at the time indicated that most of the spread throughout the whole world was due to the spread of a small number of phage types that appeared to have evolved to a high level of contagion *(7)*. Similarly, decades later, the spread of methicillin-resistant *S. aureus* appeared, owing, in most parts of the world, to a small number of clones with intricately evolved resistance constructs *(8)*.

The first strains of genera of *Enterobacteriaceae* found to be resistant to gentamicin at eight hospitals in widely separated U.S. cities and in a hospital in Venezuela, and probably also in numerous other unsurveyed hospitals, had been made resistant by acquisition of the same epidemic plasmid, pLST 1000, spreading between them in the late 1970s *(9)*. The first extended-spectrum-lactamase to produce resistance to a third-generation cephalosporin in one of those hospitals a decade later arose from a point mutation within the same plasmid. When a variant of that plasmid was later found also to express resistance to amikacin, which it had never done earlier, that resistance was found to be encoded on a 3.2-kb segment of DNA inserted in the plasmid. The nucleotide sequence of this segment proved to be identical to that of a transposon described earlier in isolates from Chile and Argentina.

Each of three different serotypes of *Salmonella* resistant to multiple antimicrobials isolated from multiple patients in Massachusetts, California, and Wisconsin was found to carry a plasmid that was also present in isolates of the corresponding serotype with similar resistance isolated from large numbers of farm animals in other states *(10)*.

The increase in the prevalence of penicillin-resistant *Streptococcus pneumoniae* observed in many parts of the world in recent decades appears to be largely accounted for by the acquisition of penicillin-binding proteins imported by transformation from other species of bacteria into a small number of strains of *S. pneumoniae* of certain serotypes. These relatively few clones, now made resistant by their newly acquired genetic complexes, spread widely under antimicrobial selection throughout the world *(11)*. A single clone imported into Iceland, for example, came to account for all of the increasing number of penicillin-resistant *S. pneumoniae* seen there *(12)*.

Global Commerce in Epidemic Antimicrobial Resistance Elements

The foregoing examples illustrate an important aspect of the increase in antimicrobial resistance over the past six decades, from a period when bacteria isolated from patients had almost no antimicrobial resistance genes to the present time, when such isolates are known to carry hundreds of different resistance genes. One component of this change may be the result of the local emergence and spread of resistant strains, e.g., in one community, one hospital, or even one human or animal host. What has become increasingly apparent, however, is the predominant role, particularly in the most troublesome types of resistance, of a small number of multiresistant genetic complexes. These complexes, as a group or individually, are epidemic across strains and species, and have evolved and spread widely under selection to prevail in many parts of the world.

In terms of commerce, an analogy might be a hypothetical city that is completely isolated from the rest of the world, which would generate its own resistance problems to some degree as a consequence of its own antimicrobial use. These problems, however, would probably be far less severe than if it had not been isolated, in which case it would have imported and distributed many of the intricate genetic resistance complexes and resistant strains that have evolved under selection by the worldwide use of antimicrobials. Similarly, if the city had been completely isolated from the rest of the world's commerce, it would have had some type of economy but not the economy it would have had with the importation and exchange of products invented and manufactured all over the world.

For these reasons, we can expect that the levels and types of resistance observed in any one place—a country, city, community, hospital, intensive care unit, and so on—reflect in part the amount of selection via antimicrobial use in that place. Additionally, we can expect that they also reflect the total amount of selection by the whole world's use of antimicrobials. That is, the use of an antimicrobial anywhere in the world may eventually lead to increased resistance to that or any other antimicrobial anywhere else.

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RESISTANCE IN *SALMONELLA* NEWPORT

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An estimated 1.4 million human cases of salmonellosis occur in the United States each year. In 2001, *Salmonella* Newport was the third most common laboratory-confirmed *Salmonella* serotype reported to the Centers for Disease Control and Prevention. In the past 5 years, the proportion of reported serotyped, nontyphoidal *Salmonella* infections from serotype Newport doubled, from 5% in 1997 to 10% in 2001. The increasing number of *S.* Newport infections in the United States appears to be associated with the emergence and rapid dissemination of highly antimicrobial-resistant strains of *S.* Newport. Since 1996, the National Antimicrobial Resistance Monitoring System has identified an increasing number of *S.* Newport isolates that are multidrug resistant. Of particular concern is the increasing frequency of isolates resistant to at least nine of 17 antimicrobials tested, including ampicillin, chloramphenicol, sulfamethoxazole, streptomycin, tetracycline (ACSSuT), amoxicillin/clavulanate, cephalothin, cefoxitin, and ceftiofur (a veterinary third-generation cephalosporin). These highly resistant isolates also exhibit decreased susceptibility to ceftriaxone, an antimicrobial important in the treatment of severe human salmonellosis, particularly in children.

Concurrent with the increase of antimicrobial-resistant *S.* Newport observed in human *Salmonella* surveillance is an apparent increase in multidrug-resistant *S.* Newport isolated from ill animals and from ground beef samples. Veterinary diagnostic laboratories in the Northeast, the Midwest, and the Western regions of the United States have identified outbreaks of animal illnesses, particularly in dairy cattle. Recent public health investigations indicate that the bovine reservoir is an important source for human infections, with vehicles such as undercooked ground beef, soft cheese made from unpasteurized milk, and direct contact with cattle being implicated.

The history of antimicrobial-resistant *S.* Newport in the United States will be discussed, along with what is currently known about the emergence of highly resistant strains. The full details of *S.* Newport are detailed in the published article *(1)*.

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MULTIPLE DRUG RESISTANCE: TRENDS AND IMPLICATIONS

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Antimicrobial resistance has emerged as a global problem. Although it occurs shortly after the introduction and use of an antimicrobial, resistance levels vary over time. Historically, antimicrobials were regarded as wonder drugs, and for years resistance to a single antimicrobial was overcome by the use of newer, more effective antimicrobials. However, drug development has slowed and multiple antimicrobial resistance (MAR) has developed. MAR has become a serious concern in the animal and human health communities because it compromises treatment and impacts outcome, potentially leading to increased morbidity and mortality.

An overview of MAR will be presented, including what we know about the development of MAR, what we think we know, and what we do not know (but need to). MAR as observed in data from the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS) will also be presented. NARMS tracks the emergence of antimicrobial resistance in *Salmonella*, *Campylobacter*, *Escherichia coli*, and enterococci. Since the inception of NARMS in 1996, more than 30,000 isolates originating from animals or the production environment have been tested for antimicrobial resistance, and MAR analysis was conducted.

To summarize, MAR has emerged in foodborne and commensal bacteria. Many factors, including (but not limited to) serotype, species, resistance to compounds other than antimicrobials, and movement of mobile genetic elements, influence the development of MAR.

ANTIBIOTIC RESISTANCE TRENDS IN EUROPE

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The most important organisms causing food poisoning in European countries are *Salmonella enterica* and *Campylobacter* spp. If meaningful comparison of resistance patterns among both laboratories and countries is to be achieved, there must be international surveillance of antimicrobial resistance for salmonellas. Within Europe this has been achieved for salmonellas by the adoption of harmonized methods by the 18 European national human *Salmonella* reference laboratories, following the distribution of a panel of 48 strains ranging from fully drug sensitive to resistant to up to 10 antimicrobials *(1)*. As a result, it is now possible to compare drug resistance results for salmonellas for all countries within the European Union.

In 2001 the harmonized results of sensitivity tests for more than 25,000 salmonellas isolated from cases of human salmonellosis in the European Union were transferred electronically to the Enter-net surveillance hub at the Public Health Laboratory Service Communicable Disease Surveillance Centre at Colindale, London *(2)*. The findings demonstrated that almost 60% of isolates were resistant to at least one antimicrobial, with more than 17% multiresistant (to four or more antimicrobials). Resistance to ampicillin, sulfonamides, streptomycin, and tetracyclines was common, with more than 20% of isolates resistant to at least one of these antimicrobials. For individual serotypes, both resistance and multiple resistance were most common in *S. enterica* serotype Typhimurium. More than 75% of *S.* Typhimurium isolates were resistant to at least one antimicrobial, and 55% were multiresistant (to four or more antimicrobials), with resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines predominating (> 40% of isolates). An important factor has been the dissemination throughout Europe of a multiresistant clone of definitive phage type (DT) 104, with resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (ACSSuT). An increasing number of isolates are additionally resistant to trimethoprim and/or have decreased susceptibility to ciprofloxacin *(3)*. In these strains, decreased susceptibility to ciprofloxacin has been demonstrated to result from one of several mutations in *gyrA (4, 5)*.

Drug resistance was also found to be common in several other serotypes, although not to the same extent as in *S.* Typhimurium. There were, however, considerable regional differences in the predominance of certain drug-resistant serotypes. For example, both resistance and multiple resistance were common in strains of *S.* Virchow and *S.* Hadar isolated from humans in England and Wales, where more than 40% of isolates were multiresistant, whereas in other countries resistance was common in such serotypes as *S.* Bovis-morbificans, *S.* Heidelberg, and *S.* Bareilly. In Greece, multiresistant strains of *S.* Blockley have caused outbreaks of infection since the late 1990s *(6)*. Resistance to high levels of ciprofloxacin was uncommon, with only 0.5% of isolates exhibiting resistance to ciprofloxacin at >1 mg/l. However, decreased susceptibility to ciprofloxacin was more common, with 10% of isolates resistant at 0.125–0.5 mg/l. Such decreased susceptibility was particularly common in strains of *S.* Blockley, *S.* Hadar, *S.* Virchow, *S.* Enteritidis, and *S.* Typhimurium. For *S.* Enteritidis, 14% of isolates showed such resistance *(2)*. Most of these strains were associated with countries in Southern Europe. More than 50% of isolates of *S.* Hadar exhibited decreased susceptibility, and 45% of *S.* Virchow isolates were resistant at 0.125 mg/l. This is of particular concern because of the invasive potential of *S.* Virchow and because ciprofloxacin is the drug of choice in such circumstances. Resistance to third-generation cephalosporins was rare, with only 0.4% of isolates showing such resistance. In almost all cases, such resistance was observed in strains that had probably originated in countries outside the European Union.

An important feature of the development of multiple resistance in *S.* Typhimurium is the international spread not only of multiresistant *S.* Typhimurium DT104 but also of other phage types. In 2000, a strain of *S.* Typhimurium DT204b with resistance to ampicillin, chloramphenicol, gentamicin, kanamycin, streptomycin, sulfonamides, tetracyclines, and trimethoprim, which also exhibited decreased susceptibility to ciprofloxacin, caused extensive outbreaks in five European countries *(7)*. In this instance the epidemiologic investigations implicated contaminated lettuce as the vehicle of infection *(8)*. This outbreak of *S.* Typhimurium DT204b coincided with another large outbreak of multiresistant *S.* Typhimurium DT104 in England and Wales, again associated with contaminated lettuce *(9)*. Other multiresistant strains of epidemiologic importance include a strain of *S.* Typhimurium 4,5,12:i:-, which reacts with the *S.* Typhimurium typing phages to give a phage type that has been designated U302 *(10)*. This strain originated in Spain, where it has been responsible for numerous infections associated with pork products *(11)*, and also caused an outbreak in Denmark associated with pork products originating in Spain (M. Skov, personal communication, 2002.

For campylobacters, there has not yet been agreement on an international approach for susceptibility testing. Within the European Union, however, there have been several reports of an increase in the proportion of strains with resistance to fluoroquinolones. Such increases have been observed in Denmark, the United Kingdom, Finland, France, Austria, the Netherlands, Greece, Italy, and Spain. Up to 30% of *Campylobacter jejuni* isolates in some countries now show high-level resistance to ciprofloxacin, and more than 80% of isolates in Spain now exhibit such resistance *(12)*.

Both *S. enterica* and *Campylobacter* spp. are primarily zoonotic in origin, with the principal food animal hosts being cattle, poultry, and pigs for *S. enterica*, poultry for *C. jejuni*, and pigs for *C. coli*. There are important differences in the food animal reservoirs for different serovars of *S. enterica*, with *S.* Enteritidis, *S.* Virchow, and *S.* Hadar associated predominantly with poultry. By comparison, *S.* Typhimurium, which is found in cattle, pigs, and poultry, is ubiquitous.

It is now widely accepted that the use of antimicrobials in food animals has played an important role both in the acquisition of resistance by *S. enterica* and *Campylobacter* spp. and in the subsequent establishment of resistant strains in the food chain. For example, there is a strong temporal association between the development of resistance to ciprofloxacin in *C. jejuni* and the introduction of fluoroquinolone-containing products for use in poultry *(12)*. Similarly, *S.* Typhimurium DT104 in England and Wales coincided with the decreased susceptibility to ciprofloxacin that emerged subsequent to the licensing of fluoroquinolone for use in food animals in the United Kingdom in late 1993 *(13)*.

For other *Salmonella* serovars, the situation is more complex. Recent collaborative investigations between the Public Health Laboratory Service and the Veterinary Laboratories Agency have demonstrated that poultry reared in England and Wales may not be the source of strains of *S.* Virchow and *S.* Hadar with

decreased susceptibility to ciprofloxacin from cases of human infection in the United Kingdom *(14)*. It is therefore essential that controls targeted at reducing the incidence of drug resistance in zoonotic pathogens be adopted on a worldwide basis, and not just in countries in the European Union and North America.

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PERSPECTIVES IN ADDRESSING THE SAFETY OF CEPHALOSPORIN USE IN FOOD ANIMAL MEDICINE

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The occurrence in the United States of nontyphoidal salmonellas that are resistant to late-generation cephalosporins raises complex challenges in identifying effective strategies to mitigate their expansion and dissemination. These isolates are resistant to a number of ß-lactams, and the vast majority of them are resistant to other antibiotic classes as well. It is unclear which practices affect the dissemination of these organisms. The problem is further compounded by the complex nature of *Salmonella* epidemiology and its relationship to animal management and distribution practices (independent of antimicrobial use practices), as well as *Salmonella* transmission and dissemination in human and animal environments and their interface. The following overview of cephalosporin use in animal medicine highlights relevant characteristics in safety evaluation and the research needed to better understand the implications of antimicrobial use in resistance emergence.

Cephalosporin Use in Food Animals

Globally, there are intramammary formulations of five first-generation cephalosporins (cefazolin, cephalexin, cephalothin, cephalonium, and cephapirin) and one second-generation cephalosporin (cefuroxime) to treat mastitis in cattle. Parenteral formulations of a third-generation (ceftiofur) and a fourth-generation (cefquinome) cephalosporin, developed solely for animals, have been approved for the treatment of respiratory infections in cattle and swine. Ceftiofur also has been approved for metritis and foot rot in cattle, respiratory disease in sheep and goats, and *Escherichia coli* infections that cause early mortality in 1-to-2-day-old poultry. Cefquinome also has been approved for the treatment of metritis, mastitis, and septicemia from *E. coli* infections in cattle.

Approvals for use vary among countries. Cephapirin and ceftiofur are the only cephalosporins approved for use in food animals in the United States, although veterinarians may prescribe other cephalosporins under the Animal Medicinal Drug Use Clarification Act. Because the economics of food animal production drives producer decisions to remove animals from the production site if an animal's illness is not rapidly resolved, it is not surprising that drug sponsors have worked to develop efficacious cephalosporin treatment regimens (required for drug approval) that produce a cure in 5 days or less for these indications. Food animals are slaughtered within a few months to years of birth, limiting each animal's potential lifetime exposure to cephalosporins.

Late-Generation Cephalosporin Resistance in Target-Animal Pathogens

The pharmacokinetic and pharmacodynamic profiles of ceftiofur support its efficacy for label pathogens *(1–5)*. The prevalence of resistance to ceftiofur, which was first marketed in 1988 in the United States, has remained very low for label pathogens. Among diagnostic isolates of the bovine respiratory pathogens *Pasteurella multocida*, *Mannheimia haemolytica*, and *Haemophilus somnus* and the swine pathogens *P. multocida*, *Actinobacillus pleuropneumoniae*, and *Salmonella choleraesuis*, > 98% of the ceftiofur minimal inhibitory concentration (MIC) have remained below the breakpoints for susceptibility established by the National Committee for Clinical Laboratory Stan-

dards (NCCLS) *(6–8)*. NCCLS breakpoints have not been established for *E. coli* isolated from poultry, and there is little published information on resistance in *E. coli* pathogen for poultry. High ceftiofur MICs were detected in *E. coli* pathogens from turkeys before ceftiofur was approved for use in turkeys *(10)*.

Cephalosporin Resistance in *Salmonella* Associated with Animals

Among *Salmonella* isolated from people in the United States, cephalosporin resistance was rarely encountered before 1996 *(9, 11)*. Few data are available to assess prevalence in animal isolates before 1996. Prevalence varies in recent surveys for cephalosporin-resistant salmonellas isolated from animals, including cats, dogs, horses, poultry, cattle, and swine *(8,12–21)*. The proportion of isolates resistant to third-generation cephalosporins generally ranges from 0% to 5% of all *Salmonella* isolates, depending on the survey time period, survey size, sampling methods, animal species, geographic area, and method used to detect and score cephalosporin resistance.

Among the cephalosporin-resistant isolates characterized to date in the United States, most produce a cephalomycinase (CMY) encoded by variants of the bla_{cmy} gene, frequently located on a plasmid that encodes resistance to other classes of antimicrobials *(22–26)*. Like other types of broad-spectrum ß-lactamases reported worldwide in salmonellas from various epidemiologic sources *(27–36)*, most CMYs are found in *Salmonella* isolates that are multiresistant to other antibiotic classes, including various combinations of chloramphenicol, streptomycin, sulfonamides, tetracycline, aminoglycosides, and trimethoprim/sulfonamides. CMY expression alone results in a significant increase in MICs for, and in many cases resistance to, penicillins, monobactams, and some ß-lactamase inhibitor combinations, as well as first-, second-, and third-generation cephalosporins. The gene has been identified in different serotypes, strains of serotypes, and various plasmid backbones *(22)*, suggesting that the gene is transferred horizontally. The appearance of genetically related serotypes of *Salmonella* with similar antibiograms in geographically diverse and segmented areas may be due to animal movement (i.e., transport) and commingling and/or the result of antibiotic use in each locale.

Supportive therapy, *not* antimicrobial use, is routinely recommended for nonsystemic salmonellosis in food animals. Depending on the drug, the route of administration, and the age and immune status of the animal, antimicrobials may or may not alter shedding or the course of disease. In the case of systemic disease, there are no approved label indications for any antimicrobials for treatment. Thus, any treatment of salmonellosis in food animals is necessarily extralabel in the United States. More research and strengthened local surveillance programs are needed to better understand the implications of extralabel uses and to produce alternative mitigation strategies on *Salmonella* prevalence and resistance in herds and individuals.

Mitigation Considerations

Many factors must be considered in identifying effective mitigation strategies for multiresistant salmonellas: antibiotic exposure appropriate antimicrobial use to support effective herd health management practices, animal distribution, and slaughter practices (including holding pens or lairage) that affect *Salmonella* expansion and dissemination among animals.

Bacterial Exposure to Cephalosporins and Safety

Although it is generally accepted that many ß-lactams are relatively unstable compared with other antibiotic classes *(37–39)*, there is little specific information regarding their degradation and ultimate fate. The instability of ceftiofur has been examined *(40–43)*. Upon administration, ceftiofur is metabolized to desfuroylceftiofur metabolites, which retain an intact ß-lactam ring and microbiological activity. Ceftiofur residues excreted in feces represent a minor percentage of the total dose, and concentrations of active residues in feces are below detection limits of microbiological assays (0.2 g/g). Microbiologically active residues in urine comprise a minor percentage of the total residue. They are degraded in urine and are degraded more rapidly in mixtures of urine and feces. Furthermore, ceftiofur is degraded to inactive metabolites within minutes of addition to anaerobic incubations of minimally diluted fecal specimens collected from humans. When added to soil samples, ceftiofur is inactivated within hours without the accumulation of metabolites, and is metabolized to carbon dioxide within weeks. Thus, the potential for animal and environmental bacteria exposure to ceftiofur, microbiologically active metabolites, and degradates is low with label use practices. The instability demonstrated for this molecule may be important generally for ß-lactams with respect to environmental considerations and appropriate use practices.

Mitigation Through Animal Husbandry and Production Practices

Risk factors for salmonellosis in dairy cattle (and other animal species) include introduction of infected animals into existing herds, animal grouping and housing, contaminated feed and water, and transfer of pathogens by movement of vehicles, people, rodents, birds, and other animals. Transport and holding pose an off-farm, preharvest risk for *Salmonella* infection *(44, 45)*. A recent study *(46)* indicated that *Salmonella* infection can occur rapidly during transport and preslaughter holding at the slaughterhouse (compared with on-farm slaughter), and is a major factor in the prevalence of *Salmonella* shedding in swine. Furthermore, the identification of additional serotypes in swine at the slaughterhouse indicate that these serotypes are probably acquired after the animals leave the production site.

Mitigating the exposure of cows and calves to *Salmonella* through environmental control, monitoring, and isolation is aimed at breaking fecal-oral transmission, particularly in the more susceptible populations (e.g., late-gestation and fresh cows, newborn calves), including segregating them from more resistant, sick, and/or newly introduced livestock (e.g., older calves, replacement animals, cows in the sick pen, etc.). Good hygiene practices at calving, including cleaning the perineum and udder of cows at delivery and harvesting clean colostrum, are critical. Pasteurization of milk replacer reduces bacteria from a source that can serve as a multiplier for bacterial dissemination to many calves. However, more information is needed regarding the use of antimicrobials in milk replacers and their implications on selection of cephalosporin-resistant salmonellas. Furthermore, the management of site personnel and waste and effluent management are important. Equipment should be segregated such that "dirty" equipment is not used in "clean" areas, and people are moved from clean to dirty areas, and not vice versa. When people enter clean areas, their movement must be preceded by thorough disinfection and change into clean work clothes. Movement of farm visitors should be strictly controlled. Waste removal and runoff should always be one way, and should be directed away from the youngest and most susceptible animals. Finally, because the degree of fecal-oral contact is proportional to stocking density, reducing stocking density can mitigate exposure.

CONCLUSIONS

Salmonellas are endemic to food animal populations worldwide, and their epidemiology is complex. Expansion and dissemination of salmonellas, whether multiresistant or

pansusceptible, can occur by routes independent, dependent, or codependent on antimicrobial use. As a generalized approach to curtailing further expansion of multiresistant organisms, we must continue to support and strengthen local, regional, and national disease and antimicrobial resistance surveillance, as well as strengthen herd health management, biosecurity, and appropriate and judicious antimicrobial use programs. There is a clear research need for studies that examine antimicrobial extralabel and label use practices for their impact on *Salmonella* shedding, disease outcome, and antimicrobial resistance reservoirs, not only in terms of the local environment of the animal at the time of drug administration but also in a broader, integrated context of current animal herd management, production, transport, distribution, and slaughter practices.

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CONSEQUENCES OF THE REMOVAL OF SUBTHERAPEUTIC ANTIBIOTICS FROM DANISH FARMS

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The Danish Minister of Food, Agriculture and Fisheries banned the antimicrobial growth promoters (AGPs) avoparcin and virginiamycin in May 1995 and January 1998, respectively. The Danish food animal industry subsequently voluntarily discontinued the use of all AGPs. Beginning in March 1998, AGPs were withdrawn from broilers and cattle and from pigs heavier than 35 kg (finisher pigs); the use of AGPs in pigs below 35 kg (growing pigs) was phased out during the last half of 1999. In 1997, avoparcin was banned in the European Union. This was followed in 1999 by a temporary suspension in the European Union of four more AGPs—tylosin, spiramycin, bacitracin, and virginiamycin. A proposal issued by the European Commission in 2002 calls for phasing out the use of all AGPs by 2006. This presentation explores the effects thus far of the discontinued use of AGPs on food animal production and antimicrobial resistance.

The Effects of AGP Withdrawal on Broiler and Pig Production

Broiler and pig producers and processors were concerned that the discontinued use of AGPs would result in decreased growth rates and increased feed conversion ratios. There also were concerns that mortality, morbidity, and the number of *Salmonella*-infected flocks would increase as would the use of antimicrobials for disease treatment.

The Danish Poultry Council has collected productivity data since 1975. For the current analysis, data from November 1995 to July 1999 on broiler productivity in 6,815 flocks before and after AGP withdrawal (approximately 2 years before and 1.5 years after withdrawal) were examined for three parameters: (1) kilograms of broilers produced per square meter, (2) feed conversion ratio (i.e., grams of feed needed to produce 1 kg broiler), and (3) animal mortality. After AGP withdrawal, production held steady except for the feed conversion ratio that increased marginally by 16 g to produce 1 kg broiler. Veterinarians have also reported that AGP withdrawal appears not to have resulted in increased consumption of therapeutic antimicrobials.

The National Committee for Pigs maintains productivity data in a representative subsample of Danish herds. For the present study, data from October 1994 to 2001 were used to compare pig productivity before and after AGP withdrawal. Although a temporary decrease in daily weight gain in grower and finisher pigs was observed shortly after AGP withdrawal, data from October 1999 to October 2001 show no difference in daily weight gain compared with data from before AGP withdrawal. The feed conversion ratio for grower and finisher pigs also did not differ significantly before and after AGP withdrawal. Among weaning pigs, however, one-third of the pig herds had problems with postweaning diarrhea 1 to 3 months after AGP withdrawal, and the problem persisted beyond 3 months for 10% of the herds. This probably accounts for the decrease in daily weight gain, accompanied by increased mortality, observed in weaning pigs shortly after AGP withdrawal. Data from October 1999 to October 2001, however, show that average daily weight gain and postweaning mortality in young pigs are approaching the levels observed before AGP withdrawal.

Veterinarians representing pig producers believe that the postweaning diarrheal problem was temporary owing to insta-

bility of the gut flora, and they expect that the situation will be remedied with improved feed and management measures. No increase in the consumer prices of broilers and pork resulted from AGP withdrawal.

It was anticipated that there would be an overall increase in the consumption of therapeutic antimicrobials in meat animals to compensate for the prophylactic effect of AGPs. A comparison of data on total consumption of AGPs and therapeutic microbials in Denmark from 1990 to 2001, however, found that therapeutic consumption was constant from 1997 to 1999, although it did increase from 1999 to 2001, mainly because of the need to treat postweaning diarrhea in pigs. Month-to-month comparisons of 2001 data on tetracycline and macrolide consumption suggest that the increase in therapeutic use has likely been reversed. Overall, the consumption of antimicrobials in Danish food animals has dropped by more than 50% between 1994 and 2001.

The Impact of AGP Withdrawal on Antimicrobial Resistance

The Danish Integrated Antimicrobial Resistance Monitoring and Research Program (DANMAP) was initiated in 1995 to monitor antimicrobial resistance in bacteria of animal, food, and human origin and to monitor the use of antimicrobials both as growth promoters and as therapeutics. DANMAP also monitors the impact of AGP withdrawal on antimicrobial resistance.

A comparison of DANMAP data from 1994 to 2001 found that decreased consumption of the AGP avoparcin was followed by decreased resistance to avoparcin by *Enterococcus faecium* in broilers and broiler meat. The impact of microbial resistance to the antimicrobials tylosin and spiramycin used in animals and to erythromycin, an antimicrobial used to treat humans, is of great interest because of the cross-resistance observed in the three antimicrobials. A comparison of DANMAP data from 1994 to 2001 found that decreased use of tylosin and spiramycin in pigs was followed by a decrease in erythromycin resistance in *E. faecium* in live pigs. However, resistance to erythromycin in *E. faecium* from pork meat was found to be more variable, with no apparent clear trend discerned. A comparison of data on virginiamycin consumption and resistance to the antimicrobial by *E. faecium* in live broilers, broiler meat, live pigs, and pork from 1994 to 2001 found a correlation between decreased virginiamycin consumption and decreased resistance by *E. faecium* in live broilers, broiler meat, and live pigs. However, again, the resistance trend in pork meat was variable. Most broiler meat products sold in Denmark are packed at the processing plant, with no further handling before the product reaches consumers. There is, however, further handling of pork meat in retail outlets. Because enterococci can survive in the environment, it is likely that the population of *E. faecium* in pork meat only partly reflects its population in live pigs.

Salmonella in Broiler Flocks and Finisher Pigs

Another concern regarding AGP withdrawal was that the number of *Salmonella* infected broiler flocks would increase due to a possible instability of the gut micro flora. A comparison of data from 1989 to 2001, however, showed that after the AGP withdrawal a further decrease in the number of *Salmonella* infected broiler flocks was observed. There also was a reduction in *Salmonella* prevalence in broiler meat.

Analysis of data from 1995 to 2001 showed that the level of *Salmonella* in Danish pig herds has decreased further after AGP withdrawal. The *Salmonella* prevalence in pork was unchanged before and after the withdrawal.

CONCLUSION

The withdrawal of antimicrobial growth promoters in Denmark has had no or only minor effects on growth rate, feed conversion ratio, morbidity, and mortality in broiler chickens and in growing and finisher pigs. Problems with postweaning diarrhea, as observed in one-third of the pig herds 1 to 3 months and for 10% of the herds beyond 3 months after AGP withdrawal, will likely be remedied by improved feed and management practices. AGP withdrawal did not increase consumer prices, and exports of broiler meat and pork were unaffected. The level of *Salmonella* in Danish pig herds has decreased further, although the level in pork meat has remained unaffected since AGP withdrawal. A moderate increase in the consumption of therapeutic antimicrobials was observed.

It should also be noted that studies from Germany, the Netherlands, and Belgium indicate that after the prohibition of avoparcin as an AGP in those countries, the occurrence of vancomycin-resistant enterococci has decreased in healthy and hospitalized people.

LISTERIA RESEARCH UPDATE

USE OF SEQUENCE TYPING FOR CHARACTERIZATION OF VIRULENCE FACTORS AND FOR THE DEVELOPMENT OF A NOVEL MOLECULAR TYPING SCHEME FOR *LISTERIA MONOCYTOGENES*

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In the food industry, typing methods are often used in conjunction with epidemiologic information to monitor potential foodborne outbreaks *(1, 2)*. The need for effective methods to identify bacterial strains and track their spread is critical to the control and possible prevention of major outbreaks. Current typing methods focus on intraspecies genetic variation to determine the degree of genetic strain relatedness to identify the individual bacterial agent responsible for an outbreak *(3)*. To accurately determine the source of the outbreak, it is essential to use methods with high discriminatory potential *(3)*. For obvious public health and legal reasons, it is imperative to accurately identify and "match" the bacterial strain isolated from the food or environment to the strain isolated from the patient *(1, 2)*. A variety of sophisticated typing methods exist, each with advantages and disadvantages. Current methodologies are classified as either phenotypic (biotyping, antimicrobial susceptibility testing, serotyping, and bacteriophage typing) or genotypic (plasmid profile analysis, multilocus enzyme electrophoresis [or MLEE, which is closer to genotypic than to phenotypic methodology as genetic changes result in protein size shifts), restriction endonuclease analysis, ribotyping, pulsed-field gel electrophoresis [PFGE], polymerase chain reaction [PCR], PCR restriction digestion, random amplified polymorphic DNA, and nucleotide sequence analysis). Currently, serotyping *(4)* and PFGE are the most common methods used in listeriosis outbreak investigations *(2)*.

To clearly demonstrate whether a close genomic relationship exists among outbreak strains, epidemiologic investigations should include some form of molecular typing *(1)*. PFGE is described elsewhere as providing clear and discriminatory fingerprints of *Listeria monocytogenes* strains *(5)*. The PFGE protocol relies on a comparison of DNA fragment patterns on agarose gels; bacterial isolates with the same DNA fingerprint (digestion

pattern) are assumed to be the same strain. This method is considered highly discriminatory, and because all microorganisms can be typed this way, PFGE has become the method of choice in the investigation of many foodborne outbreaks, including those caused by *L. monocytogenes (3, 6)*. Yet, in studies of isolates that belong to the same species, even this method may sometimes lack discriminatory potential. A difference between DNA sequences of two bands of similar size will go unnoticed, as will insertions/deletions.

A nucleotide-based approach, such as multilocus sequence typing, might better overcome the limitations of PFGE. Serotyping has a further disadvantage in that more than 90% of listeriosis outbreaks are attributable to three of the 13 serovars (1/2a, 1/2b, and 4b) *(1)*.

In MLEE, bacterial isolates are characterized by the electrophoretic mobilities of 20 of their housekeeping enzymes *(2)*. Many pathogens have been successfully clustered by this method *(7)*, which has provided valuable information that has greatly contributed to improved understanding of global epidemiology. Results obtained with this method, however, are sometimes hard to compare between laboratories. Multilocus sequence typing (MLST), which classifies bacterial isolates directly from their nucleotide sequence, solves this problem *(8)*.

In the MLST method, internal fragments (approximately 450–500 bp) of generally seven housekeeping genes are amplified and sequenced to determine the allelic profile of each isolate. For each housekeeping gene, each sequence difference found between all of the isolates is assigned a distinct allele. Each isolate is therefore unambiguously characterized by the combination of alleles for the seven housekeeping gene loci [8–10]. Because nucleotide sequences reveal all possible variation that might exist at a locus, MLST, compared with MLEE, allows a greater number of alleles per locus and thus a greater discriminatory potential between isolates than is provided with PFGE *(10)*. Given the fact that MLST is based on sequence data, this method is not only unambiguous but also highly discriminatory. Other advantages include the ease of sequence data exchange between laboratories through the use of the Internet, making MLST a powerful tool for global epidemiology *(8, 10)*.

Housekeeping genes are not subject to unusual selective forces, and therefore diversification occurs slowly by the accumulation of neutral variations. As a result, they are conserved within a species *(11)*. Rapid accumulation of variation within a clone makes it difficult to discern whether its descendants are derived from a common ancestor. Studying long-term epidemiologic questions using housekeeping gene data thus provides more reliable information about the relationship between isolates *(10)*.

However, in short-term epidemiology, when the source of a sporadic case of listeriosis needs to be determined, more variation between isolates may be required to properly identify the outbreak strain. In the event that MLST itself is not sufficiently discriminatory in an outbreak setting, the inclusion of genetic information from virulence genes may provide an appropriate level of discrimination. Surveying the variation seen in virulence genes may also allow a better understanding of the pathogenic potential of the organism.

Objectives

The objectives of this research study were to develop both a traditional MLST scheme using housekeeping genes and a new typing system based on multilocus analysis of virulence genes.

Metholodology

To identify appropriate target genes for *L. monocytogenes* MLST development, potential loci were chosen by searching the GenBank database (http://www.ncbi.nlm.nih.gov/) for ~20 housekeeping genes from both *L. monocytogenes* and *L. innocua*. Genes of the latter were blasted against the genome sequence from *L. monocytogenes* strain EGD-e (http://www.ncbi.nlm.nih.gov) to identify *L. monocytogenes*-specific genes. Potential loci were chosen on the basis of (1) genome location by mapping the gene locations against genome sequence, (2) identification of primers spanning 500–750-bp regions of coding region sequence, and (3) common PCR conditions (i.e, 3 mM, $MgCl_2$, 60°C annealing temperature). The housekeeping genes that provided appropriate coverage of the *L. monocytogenes* genome are listed in Table 1.

To test PCR conditions, single-copy amplification, and feasibility of the typing scheme, a small subset of isolates ($n = 29$) were chosen for the initial screening. Nucleotide sequence was obtained by PCR amplification of coding regions from eight genes. Cycle sequencing of each purified product (10–20 ng) was performed, using the same forward (P1) and reverse (P2) primer in 10-L reaction volumes. Sequencing reactions were resolved by capillary electrophoresis. Data were imported into BioNumerics version 2.5 (Applied Maths, Kortrijk, Belgium), where sequence alignment and editing were performed. Allele sequence types were identified from 450–550 bp of edited sequence for the seven different loci identified for each strain. UPGMA (unweighted pair group method average) analysis of categorical information based on the seven different allele sequence types for each isolate was performed.

For each isolate, a single colony grown on a Tryptose agar plate was suspended in 50 µL TE (Tris HCl, 10 mM, pH 8, EDTA 1 mM, pH 8). The cell suspension was heated at 100°C for 20 minutes. The GenBank database was searched for all available *L. monocytogenes* virulence and traditional housekeeping gene sequences. The genes chosen for this study are listed in Table 1. Forward and reverse 20-mer primers were designed to amplify the most variable region (approximately 450–500 bp) of each gene. PCR reactions were optimized for each gene.

Data Analysis

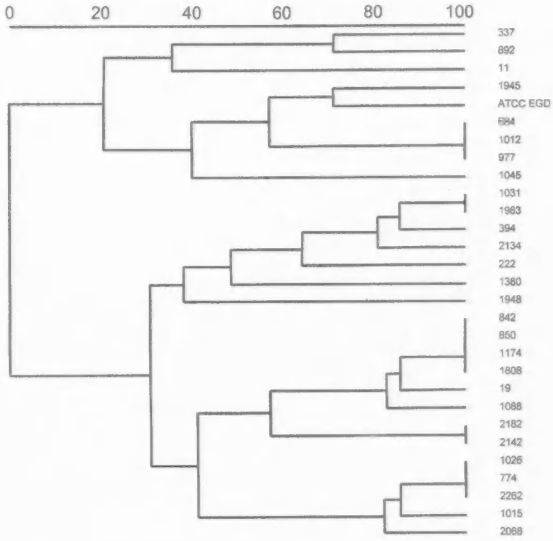
For each isolate and each gene, a consensus sequence was determined using the Genetics Computer Group (GCG) sequence analysis package version 10.1 (Madison, WI). Searches for open reading frames and similarity were performed by use of GenBank release 127.0, EMBL release 69.0, PIR-protein release 71.0, and SWISS-PROT release 40.7. The consensus sequence was entered in the BioNumerics software package (Applied Maths, Austin, TX). Based on the consensus sequence of each virulence gene, *L. monocytogenes* isolates were clustered by the UPGMA technique, and dendrograms were constructed. To assess the overall similarity between isolates, a composite dendrogram was constructed, using the nucleotide sequences of all virulence genes for all isolates. Categorical clustering analysis for the virulence genes was also done to compare dendrograms generated and to see which of the methods would be more discriminatory. The DNA sequences obtained for the housekeeping genes were analyzed according to established MLST protocols *(8, 12)*.

RESULTS AND DISCUSSION

The subset of isolates was initially chosen from a variety of sources, including veterinary, clinical, food, and common reference strains. To ensure serodiversity, 1/2(a,b,c), 3b, 4(b,bx,d,e), and 7 were included. The dates of isolation spanned 28 years (1971–1999) and included geographic representation from Canada, Denmark, France, Italy, Peru, Scotland, Sweden, Switzerland, Trinidad, the United Kingdom, and the United States.

Preliminary analysis of the subset of isolates chosen for housekeeping gene amplification allowed for the generation of 20 unique sequence types, based on the seven alleles chosen at the time of analysis (the housekeeping gene *caa* was not used). The numbers of sequence types for each allele obtained were 5 (*abs*), 7 (*ptsI*), 9 (*lisK*), 7 (*lhkA*), 6 (*dhk*), 11 (*lapC*), and 7 (*abcZ*). Allelic variation in the seven housekeeping genes was observed and ranged from 11 (*lhkA*) to 26 (*lapC*). A dendrogram of the MLST scheme is presented in Fig. 1.

FIGURE 1. Dendrogram (categorical, UPGMA) showing the genetic relatedness among the sequence types of the *L. monocytogenes* diversity set. The percentage similarity (categorical coefficient) scale is indicated above the dendrogram



Categorical clustering (MLST analysis) of the housekeeping genes allowed the grouping of certain isolates into similar sequence types. Although not related epidemiologically, isolates 842 (4b, clinical), 850 (4b, clinical), 1174 (4b, food), and 1808 (4b, food) were grouped together. This cluster could be further divided using the virulence genes *sod*, *hlyA*, and *iap*. The *iap* gene separated isolate 842 from the others; *hlyA* grouped 1174 and 1808. Using the *hlyA* gene, strain 850 branched off of the 1174/1808 cluster, with strain 842 having the greatest linkage distance. The *sod* gene grouped 1174, 842, and 850 together.

A second MLST cluster included the unrelated strains 774 (4b, clinical), 1026 (4b, clinical), and 2262 (4b, clinical). The *flaA* gene was able to distinguish isolate 774 from the other two, which formed a cluster because the sequences were identical. When the *sod* gene was used, however, isolates 2262 and 774 clustered together, with 1026 being on a separate branch of the dendrogram.

The third and fourth MLST clusters using housekeeping genes involved the unrelated isolates 1031 (3b, food)/1983 (1/2b, clinical) and 2142 (4b, food)/2182 (4b, clinical), respectively.

Virulence gene data were unable to further distinguish isolates 1031 and 1983, although the *actA* gene was able to separate the 2142 and 2182 clusters based on nucleotide differences.

Virulence data were available for all of the isolates used in the MLST analyses, with one exception. Using nucleotide analyses of the *plcB*, *prfA*, or *flaA* gene allowed for the separation of the b serovars from the others (c, d, and e).

To summarize, a novel method that uses housekeeping genes to refine traditional MLST techniques was initiated and is under development. Multivirulence gene bioinformatic analysis might also be useful in further classifying and typing isolates of *L. monocytogenes*. There has been much interest recently in the division of isolates into various epidemic clones or lineages. Degrees of pathogenicity (i.e., virulence potential), tropism for foods versus humans/animals, and so on, based on assays that include animal experiments (LD_{50}), cell culture invasion and cytopathic studies, ribotyping and PFGE analyses, to name a few, are all labor intensive and are hard to relate to each other. Our studies, using a bioinformatics-based classification system of the building blocks of life (i.e., DNA), may resolve some of the current issues surrounding foodborne listeriosis.

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IDENTIFICATION OF POTENTIALLY UNIQUE GENETIC MARKERS AND VIRULENCE ATTRIBUTES OF EPIDEMIC-ASSOCIATED STRAINS OF *LISTERIA MONOCYTOGENES*

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An important feature of *Listeria monocytogenes* is its implication in outbreaks of foodborne illness (listeriosis). Several studies indicate that a relatively small number of strains, commonly of serotype 4b, appear to have been implicated in most outbreaks of listeriosis that have been characterized (reviewed elsewhere *(1)*). Specifically, numerous outbreaks in Europe and North America have involved a cluster of closely related strains, designated epidemic clone I (ECI). ECI strains were responsible for outbreaks in Nova Scotia (coleslaw, 1981), California (Mexican-style cheese, 1985), France (pork tongue in aspic, 1992), and several other outbreaks. The reasons for the repeated involvement of this clonal lineage in foodborne outbreaks remain unidentified. Recently, the genome of one ECI strain (implicated in the outbreak associated with Mexican-style cheese in California in 1985) was sequenced, with funding from the U.S. Department of Agriculture (see: www.tigr.org), and the analysis of the genome is likely to reveal potentially unique features of this clone's ecology, physiology, and virulence.

In 1998–1999, a previously unrecognized group of serotype 4b strains was implicated in a multistate outbreak of listeriosis traced to contaminated hot dogs *(2, 3)*. The ribotype and pulsed-field gel electrophoresis (PFGE) profiles of these strains were not commonly found among clinical isolates before this outbreak, and the strain complex has been designated epidemic clone II (ECII). Unlike ECI, strains of ECII remain poorly characterized. In this study, we pursued the identification of potentially unique genetic markers and bacteriologic attributes of these strains.

MATERIALS AND METHODS

Bacterial Strains and Growth Media

The cultures analyzed in this study included clinical isolates from the 1998–1999 outbreak associated with hot dogs, as well as isolates from previous outbreaks and sporadic clinical cases, and were obtained from the *L. monocytogenes* culture collection at the Centers for Disease Control and Prevention. The media and conditions for growth of the bacteria are described elsewhere *(4)*.

Polymerase Chain Reaction (PCR) and Southern Blot Technology

Genomic DNA from *L. monocytogenes* was isolated with the DNeasy kit (Qiagen), according to the instructions of the vendor. Restriction endonuclease digestions were routinely done at 37°C overnight. Restriction enzymes were purchased from New England Biolabs and used as suggested by the vendor. Southern blots employed the nonradioactive digoxigenin-based label and detection system (Roche) and were done as described elsewhere *(4)*. PCR analysis employed primers derived from the sequences of DNA fragments found to be specific to strains of serotype 4b *(5)* as well as from serotype 4b–specific sequences identified in our laboratory *(4, 6)*. The thermostable DNA polymerase used in the PCR reactions was X-Taq (Fisher); the PCR conditions are described elsewhere *(4)*.

RESULTS

Identification of a Restriction Fragment Length Polymorphism That Differentiates ECII Strains from Other Strains of Serotype 4b

Basic characterization of the ECII strains included their reactivity with serotype 4b–specific monoclonal antibodies and with the serotype 4b–specific genes *gltA*, *gltB*, and *gtcA*, previously identified and characterized in our laboratory *(4, 6)*. The strains produced results typical of serotype 4b isolates: they reacted normally with the serotype 4b monoclonal antibodies and harbored the serotype 4b-specific genes *gltA*, *gltB*, and *gtcA* (data not shown). However, further characterization of these strains with a probe derived from another genomic region and employed in our laboratory *(7)* was successful in identifying a restriction fragment length polymorphism (RFLP) that appeared to be able to differentiate the ECII isolates from other serotype 4b strains, including strains of ECI. These results suggested that the outbreak strains had a distinct DNA sequence polymorphism in the genomic region probed in these experiments.

Such a polymorphism is useful as a genetic marker, but it does not on its own yield any additional information on potentially unique genetic and physiologic attributes of these strains. Specifically, it would be desirable to identify genes that are uniquely present in these outbreak strains, or genes that have diverged significantly in comparison with their counterparts in other strains of *L. monocytogenes*. Such genes may be especially attractive targets for involvement in virulence or in other attributes that may be of relevance to the epidemic potential of the ECII strains. In addition, such genes would be good candidates for simpler detection and monitoring systems, such as those employing PCR reactions.

Identification of a Serotype-specific Genomic Region Markedly Divergent or Absent in ECII Strains

We characterized the ECII strains further in terms of the presence of DNA sequences that have been described as being specific to strains of ECI or to serotype 4b as a whole. None of the sequences reported as specific to ECI *(5)* were found to be harbored by ECII strains. This included the genes that appear to be involved in methylation of genomic DNA at GATC sites, a feature unique to ECI strains. Such genes are absent from ECII, in agreement with our observation that the DNA of ECII strains lacks such methylation.

Characterization of sequences that had been reported to be present in ECI as well as other serotype 4b–specific strains (i.e., sequences specific to serotype 4b as a whole) *(5)* showed that the majority of these sequences were also shared by the ECII strains, in agreement with the results obtained with the serotype 4b–specific genes *gltA*, *gltB*, and *gtcA* described above. An important exception, however, was noticed with two DNA fragments (4bSF7 and 4bSF18), which could be detected by PCR in ECI and in other serotype 4b strains, but not in strains of ECII.

Analysis of the DNA sequence in the region where the fragments were located revealed that the two fragments 4bSF7 and 4bSF18 were close to each other. In addition, comparison of this region with the corresponding region in the genome of strain EGD (serotype 1/2a) *(8)* suggested that the region harbored several genes that appeared to be specific to serotype 4b.

We have utilized the available nucleotide sequence data from this region to generate DNA probes for several of these genes, including the genes that harbored 4bSF7 and 4bSF18.

Our data suggest that these genes are either absent from the genome of ECII strains or have undergone pronounced divergence. To date, we have been unable to obtain a signal in Southern blots with these probes, even under low-stringency conditions. Similarly, PCR reactions with primers derived from several of the genes in this region failed to give a product with ECII genomic DNA as template, whereas the expected product was obtained with the DNA of all other screened serotype 4b strains (data not shown). Our combined PCR and Southern blot data thus suggest that the putative serotype 4b–specific genes that are clustered in this genomic region have undergone extensive divergence between the ECII outbreak strains and other serotype 4b strains of the pathogen.

DISCUSSION

Although the ECII (hot dog outbreak) strains have the fundamental surface antigenic features typical of serotype 4b, these strains appear to have a unique assortment of genetic markers that differentiate them from other serotype 4b isolates, including strains of ECI. Such a genetic marker is represented by the RFLP that we identified. In addition, these strains differed from other serotype 4b strains in their apparent divergence in the serotype 4b–specific 4bSF7–4bSF18 genomic region.

We were surprised to find that the ECII strains either lacked or underwent pronounced divergence in this genomic region, which is otherwise highly conserved among all other serotype 4b strains that we screened. Several probes derived from this region failed to produce a signal in Southern blots, even under low-stringency conditions. Our experience with Southern blot hybridizations of divergent regions would suggest that if the genes are present, their sequence identity with their counterparts in other serotype 4b strains would likely be less than 80%. If the genes are absent, the question would arise as to whether ECII harbors a genomic counterpart in this region, with sequence content unrelated to that found in other serotype 4b strains. Our combined Southern blot and PCR data suggest that this region constitutes a serotype 4b-specific genomic "island" that either is absent or has unique sequence content in the ECII strains. The evolutionary mechanisms that have driven the apparent divergence of ECII in this genomic region remain to be elucidated.

At this time, the functional involvement of the genes in this region is not known. It is also not known how many other serotype 4b–specific genomic regions have diverged in the ECII strains. However, we have examined the presence of all nine other regions currently known to us, including those involved in the glycosylation of the cell wall-associated polymer teichoic acid *(4, 6)*. All of these regions were conserved in the ECII hot dog outbreak strains, suggesting that the divergence that we observed in the genomic island mentioned above was unusual. However, we cannot exclude the possibility that there may be additional, currently unidentified serotype-specific regions that may be significantly divergent (or absent) in these strains.

In conclusion, our current objectives are to determine the precise boundaries of the divergent region that we identified in the outbreak strains and to obtain sequence information for at least certain of the genes in the region, presuming that the ECII genome does not simply lack the genes. The available sequence data from this region will be utilized to design PCR-based protocols that may amplify the equivalent genomic fragments from the ECII genome. Such PCR-based detection would facilitate the monitoring of these strains among food, environmental, and clinical isolates of *L. monocytogenes*. Having access to the sequence of the genes also will facilitate mutagenesis approaches that will aid in the functional analysis of the genes in this genomic region.

The results that we have obtained in the course of this project clearly suggest that the ECII strains do indeed have genetically unique attributes. Further studies are needed to determine whether the genomic island that we have identified is the only one that clearly differentiates these strains from other serotype 4b isolates, and whether it is involved in the virulence and pathogenicity of these bacteria.

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MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF *LISTERIA MONOCYTOGENES* ISOLATES FROM HUMANS AND FOODS TO DEFINE HUMAN PATHOGENIC STRAINS

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Listeria monocytogenes is a foodborne pathogen associated with potentially serious diseases in humans and animals. Current directives in the United States that specify zero tolerance for the presence of any *L. monocytogenes* subtypes in ready-to-eat (RTE) foods are based on historical taxonomic classification schemes. These classical taxonomic definitions of bacterial species do not necessarily correlate with the ability of a group of bacteria to cause human foodborne disease. Rather, related bacteria that differ in their abilities to cause human and/or animal disease and in other phenotypic characteristics relevant to foodborne transmission may be grouped together into the same species. Molecular subtyping methods provide a unique opportunity to explore the population genetics and evolution of *L. monocytogenes (1)*. Subtyping methods not only have the potential to differentiate bacterial strains, but also facilitate the definition of subtypes and clonal groups that differ in phenotypic characteristics relevant to foodborne virulence, such as ability to infect human and animal cells and ability to multiply and survive

outside human and animal hosts, e.g., in foods or food-processing environments.

Although the majority of human clinical infections occur as sporadic cases, human listeriosis can also occur in large epidemics. Most sporadic human listeriosis cases and large human foodborne listeriosis epidemics have reportedly been caused by *L. monocytogenes* serotype 4b *(2)*. The 4b strains isolated from most epidemic outbreaks form two closely related homogeneous groups (so-called epidemic clones) *(3, 4)*. Serotypes 1/2a and 1/2b are also responsible for significant numbers of sporadic cases of human illness, and a serotype 1/2a strain was responsible for a recent multistate human listeriosis outbreak in the United States *(5)*. Serotyping data collected by the CDC in 1986 showed that serotypes 1/2a (30%), 1/2b (32%), and 4b (34%) represented the majority of isolates from 144 human sporadic cases *(6)*. Of 1,363 human isolates collected in the United Kingdom, 15% were 1/2a, 10% were 1/2b, and 64% were 4b *(7)*. The remaining currently recognized *L. monocytogenes* serotypes have been linked only rarely to human disease.

The apparent association between a few specific *L. monocytogenes* strains and most cases of human listeriosis raises the intriguing challenge of identifying the unique characteristics that enable these strains to be more effective than others in causing human disease. Two hypotheses could explain the apparent predominance of serotype 4b strains in human epidemic listeriosis and of 4b, 1/2a, and 1/2b strains in sporadic human cases: (1) Humans are more commonly exposed to these subtypes than to other *L. monocytogenes* serotypes; i.e., these strains are found in foods more frequently than are other serotypes. (2) These subtypes have a unique pathogenic potential for humans.

Surveillance programs using different subtyping strategies to differentiate *L. monocytogenes* strains in conjunction with population genetic and pathogenesis studies have the potential to yield a better understanding of the transmission dynamics of *L. monocytogenes* and to help us probe why specific subtypes appear to be the predominant cause of human infections.

Development and Selection of Strain Collections to Characterize *Listeria monocytogenes* Isolates From Humans and Foods

Studies of the population genetics of *L. monocytogenes* critically depend on the development of appropriate isolate collections representative of the *L. monocytogenes* strains found in foods and responsible for human invasive infections. For more than 7 years, our research group has collected human clinical and animal *L. monocytogenes* isolates with the goal of establishing a phylogenetic framework for probing relationships among *L. monocytogenes* strains.

We have developed a network for the collection of human and animal *L. monocytogenes* isolates which includes the health departments in New York State, New York City, Michigan, Connecticut, Ohio, Maryland, and California and the New York State Veterinary Diagnostic Laboratory at Cornell. Our collection already includes more than 700 human and more than 150 animal isolates, most of which have been characterized by different molecular subtyping methods.

In addition, we have collaborated with researchers at the National Food Processors Association (NFPA) who have conducted an independent prospective survey of *L. monocytogenes* for various RTE food products (sliced deli meats, prepared deli salads, smoked seafood, prepared seafood salads, bagged salads, blue-veined and soft mold–ripened cheeses, Hispanic-style soft cheeses) collected in Maryland and in the San Francisco area in 2000 and 2001. Through this collaboration, we have obtained a total of 502 randomly collected *L. monocytogenes* food isolates. Forty-two clinical isolates from human listeriosis cases reported over the same time period were also obtained. We report below on the comparative characterization of these food and human isolates.

Listeria monocytogenes Isolates Characterization by Molecular Subtyping

All human and food isolates were characterized by automated *Eco*RI ribotyping as well as by PCR restriction fragment length polymorphism analysis of the virulence gene *hly* as described and reported elsewhere *(4, 8)*. Our group also used these data to classify isolates into the three phylogenetic *L. monocytogenes* lineages, described elsewhere *(4)*. A total of 36 *Eco*RI ribotypes were differentiated among the 502 food isolates obtained through our collaboration with NFPA. Of these ribotypes, seven (representing 45 isolates) were not represented among any of the more than 700 human isolates in our collection, including 42 clinical isolates collected from human cases in Maryland and California in 2000. Interestingly, ribotype DUP-1042B, which was linked with listeriosis outbreaks from contaminated pate in the United Kingdom and milk in Massachusetts, represented only 4% of food isolates, whereas it represented 10.7% of the isolates in our collection of more than 700 human isolates and four out of 20 human clinical isolates from Maryland and California. Statistical analyses of our data have not yet been completed, but limited evidence suggests considerable differences in the distribution of different ribotypes among the food isolates from the two.

When the food isolates were classified by lineage, 37.3% represented lineage I, 62.3% represented lineage II, and 0.4% represented lineage III. Among the more than 700 human isolates, 59.3%, 35.3% and 2.2% represent lineages I, II, and III, respectively. These data are consistent with previous observations that *L. monocytogenes* serotype 1/2b and 4b are more common in human listeriosis cases; these serotypes are grouped into lineage I, whereas lineage II contains predominantly serotypes 1/2a and 1/2c *(9)*.

Listeria monocytogenes Isolate Characterization By Tissue Culture and Other Phenotypic Assays

Selected representative *L. monocytogenes* food isolates were also characterized by a tissue culture plaque assay using mouse L cells to define specific isolates and strains that have no or attenuated ability to invade and multiply in these animal cells. This assay can be used to define cytopathogenicity-related parameters, including (1) relative plaque size (expressed as percentage of the plaque size formed by the internal control strain *L. monocytogenes* 10403S) and (2) invasion efficiency (CFU/plaque-forming units (PFU)). This assay was previously shown to correlate well with mouse infections; isolates shown to be attenuated in this tissue culture assay were also attenuated in mouse infections *(4)*. So far, 44 of the *L. monocytogenes* food isolates described above have been characterized in this assay. Three of these isolates did not form any plaques at the *Listeria* numbers used for infections, and one strain formed extremely small plaques (< 60%); the cytopathogenicity of these four isolates should be considered attenuated. Three isolates showed plaque sizes of between 60% and 79%, whereas 12 and 25 isolates showed plaque sizes of 80% and 100% to > 100%, respectively.

Previous results from our group showed that lineage I strains on average form larger plaque sizes, compared with lineage II strains *(4)*. These results were confirmed in this study: the average plaque size of the lineage I food isolates was 117%, compared with 102% for the lineage II isolates. The fact that lineage I strains are more commonly associated with human cases but

are less prevalent than lineage II strains in contaminated foods may reflect the fact that lineage I strains have increased cytopathogenicity.

Development of A Subtype and Strain Database

In collaboration with bioinformatics experts at Cornell, we recently developed a publicly accessible database of bacterial strain subtypes. Named PathogenTracker, the database is available via the Internet (www.pathogentracker.net). It provides a unique platform for the exchange of bacterial subtyping information, particularly for molecular subtyping data. The database will be a key link that allows the research results described above to be used not only by other researchers but also by public health agencies around the world. A large database linking bacterial phenotypes and virulence characteristics with molecular subtyping data may bring us closer to a future when genetic characteristics rather than traditional species definitions are used to differentiate and define bacterial organisms that show distinct phenotypic characteristics.

CONCLUSIONS

Listeria monocytogenes can be separated into three genetic lineages that appear to differ in their prevalence among human, food, and animal isolates. Preliminary experiments indicate that isolates of these lineages may differ in the phenotypic characteristics relevant to their foodborne transmission potential, including tissue culture cytopathogenicity and growth capabilities at refrigeration temperatures. Continued large-scale population genetic studies of *L. monocytogenes* and other foodborne pathogens will provide an important opportunity to develop a better understanding of subtype characteristics and will allow the development of subtype-specific intervention strategies. Databases of subtypes and strains that are broadly accessible via the Internet, such as the PathogenTracker database developed in our laboratory, will be crucial in allowing regulatory agencies and the food industry to take advantage of improved knowledge of the population genetics of foodborne pathogens.

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RAPID NUCLEIC ACID–BASED DETECTION AND ENUMERATION OF *LISTERIA* SPP. BY FLOW CYTOMETRY

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The genus *Listeria* comprises six species: *monocytogenes*, *grayi*, *innocua*, *ivanovii*, *seeligeri*, and *welshimeri (1)*. Of these species, only one, *L. monocytogenes*, is pathogenic for humans, another, *L. ivanovii*, is an important animal pathogen. Members of the genus can be found in association with soil, water, and vegetation and are able to grow at refrigeration temperatures. Compared with many other foodborne pathogens, *Listeria* spp. have relatively high resistance to food-processing procedures and antimicrobials, and are difficult to control in foods and food-pro-cessing environments. Because the avirulent species share an ecological niche with *L. monocytogenes*, their presence in a food production facility can serve as an indicator for conditions that allow the growth and survival of this species *(2)*. The high mortality rate (~25–30%) associated with foodborne cases of listeriosis.

Fluorescence in situ hybridization (FISH) is a rapid and highly specific nucleic acid–based method for whole-cell identification of bacteria *(3)*. In the FISH technique, fluorescently labeled nucleic acid probes complementary to genus- or species-specific ribosomal RNA (rRNA) sequences are hybridized to whole bacterial cells, leading to the selective staining of target cells *(3)*. Recently, two DNA-based FISH probes were developed for the detection of *Listeria*. The first, Lis-1255, was originally reported for use as a polymerase chain reaction (PCR) primer *(4)*, but has been adapted for use as a FISH probe *(5)*. This probe is complementary to the 16S rRNA of all six species of *Listeria*, but has also been reported to react with *Brochothrix* spp. *(5, 6)*. Exact matches for other nontarget strains, including several marine species of *Bacillus*, an unidentified green nonsulfur bacterium, and a hydrocarbon-degrading bacterium, can also be found in the GenBank database. Although it is unlikely that these species would be present in the same environmental niche as *Listeria*, exact matches with members of other genera, especially *Brochothrix* spp., limit the diagnostic information available from this probe for the detection of *Listeria*. The other probe, Lis-637 *(5)*, reacts with all members of the genus *Listeria* except *L. grayi*. Because its reactivity is restricted to the target genus, Lis-637 is a much more useful tool than is Lis-1255. However, an ideal probe for detection of generic *Listeria* would meet two criteria: it would be restricted to the genus and it would react with all six species.

Because of the permeability barriers posed by their thick and highly anionic cell walls, Gram-positive bacteria also present unique challenges to the use of DNA-based FISH probes *(7, 8)*. As a result, DNA-based FISH analysis of Gram-positive cells often requires extensive preparatory steps, including the use of acid hydrolysis or lysozyme and proteinase K digestions *(6, 8)*. Because an unknown sample may contain cells that differ markedly in their requirements for permeabilization, the use of such steps may lead to cell lysis and reduced assay sensitivity *(9)*. Extensive processing may also result in the degradation of cellular light-scatter properties, which can interfere with analyses by microscopy or flow cytometry that are often used in conjunction with FISH.

The use of peptide nucleic acid (PNA) probes offers a solution to many of the problems associated with DNA-based FISH detection of Gram-positive bacteria. PNA is a peptide-like DNA mimic with an uncharged, achiral backbone *(10)*. The unique chemical makeup of PNA probes confers a number of beneficial properties, including rapid hybridization kinetics, resistance to nucleases, and the ability to hybridize to positions on the ribosome that are inaccessible to DNA probes *(11)*. PNA probes are also able to penetrate recalcitrant biological structures such as mycobacterial and Gram-positive cell walls *(10)*.

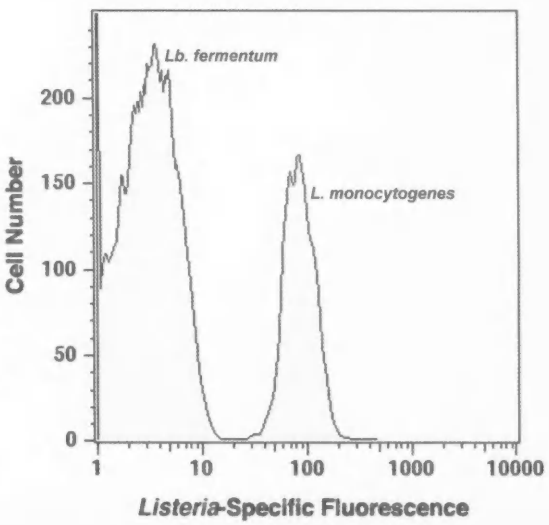
In collaborative work with Applied Biosystems, Inc. several PNA oligomers designed for the detection of *Listeria* spp. were synthesized, end-labeled with fluorescein, and examined by use of a FISH assay for their abilities to hybridize to whole cells. Probes yielding weak or no fluorescent signals were not investigated further. The remaining FISH-compatible probes were evaluated for their reactivities against the six members of the genus *Listeria* and against a number of species from closely related genera, including *Brochothrix*, *Bacillus*, and *Staphylococcus*. The *Listeria* strains tested included the type strains for all six members of the genus as well as several epidemiologically important serotypes of *L. monocytogenes*. The identities of all strains were confirmed through sequencing of polymerase chain reaction products. Hybridization with a domain-specific eubacterial probe was used as a positive control for target accessibility in both *Listeria* spp. and nontarget cells.

RNase treatment of selected strains was used to confirm that positive reactions were rRNA dependent and to examine the extent of nonspecific staining of nontarget cells. Two PNA probes yielding bright, rapid, and genus-specific hybridizations were identified. Of these two probes, LisUn-11 was the brightest and stained all six *Listeria* species. The other probe, LisUn-3, hybridized with all *Listeria* spp. except for *L. grayi*, for which it had two mismatched bases. Probe specificities were not dependent on the use of toxic denaturants such as formamide, and combined hybridization and washing steps were completed within 1.5 hours. Cell preparation was simple and the use of toxic fixatives was avoided. No special preparatory treatments, such as acid hydrolysis or digestions with lysozyme, lysostaphin, or proteinase K, were required prior to hybridization. To our knowledge, this is the first report of peptide nucleic acid probes for use in whole-cell detection of *Listeria* spp. and the first report of any genus-specific FISH probe that reacts with all six species of *Listeria*.

A separate goal of this project is to combine these newly developed methods for nucleic acid–based identification of *Listeria* spp. with flow cytometry. Flow cytometry provides a rapid and precise means for fluorescence-based detection and enumeration of individual cells from mixed populations. Most commercial flow cytometers, however, are optimized for the detection of relatively large mammalian cells. Although the use of microscope-based or custom-built cytometers represents one approach for obtaining superior resolution of bacterial cells, we have sought to develop methods that can extend the detection capabilities of more widely available, nonspecialized machines. Toward this end, we have found that mismatches in refractive indices between the sample suspension buffer and the cytometer's sheath fluid can give rise to potentially useful "lensing" effects. This phenomenon can be used to obtain enhanced resolution of the light scatter and fluorescence properties of smaller bacteria, including *Listeria* spp., and is now routinely used in this laboratory for flow cytometric analysis of *Listeria* spp.

In initial experiments with cocultures of *L. monocytogenes* and *Lactobacillus fermentum* in *Listeria* enrichment broth (LEB), nonspecific green fluorescence from the selective dye acriflavine lowered signal-to-noise ratios for fluorescein-labeled PNA probes. However, with the combination of PNA-FISH and flow cytometric analysis, we were able to detect a minority of *L. monocytogenes* against a high background of *L. fermentum* when the two were cocultured in nonselective media (MRS broth) (Fig. 1). The ability to differentiate *Listeria* spp. from competitive microflora under nonselective conditions suggests the potential use of combined PNA-FISH and flow cytometry for early detection of *Listeria* spp. in primary enrichment media inoculated with food or environmental samples.

Figure 1. Combination of peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH) and flow cytometry for the rapid detection of *Listeria monocytogenes* in nonselective coculture. *Lactobacillus fermentum* and *L. monocytogenes* were grown together in MRS broth at 30°C. At various intervals, samples were taken and fixed by resuspending pelleted cells in a 50:50 mixture of phosphate-buffered saline (PBS) and absolute ethanol. Samples were hybridized for 30 minutes with 200 pmol/mL of the PNA-FISH probe LisUn-11 and analyzed by flow cytometry. These data demonstrate the power of combined PNA-FISH and flow cytometry for detecting low levels of *Listeria* (19.1% of total population analyzed) against a high background of nontarget bacteria (80.9% of total population analyzed)



The use of nonselective media may also facilitate earlier recovery of injured cells from these samples. Mixtures of live and ethanol-killed cells of *L. monocytogenes* and *L. fermentum* subjected to several days of starvation in phosphate-buffered saline (PBS) did not yield a *Listeria*-specific FISH signal. However, after brief (1.5 hours) exposure to a dilute nutrient source, a small population of *L. monocytogenes* was readily stained after only 15 minutes of hybridization with LisUn-11. These results suggest that rRNA levels in *L. monocytogenes* were degraded to below FISH-detectable levels during starvation, but that detectable levels of new rRNA were readily synthesized upon exposure to nutrients.

This research provides new tools for the rapid detection of *Listeria*, and the technology could be expanded for detection of other foodborne pathogens. The novel feature of this work is the use of PNA probes, which have considerable advantages over DNA probes. PNA-targeted listerias can be detected by various techniques, including microscopy and flow cytometry. PNA-FISH technology has excellent potential for disease intervention and regulatory compliance in food safety.

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CHRONIC WASTING DISEASE AND OTHER TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

OVERVIEW OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

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Students of food safety who thought they knew about all the hazards associated with foods had a rude awakening in 1995–1996 *(1, 2)*. The idea that agents called prions might be transmissible via foods was novel, and alarming. For simplicity, one can consider prions to be low-molecular-weight glycoproteins that occur in the central nervous system and some other tissues and may play a role in the conduction of nerve impulses. The normal folding of these molecules is determined by their amino acid sequence, which in turn is genetically determined. Some prions, however, are capable of folding into an alternative configuration that makes them resistant to protease digestion and apparently results in their accumulation in the brain, leading to spongiform degeneration of the brain tissue. This alternative configuration is sometimes designated $PrPS^c$, meaning prion protein that is resistant to protease digestion. Unfortunately, such prions are also extremely resistant to heat, irradiation, and most chemical disinfectants.

The $PrPS^c$ have the peculiar property of conferring their abnormal configuration onto normal prions of the same species with which they come in contact. This is the recognized mode of pathogenesis in scrapie of sheep and in classical Creutzfeldt-Jakob disease (CJD) of humans. Sheep scrapie has not been known to be transmitted to species other than sheep and goats, and CJD has been transmitted among humans only by transplantation of certain tissues (e.g., dura mater from cadavers, and perhaps in human growth hormone from cadavers). Interspecies transmission, however, was not observed or expected, because prions have amino acid sequences that differ significantly among species. As will be described below, past experience was contradicted in the case of bovine spongiform encephalopathy (BSE). It is now known that in certain instances, $PrPS^c$ from one species can confer abnormality on prions of another species, assuming that the two types of prions come in contact.

Transmissible spongiform encephalopathies (TSE) are diseases that result from the accumulation of $PrPS^c$. All are fatal, and some are "contagious." Some TSEs have been known for many years, including scrapie in sheep and goats, CJD and various related illnesses in humans, transmissible mink encephalopathy, and chronic wasting disease (CWD) in deer and elk. CJD could be familial, iatrogenic, or sporadic. The sporadic form occurs spontaneously and worldwide at a rate of approximately one case per million people, typically in people > 60 years. New TSEs include bovine spongiform encephalopathy (BSE, sometimes called "mad cow disease"), feline spongiform encephalopathy, and new variant CJD (vCJD) in humans.

It is thought that BSE first appeared in cattle in the United Kingdom in April 1985. By December 2001, more than 180,000 cases had been recorded there. An incubation period of 3–5 years had been inferred, and control was attempted by prohibiting feeding of rendered ruminant meat-and-bone meal (MBM) to cattle. The origin of BSE remains unknown. Some suggest that scrapie prions in MBM fed to cattle became, by chance, infectious for cattle. Others think that a spontaneous occurrence in

cattle (analogous to sporadic CJD in humans) may have been the source. A contributing factor may have been the adoption of a less rigorous rendering process in the United Kingdom by the end of the 1970s, permitting the persistence of $PrPS^c$ that would otherwise have been inactivated. The outbreak led to the slaughter of affected herds, imposition of special precautions in the disposal of the carcasses, and an enormous research effort. Risk materials called "specified bovine offals" (especially brain and spinal cord) were not to be used for human food. Even so, it was thought that the disease was confined to cattle (and perhaps other ruminants) until an outbreak of TSE was recognized in domestic and zoo species of cats.

Then, in 1995, an outbreak of what came to be called vCJD was perceived in humans. It was distinguished from CJD because young people (often in their 20s) were affected, although other distinctive clinical features were noted (Table 1). The realization that humans were at risk led to a much higher level of alarm, and more resources were applied to controlling the disease in animals and to preventing transmission to humans *(4)*. Animal-to-human transmission was implicitly via food, but to date no specific food item has been incriminated. High levels of $PrPS^c$ are known to occur in bovine brain and spinal cord, which traditionally have been classified as edible tissue, whereas no $PrPS^c$ have yet been detected in voluntary muscle (red meat) or in milk. Extensive food histories have been obtained from or for vCJD victims in the United Kingdom, with no clear resolution of pathogenicity. Some research suggests that "vertical" (cow-to-calf) transmission of BSE is unlikely. In addition to banning specified bovine offals from the human food supply, no cattle older than 30 months at slaughter may be used as food. There also was a transitory effort to require that no beef be served on the bone.

By the end of May 2002, 122 people in the United Kingdom were recorded as having been affected with vCJD. This figure is considerably below the rate of sporadic CJD there, but represents far fewer deaths than from other foodborne diseases. Nevertheless, the public reaction in the United Kingdom and elsewhere has been profound. The combination of the younger affected age group, tragic symptoms, and incurability of the disease have weighed heavily on people's minds. There is a genetic aspect to vCJD susceptibility: to date, all vCJD patients tested have been homozygous for methionine at codon 129 of their prion genes (this is true of only 40% of the population from which the cases derive.

Until 1996, some cattle, much beef, and a great deal of MBM were exported from the United Kingdom to other countries. BSE began to be noted in other countries (all in Europe to date, with the exception of Japan and the Occupied Territories of Palestine), and a few vCJD cases have been recorded outside the United Kingdom. Slaughter cattle older than 30 months (24 months in some countries) are being tested in other BSE countries. If the rapid test indicates that the animal is BSE-positive, the carcass is held until further testing either negates or confirms the result; in the latter case, the carcass is destroyed. In Japan, even veal calves are tested, even though the $PrPS^c$ is unlikely to reach a detectable level until much later in an animal's life. There is also concern in the United Kingdom that BSE may have infected sheep, whereby it might cause a disease indistinguishable from scrapie but be transmissible to humans via lamb and mutton. This is being investigated, but no evidence to support the hypothesis seems to have emerged.

The epidemic in humans has killed fewer than 120 in the United Kingdom and around 10 in other countries, but there is great disagreement about where the epidemic curve is headed. Although there is still no direct evidence that vCJD is transmissible by blood transfusion, the United Kingdom now collects blood only from persons judged to be at relatively low risk, and the blood is either used by its expiration date or discarded. That is, pooling of expired blood units for manufacture of other products, such as clotting factors for hemophiliacs, is no longer done in the United Kingdom. Instead, such products are purchased from safe sources (e.g., North America).

TABLE 1. Summary comparison of variant Creutzfeldt-Jakob disease [vCJD] and Creutzfeldt-Jakob disease [CJD]

Clinical features	vCJD	CJD
Age of onset	Earlier (12–75 years)	Later (>60 years)
Median duration	13 months	4 months
Median age at death	28 years	68 years
Psychiatric and sensory symptoms	Frequent early in the course of illness	Appear later in the course of illness
EEG changes	Absent	Common

Obviously, BSE-free countries are at great pains to stay that way, and the United States is no exception. Importation of cattle from the United Kingdom has long been banned, and other countries of origin are added to the embargo list as BSE cases are diagnosed in them. Bovine products from these countries are also generally embargoed, but it must be recognized that products have probably come from many of these countries while their cattle were in the incubation phase of BSE. All the same, MBM and specified bovine offals have generally not been imported to the United States regardless of the BSE concern. The United States has also banned feeding MBM of mammalian origin to ruminant food animals, and this prohibition seems likely to be broadened even as enforcement of the basic regulation is tightened. Additionally, the heads of slaughter cattle that show possible neurological abnormalities at antemortem inspection are supposed to be submitted to the U.S. Department of Agriculture for BSE testing. Apparently the proportion of such cattle heads being tested falls short of 100%. There may in time be restrictions on the use of bovine offals as food in the United States, but at present bovine brains and spinal cords from USDA-inspected animals remain food.

Perhaps most significant, from the standpoint of public health, is the enactment of several restrictions on the donation of blood in the United States *(6)*. Obviously, no blood or blood products may be imported from BSE countries; also, biologicals (e.g., vaccines) are being closely scrutinized for the presence of bovine blood derivatives from BSE countries. U.S. donors are "deferred" (excluded) if they have vCJD or CJD or are thought to be at heightened risk of CJD, if they have spent ≥3 months cumulatively in the United Kingdom between 1980 and 1996 or ≥5 years cumulatively in Europe from 1980 to the present, if they have received a transfusion of blood or blood products in the United Kingdom from 1980 to the present, or if they have injected bovine insulin since 1980 unless it can be shown that the source cattle were not in the United Kingdom *(3, 5)*. Military personnel, their dependents, and civilian military employees are

also excluded from blood donation on the basis of whether the beef served or sold at their duty stations derived from the United Kingdom. Some of these measures supplanted earlier, less rigorous measures on May 31, 2002, and Phase II measures are scheduled to be implemented on October 31, 2002.

In the aftermath of these events, there is great fear as to whether other TSEs (e.g., CWD) can be transmitted to humans. The United States has lived with scrapie and CWD for many years, and because the latter is the topic of this symposium, I defer to other speakers to address this problem. It appears that there is a serious effort to mount a scrapie eradication program in the near future, but one always wonders to what degree resources will be made available for this, given the many competing priorities at this time.

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DIAGNOSTIC TESTS FOR CHRONIC WASTING DISEASE: WHAT IS NEEDED? WHAT WILL BE AVAILABLE?

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Chronic wasting disease (CWD) is a family of disorders affecting members of the Cervidae group, including mule deer, white-tailed deer, and Rocky Mountain elk. The disease has been reported in captive animals raised in game farm settings and in free-ranging animals. Diagnosis of CWD is complicated by species differences in tissue distribution of the marker protein (PrP^{CWD}) during the preclinical phase of the disease. Furthermore, diagnostic samples vary from individual game-raised animals submitted to veterinary laboratories for necropsy to groups of several hundred thousand deer harvested by hunters over a single hunting season. Relative sensitivity, specificity, throughput, technical demands, and cost are factors in the development and implementation of diagnostic tests for CWD. A variety of test formats are being evaluated to meet the needs of game management, agricultural regulatory, research, and hunter groups.

The transmissible spongiform encephalopathies (TSE) are a heterogeneous group of fatal neurological disorders. TSEs are notable for their presumed etiology, a particularly stable heat- and denaturation-resistant mammalian protein, generically termed PrP^d. PrP^d is a primary component of infectious tissue extracts and is thought to initiate the disease process when ingested by or injected into a susceptible host. Exogenous PrP^d forms aggregates with the endogenous, ubiquitously expressed host cell isoform ($PrP^{Cellular}$, or PrP^C), inducing the conversion of PrP^C to PrP^d through poorly defined changes in secondary structure. Amplification of the input PrP^d and axonal transport to the brain occurs during the prolonged preclinical incubation period. Deposition of PrP^d in the central nervous system precedes the appearance of the classical lesions, including spongiform change, gliosis, and astrocytosis. Widespread neuronal loss is observed in the clinical stage of the disease. Diagnosis of the ruminant TSEs is typically made by detection of PrP^d in brain tissue, using immunohistochemistry, Western blot, or enzyme-linked immunosorbent assays.

The TSEs present in hoofed stock and humans represent a spectrum of disorders that vary by host range, epidemiology, presumed etiology, and pathogenesis. Only bovine spongiform encephalopathy (BSE) is considered a zoonotic disease. Although a number of relatively rare human TSEs have been described, only one disorder is linked to exposure to infected hoofed stock. Variant Creutzfeldt-Jakob disease (vCJD), first reported in 1996, is associated with BSE, based on similarities in biochemical profiles and rodent bioassays. The possibility that the food supply may contain a heat-resistant transmissible agent fatal to humans has led to an increased awareness of the TSEs by the public and a call for worldwide eradication of the animal TSEs.

Chronic wasting disease is a TSE of North American deer and elk, first reported in 1980. The disease has now been reported in mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and elk (*Cervus elaphus*) in limited geographic regions of the United States and Canada. Although the disorder shares certain characteristics with the better-defined TSEs of ruminant herbivores, notably scrapie of sheep and goats and BSE, there are important differences between these diseases and between deer-associated and elk-associated CWD. These differences contribute to the difficulty of designing specific, sensitive diagnostic and surveillance tests.

Deer-associated CWD is characterized by early accumulation of PrP^d in lymphoid tissue. PrP^d is readily detectable in peripheral lymphoid tissues of experimentally and naturally infected deer. Early lymphoid accumulation of PrP^{Sc} is typical in ovine scrapie as well, and is the basis for preclinical antemortem and postmortem diagnosis. The abundant deposition of PrP^d in tonsil and retropharyngeal lymph nodes of deer suggests that similar approaches will be feasible in these species. Live animal testing by tonsil biopsy and postmortem testing by immunohistochemistry or other bioassay will contribute to surveillance and game management programs. The capacity of these test formats to meet testing needs will depend in large part on public perception of the safety of food products from animals in a CWD-endemic area. Live animal testing by examination of peripheral lymph nodes has some value in surveillance programs but is impractical for the huge number of deer—in the millions annually—harvested by hunters. If the public perceives a threat to human health from contact with CWD-infected deer, the demands for preclinical, postmortem testing will probably require a laboratory network using robotically controlled, high-throughput tests.

Elk-associated CWD is characterized by scant accumulations of PrP^d in lymphoid tissue, particularly in the preclinical phase

of the disease. In contrast with scrapie and deer-associated CWD, the tonsil and retropharyngeal nodes of some infected elk (diagnosed by PrP^{d} in the brain) do not contain detectable PrP^{d}. As with the other ruminant TSEs, the earliest site of accumulation in the brain is the dorsal motor nucleus of the vagus nerve (DMNV), small paired areas in the medulla. The limited distribution of PrP^{d} suggests that antemortem testing may require a different disease marker and that postmortem testing will require careful sampling of the brain to collect the DMNV. The need for live animal testing is more acute in elk than it is in deer. Elk are raised domestically in many parts of the United States and Canada, and movement of infected animals that do not appear to be infected may contribute to spread of the disease. Mandatory necropsy laws, now in effect in many states, and voluntary or mandatory testing of hunter-harvested animals in an endemic area will require a substantial diagnostic test capability. Immunohistochemistry will remain the gold standard for elk-associated CWD testing.

Federal and state regulatory agencies are establishing CWD control programs to coordinate diagnosis, research, information gathering and dissemination, and education. The challenge of controlling a transmissible disease with a long incubation period in free-ranging and captive wildlife is formidable, and success will require a partnership of governmental agencies, the research community, and the private diagnostic testing industry.

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IN VITRO AND IN VIVO MODELS FOR THE BIOLOGY, PATHOGENESIS, AND TRANSMISSION OF CHRONIC WASTING DISEASE

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The transmissible spongiform encephalopathies (TSE) are fatal neurodegenerative diseases that include scrapie in sheep, Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE), and chronic wasting disease (CWD) in mule deer and elk. It is well known that TSE infectivity can cross species barriers. The fact that BSE has crossed species barriers to cause new variant CJD in young people in the United Kingdom, in combination with concerns that a similar situation could arise with CWD in the United States, underscores the importance of understanding the basis of cross-species transmission of TSE infectivity.

A key event in TSE pathogenesis is the conversion of the normal host cellular prion protein (PrP^{sen}) to an abnormal form (PrP^{res}) associated with TSE infectivity and disease. Experiments with transgenic mice have shown that efficient cross-species transmission of TSE infectivity is strongly influenced by the amino acid sequence of PrP. Furthermore, both cell-associated and cell-free assay systems have provided compelling evidence that amino acid homology between PrP^{sen} and PrP^{res} is also critical for the species-specific formation of PrP^{res}. In these in vitro systems, the species-specific formation of PrP^{res} correlates well with the cross-species transmissibility of TSE agents.

Using a cell-free assay of PrP^{res} formation, it has been demonstrated that both BSE- and CWD-derived PrP^{res} convert noncervid PrP^{sen} to PrP^{res} less efficiently than cervid-derived PrP^{sen}. These results suggest that the susceptibility of humans and other noncervids to CWD and BSE may be limited. In vitro systems therefore provide a means to assess, at the molecular level, the potential susceptibility of one species to infection with the TSE agent of another species.

EPIDEMIOLOGY OF CHRONIC WASTING DISEASE IN WILDLIFE

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Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy or prion disease of deer (*Odocoileus* spp.) and Rocky Mountain elk (*Cervus elaphus nelsoni*). Although the disease has been recognized in a core endemic area of Colorado and Wyoming for more than 35 years, it is only within the last decade that concern about the disease has greatly expanded. This increased concern follows on the epidemic of bovine spongiform encephalopathy (BSE) in the United Kingdom, continental Europe, and Japan and its consequent effect on livestock industries; recognition of the link between BSE and variant

Creutzfeldt-Jacob disease in people; and increased interest in the transmissible spongiform encephalopathies in general. Although there are many questions about CWD, numerous features of the disease have been defined and many are currently under study. The purpose of this review is to present current information on the epidemiology and host range of CWD. More detailed recent reviews of CWD are available elsewhere *(1, 2)*.

History and Distribution

Our current knowledge of the distribution of CWD is based on a combination of surveillance methods, including investigation of clinical cases fitting the case definition for CWD (deer or elk >16 months showing weight loss and/or behavioral changes) and active surveillance of clinically healthy animals (hunter harvested, agency collected, roadkill, accidental deaths, slaughtered, etc.) *(3)*. Techniques used for CWD surveillance vary among the states and provinces, depending on the populations of interest (i.e., farmed or free-ranging animals) and the resources available. Federal programs for CWD surveillance of farmed cervids (members of the deer family) have been proposed *(4)*.

The first recognition of CWD in the late 1960s in Colorado was based on observations of chronic progressive unexplained illness in captive deer. By the late 1970s this syndrome was diagnosed as a spongiform encephalopathy, and it was found in additional wildlife facilities in Colorado and Wyoming that exchanged research animals *(5, 6)*. Modeling the history of CWD based on parameters estimated from CWD surveillance in Colorado and Wyoming suggests that CWD may have been present in northeastern Colorado and southeastern Wyoming as early as the 1950s *(7)*.

By the late 1980s and early 1990s, CWD had been found in free-ranging mule deer (*O. hemionus*), white-tailed deer (*O. virginianus*), and Rocky Mountain elk in Colorado and Wyoming *(7, 8)*. Clinical CWD was first diagnosed in farmed elk in Saskatchewan in 1996 and was subsequently identified in a herd of farmed elk in South Dakota. These diagnoses greatly expanded the geographic distribution of CWD; epidemiologic investigations have linked the occurrence of most of the cases of CWD in farmed elk to the movement of animals in commerce. However, a few unexplained cases of CWD in farmed elk, without clear epidemiologic links to affected herds, remain. As of June 2002, CWD had been identified in farmed elk herds in South Dakota, Oklahoma, Nebraska, Colorado, Montana, Kansas, Saskatchewan, and Alberta. Most affected herds have been depopulated and indemnity paid. CWD was identified or suspected in zoologic gardens in Ontario, Colorado, and Wyoming prior to the mid-1980s; there is no evidence that CWD has persisted in any of these premises.

CWD in free-ranging deer and elk remained geographically limited to the core CWD endemic area in Colorado and Wyoming until cases were found in the southwestern corner of the panhandle of Nebraska in 2000–2002. This geographic extension was expected and was most likely due to the movement of affected deer along major drainages from the contiguous CWD core areas eastward. However, unexpected cases of CWD in free-ranging deer were found in western Saskatchewan, northwestern Nebraska, southwestern South Dakota, south central Wisconsin, northwestern Colorado, and southern New Mexico in 2000–2002. Most of these foci were well removed from the CWD endemic area. In some cases, affected deer were associated with farmed elk, although routes of transmission or even direction of transmission remain under study. The occurrence of CWD in white-tailed deer in Wisconsin and in mule deer from New Mexico has not yet been accounted for. As awareness of CWD increases and there is implementation of expanded state-federal-industry surveillance and herd certification programs, the geographic distribution of CWD will be more clearly defined.

Epidemiology

There is good epidemiologic evidence that CWD is laterally transmitted, although the details of this transmission are not yet clearly understood and are under study. CWD disease transmission can be examined in at least three ways: (1) transmission from animal to animal (lateral transmission), (2) transmission via environmental contamination, and (3) geographic expansion.

Evidence of early and widespread involvement of the lymphoid tissues, including tonsil, retropharyngeal lymph node, mesenteric lymph nodes, and Peyer's patches, has been obtained experimentally in mule deer orally exposed to CWD *(9;* E. S. Williams et al., unpublished data, 2002). These lymphoid organs accumulate the abnormal protein associated with CWD (PrP^{CWD}), which can be detected by immunohistochemistry as early as 42 days postinoculation. This is well before PrP^{CWD} can be detected in the brain. Involvement of the lymphoid tissues lining the alimentary tract provides a possible route of excretion of the CWD agent in the feces and possibly saliva. Thus, a potential route of lateral transmission is via the fecal-oral route. This hypothesis is being studied.

Anecdotal observations provide strong evidence that CWD agent may accumulate in the environment and that contaminated facilities may retain infectivity for susceptible deer and elk. Because TSE agents, and no doubt CWD agent, are extremely resistant to environmental conditions, excretions and secretions from affected deer and elk, particularly in confined or high-density situations, could increase over time to provide an environmental reservoir of infectivity. Controlled studies to evaluate the role of environmental contamination are under way. Although unlikely to be important in captive herds, the contribution of decomposing carcasses of deer and elk with CWD that contain organs known to contain high levels of infectivity, such as brain and spinal cord, could be substantial in free-ranging herds.

Finally, gross geographic extension of CWD is most likely associated with movement of live animals affected subclinically or clinically with CWD. This is the probable mode of movement of CWD in farmed elk commerce. Transplantation and anthropogenic movement of publicly owned deer and elk in the CWD core endemic areas have been banned for many years. The spread of CWD via movement of carcasses or fomites is theoretically possible but seems less likely than movement via an animal actively shedding the agent.

Host Range

The only species known to be naturally susceptible to CWD are mule deer, white-tailed deer, and Rocky Mountain elk. It is very likely that subspecies of these cervids will also prove to be susceptible. Because of significant concerns about BSE and questions about the susceptibility of cattle to CWD, cattle were experimentally exposed to CWD 7 years ago (with these studies planned for another 3 years). Of 13 cattle inoculated intracerebrally with CWD agent, transmission occurred in 5 animals but not in the remaining 8 cattle *(10)*. In the few affected animals, the lesions were different from those associated with BSE, and the susceptibility of cattle to CWD was less than that of cattle to scrapie agent in similar studies *(10)*. Other species susceptible to CWD agent by intracerebral inoculation include domestic goat, domestic ferrets, farmed mink, mice, hamsters (after passage in ferrets), squirrel monkey, and mule deer *(11–13;* R. Marsh et al., unpublished data, 2002). Experimental oral transmission of CWD has been accomplished in mule deer *(9)*, white-tailed deer, and elk (E.S. Williams et al., unpublished data, 2002); ongoing studies include pronghorn antelope (*Antilocapra americana*), moose (*Alces alces*), and cattle. Cattle living in contact with deer and elk with CWD have remained healthy for 7 years. These preliminary studies thus suggest that cattle are not naturally susceptible to the CWD agent.

SUMMARY

Although many questions remain about the epidemiology and host range of CWD, in addition to questions about control and management, it is clear that CWD is both infectious and contagious. The epidemiology of CWD is under considerable study, and results of increased surveillance over the next few years should provide a basis for developing and implementing CWD management plans. The studies of CWD have progressed since this short review was compiled (2002) and interested readers should consult more recent literature.

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CONTROL MEASURES FOR CHRONIC WASTING DISEASE

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The Veterinary Services (VS) arm of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service is responsible for protecting the health of animals involved in agriculture. Agricultural animals include not only horses and domestic livestock but also a number of alternative livestock species such as farmed elk and deer. The existing jurisdictional frameworks for farmed elk and deer are highly fragmented. Consequently, the development of formal VS disease control programs for these animals requires more jurisdictional cooperation than do programs for typical domestic livestock. The major disease issue currently facing the farmed elk and deer industries is chronic wasting disease (CWD). Since 1999, VS has collaborated with both the state agencies and industry to develop and implement a national program to eliminate CWD from farmed elk. CWD presents unique challenges for program development because of critical gaps in disease knowledge, limited diagnostic tools, and involvement of multiple cervid species industries. The proposed USDA program, as well as recent VS activities, in response to positive farmed elk herds will be discussed.

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11. Abstract — Type abstract, double-spaced, in the space provided or on a separate sheet of paper, using a 12-point font size. Use no more than 250 words.

Abstract Submission

Abstracts submitted for IAFP 2005 will be evaluated for acceptance by the Program Committee. Please be sure to follow the format instructions above carefully; failure to do so may result in rejection. Information in the abstract data must not have been previously published in a copyrighted journal.

Abstracts must be received no later than January 12, 2005. Return the completed abstract form through one of the following methods:

1. Online: Use the online abstract submission form located at www.foodprotection.org. You will receive an E-mail confirming receipt of your submission.
2. E-mail: Submit via E-mail as an attached text or MS Word™ document to abstracts@foodprotection.org.

Selection Criteria

1. Abstracts must accurately and briefly describe:
   (a) the problem studied and/or objectives;
   (b) methodology;
   (c) essential results; and
   (d) conclusions and/or significant implications.
2. Abstracts must report the results of original research pertinent to the subject matter. Papers should report the results of applied research on: food, dairy and environmental sanitation; foodborne pathogens; food and dairy microbiology; food and dairy engineering; food and dairy chemistry; food additives and residues; food and dairy technology; food service and food administration; quality assurance/control; mastitis; environmental health; waste management and water quality. Papers may also report subject matter of an educational and/or nontechnical nature.
3. Research must be based on accepted scientific practices.
4. Research should not have been previously presented nor intended for presentation at another scientific meeting. Papers should not appear in print prior to the Annual Meeting.
5. Results should be summarized. Do not use tables or graphs.

Rejection Reasons

1. Abstract was not prepared according to the "Instructions for Preparing Abstracts."
2. Abstract does not contain essential elements as described in "Selection Criteria."
3. Abstract reports inappropriate or unacceptable subject matter or is not based on accepted scientific practices, or the quality of the research or scientific approach is inadequate.
4. Work reported appears to be incomplete and/or data are not presented. Indication that data will be presented is not acceptable.
5. Abstract was poorly written or prepared. This includes spelling and grammatical errors.
6. Results have been presented/published previously.
7. Abstract was received after the deadline for submission.
8. Abstract contains information that is in violation of the International Association for Food Protection Policy on Commercialism.

Projected Deadlines/Notification

Abstract Submission Deadline: January 12, 2005. Submission Confirmations: On or before January 13, 2005. Acceptance/Rejection Notification: February 16, 2005.

Contact Information

Questions regarding abstract submission can be directed to Bev Brannen, 515.276.3344 or 800.369.6337; E-mail: bbrannen@foodprotection.org.

Program Chairperson

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Abstract Form

DEADLINE: Must be Received by January 12, 2005

(1) Title of Paper _____

_____

(2) Authors _____

_____

(3) Full Name and Title of Presenter _____

_____

(4) Institution and Address of Presenter _____

_____

(5) Phone Number _____

(6) Fax Number _____

(7) E-mail _____

(8) Format preferred: ☐ Oral ☐ Poster ☐ No Preference

The Program Committee will make the final decision on presentation format.

(9) Category: ☐ Produce ☐ Foods of Animal Origin ☐ Seafood ☐ Other Food Commodities

☐ Risk Assessment ☐ Education ☐ General Microbiology and Sanitation

☐ Antimicrobials ☐ Pathogens ☐ Dairy

(10) Developing Scientist Awards Competition ☐ Yes Graduation date _____

Major Professor/Department Head approval (signature and date) _____

(11) TYPE abstract, DOUBLE-SPACED, in the space provided or on a separate sheet of paper, using a 12-point font size. Use no more than 250 words.

Call for Entrants in the Developing Scientist Awards Competitions

Supported by the International Association for Food Protection Foundation

The International Association for Food Protection is pleased to announce the continuation of its program to encourage and recognize the work of students and recent graduates in the field of food safety research. Qualified individuals may enter either the oral or poster competition.

Purpose

1. To encourage students and recent graduates to present their original research at the Annual Meeting.
2. To foster professionalism in students and recent graduates through contact with peers and professional Members of the Association.
3. To encourage participation by students and recent graduates in the Association and the Annual Meeting.

Presentation Format

Oral Competition – The Developing Scientist Oral Awards Competition is open to graduate students (enrolled or recent graduates) from M.S. or Ph.D. programs or undergraduate students at accredited univesities or colleges. Presentations are limited to 15 minutes, which includes two to four minutes for discussion.

Poster Competition – The Developing Scientist Poster Awards Competition is open to students (enrolled or recent graduates) from undergraduate or graduate programs at accredited universities or colleges. The presenter must be present to answer questions for a specified time (approximately two hours) during the assigned session. Specific requirements for presentations will be provided at a later date.

General Information

1. Competition entrants cannot have graduated more than a year prior to the deadline for submitting abstracts.
2. Accredited universities or colleges must deal with environmental, food or dairy sanitation, protection or safety research.
3. The work must represent original research completed and presented by the entrant.
4. Entrants may enter only one paper in either the oral or poster competition.
5. All entrants must register for the Annual Meeting and assume responsibility for their own transportation, lodging, and registration fees.
6. Acceptance of your abstract for presentation is independent of acceptance as a competition finalist. Competition entrants who are chosen as finalists will be notified of their status by the chairperson by May 27, 2005.
7. All entrants with accepted abstracts will receive a complimentary, one-year Student Membership. This membership will entitle you to receive *JFP* Online.
8. In addition to adhering to the instruction in the "Call for Abstracts," competition entrants must check the box to indicate if the paper is to be presented by a student in this competition. A signature and date is required from the major professor or department head.

Judging Criteria

A panel of judges will evaluate abstracts and presentations. Selection of up to ten finalists for each competition will be based on evaluations of the abstracts and the scientific quality of the work. All entrants will be advised of the results by May 27, 2005. Only competition finalists will be judged at the Annual Meeting and will be eligible for the awards.

All other entrants with accepted abstracts will be expected to be present as part of the regular Annual Meeting. Their presentations will not be judged and they will not be eligible for the awards.

Judging criteria will be based on the following:

1. Abstract – clarity, compre hensiveness and conciseness.
2. Scientific Quality – Adequacy of experimental design (methodology, replication, controls), extent to which objectives were met, difficulty and thoroughness of research, validity of conclusions based upon data, technical merit and contribution to science.
3. Presentation – Organization (clarity of introduction, objectives, methods, results and conclusions), quality of visuals, quality and poise of presentation, answering questions, and knowledge of subject.

Finalists

Awards will be presented at the International Association for Food Protection Annual Meeting Awards Banquet to the top three presenters (first, second and third places) in both the oral and poster competitions. All finalists are expected to be present at the banquet where the awards winners will be announced and recognized.

Awards

First Place – $500 and an engraved plaque
Second Place – $ 300 and a framed certificate
Third Place – $100 and a framed certificate

Award winners will receive a complimentary, one-year Student Membership including *Food Protection Trends, Journal of Food Protection,* and *JFP* Online.

Policy on Commercialism

for Annual Meeting Presentations

1. INTRODUCTION

No printed media, technical sessions, symposia, posters, seminars, short courses, and/or other related types of forums and discussions offered under the auspices of the International Association for Food Protection (hereafter referred to as to Association forums) are to be used as platforms for commercial sales or presentations by authors and/or presenters (hereafter referred to as authors) without the express permission of the staff or Executive Board. The Association enforces this policy in order to restrict commercialism in technical manuscripts, graphics, oral presentations, poster presentations, panel discussions, symposia papers, and all other type submissions and presentations (hereafter referred to as submissions and presentations), so that scientific merit is not diluted by proprietary secrecy.

Excessive use of brand names, product names or logos, failure to substantiate performance claims, and failure to objectively discuss alternative methods, processes, and equipment are indicators of sales pitches. Restricting commercialism benefits both the authors and recipients of submissions and presentations.

This policy has been written to serve as the basis for identifying commercialism in submissions and presentations prepared for the Association forums.

2. TECHNICAL CONTENT OF SUBMISSIONS AND PRESENTATIONS

2.1 Original Work

The presentation of new technical information is to be encouraged. In addition to the commercialism evaluation, all submissions and presentations will be individually evaluated by the Program Committee chairperson, technical reviewers selected by the Program Committee chairperson, session convenor, and/or staff on the basis of originality before inclusion in the program.

2.2 Substantiating Data

Submissions and presentations should present technical conclusions derived from technical data. If products or services are described, all reported capabilities, features or benefits, and performance parameters must be substantiated by data or by an acceptable explanation as to why the data are unavailable (e.g., incomplete, not collected, etc.) and, if it will become available, when. The explanation for unavailable data will be considered by the Program Committee chairperson and/or technical reviewers selected by the Program Committee chairperson to ascertain if the presentation is acceptable without the data. Serious consideration should be given to withholding submissions and presentations until the data are available, as only those conclusions that might be reasonably drawn from the data may be presented. Claims of benefit and/or technical conclusions not supported by the presented data are prohibited.

2.3 Trade Names

Excessive use of brand names, product names, trade names, and/or trademarks is forbidden. A general guideline is to use proprietary names once and thereafter to use generic descriptors or neutral designations. Where this would make the submission or presentation significantly more difficult to understand, the Program Committee chairperson, technical reviewers selected by the Program Committee chairperson, session convenor, and/or staff, will judge whether the use of trade names, etc., is necessary and acceptable.

2.4 "Industry Practice" Statements

It may be useful to report the extent of application of technologies, products, or services; however, such statements should review the extent of application of all generically similar technologies, products, or services in the field. Specific commercial installations may be cited to the extent that their data are discussed in the submission or presentation.

2.5 Ranking

Although general comparisons of products and services are prohibited, specific generic comparisons that are substantiated by the reported data are allowed.

2.6 Proprietary Information (See also 2.2.)

Some information about products or services may not be publishable because it is proprietary to the author's agency or company or to the user. However, the scientific principles and validation of performance parameters must be described for such products or services. Conclusions and/or comparisons may be made only on the basis of reported data.

2.7 Capabilities

Discussion of corporate capabilities or experiences are prohibited unless they pertain to the specific presented data.

3. GRAPHICS

3.1 Purpose

Slides, photographs, videos, illustrations, art work, and any other type visual aids appearing with the printed text in submissions or used in presentations (hereafter referred to as graphics) should be included only to clarify technical points. Graphics which primarily promote a product or service will not be allowed. (See also 4.6.)

3.2 Source

Graphics should relate specifically to the technical presentation. General graphics regularly shown in, or intended for, sales presentations cannot be used.

3.3 Company Identification

Names or logos of agencies or companies supplying goods or services must not be the focal point of the slide. Names or logos may be shown on each slide so long as they are not distracting from the overall presentation.

3.4 Copies

Graphics that are not included in the preprint may be shown during the presentation only if they have been reviewed in advance by the Program Committee chairperson, session convenor, and/or staff, and have been determined to comply with this policy. Copies of these additional graphics must be available from the author on request by individual attendees. It is the responsibility of the session convenor to verify that all graphics to be shown have been cleared by Program Committee chairperson, session convenor, staff, or other reviewers designated by the Program Committee chairperson.

4. INTERPRETATION AND ENFORCEMENT

4.1 Distribution

This policy will be sent to all authors of submissions and presentations in the Association forums.

4.2 Assessment Process

Reviewers of submissions and presentations will accept only those that comply with this policy. Drafts of submissions and presentations will be reviewed for commercialism concurrently by both staff and technical reviewers selected by the Program Committee chairperson. All reviewer comments shall be sent to and coordinated by either the Program Committee chairperson or the designated staff. If any submissions are found to violate this policy, authors will be informed and invited to resubmit their materials in revised form before the designated deadline.

4.3 Author Awareness

In addition to receiving a printed copy of this policy, all authors presenting in a forum will be reminded of this policy by the Program Committee chairperson, their session convenor, or the staff, whichever is appropriate.

4.4 Monitoring

Session convenors are responsible for ensuring that presentations comply with this policy. If it is determined by the session convenor that a violation or violations have occurred or are occurring, he or she will publicly request that the author immediately discontinue any and all presentations (oral, visual, audio, etc.) and will notify the Program Committee chairperson and staff of the action taken.

4.5 Enforcement

While technical reviewers, session convenors, and/or staff may all check submissions and presentations for commercialism, ultimately it is the responsibility of the Program Committee chairperson to enforce this policy through the session convenors and staff.

4.6 Penalties

If the author of a submission or presentation violates this policy, the Program Committee chairperson will notify the author and the author's agency or company of the violation in writing. If an additional violation or violations occur after a written warning has been issued to an author and his agency or company, the Association reserves the right to ban the author and the author's agency or company from making presentations in the Association forums for a period of up to two (2) years following the violation or violations.

NEW MEMBERS

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São Pãulo

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Jennifer Crossley
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Aamir M. Fazil
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UPDATES

Leslye M. Fraser, S.M., J.D., Named Director of FDA's Office of Regulations and Policy, Center for Food Safety and Applied Nutrition

The Food and Drug Administration (FDA) has announced that its Center for Food Safety and Applied Nutrition (CFSAN) has appointed Leslye M. Fraser, S.M., J.D., as the director of the Office of Regulations and Policy.

In this capacity, Ms. Fraser will provide leadership for FDA's food and cosmetic regulations, guidance documents and policy development and will provide management oversight for international activities.

Prior to acceptance of this appointment, Ms. Fraser served as CFSAN's associate director for regulations, Office of Regulations and Policy, since May 2001. Before joining FDA, Ms. Fraser was assistant general counsel for Regulatory Issues at the United States Environmental Protection Agency. There she provided legal counsel to senior agency officials and led a group of staff attorneys who counseled all agency program and regional offices on rulemaking requirements contained in regulatory statues and Presidential executive orders. She also worked at the international law firm Gibson, Dunn and Crutcher as an associate attorney and at a large aerospace company, TRW, as a research engineer and a section and project manager.

Ms. Fraser received her Bachelor and Master of Science Degrees in chemical engineering from the Massachusetts Institute of Technology, and her Juris Doctor degree with honors from the University of California at Los Angeles School of Law. She is a member of the State Bar of California and the Bar of the District of Columbia.

Ms. Fraser replaces L. Robert Lake, Esq., who retired on September 3, 2004.

Christopher G. Toomey Joins Nilfisk-Advance America as Application Engineer

Nilfisk-Advance America announced that Christopher G. Toomey has joined its technical department as application engineer.

In this position, Toomey will assist the sales department by designing process-integrated vacuum systems and central vacuum systems that meet customers' application-specific needs. In addition, he will work to expand the company's engineering capabilities by developing new vacuums and enhancing existing vacuums with new features.

Prior to joining Nilfisk-Advance America, Toomey spent six years at Lutron Electronics, Coopersburg, PA, where he held a number of positions, including technical support engineer, application engineer, and inside sales representative. In these roles, Toomey supported customers with a variety of Lutron product applications.

Toomey, who resides in Coopersburg, PA, graduated with a Bachelor of Science degree in mechanical engineering from Lafayette College, Easton, PA. He also holds a Pennsylvania Engineer-in-Training certification from the State Registration Board of Professional Engineering, Land Surveyors, and Geologists.

Mark Lichter Promoted to National Sales Manager for Fortitude Brands

Fortitude Brands LLC is proud to announce the recent promotion of Mark Lichter to national sales manager. Mark's experience includes working as a broker, representative, a regional sales manager and a national sales manager for a variety of companies, such as Chiquita Brands, Matlaw's Food Products and Caesar Cardini Salad Dressings, encompassing frozen, grocery, retail, foodservice, specialty for over three decades.

As national sales manager, Mark will manage the national expansion of Fortitude Brands LLC, through the establishment of a broker network concentrating on specialty and natural food distributors and their retail customers. He will be responsible for the management, development, mentoring and coaching of the sales team.

The Continual Challenge of Emerging Infectious Diseases

Emerging infectious diseases, which have shaped the course of humanity and caused incalculable suffering and death, will continue to confront society in unpredictable ways as long as humans and microbes co-exist, write authors from the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health in a review article published in the July 8 issue of the journal *Nature*.

In their paper, the authors classify three types of emerging infections and consider methods for their control: newly emerging infections (e.g., HIV, SARS); re-emerging/resurging infections (e.g., influenza, West Nile virus); and deliberately emerging infections (e.g., microbes used for bioterror).

The authors note that emerging infectious diseases are superimposed on a constant backdrop of established infections. Approximately 15 million deaths in 2002 were directly attributable to infections, according to the World Health Organization. Tragically, the authors point out, the burden of all infections falls most heavily on those least able to manage them: people living in developing countries, especially infants and children, and indigenous and disadvantaged minorities in developed countries.

Why do infectious diseases emerge and re-emerge? The viruses, bacteria and parasites that cause these diseases continually and sometimes dramatically change over time. The authors note that emergence results from "...dynamic interactions between rapidly evolving infectious agents and changes in the environment and in host behavior that provide such agents with favorable new ecological niches." As a result, new pathogens arise, and familiar ones re-emerge with new properties or in unfamiliar settings.

Historically, the authors write, the results have been devastating. For example, importation of smallpox into Central America caused 10–15 million deaths in 1520–1521, effectively ending Aztec civilization. AIDS, first recognized in 1981, now threatens to surpass in global fatality the "Black Death" of the 14th century and the influenza pandemic of 1918–1919, two notable infections that emerged to each kill tens of millions of people.

In the past five years alone, two pathogens well known to countries on other continents were seen in the Unites States for the first time — West Nile virus and monkeypox virus. In addition, a new infectious disease, SARS, emerged in 2003 and has since caused more than 8,000 cases of illness and nearly 800 deaths around the world. In addition, in 2001 the United States was confronted with a third, extremely disquieting category of threat: a disease resulting from the deliberate release of an infectious agent, anthrax, by a terrorist(s).

The authors write that an effective response to any new infectious disease threat, whether it emerges, re-emerges, or is deliberately introduced, involves mobilizing many different types of public health activities. In particular, frontline surveillance and response is critical and depends on rapid detection, clinical diagnosis and containment. Concomitantly, basic and applied research enables the development of medical countermeasures such as surveillance tools, diagnostic tests, vaccines and therapeutics. The authors note that these efforts have been accelerated by advances in fields such as genomics/proteomics, nanotechnology, direct and computational structural determination, immunology, and geographical information systems and satellite imaging.

USDA Awards More Than 12 Million in Integrated Food Safety Grants

Agriculture Secretary Ann M. Veneman has announced that more than $12 million has been awarded to 19 colleges and universities throughout the US and its territories through the National Integrated Food Safety Initiative (NIFSI). "The selection of these projects supports the Bush Administration's efforts to enhance the protection and safety of agriculture and the food supply," said Veneman during a visit here to dedicate a National 4-H Monument where she discussed the importance of today's youth choosing careers in food and agriculture fields, including the areas of research. "Targeted research is one of several key initiatives we are implementing to enhance food safety and improve food inspection systems."

These projects were selected for funding under USDA's unified food safety research agenda announced November 2003, to improve the efficiency and effectiveness of food safety programs. The unified agenda prioritizes research needs and maximizes use of available resources and involves coordination among the Food Safety and Inspection Service, (FSIS) the Agricultural Research Service (ARS) and the Cooperative State Research, Education and Extension Service (CSREES). FSIS is a public health regulatory agency that protects consumers by ensuring that meat, poultry and egg products are safe, wholesome and accurately labeled. FSIS does not conduct its own research. Rather, the Agency identifies research necessary to fulfill its public health mission. The Agricultural Research Service is USDA's chief in-house scientific research agency and the Cooperative State Research, Education and Extension Service is USDA's chief research funding agency.

The purpose of the NIFSI, which is managed by CSREES, is to support competitive projects that address priority issues in food safety that are best solved using an integrated approach. These projects address a broad spectrum of food safety concerns from on-farm production, post-harvest processing and distribution, to food selection, preparation and consumption.

The grants make sure that food safety information is passed on to people who operate various parts of the food chain. Twenty-six grants have been awarded for Fiscal Year 2004. Each year NIFSI awards these funds to faculty at land-grant and non-land grant colleges and universities to ensure that valuable research, education and extension knowledge is transferred to teachers, scientists, health professionals, researchers, farmers, food processors, foodservice workers, consumers and all others making crucial decisions about the safety of the US food supply. An average of approximately $630,000 was awarded to each university to support integrated food safety projects.

To learn more about the integrated food safety program at CSREES, visit http://www.csrees.usda.gov/nea/food/in_focus/safety_if_national.html.

Listeria Can Persist in Stores and Processing Plants

Despite the efforts of food retailers and food-processing plant managers to maintain a clean, safe environment, strains of the deadly pathogen *Listeria monocytogenes* can persist for up to a year or longer, according to Cornell University food scientists in the latest issue of *Journal of Food Protection* (July 2004).

"This is disturbing because this points the finger at retail stores and some processors as a continuing source of food contamination," says Brian D. Sauders, a Cornell doctoral candidate in food science, who worked on the study with Martin Wiedmann, D.V.M., Cornell assistant professor of food science.

Sauders and Wiedmann examined specific strains of *L. monocytogenes* that had been found in 125 foods in 50 retail food stores and seven food-processing plants in New York state examined by inspectors of the New York State Department of Agriculture and Markets. The inspectors found the bacteria during routine surveys, sanitary inspections and as a result of consumer complaints between 1997 and 2002. *Listeria* can cause listeriosis, a deadly disease that primarily affects pregnant women, newborn children, and adults with weakened immune systems.

Each year in the United States about 2,500 people are infected, of which one-fifth die. Pasteurization and cooking kill the bacterium. The foods in which *Listeria* was found included ready-to-eat delicatessen foods like ham, beef bologna, chicken, pastrami, roast beef and smoked fish.

It also was found in hummus, imitation crab, cheeses and in foods requiring cooking before consumption, such as hot dogs and raw foods including beef, ground chuck, turkey, lobster tails and shrimp. The bacterium was found directly on food in 47 of 50 retail food stores, including 20 food stores where the bacterium was found on several foods.

When the 50 stores were re-inspected weeks, months or even a year later, about 34 percent had persistence of the same strains of *Listeria*. Of the seven food-processing plants where *Listeria* was found, three had persistent strains of the bacterium.

Wiedmann explains that food retailers have a harder time controlling for *Listeria* than do food processors. Food processors can control for people entering the plant, while retailers cannot always control the pathogens introduced by customers and employees. "*Listeria* is a very hardy organism. Even if you think you're doing a good job of cleaning and getting rid of *Listeria*, it is likely to return. Normal cleaning and even super cleaning does not always get rid of

it. It's a tribute to *Listeria's* ability to survive," says Wiedmann.

The study is intended to help state health departments track the origins of listeriosis. "While our understanding of the ecology of (*Listeria*) has clearly improved over the last decade, considerable gaps still exist in our understanding of the transmission of human listeriosis. For example, our knowledge of the contributions of food contamination with *Listeria* at retail, at restaurants, and at home is extremely limited," writes Sauders in the study.

In addition to Sauders and Wiedmann, the article (titled "Distribution of *Listeria monocytogenes* Molecular Subtypes Among Human and Food Isolates from New York State Shows Persistence of Human Disease-Associated *Listeria monocytogenes* Strains in Retail Environments") was authored by Kurt Mangione, Curtis Vincent, Jon Schermerhorn and Claudette M. Farchione of the New York State Department of Agriculture and Markets; Nellie B. Dumas and Dianna Bopp of the New York State Department of Health; Laura Kornstein of the New York City Department of Health; and Esther Fortes and Katy Windham of Cornell. Funding for the research came from the US Department of Agriculture and the National Institutes of Health.

Certain Symptoms May Predict Fatal Foodborne Botulism

A distinctive group of symptoms—shortness of breath, impaired gag reflex, and absence of diarrhea—may be predictive of severe outcomes, including death, from foodborne botulism, a group of researchers reported recently. The authors, from the Centers for Disease Control and Prevention, National Center of Disease Control, Tbilisi, and the Republic of Georgia, collected data from the medical records of 706 patients hospitalized in the Republic of Georgia with botulism from 1980 through 2002. The country has the highest reported incidence of foodborne botulism in the world (0.9 cases per 100,000 population), according to the report.

Patients were considered to have botulism if this was listed as the final diagnosis. A trained epidemiologist completed a form about each patient that included patient demographic characteristics; medical history; history of present illness; physical examination findings at admission; clinical course, including complications, adverse reactions, and death; suspected source of disease; and results of laboratory tests.

The most common symptoms at admission were found to be fatigue (90%), muscle weakness (89%), and difficulty swallowing (81%). Ophthalmoplegia, ptosis, and slurred speech were the most common physical examination findings, present in 79%, 76%, and 58% of patients, respectively. Among the 705 patients for whom final outcome was known, 54 (8%) died. The group of symptoms classically considered to be predictive of botulism — nausea and vomiting; dysphasia; diplopia; dry mouth; and fixed, dilated pupils, was present in only 2% of patients.

Classification and regression tree (CART) analysis was used to find clinical syndromes at presentation that were predictive of survival or death. In an analysis limited to patient age, signs, and symptoms, it was found that a history of shortness of breath or vomiting and normal facial muscle strength on physical examination at admission was 100% predictive of survival (odds ratio for death, 0%, $P<.01$). The clinical syndrome most predictive of death included shortness of breath, impaired gag reflex, and absence of diarrhea; patients who died were 22.6 times more likely to have this syndrome than were those who survived (95% confidence interval, 22–48).

Because botulism is among the diseases considered most likely for use as a bioterrorist weapon, any clues to outcome, such as those in this study, could be useful in triage in a mass-casualty setting. For example, in such an emergency situation, rapid transport to a facility providing higher-level care might be justifiably reserved for patients with the triad of symptoms identified as predictive of death.

The authors point out a number of limitations to their study and stress that validation outside of Georgia is needed. Varma, J. K., G. Katsitadze, and M. Moiscrafishvili, et al. Signs and symptoms predictive of death in patients with foodborne botulism — Republic of Georgia, 1980–2002. Clin. Infect. Dis. 2004 Aug 1:39(3):357–62 [abstract].

Targeting *E. coli* Bacteria at Their Source

Agricultural Research Service scientists and colleagues are looking inside the cow in order to spot—and to stop—bacteria that cause a particularly nasty *E. coli*-related disease. Microbiologist Evelyn Dean-Nystrom and Veterinary Medical Officer William Stoffregen of the ARS National Animal Disease Center (NADC) in

Ames, IA, are pinpointing where microbes called enterohemorrhagic *Escherichia coli* O157:H7 lurk in calves.

Also, Nystrom is helping researchers at the Uniformed Services University of the Health Sciences in Bethesda, MD, develop and test an oral vaccine that eliminates these bacteria from cattle. *E. coli* O157:H7 is the most common infectious cause of bloody diarrhea in people in the United States. Hemolytic uremic syndrome, a potential consequence of its infection, is the primary cause of acute kidney failure in US children.

Undercooked or raw ground beef has been implicated in many *E. coli* O157:H7 outbreaks in humans. However, the causative bacteria have almost no discernable effect in cattle, making them hard to detect there.

Nystrom and Stoffregen found that, in addition to intestines, calves' gall bladders may be a good place to check whether an *E. coli* O157:H7 infection has taken place. This finding indicates that including gall bladders in samples cultured for *E. coli* O157:H7 may help identify infected cattle at slaughter.

The oral vaccine, developed at the Bethesda University by graduate student Nicole A. Judge, uses intimin, a protein on the outer membrane of the O157:H7 strain that the *E. coli* bacteria need for attaching themselves to intestinal tissue. Nystrom assisted with development of the vaccine — supervised by microbiologist and department chair Alison O'Brien — early on, by showing that calves injected with purified bacterial intimin would develop antibodies against it. Nystrom works in NADC's Preharvest Food Safety and Enteric Diseases Research Unit, while Stoffregen works in the center's Bacterial Diseases of Livestock Research Unit.

Read more about the research in the August issue of *Agricultural Research* magazine, available online at: http://www.ars.usda.gov/is/AR/archive/aug04/ecoli0804.htm.

Wine-making Waste: A Natural Weapon to Beat Bacteria

Pomace consists of grape seeds, skin and stems, and is a rich source of polyphenols. Phenolic substances are known to reduce the risk of heart disease and cancer by inhibiting human low-density lipoproteins. Pomace is already used as an important by-product of wine-making in the production of foods such as vinegar and molasses.

Bacterial tests — Pomace from the most popular Turkish grape cultivars, Kalecik karasi and Emir, was collected and tested against 14 types of common bacteria, including *Escherichia coli and Staphylococcus aureus* species, by Osman Sagdic and his team at Erciyes University and Suleyman Demirel University, Turkey. The grape pomace extracts gave effective anti-bacterial results when tested on all bacteria species at a concentration of five percent, although the effects varied according to concentration, method and cultivars used.

Natural preservatives — "The extracts can be used in food formulations to protect food against spoilage bacteria. People prefer natural preservatives in the place of synthetic counterparts in food," says researcher Dr. Sagdic.

"The world is always ready for better and more natural food preservatives. What we need to do now is to find a suitable food to put it in. The appearance and taste of the final product must be acceptable to the consumers," says Dr. Yiu-Wai Chu, Biotechnology Group, Society of Chemical Industry.

FSIS Establishes New Institute to Promote Food Safety Education, Information, Communication and Outreach in the Americas

Under Secretary for Food Safety Dr. Elsa Murano has announced the establishment of the Food Safety Institute of the Americas, a cooperative educational and research organization designed to promote food safety and identify and develop educational programs throughout the Americas.

Murano said the institute demonstrates the commitment of Agriculture Secretary Ann M. Veneman to improving public health throughout the hemisphere by making meat, poultry and egg products safer. "Secretary Veneman challenged us to think broadly when it comes to improved food safety standards," Murano said. "This institute will become a forum for scientific discussion and educational opportunities for government and industry in all countries to improve the safety of imported and exported meat, poultry and egg products."

Murano has worked hard to improve food safety programs in the Western Hemisphere since being named to her post in the fall of 2001. Through speeches and personal contacts, she has worked to convince governments to raise the level of food safety and to become active participants in international food standard setting bodies like the Codex Alimentarius Commission.

In June, Murano signed a Memorandum of Understanding with the Pan American Health Organization to improve the safety of meat and poultry products that are traded among the nations of the Western Hemisphere. The establishment of the Institute was also one of the goals listed in the recently released "Fulfilling the Vision: Initiatives in Protecting Public Health," a document that reviews recent successes and builds on the course the Bush Administration set last year to improve the prediction and response to food safety challenges and further reduce the rate of foodborne illness.

Murano said that many organizations–academic, governmental and nongovernmental will be active partners in the FSIA. Food safety subject matter areas like public health, food security, Codex and animal and food production will be grouped into "colleges and departments" within the FSIA and entrusted to centers of academic expertise. The FSIA will also tap into existing networks of universities and organizations within North America, Central America, South America and the Caribbean.

"We want to bring people together and incorporate the best existing training and education programs available to promote efficiency and avoid duplication. By using existing expertise, we can place a greater emphasis on developing materials to fill gaps in food safety education and information," Murano said.

The Food Safety Institute of the Americas will be located in Miami, FL, which is recognized as the gateway to the Americas. This location will enhance the institute's ability to bring together experts quickly and to develop and carry out programs efficiently. Linda Swacina, presently deputy administrator of FSIS, will serve as the senior agency representative and federal coordinator of all FSIA activities. Ms. Swacina, who holds degrees in Spanish and Latin American studies, has traveled extensively throughout Central and South America on behalf of FSIS food safety programs and has extensive experience and understanding of international food safety standard setting activities.

INDUSTRY PRODUCTS

Anver Corporation

Anver Corporation Fiber Drum Lifter Designed for Clean Rooms

A new vacuum tube lifter designed for handling fiber drums in clean rooms and other environments requiring all-stainless steel construction is being introduced by Anver Corporation.

Anver VT180 Clean Room Drum Handlers are vacuum tube lifters that feature electropolished 304 stainless steel construction, a white hose cover, and can include pick-up heads with an adjustable side-support for fiber drums. Featuring interchangeable vacuum heads with quick-disconnects, they can adapt to a variety of drum sizes weighing up to 500 lbs (227 kg).

Equipped with an ergonomic handle with adjustable up/down controls for easy operation, Anver VT180 Clean Room Drum Handlers swivel 360° under vacuum for optimum maneuverability and provide a 100% continuous duty cycle. Vacuum pumps are remote and have a wash-down coating for easy cleaning, while a wide range of vacuum pad materials, shapes, and sizes are offered.

Anver Corporation
800.654.3500
Hudson, MA
www.anver.com

Chr. Hansen Expands Probiotic Product Portfolio with Probio-Tec® AB-tab-4-Tablets

In response to consumer demands, Chr. Hansen expands their probiotic product and introduces Priobio-Tec® AB-tab-4 tablets. The formulation contains two clinically documented probiotic cultures, LA-5® *Lactobacillus acidophilus* and BB-12® *Bifidobacterium*, and assists in strengthening, maintaining and restoring the intestinal microflora.

"Our goal is to offer a broad scope of products to satisfy consumers all over the world," states Margaret Connor, VP of sales for Human Health & Nutrition at Chr. Hansen. "Consumers are increasingly become aware of probiotics and their positive effect on gastrointestinal health. Our line of Probio-Tec probiotics in capsule form are very well received by consumers, but some consumers prefer tablets, and several of our dietary supplement and pharmaceutical customers have requested a tablet formulation. That's why we decided to extend the product line and develop a formulation containing our premier probiotic strains in this new product form."

"Developing a probiotic tablet can be more sophisticated than producing a sample. The challenge was to formulate a tablet that was fairly small and easy to swallow — and at the same time allow the live probiotic bacteria to survive passage through the stomach and into the small intestine. Our new Probio-Tec tablet is a targeted delivery system that keeps the probiotic bacteria within the tablet matrix and protected against gastric acid during passage through the stomach. When the tablet reaches the small intestine, the viable probiotic bacteria are released and start working. Another challenge was to ensure that the probiotic bacteria survive the tableting process, which can be detrimental to live microorganisms. We have successfully overcome this obstacle, and have applied for a patent on our process," says Pete Budde, technical manager for probiotics for Chr. Hansen.

Chr. Hansen, Inc.
800.558.0802
Milwaukee, WI
www.chr-hansen.com

EnviroTrans™ from Hardy Diagnostics

The Hardy Diagnostics EnviroTrans™ is a ready-to-use sampling system with everything you need to collect samples from work surfaces and machinery. The EnviroTrans™ is designed for

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sampling surfaces as a part of your HACCP program, for environmental monitoring, and other microbiological studies. EnviroTrans™ makes sampling easy and convenient. Simply unscrew the cap and pull out the affixed Dacron-tipped swab, sample your surface, recap, and send it to the lab. The sterilized EnviroTrans™ comes ready-to-use and pre-filled with your choice of four formulas; Neutralizing Buffer, Letheen Broth, De-Neutralizing Broth, or 0.85% Saline.

Hardy Diagnostics
800.266.2222 ext. 7696
Santa Maria, CA
www.hardydiagnostics.com

Labconco Corporation Purifier® Class I Safety Enclosures Protect Users from Agents That Require Biosafety Level 1, 2 or 3 Containment

Labconco Corporation presents the Purifier Class I Safety Enclosures, which provide user protection from agents that require Biosafety level 1, 2 or 3 containment. Additionally, it serves as an economical alternative to Class II biological safety cabinets when applications do not require product protection.

Class I Enclosures include an ultraviolet light that may be used in conjunction with surface disinfection to ensure thorough decontamination while the enclosure is unattended.

Class I Enclosures include an Exhaust HEPA filter proven to be 99.99% efficient on 0.3 micron particles and patented Clean-Sweep air foil and rear baffle to direct airflow for maximum containment. They are available in 2-, 3- and 4-foot widths.

Tests conducted to confirm the performance of the Purifier Class I Enclosure include a biological challenge per NSF Standard number 40 Personal Protection Test, independent particulate containment resting and tracer gas containment conforming to ASHREA 110-1995.

Labconco Corporation
800.821.5525
Kansas City, MO
www.labconco@labconco.com

Sigma-Aldrich Introduces the RapidTransit™ Transformation Kit

Sigma-Aldrich Corporation has completed development on the new RapidTransit™ Transformation Kit. RapidTransit provides a method to quickly prepare custom competent cells at efficiencies suitable for standard cloning purposes.

The RapidTransit system allows for the streamlined and rapid preparation of chemically competent cells from overnight cultures. The key to the system is the RapidTransit Transformation Buffer, which quickly prepares cells for uptake of DNA without the traditionally heat shock step. RapidTransit is particularly useful for large format studies that require large amounts of chemically competent cells. In addition, RapidTransit may be used in the preparation and transformation of competent cells from a variety of *Escherichia coli* strains such as K12 cloning strains and B strains used for recombinant protein expression. With RapidTransit, researchers are not limited to commercial strains if convenience is required. The system eliminates complex buffer preparation and lengthy incubation periods found in traditional methods while reducing the number of procedural steps. With few steps and no heat shock, reproducible transformation efficiencies suitable for cloning are achieved, making RapidTransit a convenient method for standard cloning and transformation methods.

Sigma-Aldrich Corporation
800.521.8956
St. Louis, MO
www.sigma-aldrich.com

Xenon Corporation Pulsed UV Sterilization System Employs Fast, Mercury-Free UV Kill Technology

A pulsed UV system for R/D laboratories involved with the bio-reduction and sterilization of air, water, food, pharmaceuticals, and surface decontamination is available from Xenon Corporation.

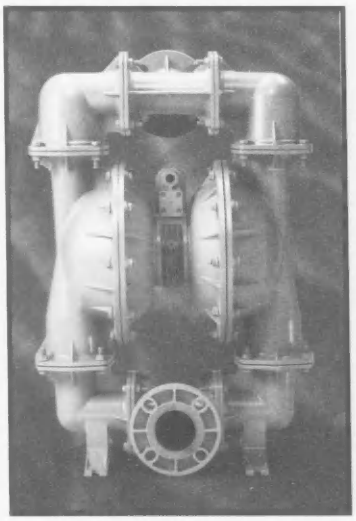
The SteriPulse-XL® Pulsed UV/Visible Light System is a bench-top unit that delivers high peak energy which is 100,000 times more powerful than the sun and employs mercury-free, UV kill technology. Featuring a rapid, low-heat process, three pulses in under 1 s produced a >log 6 kill for *Bacillus subtilis*, demonstrating how this benign technology provides a much higher rate of sterilization than standard continuous UV lamp exposure.

Suitable for a broad range of sterilization and decontamination research involving a wide variety of products, the SteriPulse-XL® Pulsed UV/Visible Light System has a slide-out tray and permits the use of small laboratory samples to provide proof-of-principle validation.

Be sure to mention, "I read about it in *Food Protection Trends*"!

Applications include biological warfare, terminal sterilization, medical devices, barrier isolators, closed containers, involving air, water, food, and pharmaceuticals.

Xenon Corporation
800.936.6695
Woburn, MA
www.xenoncorp.com



Versa-Matic Pump Company

Versa-Matic's New E3 Aluminum Bolted Pump Ensures Reliability in the Handling of Large Fluid Volumes

Versa-Matic® announces the new E3 Aluminum Bolted Pump offering a leak-free design to enhance reliability and reduce downtime when pumping large volumes of fluid. Part of Versa-Matic's line of dependable, self-priming E3 3" pumps, the aluminum bolted pump ensures continuous operation by employing an innovative air valve system that eliminates stalling, icing, and the need to lubricate.

The pump's leak-free bolted design provides a greater positive seal, effectively handling large volumes in fluid in applications such as bulk chemical transfer, resins transfer, wastewater treatment, filter press applications, and tanker car unloading.

Capable of handling infinite variable flows up to 90% solids, the E3 Aluminum Bolted Pump features a maximum particle clearance of 3/4" (19.1 mm). Flange connections that conform to both ANSI 150# and DIN #80 standards enable the pump to handle a flow rate that's adjustable to 230 gpm (871 lpm).

Versa-Matic Pump Company
724.327.7867
Export, PA
www.versamatic.com

First New Food Products Introduced with Cognis' TONALIN® CLA for Optimal Body Composition

Cognis Nutrition and Health, announced that the TONALIN® brand of CLA (Conjugated Linoleic Acid) is being introduced for the first time in food. Two recent developments have generated interest in TONALIN® among food manufacturers: Self-affirmed Generally Recognized As Safe (GRAS) status, and publication of a new landmark study. Cognis now offers the food industry one of the most extensive lines of CLA ingredients available for a broad range of approved food applications.

TONALIN® helps consumers reduce body fat, maintain lean body mass, prevent fat regain and contribute to improved overall health.

A panel of independent experts has found that TONALIN® is safe for use in yogurt, meal replacement bars and drinks, fruit juices, chocolate, milk-based beverages and coffee cream substitutes. TONALIN® CLA was introduced for the first time in food products in a new line from the Spanish dairy, Corporacion Alimentaria Penasanta (CAPSA), called NATURLINEA, including a milk and orange juice-milk blend. The TONALIN® logo is featured on the milk carton that contains a claim for helping to reduce body fat.

Franz Timmerman, Cognis' global market segment manager, functional food and medicinal nutrition, explained that Spain has a sizeable fortified milk market. "We are focusing on the dairy industry as CLA occurs naturally in milk and works well with milk-based products."

Interest in TONALIN® CLA has been spurred by consumers' increased demand for safe, natural, non-stimulant products for reducing unwanted inches. Cognis produces TONALIN® CLA through a new proprietary process that converts linoleic acid from safflowers into CLA which provides the highest quality of CLA available. TONALIN® CLA contains the lowest amount of non-naturally occurring isomers, a patent-protected attribute that provides food manufacturers with the highest levels of ingredients known to be beneficial. CLA is available in several product forms – oil, water-dispersible powder and

emulsions – that offer food manufacturers a broad range of options for their new products.

Cognis Nutrition and Health
847.945.0101
Las Vegas, NV
www.cognis.com

Thermo Electron's Orion ROSS Ultra® Screw Cap pH Electrodes Extended Line is Now Available

Thermo Electron Corporation introduces the Orion ROSS Ultra screw cap electrodes, extending the premium Orion ROSS Ultra combination pH and reference electrode line.

Researchers, QA/QC and general lab technicians are finding the virtually drift-free ROSS Ultra electrode is the best electrode they have ever used. The fast response time and equilibration provide faster sample throughput. The drift-free reference assures fewer calibrations, again translating to faster throughput and cost savings per test. The ROSS Ultra pH line has grown again to accommodate applications such as titrations, where a screw-capped electrode is preferred. ROSS Ultra electrodes use a unique reference system developed by Thermo that offers longer life, greater stability, and fast results, regardless of sample composition or temperature. ROSS Ultra-pH electrodes are available in a rugged and standard glass bulb, flat surface, semi-micro and epoxy bodied styles to best determine the pH of a variety of sample types, now with a screw cap option for time saving in the laboratory.

A ROSS Ultra half-cell reference electrode is also available for applications where separate sensor and reference electrodes are preferred. ROSS Ultra combination pH electrodes are also available as Orion meter and electrode packages. Due to the outstanding innovations and performance of these electrodes, the ROSS Ultra line carries twice the warranty due to greater stability than its predecessor electrodes.

The ROSS Ultra pH line includes the following features: (1) Unmatched drift-free reference system; (2) Unparalleled pH response to temperature changes, (3) Handles even the most difficult samples, and (4) Extended 24 month warranty.

Thermo Electron Corporation
978.232.6057
Chicago, IL
www.thermo.com

Intervent Offers Anti-Microbial Technologies for Food and Beverage Manufacturers

Intervent, the new food safety technology and consulting arm of BOC is providing food and beverage manufacturers with a single source for addressing their plants' food safety needs.

Intervent offers proven ozone and ultraviolet light technologies specifically designed for use in food and beverage plants, and also provides HACCP and food science consulting services.

"Intervent focuses on a key area of concern to food and beverage processors — food safety. Intervent offers processors a range of validated technologies that are integrated seamlessly into a plant's total environment to treat atmospheres, food, food contact surfaces, including beverage bottles, and processing fluids for protection against pathogens and spoilage organisms. We also facilitate processors' compliance with USDA regulations by evaluating their plant's risk factors, incorporating technologies to lower that risk and assisting with USDA acknowledgment of their HACCP and GMP plans," Mark DiMaggio, BOC business manager said.

"These consulting services, combined with Intervent's anti-microbial technologies provide a powerful weapon for food processors to meet HACCP performance thresholds and federal directives for *Listeria* and *E. coli*," DiMaggio said.

BOC
908.771.1510
Murray Hill, NJ
www.boc.com

Be sure to mention, "I read about it in *Food Protection Trends*"!

COMING EVENTS

NOVEMBER

- **1–3, Basic HACCP,** Davis, CA. For more information, contact Jennifer Epstein at 202.637.4818; E-mail: fpi@nfpa-food.org.
- **3–4, Implementing *Listeria* Intervention and Control Workshop,** Chicago, IL. For more information, contact American Meat Institute Foundation at 703.841.2400 or go to www.meatami.com.
- **3–4, Sanitary Design: A Practical Perspective,** GFTC, Guelph, Ontario, Canada. For more information, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.
- **3–5, The Dairy Practices Council® 35th Annual Conference,** Radisson Hotel Indianapolis Airport, Indianapolis, IN. For more information, call The Dairy Practices Council® at 732.203.1947; E-mail: dairypc@dairypc.org.
- **4–5, HACCP Verification and Validation,** Davis, CA. For more information, contact Jennifer Epstein at 202.637.4818; E-mail: fpi@nfpa-food.org.
- **4–5, Lead Auditor,** Atlanta, GA. For more information, contact ASI Food Safety Consultants at 800.477.0778 ext. 113; E-mail: jhuge@asifood.com.
- **5, SQF Systems Awareness,** GFTC, Guelph, Ontario, Canada. For more information, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.
- **5–6, Mexico Association for Food Protection Annual Fall Meeting,** Guadalajara, Jalisco, Mexico. For more information, contact Lydia Mota De La Garza at 01.5794.0526; E-mail: dra_lydia_mota@lei.com.mx.
- **7–11, FPMA (Food Processing Machinery Association) Expo,** McCormick Place, Chicago, IL. For more information, call 800.331.8816 or go to www.foodprocessingmachinery.com.
- **9–10, Principles of Food Safety Auditing/Inspection,** Atlanta, GA. For more information, contact AIB at 785.537.4750 or go to www.aibonline.org.
- **9–10, Principles of Food Safety Auditing/Inspection,** Four Points Sheraton Hotel Chicago O'Hare, Chicago, IL. For more information, contact AIB at 785.537.4750; or go to www.aibonline.org.
- **17, HACCP: A Management Summary,** GFTC, Guelph, Ontario, Canada. For more information, contact Marlene Inglis at 519.821.1246; E-mail: minglis@gftc.ca.
- **18, Ontario Food Protection Association Annual Fall Meeting,** Stage West, Mississauga, Ontario. For more information, contact Gail Evans Seed at 519.463.6320; E-mail: ofpa_info@worldchat.com.

DECEMBER

- **1–2, Food Plant Sanitation,** GFTC, Guelph, Ontario, Canada. For more information, contact GFTC at 519.821.1246; E-mail: gftc@gftc.ca.
- **2–5, ALEXPO 2004,** Hilton Green Plaza, Alexandria, Egypt. For more information, call at 011.203.358.0139; E-mail: abi2000@link.net.
- **13–15, Microbiology III: Foodborne Pathogens,** GFTC, Guelph, Ontario, Canada. For more information, contact GFTC at 519.821.1246; E-mail: gftc@gftc.ca.

FEBRUARY

- **15–17, NFPA's 2005 Food Claims and Litigation Conference,** Ojai, CA. For more information, call 202.639.5950; E-mail: dherman@nfpa-food.org.
- **22–25, Kentucky Association of Milk, Food & Environmental Sanitarians Annual Spring Meeting,** Executive Inn West, Louisville, KY. For more information, contact Laura Strevels at 859.363.2022; E-mail: laura.strevels@ky.gov.

ADVERTISING INDEX

CAREER SERVICES SECTION

Neogen Corporation is an internationally recognized leader in diagnostic test kits for food safety. We have excellent benefits, relocation assistance may be available and compensation packages commensurate with experience. We are currently seeking exceptional individuals for the following positions.

Research Scientist – Biochemistry

Neogen Corporation is currently seeking a research scientist with a protein biochemistry background. Successful candidate will perform laboratory tests to support research efforts in developing new diagnostic tests for food and agricultural industries. Applicant must hold Bachelor's Degree in a science discipline (chemistry, microbiology, biochemistry, immunology) from a four-year college or university or have five years related experience and/or training.

Marketing Specialist – Molecular Biology Products

Neogen Corporation is currently seeking a Marketing Specialist to develop and implement marketing plans to maximize the sales of current and future microbiological products. Key products include qualitative diagnostic tests for sales of current and future microbiological products. Key products include qualitative diagnostic tests for *Salmonella, Listeria, L. mono* and *E. coli* O157:H7. It is expected that markets for these products will continue to grow and at the same time become more sophisticated. Extensive industry involvement (trade associations, food processors, food marketers, etc.) will be required to determine market needs (current and expected). Sales of Neogen Corporation's microbiological diagnostic testing products are expected to grow and contribute significantly of the revenue and earnings for the Food Safety Division. This position is expected to be a major contributor to the development of the category sales and profitability through the achievement of optimum market shares, strategic positioning and expense control. A strategic analysis of customer needs/desires and the regulatory environment will be used in developing Strategic Plans, Marketing Plans and Market Support Activities.

Qualifications:

- College degree in life sciences discipline such as Agriculture, Microbiology, Immunology, Chemistry, etc. (preferred).
- Sales experience in a company that operated with a Product Management structure and knowledge of marketing principles, product launch activities and sales support.
- Strong communication skills, including interpersonal, writing and presentation.

Postdoctoral Scientist – Immunoassay Development

Neogen Corporation is currently seeking a postdoctoral scientist. Candidate will be responsible for developing research plans, designing experiments, conducting research and providing training to laboratory staff, as well as providing technical information and reporting on research goals. Qualified applicant will have a Ph.D. in immunology, biology, biochemistry, microbiology or related fields with 0-2 years of relevant work experiences post-degree. A strong background in immunology and/or immunochemistry is required and experience in antibody development and immunoassay development is preferred.

Process Chemist

Neogen, is currently seeking a Molecular Diagnostics Manufacturing Manager for our corporate campus located in Lansing, MI. Minimum of BS degree in Biochemistry, Microbiology or related field with a strong background in the manufacture, testing and new product start ups of molecular probes, antibody based reagents, microwell assays and protein conjugates is desired. This position also requires a minimum of 2 years management level experience of both technical and non-technical staffs.

Please send resume to: Human Resources
Neogen Corporation
620 Lesher Place
Lansing MI 48912
Fax: 517-372-3480
E-mail: hr1@neogen.com
E.O.E.

CAREER SERVICES SECTION

Position available: Assistant/Associate/Full Professor of Microbial Food Safety. Salary dependent on qualifications and experience. PhD required with research expertise in microbial food safety, especially of foods of animal origin. DVM or equivalent preferred. Demonstrated aptitude/experience or potential in teaching required. Documented research program in microbial food safety. In order to complement the department's existing strength in pre-harvest food safety and epidemiology, the successful candidate will possess strength in food safety beyond the pre-harvest stage (e.g., animal transport, slaughter, processing, product handling or distribution). Demonstrated record or evidence of potential in acquisition of extramural funding. Familiarity with food animal production and processing systems. Knowledge of use of applied epidemiological methods is desirable. Must possess excellent interpersonal and communication skills and a demonstrated ability to work with others in a collegial team atmosphere. Evidence of leadership and initiative is required. Teaching responsibilities include: 1) participation in lectures, laboratories and discussions in the DVM professional curriculum and graduate professional curricula (MPVM, MPH, and planned MEH), and 2) participation in the graduate academic programs (MS and PhD) of the campus.

Research responsibilities include the development of a creative, independent and productive research program in microbial food safety is a fundamental and indispensable requirement of the position, including publication of results in professional/scientific journals. The successful candidate will be expected to develop an on-going research program in food-borne pathogens at the molecular, organismal or host-population level. Individual will provide leadership in directing research projects of graduate students.

Service: The successful candidate is expected to work with state agencies and campus groups in identifying research needs in microbial food safety and to be a consultative resource for those agencies. University and public service through committee work, participation in professional organizations, continuing education and other appropriate means is required. To receive fullest consideration, applications must be received by October 15, 2004; position open until filled. Interested applicants should submit 1) a letter of intent outlining special interest in the position, overall related qualifications and experience and career goals; 2) curriculum vitae; and 3) the names and addresses of four professional references to: Dr. R.H. BonDurant, Chair, Attn: Terry Davison, MSO, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616.

The University of California is an Affirmative Action/Equal Opportunity Employer.

TECHNICAL SUPPORT SPECIALIST

When it comes to "clean" Ecolab is the world leader, with $3.2 billion in global sales and the respect of customers in more than 170 countries. For over seven decades, we have developed and marketed cleaning solutions and systems making the world a cleaner, safer, better place to live. It all takes teamwork and commitment to customer satisfaction. This is where you come in.

Ecolab has an opening for a Technical Support Specialist. This position is responsible for troubleshooting customer sanitation or related problems, especially microbial quality problems, which are unusual, complex, or beyond the expected ability of our Account Managers or Quality Management Consultants; Provide effective, accurate, courteous and prompt consultation and routine phone technical service to both internal and external customers with commitment to follow-up and resolution; Communicate to the sales force, staff and management, techniques and general information regarding products, services, equipment and procedures – both Ecolab and competitor information. Identifying and reporting product deficiencies, new products needs, and competitor activities; Evaluate and contribute programs and/or procedures for improved customer quality through sanitation products, procedures and services; Training of Food & Beverage personnel and customers in sanitation programs, technical application of sanitation products and methods of problem solving; liaison with vendors to the food industry, universities and customers; Keep current on new technologies, technical information and government regulations and regulatory activity. The successful candidate will be an independent and highly motivated collaborative individual who has excellent communication skills, professional manner and solid industry and technical experience.

This position includes necessary travel, anticipated to be 30%, to deliver field technical support to employees and customers.

POSITION REQUIREMENTS

This position requires an incumbent with a Bachelor Degree in Chemistry, Microbiology, or Food Science with strong (3-5 years) experience in the food industry or sanitation. Must be familiar with Dairy, Food and Beverage processing.

Please apply directly on-line by visiting our website at www.Ecolab.com/careers You will also reference job #3690BR.

Ecolab is an Equal Opportunity/Affirmative Action Employer

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New England Overshoes
Suite 3F
208 Flynn Avenue
Burlington, VT 05401
802-846-8880
chuck@overshoe.com

Division – NEOS Industrial
U.S. Sales Representative

New England Overshoes (NEOS) — the leader in performance overshoes — is looking for an experienced entrepreneurial-minded sales representative to successfully introduce its new Industrial Overshoe brand.

The NEOS Industrial Overshoe helps maintain hygienic zones and reduce biohazard contamination in all types of industrial environments, while providing greater comfort and safety for employees.

This sales position will be specifically responsible for direct sales growth to major food manufacturers. This integral part of the team will build company brand recognition, identify and penetrate new major accounts while further developing existing client base. Experience with sanitizing plastics, chemical resilience and work wear sanitizing requirements in food processing environments is critical.

Qualifications:

- Ideal profile combines experience building and managing pipeline with identifying and penetrating new major accounts.
- Must be proven in identifying and building appropriate, profitable relationships with the decision makers.
- Proven skills of superior networking capabilities.
- Must be self-motivated, with a passion for pioneering.
- Must work well without significant infrastructure and support, with the ability to make things happen.
- Outstanding sales skills and complete confidence in initiating potential customer contacts.
- Extensive background and experience in food and pharmaceutical sanitation supply of apparel or accessories.
- Proven ability to meet / exceed annualized sales targets
- Excellent analytic, writing and presentations skills.
- Experience selling to large, multifaceted organizations
- Undergraduate degree in a related discipline is required, graduate program is a plus

Compensation: Based on experience

Burlington, VT, although relocation may not be necessary.

New England Overshoes
Suite 3F
208 Flynn Avenue
Burlington, VT 05401
802-846-8880
scott@overshoe.com

Division – NEOS Industrial
Director of New Business Development

New England Overshoes (NEOS) – the leader in performance overshoes – is looking for an experienced entrepreneurial-minded leader to successfully direct the establishment of its new Industrial Overshoe brand.

The NEOS Industrial Overshoe helps maintain hygienic zones and reduce biohazard contamination in all types of industrial environments, while providing greater comfort and safety for employees.

The Director of New Business Development will be responsible for the formulation of a five year business plan, business unit strategy, sales growth, marketing and continual new product line development. This integral part of the executive team will build company brand recognition, identify and penetrate new major accounts while further developing existing client base. Experience with sanitizing plastics, chemical resilience and work wear sanitizing requirements in food processing environments is critical.

This exciting opportunity includes splitting the Industrial Overshoe business from the parent company incubation into a self standing, self funding brand.

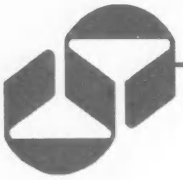
Qualifications:

- Ideal profile combines experience building and managing pipeline with identifying and penetrating new major accounts.
- Must be proven in identifying and building appropriate, profitable relationships with the decision makers.
- Track record in building strategic product development plans that have helped set the standard in a niche market.
- Proven skills of superior creative research, networking capabilities, and opportunity assessment in new product development.
- Must be self-motivated, highly collaborative, dogged and reliant, with a strong intellect and a passion for pioneering.
- Must work well without significant infrastructure and support, with the ability to make things happen.
- Outstanding sales/marketing skills and complete confidence in initiating potential customer contacts.
- Extensive background and experience in food and pharmaceutical sanitation supply of apparel or accessories
- High degree of knowledge with chemicals and material used in the sanitation process or in protective gear
- Proven ability to meet / exceed annualized sales targets
- Strong proposal development and negotiating skills. Excellent analytic, writing and presentations skills.
- Senior level experience (minimum 7 years) selling to large, multifaceted organizations
- Undergraduate degree in a related discipline is required, graduate program is a plus

Compensation: Competitive including bonus and equity opportunities

Burlington, VT, although relocation may not be necessary.

CAREER SERVICES SECTION



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We seek an acknowledged leader to join our organization in the position of Laboratory Director.

The ideal candidate will have a Masters in Food Science, or equivalent major, as well as, 7-10 years of food testing experience in a laboratory setting with supervisory and operational experience, and 3-5 years proven management experience.

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MICROBIOLOGY LABORATORY MANAGER
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We seek an experienced Microbiology Laboratory Manager.

This individual must possess a Bachelors in the Sciences and have a minimum of 8 years industrial experience; 3–5 years experience in a managerial capacity.

This individual must possess strong quantitative skills; proven attention to detail with effective organizational skills and proven ability to develop, adapt and validate testing methods. The Microbiology Laboratory Manager must also have proven problem solving and trouble shooting skills along with strong verbal and written communication skills.

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Research Notes

Supplement

* Asterisk indicates author for correspondence.

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The use of the Audiovisual Library is a benefit for **Association Members only.** Limit your requests to five videos. Material from the Audiovisual Library can be checked out for 2 weeks only so that all Members can benefit from its use.

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Invite a Colleague to Join

The International Association for Food Protection, founded in 1911, is a non-profit educational association of over 3,000 food safety professionals with a mission ***"to provide food safety professionals worldwide with a forum to exchange information on protecting the food supply."*** Members belong to all facets of the food protection arena, including Industry, Government and Academia.

Benefits of Membership

- ***Food Protection Trends*** — Published as the general Membership publication, each issue contains refereed articles on applied research, applications of current technology and general interest subjects for food safety professionals. Regular features include industry and association news, an industry-related products section and a calendar of meetings, seminars and workshops.
- ***Journal of Food Protection*** — First published in 1937, the Journal is a refereed monthly publication. Each issue contains scientific research and authoritative review articles reporting on a variety of topics in food science pertaining to food safety and quality.
- ***Journal of Food Protection* Online** — Internet access to abstracts and full text articles. Full text searching, active reference links, multiple delivery options, and table of contents alerting at your fingertips.
- **The Audiovisual Library** — As a free service to Members, the Library offers a wide variety of quality training videos dealing with various food safety issues.
- **The Annual Meeting** — With a reputation as the premier food safety conference, each meeting is attended by over 1,500 of the top industry, academic and government food safety professionals. Educational sessions are dedicated to timely coverage of key issues and cater to multiple experience levels.

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If you know someone who would prosper from being a Member, share with them the benefits of Membership, send them to our Web site, or provide us with their mailing address and we will send them information as well as sample journals. Together we are *Advancing Food Safety Worldwide*!

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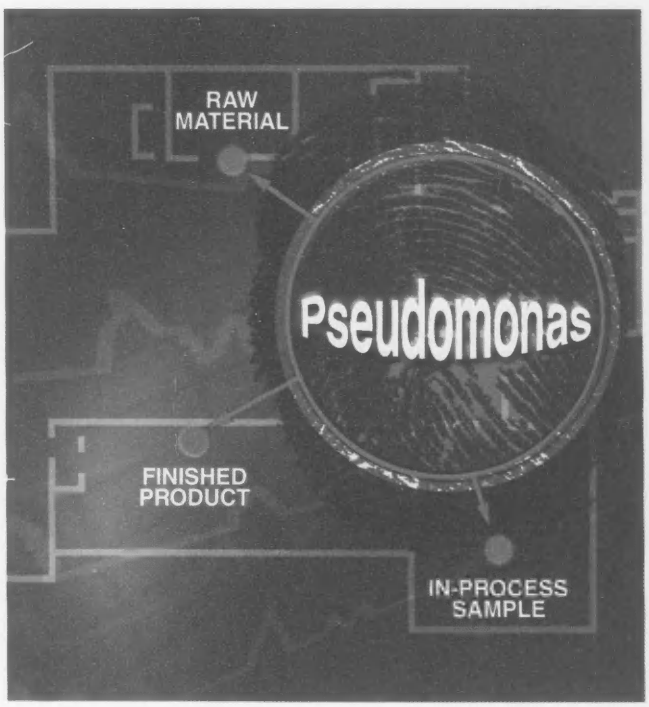

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