Future challenges to microbial food safety

Havelaar, A. H., Brul, S., de Jong, A. E. I., de Jonge, R., Zwietering, M. H., & ter Kuile, B. H.

Havelaar, A. H., Brul, S., de Jong, A. E. I., de Jonge, R., Zwietering, M. H., & ter Kuile, B. H. (2010). Future challenges to microbial food safety. International Journal of Food Microbiology, 139(Suppl. 1), S79-S94. https://doi.org/10.1016/j.ijfoodmicro.2009.10.015

Confidential manuscript submitted to International Journal of Food Microbiology

Future Challenges to Microbial Food Safety

Arie H. Havelaar*[1,2], Stanley Brul[3], Aarieke de Jong[4], Rob de Jonge[1], Marcel H. Zwietering[5] and Benno H. ter Kuile[3,6]

[1] Laboratory for Zoonoses and Environmental Microbiology, Centre for Infectious Diseases Control Netherlands, National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, the Netherlands

[2] Division of Veterinary Public Health, Institute for Risk Assessment Sciences, Utrecht University, PO Box 80175, 3508 TD Utrecht, the Netherlands

[3] Laboratory for Molecular Biology and Microbial Food Safety, Netherlands Institute for Systems Biology, Swammerdam Institute for Life Sciences, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, the Netherlands

[4] Dutch Food and Consumer Product Safety Authority, Hoogte Kadijk 401, 1018 BK Amsterdam, the Netherlands

[5] Laboratory of Food Microbiology, Wageningen University, PO Box 8129, 6700 EV, Wageningen, the Netherlands

[6] Dutch Food and Consumer Product Safety Authority, Office for Risk Assessment, PO Box 19506, 2500 CM The Hague, the Netherlands

* Corresponding author. Tel.: +31 30 2742826, fax: +31 30 2744434.

E-mail address: arie.havelaar@rivm.nl

Abstract

Despite significant efforts by all parties involved, there is still a considerable burden of foodborne illness, in which microorganisms play a prominent role. Microbes can enter the food chain at different steps, are highly versatile and can adapt to the environment allowing survival, growth and production of toxic compounds. This sets them apart from chemical agents and thus their study from food toxicology. We summarize the discussions of a conference organized by the Dutch Food and Consumer Products Safety Authority and the European Food Safety Authority. The goal of the conference was to discuss new challenges to food safety that are caused by microorganisms as well as strategies and methodologies to counter these. Management of food safety is based on generally accepted principles of Hazard Analysis Critical Control Points and of Good Manufacturing Practices. However, a more pro-active, science-based approach is required, starting with the ability to predict where problems might arise by applying the risk analysis framework.

Developments that may influence food safety in future occur on different scales (from global to molecular) and in different time frames (from decades to less than a minute). This necessitates development of new risk assessment approaches, taking the impact of different drivers of change into account. We provide an overview of drivers that may affect food safety and their potential impact on foodborne pathogens and human disease risks. We conclude that many drivers may result in increased food safety risks, requiring active governmental policy setting and anticipation by food industries whereas other drivers may decrease food safety risks.

Monitoring of contamination in the food chain, combined with surveillance of human illness and epidemiological investigations of outbreaks and sporadic cases continue to be important sources of information. New approaches in human illness surveillance include the use of

46 molecular markers for improved outbreak detection and source attribution, sero-epidemiology
47 and disease burden estimation.

48 Current developments in molecular techniques make it possible to rapidly assemble
49 information on the genome of various isolates of microbial species of concern. Such
50 information can be used to develop new tracking and tracing methods, and to investigate the
51 behavior of microorganisms under environmentally relevant stress conditions. These novel
52 tools and insight need to be applied to objectives for food safety strategies, as well as to
53 models that predict microbial behavior. In addition, the increasing complexity of the global
54 food systems necessitates improved communication between all parties involved: scientists,
55 risk assessors and risk managers, as well as consumers.

56

57 Keywords: food safety, microbial hazards, future trends, monitoring, surveillance, risk
58 assessment, tracking and tracing, communication

59

1. Introduction

The microbiological aspects of food safety have been studied intensively for many decades. In the Netherlands, the standard of food safety has increased in the last decades (Van Kreijl *et al.*, 2006), and political attention is shifting to other food-related problems such as obesity and unhealthy diets. However, even in industrialized countries, there is still a considerable burden of foodborne illness. For example, in the Netherlands there are an estimated 700.000 cases of illness and 80 deaths per year. The burden of foodborne disease for this country is at least 3800 Disability Adjusted Life Years and 65 million Euro per year (Havelaar *et al.*, 2008). Also other industrialized countries report a continuing burden of foodborne illness (Flint *et al.*, 2005, Hall *et al.*, 2005, Adak *et al.*, 2005, Anonymous 2007a, Jones *et al.*, 2007). Foodborne outbreaks appear to be on the rise again in some industrialized countries, with a shift from traditional problems with foods from animal origin to fresh foods such as produce (Anonymous, 2008a), shellfish (Pontrelli *et al.*, 2008) and dry products and ingredients (e.g. peanuts, Anonymous, 2009). Furthermore, new threats continue to be identified. The attention for viruses is more recent, but no less relevant. New risks are being encountered because of changing characteristics of the relevant micro-organisms, changing production methodologies, changes in the environment and the ecology, and an increase of the global trade of foodstuffs. In addition, demands on food safety increase steadily. Due to the nature of microbes and our food chain, measures to ensure food safety have to be implemented on a global scale, necessitating a global approach.

To discuss the challenges that microbes pose to food safety on the longer term, the Dutch Food and Consumer Products Safety Authority (VWA) and the European Food Safety Authority (EFSA) organized a conference on “Future Challenges to Food Safety” (Wolfheze, the Netherlands, 9-12 June 2008). The goal of the conference was to discuss new challenges to food safety that are caused by microbes and strategies and methodologies to counter these.

It was aimed at achieving conceptual breakthroughs through an imaginative combination of recent developments in microbiology, epidemiology, mathematical modeling and expert knowledge; using these to propose new approaches to analyze and control food safety issues in the future. Such tools should enable risk assessors to pro-actively address imminent problems before they cause harm. This paper provides an overview of the major themes identified during the conference. The paper starts with a discussion of food safety from the risk management perspective. A systems approach to identify and structure future developments that may help to develop a pro-active approach to food safety is introduced, followed by a more detailed discussion of relevant developments with special attention for the interaction between micro-organisms and their environment, and for microbial evolution. Despite the need for pro-active approaches, surveillance and monitoring are discussed as important cornerstones of food safety policy and new developments in the available methodology are discussed. Finally, needs related to communication between all actors in the food chain are outlined.

2. Risk management

Food safety demands

Management of microbial food safety is a balancing act involving disparate factors. A high level of safety can be achieved by rigorously heat-sterilizing the food, thereby destroying taste and nutritious value. Irradiation would be another method for virtually absolute control of microbial risks, but in addition to being expensive, it is not acceptable to the public at large in many countries. Furthermore, some bacterial and fungal toxins are not inactivated by currently used irradiation doses. The consumer demands fresh, tasty, healthy and wholesome food products. Nevertheless, safety is in this framework considered an absolute requirement; placing unsafe food on the market is not an option in the consumer's mind. Food laws

everywhere are very clear on this point. For example, the EU General Food Law (Anonymous, 2002) states that: “a high level of protection of human life and health should be assured in the pursuit of Community policies”. Still, placing chicken contaminated with *Salmonella* or *Campylobacter* on the market is tolerated, because the consumer can circumvent this risk by cooking the meat properly and taking adequate precautions against cross-contamination, illustrating that responsibility for food safety is distributed over the entire chain. Nevertheless, there is an increasing pressure on producers to reduce contamination levels of fresh meat as far as possible and economically feasible. It has been demonstrated that it is very difficult to modify consumer behavior by education campaigns (Nauta *et al.*, 2008).

Microbial food safety differs fundamentally from chemical food safety. While chemical residues and additives typically enter the food chain at more or less predictable steps, microbes can enter at any step. They grow and die and interact with the food in ways that are at best empirically described, but less understood in detail. The effects are also of a different nature. Chemical contaminants, such as dioxins, can accumulate in the human body over the years and still exert influence long after ingestion. Microbial pathogens can in some cases be dormant for a certain time, but usually cause disease in a matter of days or weeks. The public perception of microbial and chemical risks is also different. Residues of pesticides cause public outcries if they exceed the norms, but usually will not have any noticeable detrimental effect, while foodborne microbial and viral diseases are generally more accepted as facts of life, as long as death or permanent harm do not occur (Hansen *et al.*, 2003)

One of the challenges for managers of microbial food safety risks is to put in place effective controls, without unnecessarily increasing costs or reducing taste and nutritional value. Microbial hazards can be introduced at any step in the production chain and the most effective opportunity for controlling those hazards can very well be a different step. Microbial risk

management therefore requires a thorough understanding of the entire food production chain. Monitoring the presence of pathogens in the end product usually is an inefficient approach to hazard control, because it is impossible to test sufficient samples to obtain the necessary degree of statistical power to detect contaminants at levels that may create unacceptable health risks. Furthermore, by the time the potential presence of pathogens has been confirmed, the optimal moment to take measures may have passed. Therefore, a pro-active approach is required, starting with the producer ensuring a safe product and process design, and predicting where problems might arise, rather than detecting them after they have occurred.

At present HACCP (Hazard Analysis Critical Control Point) programs and GMP (good manufacturing practice) are mainly used to manage microbial hazards in foods. While these systems have proven very effective for the control of food safety (Van Der Spiegel *et al.*, 2004, Arvanitoyannis and Traikou, 2005), it must be realized that they are designed on the basis of known hazards, and do not necessarily take potential future developments in consideration. For innovations, new validations and verifications are necessary. Furthermore, it should be realized that the implications of microbial adaptability are not sufficiently taken into account (McMeekin and Ross, 2002, McMeekin *et al.*, 2006). Although documentation is a very important aspect of HACCP and GMP procedures, the real confidence in control comes for the validity of the effectiveness of the written guidelines and the adherence to them. Producers and handlers of foodstuffs are more likely to adhere to the prescribed HACCP procedures if they recognize these as useful and implementable (Taylor and Taylor, 2004).

Science-based risk-management

Within the food safety discipline the terms “risk manager” and “risk management” are not unequivocal and are used to indicate several functions and persons. As a consequence there are several persons with responsibility as risk managers, each at a different step of the food chain. Formally “the” risk manager is the government’s minister of public health and/or

his/her colleague of agriculture, as they set the standards to which food producers must adhere. Furthermore, within government supervisory agencies the persons in charge of the enforcement branch are often called risk managers. Often, however, the person responsible for compliance to procedures within a food manufacturing company is called a “risk manager” as well, as this term in fact very well describes his/her day-to-day activities. Although these functions are less clearly defined in smaller operations and in primary production, the role should be fulfilled in any food operation. Risk assessors, on the other hand, have a different function. They provide the risk manager with science-based advice on the magnitude of risks and cost-effective ways to reduce these. This advice enables the different risk managers to take decisions on measures to control risks, by setting standards, by implementing in-plant control measures and/or enforce existing regulations.

A challenge to food microbiologists in their role of risk assessors is to translate complex scientific problems in such a way as to help a risk manager to make a simple yes/no decision. The risk manager wants to know what standards to set, when to interfere in the production process, prevent a batch from reaching the market or take another measure. Under the WTO Agreements, and in particular the Agreement on the Application of Sanitary and Phytosanitary Measures, considerations of food safety and animal and plant health are the only legitimate reason for trade restrictions. The type of decisions in these cases is also of the yes/no kind. The underlying science will have to provide the decision maker with a solid rationale that will stand up in the international courts. This implies that data on the microbiological status of the foods concerned must be communicated in terms of public health risk with a limited margin of error, requiring a predictive power from food microbiology. As zero-risk is unattainable, this approach also implies that the risk manager defines a level of acceptable (tolerable) risk. In international trade, this is called the Appropriate Level of Protection, equivalent to the currently realized risk level under the food

safety system of the importing country. Food safety managers may wish to achieve a higher level of protection in future by stating additional public health targets (Anonymous 2006, Anonymous 2007b).

Food safety risk-management in the EU

Public concern about food safety increased sharply as a result of the food scandals in the last decade of the twentieth century and confidence decreased in parallel. To counter these sentiments national governments and the EU established food laws and regulations that strictly separate risk management from risk assessment. The idea behind this was to create transparency by having the risk assessor provide advice to the risk manager completely in the open. The risk assessor operates independently, based on the best available science and free from influence by politics, industry or any other stakeholder. The risk assessor should give objective advice, based on science while taking account of other considerations that the risk manager has indicated. The risk manager can then incorporate other factors such as public concern or political preferences into the decision making process.

The exact procedures for risk assessment differ considerably in the different member states. The EU itself has mandated the European Food Safety Authority (EFSA) to carry out risk assessments, either at the request of the Commission, a member state or the European Parliament, or on its own initiative. The EFSA in turn has handed this task to 10 scientific panels and the Scientific Committee, which it finances and supports scientifically and otherwise. These panels write opinions which are passed on unchanged to the EU Commission and published on the EFSA website. When strong public interest is expected a press release is issued as well. The panels are comprised of scientists who operate independently from risk management, and almost all come from EU-member states, though this is not a formal requirement. The scientists are chosen in an EU-wide application

208 procedure on the basis of their expertise and prominence in their scientific fields, and are
209 appointed for a period of three years at a time.

210 The advice of EFSA's panels is presented as a scientific opinion to assist in the formulation of
211 risk management measures by the European Commission. Measures may be decided upon by
212 the members states in a consensus procedure, allowing political, economic and other
213 considerations to influence the decision making process.

214 *Interaction between scientists and regulators*

215 Researchers, in their role as risk assessors, need to provide the regulators, in their role as risk
216 managers, with advice that can be implemented in a practical manner. Regulators may or may
217 not have a scientific background and thus results of risk assessments have to be
218 communicated in a way that can be understood by fully informed laypersons. The process of
219 risk analysis, as defined by Codex Alimentarius, consists of risk assessment, risk management
220 and risk communication. The very first step in the process, hazard identification, is a
221 component of risk assessment. The hazard identification can come from any source. In
222 practice it is often the risk manager, who "identifies" the risk by asking for a risk assessment.
223 For a good risk assessment, the problem at stake needs to be well understood by the assessor.
224 Therefore, correct phrasing of the request is essential for a successful risk assessment
225 procedure. The regulator needs to articulate his question so that it will not be misunderstood
226 by the scientist. In short, they need to "speak each others language". This seems a trivial
227 point, but experience proves that it is not.

228 The roles of the risk assessor and the risk manager need not only to be formally separated, but
229 also with respect to substance. A risk manager has to consider more issues than science only,
230 such as stakeholder interests, public concerns and political pressure. The risk assessor needs

to present a science-based advice, but may anticipate the risk manager to take other than scientific factors into account when selecting the risk management options.

3. A systems approach to analyzing future challenges to food safety

In addressing the present or future state of food safety one should investigate effects both on large scales of space and time as well as on small scales (Table 1). Certain aspects slowly change at a global scale, like climate change while others such as point mutations or the acquisition of a plasmid by a micro-organism, occur on a molecular scale and on very short time scales. In addition, certain aspects occur over longer time scales, but on a molecular level, like subsequent adaptation of micro-organisms, or on a small time scale but on a larger spatial scale, like the spread of a virulent micro-organism due to the large traffic of people or goods. Furthermore these changes occur in micro-organisms and humans, in habitats and in the environment. To accurately describe and predict processes in all these different organisms and locations, on these very different spatial scales and time scales is virtually impossible, the more because all these aspects interact. The risk framework of the UK Foresight project on infectious diseases (Tait *et al.*, 2006) offers a useful starting point for the development of scenario analysis in relation to food safety. The project has developed the following definitions, which are cited literally here:

- Disease sources/emerging hazards: phenomena or biological events that: give rise to potential new diseases; enable existing diseases to become more harmful; enable existing diseases to infect new hosts; or enable existing diseases to spread to new areas.
- Pathways: mechanisms or routes by which a disease organism can transfer from one host to another, within or between species.

- Drivers: social, economic or physical factors that affect disease outcomes by changing the behavior of disease sources or pathways.

- Outcomes: diseases of plants and animals at the individual, community and ecosystem or farming system level, and diseases of humans at individual and societal levels.

A basic risk framework (Figure 1) shows the links between these different factors.

The PERIAPT project on emerging risks (Noteborn *et al.*, 2005) identified 8 major categories of drivers, based on expert surveys. Later, we will present a tabular representation of these drivers of change in the food system, and a qualitative analysis how they affect sources, pathways and outcomes. To better explore these complex interrelationships among different drivers, mathematical models may be helpful.

Mathematical models are a representation of the essential aspects of an existing system (or a system to be constructed), presenting knowledge of that system in a usable form. A mathematical model usually describes a system by a set of numerical variables and a set of equations that establish relationships between the variables. The variables represent some properties of the system, obtained by measurements or by expert opinion. The actual model is the set of functions that describe the relations between the different variables. The purpose of modeling is to increase our understanding of the world. The usefulness of a model rests not only on its fit to empirical observations, but also on its ability to extrapolate to situations or data beyond those originally described in the model (from Wikipedia, March 21, 2008).

Some examples of models that are currently used to analyze and to support decision making on food safety:

- Microbial risk assessment (MRA) models (hazard identification, exposure assessment, hazard characterization (including dose-response), risk characterization).

  Used to understand the relationships of pathogen occurrence (both prevalence and

concentration) in different steps of the food chain, to predict health risks associated with pathogens in food, and the expected public health effects of interventions and the setting of risk-based standards for food production.

- Predictive microbiology (growth/death/survival).

  Used to understand the growth or death of micro-organisms in relation to their implicit properties and interactions, and the intrinsic properties of the food and the extrinsic factors of the (processing) environment. It is an important component of MRA models, and also used for prediction of shelf-life and intrinsic safety of foods.

- Dynamic infectious disease models.

  Used to understand the spread of diseases in human or animal populations, depending on contact patterns and mode of spread of the pathogens, in relation to the development of protective immunity.

- Risk factor models (analytical epidemiology).

  Used to relate the observed occurrence of responses (e.g. illness) to the occurrence of potential predictive factors.

- Attribution models.

  Used to estimate the contribution of putative sources to the observed occurrence of responses (e.g. illness).

- Multi-criteria analysis models.

  Used to support decision makers in making evaluations of different options, based on a combination of variables of a different nature (health, economic, societal, ….) with value-based weights.

All models are based on a set of (simplifying) assumptions and have specific data needs. They also differ in their form (linear or non-linear, deterministic vs. stochastic, static vs. dynamic)

which impacts on their use for specific purposes. It is noted that all models described above work at relatively low levels of aggregation, looking at specific (parts of) food chains in relation to public health effects. The challenge is to develop models that are able to capture the impact of different drivers on foodborne risks at higher levels of aggregation.

? The overall aim of this coupling of various aggregation levels is to better understand how drivers affect the evolution of foodborne illness and to determine the most important drivers. This can help to react with a timely and adequate response, and support a pro-active approach, targeted at future events. Generally one needs to decrease complexity in the more detailed parts and move upwards to more global aspects, but also *vice-versa*, since after identifying certain important aspects on a global scale one might need to change focus to the more detailed level. For this, intensified linking to models in other domains is necessary, like biosphere models, geospatial modeling, catastrophe modeling, climate models, remote sensing, network science, statistical physics, and data mining.

For these models, a huge amount of data is needed such as information of food production sites and global product flows (volume, origin) for high risk products (e.g. fresh meat, fresh produce, shellfish), risk maps, global atlases of food consumption and production, food categories with different levels of risk. Connecting them in dynamic systems can help to identify rates of change. However, it is necessary to begin with low granularity, with more detail based on sensitivity analysis.

In current risk evaluations considerable uncertainties already prevail, so, when modeling future risks even larger uncertainties will exist. However, important insights can still be gained using the available information, making the best informed decisions at a given point in time, that later can be detailed if more and better information becomes available. Scenario analysis will be particularly important to better understand the impact of different factors, their interrelatedness and their uncertainties. It is a process of analyzing possible future events

by considering alternative possible future developments and outcomes (scenarios), and their likelihood. These insights can then be used to develop a range of contingency plans to address the most likely or most serious scenarios. The analysis is designed to allow improved decision-making by providing more complete consideration of outcomes and their implications. Typically, scenario analysis starts with the identification of possible important drivers of change, and subsequently assigning a preliminary ranking of their importance.

4 Trends and future developments

In this section, we present an attempt to collect and structure available information on the current and future aspects of microbial food safety. Table 2 gives an overview of factors identified so far, according to the model presented by Tait *et al.*, (2006). We interpret the sources category as referring to the pathogens; the pathways category is split into the three major stages of the farm-to-fork pathway (farm, processing and consumption), and outcomes are defined at the public health level. Note that there are complex interrelationships between different drivers, sources, pathways and outcomes that are difficult to visualize in a two-dimensional table, and hence the information must be interpreted with care. Nevertheless, important insights can be gleaned from this analysis, which provides a general background against which specific situations can be analyzed. Currently, it is only possible to discuss the impact of drivers of change on sources and outcomes in general, qualitative terms and so an attempt has been made to indicate the anticipated direction of change for specific sources and overall for drivers. It is clear from Table 2 that many drivers are expected to result in an increased risk to food safety, although there are also favorable exceptions, such as the lesser consumption of meat due to higher food prices. Controlling such threats is a challenge to governments as the only drivers assumed to result in reduced threats relate to government and policies. A second important challenge is to food industries that can modulate the effects of

many drivers in such a way that a neutral or even positive effect on food safety can be expected (see drivers under science, technology and industry).

Further elaboration of the crude framework sketched in this chapter requires considerable inputs. It is suggested that to further develop the framework, historical examples be analyzed. This will provide more detailed insight regarding relevant drivers and sources, and their interaction as well as data to validate the approach. Such examples include the BSE epidemic, different meatborne zoonoses (e.g. VTEC O157), shellfish poisoning and more recent outbreaks in fresh produce.

In order to improve future responsiveness, signals about changes or breakdowns in the food safety system must be received and processed in time. This implies a pro-active approach. Process or production failures, including fraud and terrorist action need specific attention. Risk mapping (on a global scale) can be a helpful tool. This includes observation and systematically analyzing consumption patterns, processing / production changes, knowledge of international production chains (trade), and data & knowledge sharing. Where necessary, available data can be supplemented with expert opinions (global and multidisciplinary).

Trends in food processing

The trend for mildly preserved foods comes with a range of approaches being investigated, mostly using minimal heating, natural preservatives and non-thermal treatment as technologies and often combined preservation/hurdle technology as the principle in designing the overall treatment. Such processes need to be well controlled through adequate product and process design and proper implementation and monitoring through HACCP. This places a responsibility on industry, including small enterprises. There are more weak links, and overall the processes are less robust and more accident prone. As the scale of operation of food businesses continues to increase, errors may have a bigger impact.

Consumers need to be aware of the criticality of the formulations and of the need to treat manufactured foods either as perishable products needing proper refrigeration or requiring specific conditions of preparation (for instance non-Ready-To-Eat products that need to be cooked properly before consumption even though they may appear to be cooked). The concern is that many consumers do not habitually read labels and are unaware of shelf-lives and preparation requirements.

Can these new product types offer niches for concurrent, old, emerging or new microbial hazards? Classical examples are refrigeration and the niche created for *Yersinia* and *Listeria*, and for sporeformers by non-thermal treatments at the pasteurization level. As in many of these foods spoilage organisms have been removed or suppressed, there is increased opportunity for the growth of pathogens that recontaminate treated products – in the absence of the "normal" spoilage signal. Noroviruses show prolonged survival during cold storage and even freezing. The probability and extent of survival and of post-process contamination, rather than pathogen growth opportunity, may then determine the level of consumer risk.

While there would be a benefit from (and indeed a need for) for irradiation technology for certain applications (e.g. it is "safe", "invisible", no microbial issues of resistance, or of recontamination when done in-pack), consumer concerns towards the technology itself and about misuse to make spoiled food marketable, prohibit its wider use in practice. This implies considerable communication challenges, should the technology prove to be the treatment most relevant for certain applications.

While reducing packaging is a laudable initiative where the packaging is for "cosmetic" or bulk-transport purposes, industry and consumers should be aware of those situations where the packaging has a preservative function by minimizing growth and/or recontamination of microorganisms.

Consumer behavior

Several changes in food composition related to consumer health are foreseen. These may also have an impact on bacterial growth. For instance, components like salt and sugar are often used to inhibit the growth of organisms, both by their water activity lowering effect, and additionally solute specific effects. Their concentration cannot be safely reduced for health or other non-safety reasons without adapting the product design. On the other hand, fat can be seen as a vehicle for better stomach survival of pathogens, so less fat might reduce risks. Lower fat may also increase the water activity of a product by having more diluted solutes in the aqueous phase (Senhaji, 1977).

Considering the present increased level of general health of the population and better medical treatments, proportionally more elderly will be present in society. These elderly often are more vulnerable to foodborne diseases partly, as a result of a weakened immune system increasing the risks of complications and even death. Other defence systems, such as stomach acid may be impaired (achlorhydria), augmented by medication.

Due to changes in eating habits, certain risks might change in magnitude. For example an increased consumption of fish for health benefits may result in increased microbial risks. The increasing trend for fresh, pre-packaged produce or other foods that are consumed without additional heating by consumers also increases consumer risk. Exotic and ethnic foods are now trendy in the market, but do we understand the underlying preservation system? When we change these products (adapted for new markets; altered ingredients), are we clear on how this may affect safety? More and more animal species are used for food production and there is little knowledge about zoonotic risks of such foods (e.g. reptile meat, Magnino *et al.*, 2009). New culinary techniques, such as molecular gastronomy involve more and more technical creativity and exotic ingredients to improve quality and consumer acceptance, which may result in unexpected risks. In the case of small restaurants, catering establishments and

street vendors the scale of production is small and often, knowledge and sufficient technology may be lacking, resulting in avoidable errors which may lead to serious consequences for consumers.

Improving animal welfare (e.g. by increased outdoor access) and organic food production may lead to the re-introduction of pathogens with wildlife reservoirs such as *Trichinella spiralis* and *Toxoplasma gondii* and increase the prevalence of other hazards such as *Campylobacter* spp. but may reduce the prevalence of others such as *Salmonella* spp. (Gebreyes et al., 2008). Reduced usage of antimicrobial agents may have a positive impact on resistance development (Van der Giessen *et al*., 2007; Hoogenboom *et al*., 2008). Trends towards continuously increasing herd size in intensive bio-husbandry may also lead to increased public health risks by increased numbers of contacts between food animals, including purchasing of animals from more suppliers. The potential for infections to spread increases with herd size, and if such farms are concentrated in particular regions, there is also an increased risk of spread between farms. On the other hand, establishment of larger, newly designed farms may improve conditions for biosecurity and farm management, potentially reducing zoonotic risks (Kornalijnslijper *et al*., 2008).

Price

It is well known that cost is a very important consideration for the consumer when selecting foods, and that profits on food products are generally quite low. Both aspects make it difficult to be critical towards food safety and furthermore might occasionally result in fraud. Also, costs and conservatism may lead to resistance against implementing reasonable interventions or to interpret existing regulations liberally (e.g. use of approval of sick animals for consumption). Such non-compliance may increase consumer risk. The Law Enforcement Department of the Netherlands Food and Consumer Safety Authority experienced during the 2008-9 economic downturn that food producers and retailers more frequently violate

449 regulations relating to cleaning and maintenance due to cost cutting (J. van der Kooij,
450 personal communication). Increasing food prices are likely to compromise food security (in
451 terms of food availability) on a global scale. For industrialized countries, food security is less
452 at risk, but consumers may choose less costly alternatives. This may lead to less consumption
453 of animal proteins (also driven by animal welfare and environmental considerations), leading
454 to other health-related issues. Higher food prices may cause consumers to use food more
455 frequently past its shelf life, and may increase recycling of food.

456 *Global aspects*

457 Due to the increase of international travel, organisms can be spread easily and quickly over
458 the globe, and people come in contact with organisms and specific strains to which they have
459 not been exposed earlier, increasing the risk of illness. Global trade will result in a longer
460 transit distances and durations in the food chain, possibly increasing risk. Furthermore,
461 complex food chains with stakeholders in many different countries will make the management
462 of safety more difficult, especially at the initial stages of the food chain, the primary
463 production as it consists of many small farms and is increasingly global in nature. On the
464 other hand more powerful stakeholders (trade companies, supermarkets) will have the
465 intention to influence these complex chains in order to guarantee food safety. Several large
466 retailers that operate internationally have organized the GlobalG.A.P. quality control system
467 (www.globalgap.org) that aims to supervise the primary production process of all agricultural
468 products. Sourcing food from various climate areas means more variation in hazards. Border
469 controls are effective in regard to the control of only a small proportion of imported foods and
470 are less effective than hygiene controls imposed in the country of origin. Furthermore, global
471 food chains may be more vulnerable to terrorist attacks.

472 More and more harmonization will occur in international regulation of food safety by the
473 activities of e.g. the Codex Alimentarius Commission and the European Union. As a positive

effect, fairness in trade will increase but on the other hand the same level of contamination in food may result in very different health risks in different parts of the world, due to differences in e.g. demography, immune status, food preparation and consumption habits, and relevance of various routes of infection.

Climate change

Climate change is considered to be one of the greatest current challenges to mankind, affecting all sectors of society, including nutrition, food security and food safety (Anonymous, 2008b). Due to climate change various risks may change, as a result of changed ecological conditions on various places on the earth. Changing ecology is expected to affect the distribution of plant and animal diseases. Water shortages may lead to limited quantities or quality problems with irrigation water, process water or ingredient water. This may lead to shifts in production areas and cultured crops, as well as an increased use of agrochemicals. This trend may be increased by competition for land-use, e.g. for biofuels or for settlements. Flooding may lead to increased contamination of crops in the field, or increased exposure of food animals to zoonotic agents. Control of cold chains may be impeded by rising ambient temperatures. Humidity may increase production of mycotoxins, whereas certain foodborne pathogens may thrive better under warm conditions. The mechanisms by which climate change affect food safety are highly complex and interrelated with many other societal factors, The outcome of these changes strongly depends on the adequateness of societal responses, both of a technical and a political nature.

Science

More and more public health risks, physiological and ecological traits of foodborne pathogens, routes of contamination, effects of interventions, will be investigated and quantified, making it possible to better balance risks, and evaluate optimal interventions to

control risks to an appropriate level. That is, science will have a greater role in setting criteria both nationally and in international trade agreements.

The genomics revolution will facilitate easier and faster detection and identification methods, and can in particular lead to a better mechanistic understanding of the behavior of micro-organisms, both their physiology as well as their ecology. Furthermore new pathogens can be uncovered, for example better detection of injured and thus less easily culturable organisms is possible and more advanced methods to investigate cases and outbreaks become available.

Antimicrobial resistance

Usage of antimicrobial agents, both in the agricultural sector and in human health care settings, contributes to the emergence of resistant microbes. While resistance in the agricultural sector might be considered an economic problem with limited other consequences, it is developing into a cause of growing concern for human health care (see Newell et al., in an accompanying paper in this Special Issue). The overall use of antimicrobial agents in food animals is high and in certain countries largely exceeds the human use. The ban on usage of antimicrobials as growth promotors has, in some countries, barely had an influence, as it has been replaced by increased use for therapeutic purposes (Mevius and Van Pelt, 2006). Micro-organisms have an immense diversity and can easily transfer genetic information, making the emergence of new hazards, and adaptation to previously effective intervention methods possible.

At present, it is not clear what proportion of resistance encountered in human pathogens originates from selection in and transfer from animal reservoirs. It is not known if measures to reduce usage in the agricultural setting will lead to a rapid reduction of resistance, as this can also contribute in other ways to overall fitness of the micro-organism. The benefits to human health care of such measures are not quantified, as resistance may also develop due to other

factors. In spite of these uncertainties, the development of antimicrobial resistance in agricultural settings is a cause of growing concern to public health and prudent use is advocated.

5. Epidemiology and surveillance

Monitoring of contamination in the food chain, combined with surveillance of human illness and epidemiological investigations of outbreaks and sporadic cases continue to be important sources of information to evaluate the success of current food safety management systems and to identify new hazards. Surveillance is defined as "the ongoing and systematic collection, analysis, and interpretation of data about a disease or health condition; used in planning, implementing, and evaluating public health programs" (Anonymous, 2000a). Surveillance can be aimed at outbreaks or sporadic cases of foodborne disease, and continues to be a cornerstone of food safety management (see the accompanying paper by Tauxe *et al.,* in this issue).

Outbreak surveillance primarily aims to stop the outbreak by identifying incriminated products and taking them from the market. Furthermore, investigations may aim to prosecute those responsible, or to learn from outbreaks so as to avoid future outbreaks by identifying unsafe practices that had led to the outbreak. Outbreak investigations have and will continue to be an important instrument for identifying new pathogens (e.g. *Cyclospora cayatenensis*, Herwaldt, 2000), new vehicles for known pathogens (e.g. *Salmonella* Tennessee in peanut butter, Anonymous, 2007c), new disease syndromes associated with known pathogens (e.g. febrile gastroenteritis associated with *Listeria monocytogenes*, Dalton *et al.,* 1997), and the re-emergence of problems that were thought to be under control (e.g. botulinum toxins in canned foods, Ginsberg et al., 2007). They are important sources of data for establishing the

545 economic impact of foodborne illness on populations and may provide dose-response
546 information for microbial risk assessment (Teunis *et al.*, 2008).

547 Many countries have surveillance systems for outbreaks of foodborne illness and data are
548 reported at an aggregated level annually (Anonymous, 2007a) or over a number of years
549 (Wang *et al.*, 2007, Cretikos *et al.*, 2008). New tools are becoming available to detect
550 international outbreaks for foodborne viruses (Verhoef *et al*., 2009). Supranational agencies
551 such as EFSA and ECDC in Europe present regular reports on foodborne outbreaks in a larger
552 region or globally (Anonymous, 2007a). Outbreak summary reports provide important
553 insights in current and emerging food safety problems but it is essential that such summaries
554 are based on systematic surveillance activities. Reports in the peer-reviewed literature may
555 suffer from publication bias and overestimate the impacts of milk/milk products,
556 miscellaneous foods (e.g. sandwiches) and desserts while underestimating those of poultry,
557 fish and shellfish, red meat/meat products and eggs/egg products (O'Brien *et al.*, 2006).

558 Molecular tools identifying causative agents in environmental and clinical samples, and
559 molecular typing techniques identifying nucleotide sequences of single genes (i.e. *fla*-typing),
560 techniques identifying sets of genetic elements (MLST, MLVA) and various restriction
561 techniques (i.e. PFGE) have proven to be very useful aids in the epidemiology of foodborne
562 illness. Developments in genotyping of pathogens and informatics have enabled the
563 recognition of diffuse or multinational outbreaks which were previously unnoted (Gerner-
564 Smidt *et al.*, 2006, Kirk *et al.*, 2004, Kroneman *et al*., 2008).

565 Estimating the incidence of sporadic cases of foodborne illness is more complex. Most
566 existing surveillance systems are based on either notifiable disease reporting or laboratory
567 surveillance. Both systems are passive in nature, and record only a minor proportion of all
568 cases in the population. To estimate the true incidence of diseases that can be transmitted by
569 food, active surveillance is necessary and more accurate estimates are needed for under-

reported illness. The UK (Wheeler *et al.*, 1999, Tompkins *et al.*, 1999) and the Netherlands (De Wit *et al.*, 2001a, De Wit *et al.*, 2001b, De Wit *et al.*, 2001c) have carried out population-based prospective studies of infectious gastro-enteritis, combined with laboratory diagnostics to assess the proportion of cases due to specific pathogens. Currently, the UK has launched the second IID study (http://www.iid2.org.uk). Even in these large-scale projects, in a large proportion of cases (60%) it was not possible to identify a causal pathogen. However, it appears to be possible to reduce this diagnostic gap by the application of molecular methods (Amar *et al*., 2007). Population-based studies are expensive and time consuming, and several countries have attempted to develop less costly alternatives. These include FoodNet in the USA (Jones *et al.*, 2007), OzFoodnet in Australia (Kirk *et al.*, 2008), and the International Collaboration on Enteric Disease Burden of Illness Studies (Flint *et al.*, 2005, Roy *et al.*, 2006, Thomas *et al.*, 2006). As laboratory-based surveillance only detects a fraction of all illness occurring in the population, modeling approaches have been used to reconstruct the surveillance pyramid (Michel *et al*., 2000, Voetsch *et al*., 2004, Majowicz *et al*., 2005). Serosurveillance is now being explored as a new tool to provide internationally comparable estimates of the exposure of populations to foodborne pathogens (Simonsen *et al.*, 2008).

Although most surveillance activities are focused on gastro-intestinal illness, other symptoms are also commonly associated with foodborne illness. These may be more serious or of longer duration than GI illness. Furthermore, most pathogens that can be transmitted by food may also be transmitted by other pathways such as water, direct human and animal contact. Therefore, there is a need for source attribution to quantify the proportion of all cases that is foodborne, and the food vehicles that are most frequently associated with illness (Batz *et al.*, 2005). Molecular typing has successfully been used for source attribution of salmonellosis (Van Pelt *et al*., 1999, Hald *et al*., 2004) and more recently for campylobacteriosis (Wilson *et al*., 2008). Other methods being explored include case-control studies, outbreak studies, risk

595 assessment modeling, natural or deliberate intervention studies at population level and expert
596 elicitation. Each method is subject to specific biases, and may attribute illness to different
597 points in the food chain. Therefore, interpreting the results from attribution studies should be
598 done with care (Pires et al., 2009).

599 The World Health Organization has recently launched a new initiative to estimate the burden
600 of foodborne illness on a global scale (Stein *et al.*, 2007). This initiative is advised by experts
601 of the Foodborne Epidemiology Reference group (FERG), which assembles and appraises
602 global evidence on foodborne disease epidemiology. This action is considered necessary in
603 view of globalization, and to contribute towards meeting the Millennium Development Goals[1].
604 Results will be a basis for action at the global scale. Virtually no data on morbidity and
605 mortality exist in large areas of the world, and even more data gaps are expected for
606 attribution. Therefore, systematic reviews will be carried out and extrapolation will be
607 necessary. As an example approach, the estimates of death from diarrhea for children under 5
608 from the Childhood Epidemiology Reference group (CHERG) will be used (Boschi-Pinto *et*
609 *al.*, 2008); complemented with other methods, including expert opinion.

610 In addition to surveillance of human illness, systematic food chain surveillance is necessary to
611 inform food safety decision making. Recent EU-wide baseline studies on the prevalence of
612 zoonotic pathogens have illustrated the benefits of such standardized sampling and analytical
613 approaches. For example, in the baseline survey on the prevalence of *Salmonella* in slaughter
614 pigs, which took place between October 2006 and September 2007, it was demonstrated that
615 approximately one out of every ten slaughter pigs in the European Union was infected with
616 *Salmonella* in the lymph nodes, while one out of twelve pig carcasses was contaminated with
617 Salmonella. The survey also indicated large differences between Member States (Anonymous,
618 2008c). These data will be the basis for risk assessment and cost-benefit analysis of

[1] http://www.un.org/millenniumgoals/

Salmonella control in the slaughter pig chain to support decision making by European risk managers.

The previous sections have described a highly complex set of interrelated factors affecting future trends in food safety. Predicting the impact of these factors is highly complex and surrounded by uncertainties. Hence, to be able to respond timely and appropriately, active, real-time surveillance in both the human and food system and communication to professionals responsible for infection control is of utmost importance.

6. Methodology

Molecular Methods for complex food analysis

One of the key challenges in food microbiology that has always been around and can now be addressed is to assess what molecular mechanistic processes underlie the observed physiological behavior of pathogens in food (see e.g. McMeekin *et al.*, 2007). Much of this work relies on a proper identification of (a) the microorganisms in the food at hand and (b) the food components that are relevant in determining the microbial stability of such foods. The latter range from small molecules (flavor-like molecules, food preservatives and other organic molecular) to the macro-ingredients i.e. proteins (peptides), sugar (polymers) and fats.

In many foods the microbes that are to be analyzed for are non-uniformly dispersed throughout the product. This is the case for many ready-to-eat products from the chilled food chain and is equally so for liquid products such as sauces and soups in which particles, as putative sources of microorganisms, may be non-uniformly mixed.

Analysis of microorganisms in foods may be done with two objectives in mind. On the one hand it may be a direct assessment related to production processes or inspection, on the other hand it may be research-oriented in which physiological inferences are made from molecular data. Rapid analysis techniques for use in industrial practice have to be easy to perform, low

643 cost, optimally selective and must demonstrate reproducible sensitivity and specificity. Such
644 methods need to be validated and written down in standardized protocols, preferably being
645 able to provide quantitative data of use in risk assessment and in food safety management.

646 Currently they are generally based on DNA detection systems, either specific for ribosomal
647 genes, or in the more advanced systems for specific sequences that occur along the entire
648 genome (see e.g. Wattiau *et al.*, 2008; Scaria et al., 2008). Comparative genome sequencing is
649 certainly at hand nowadays. Thus, in the case of relevant, closely related bacterial isolates it is
650 increasingly easy to identify unique sequences (see e.g. the discussions in Earl *et al.*, 2008
651 and Medini *et al.*, 2008). Many of these may then be used to derive sequences amenable to
652 use in DNA chip and / or PCR based detection platforms to the benefit of the safety
653 assessment of food processing.

654 Although these methods are fast, highly specific and relatively sensitive, the application of
655 molecular-based techniques in the control of food safety seems to be limited as they suffer
656 from some serious drawbacks. The development of a horizontal method is seriously hampered
657 by the fact that food products may contain interfering components. The development of
658 horizontal methods becomes even more difficult due to a constant introduction of new
659 matrices. While they are very sensitive, low copy numbers are difficult to detect when the
660 sample size is very small. Introduction of an enrichment step preceding DNA-detection is a
661 solution, but this makes results qualitative, rather than quantitative unless cumbersome MPN
662 techniques are used. While this may not be problematic for quality assurance purposes,
663 quantitative results may be necessary for risk assessment studies. Sample preparation needs
664 close attention. Preferably, such sampling needs to be rapid and as homogeneous as possible.
665 Innovative strategies focus on the use of magnetic beads coated with cell-recognizing
666 molecules, on physical methods such as floatation, and on lysis of whole food matrices
667 (Wagner and Dahl, 2008). The latter was described originally by Hein and co-workers who

obtained enough bacteria from a complex set of food matrices in one-step approach taking only a few hours to be able to recover DNA for further study (Rossmanith *et al.*, 2007). It has yet to be established that such a procedure will also be effective for determining the concentration of bacterial spores. A limitation of currently available molecular techniques is also that they fail to discriminate between viable and inactivated organisms. Recent research may provide future practical solutions to this as transcriptional activity around bacterial cell survival / death reveals molecular markers for cell viability (Kort *et al*., 2008).

Finally, assays based on detection of multiple virulence genes can still give ambiguous results if there is a mixed culture to begin with. This may be for instance the case for the detection of the Shiga like toxin (stx) and Intimin (eaeA) genes from *Escherichia coli* in direct molecular analyses on food samples (e.g. Monday et al. 2007). The outcome will be positive with one strain having both or two strains, each having one of the virulence genes.

The issues discussed above corroborate the notion that it is necessary to properly validate such newly developed techniques. How do they compare to standard reference culture techniques described in ISO-protocols, and which controls must be used? Information is available on the efficacy of protocols using spiked samples, but little information is available on the efficacy of developed protocols in case of naturally contaminated samples. Without multi-laboratory validation, protocols for molecular techniques can be used for in-house purposes, but to be used as a standard method for the detection of pathogens, molecular techniques have to be validated, using a multi-laboratory approach, according to the ISO-16140 protocol.

In analyzing the composition of foods it is also more and more possible to detail comprehensively the full chemical spectrum of the compounds observed. To this end tools such as liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS) are increasingly successfully used (reviewed in Hounsome *et al.*, 2008). A

full analysis provides valuable information on product quality as well as the environmental parameters that can be most relevant to microbial survival (Beckmann et al., 2007). The analysis may also be used to detect the presence of microbial spoilage (Ellis et al., 2007). Pattern analysis to identify relevant compounds is the area where developments are rapid. While the costs of detection at the DNA level are increasingly reducing and interpretation of the data can now be automated to a large extent, this is not always as straightforward with the measurements of small to medium sized (mostly) organic molecules (see e.g. review by Hounsome *et al.*, 2008).

In the research area, molecular techniques are widely used. They can be used for the identification of organisms, for behavioral studies or for studying genes involved in (the regulation of) virulence and stress in response to different environmental conditions, while they can also be used in evolutionary studies. This approach marks the second objective, i.e. the use of genomics data to underpin physiological observations and to mechanistically 'explain' them. Here the analysis platform used need not be restricted to the cheaper methods, focused on biomarkers only; in fact the approach should be wider in nature while costing marginally more (though cost-effectiveness remains an important parameter). To pinpoint which type of compound has the most effect on the microorganisms at hand it is useful to analyze the genome-wide expression pattern and use the data obtained as a bioassay to identify the physiologically most sensitive environmental cues. Translation of molecular data into a biological meaning remains an essential subject for future studies. Application of techniques from other disciplines like ecology and medicine will be very useful.

Mining molecular data for new threats; metabolic capability models

New methods to screen sequenced genomes with the aim of understanding the physiological capability of microbes have led to the identification of major differences at the genome level between common laboratory strains of e.g. *E. coli* K12, enterohemorragic *E. coli* and

uropathogenic *E. coli* (Brzuszkiewicz *et al.*, 2006; Fraser-Ligget, 2005; see also Perna *et al.*, 2001). Extending the analysis with state of the art rapid sequencing technique to other pathogens such as bacilli and certain streptococci has led to the realization of the so-called 'pan' and 'core' genome concepts (see e.g. Hiller *et al.*, 2007, Ara *et al.*, 2007 and the discussion in Medini *et al.*, 2008). In this classification the pan-genome is seen as being composed of three elements: the core genome, a set of non-essential genes shared by several isolates (strains) of the species, and a set of genes unique to an isolate. The size of each can significantly differ from species to species. The core genome generally gives the basic metabolic requirements of a certain species whereas the genetic plasticity of strains is generated by the other sequences (Medini *et al.*, 2008 for general concepts and Earl *et al.*, 2008 for specifics regarding bacilli). Data on the core genome aid significantly in defining the metabolic potential of an organism. Flux Balance Analysis is then often used as a modeling approach to find the possible steady states that the organism can attain (Schilling, 2000). In a next step such models may be detailed further to incorporate molecular signaling data at various levels of complexity (see Ropers *et al.*, 2006 for a highly detailed model of carbon starvation in *Escherichia coli*). Such extension should at all times be subject to scrutiny though in assessing its use given the investment of effort needed versus the (food) microbiological problem at hand needs to be considered.

7. Interaction between micro-organisms and their environment (foods) and microbial evolution

Understanding short-term adaptations of microbes

The recent genomics revolution has facilitated the interpretation of the molecular basis of microbial behavior. Examples stem from many fields and range from bacteria to filamentous fungi. The response to high-end temperature stress conditions often characteristic of the

742 manufacturing process of savoury products is nowadays studied at the molecular level. This is
743 most relevant to aerobic bacterial spore formers. Various strains of bacilli produce spores
744 resistant to temperatures up to and well above those of classical sterilization at 121ºC (Oomes
745 *et al.,* 2007). Keijser *et al.,* (2007) showed that spores express specific stress response genes
746 during germination, some of which are likely responsible for repair of incurred thermal
747 damage (see also Setlow 2006). In order to aim at understanding spore behavior after a
748 thermal stress, in particular mechanisms of heat damage repair, we now have the possibility of
749 utilizing the genome information for *Bacillus subtilis* 168 (Kunst *et al.,* 1997).

750 Cells subjected to acidic food conditions or to low water activity environments have been
751 studied extensively in the context of food microbiology. Specific examples of food
752 preservative stresses are those where cells have to respond to the antimicrobial action of a
753 weak-organic acid such as sorbic acid. The latter is the most widely used food preservative.
754 The common view is that the cells initially use energy driven pumps to extrude the acid from
755 the cytosol while upon full adaptation they induce the synthesis of pumps specific for
756 lipophilic weak acids (discussed for yeast extensively in Mollapour *et al*., 2008; for recent
757 original research on sorbic acid stress response in vegetative bacteria see Ter Beek *et al*.,
758 2008).

759 Stress adaptation of microorganisms in foods or upon being exposed to food processing
760 conditions may lead to the induction of survival systems and could even induce virulence in
761 pathogens. Many phenomena i.e. resistance to preservatives, oxidizing agents and natural
762 extracts in foods are as important for successful infection as are mechanisms operative in ‘in
763 host’, actual infection, and survival. Erickson and Doyle (2007) have illustrated this
764 extensively for Shiga toxin-producing *Escherichia coli* and its survival on fresh produce, meat
765 and in unpasteurized juices. Successful activation of stress response systems by some but not

766 all strains may be instrumental in letting some strains adapt to the 'adverse' conditions in the
767 food chain.

768 *Presence and development of microbial virulence traits in non-human environments*

769 The following section will provide some selected examples of microbial virulence traits of
770 relevance to man of organisms present in non-human environments.

771 Foodborne pathogens may survive well in the animal production chain. Classical examples
772 include *Campylobacter jejuni*, an organism not pathogenic to avian species but highly
773 pathogenic to man (reviewed by Poly and Guerry, 2008). The organism is widely spread, as
774 was demonstrated again by, for instance, the studies of Fearnley *et al.* (2008). These authors
775 demonstrated the occurrence of hyperinvasive *Campylobacter* strains in isolates both from
776 poultry and from human sources. There is not much data yet on the molecular basis of
777 infection, be it that information on the intracellular signal transduction cascades of the
778 organism becomes more and more available (Boyd *et al.*, 2007).

779 *Salmonella* species are well-known pathogens for animals and man. Callaway *et al.* (2008)
780 have recently described their occurrence in various types of cattle. Sternberg et al. (2008)
781 described an outbreak of *Salmonella* infection in a Swedish dairy herd. Schmidt et al. (2008)
782 reported on *Salmonella enterica* infections in Swiss cattle in the summer of 2008. The various
783 serovars have meanwhile been characterized at the molecular level (Edwards *et al.*, 2002).
784 The use of such data can be in two non mutually exclusive directions. On the one hand the
785 data provide information for use in quality control settings and epidemiological surveillance.
786 On the other hand the gathered information can be used as a starting point in the formulation
787 of novel research questions such as the molecular physiological mechanisms behind the
788 observed microbial ecology. Studies aiming at answering such questions will require next to
789 kinetic data on microbial metabolism measured at the population level, also quantitative data

on cell - cell variation in microbial stress response in order to allow incorporation in next generation predictive food microbiology models (McMeekin et al., 2007). Both for non-pathogenic *B. subtilis* and for pathogenic *B. cereus*, spore formation of strains attached to naturally occurring biofilms is a well known phenomenon (Lindsay *et al.*, 2006). It has also been documented nicely that spore-formation in a biofilm-like environment, a complex colony, leads to spores with a higher thermal resistance than that observed in spores originating from liquid cultures (Veening *et al.*, 2006). The (thermal) stress resistance of spores is again not a direct virulence trait but is does contribute to survival in the animal chain as well as transfer to the human food chain (Huck *et al.*, 2008). As such it is a crucial determinant of the likelihood of intoxication of the host. Other such virulence characteristics of e.g. *Bacillus cereus* include the resistance of the spores (and vegetative cells) to acid facilitating the 'settlement' and toxin production of the organism in the intestine (Wijnands, 2008; see also Stenfors Arnesen *et al.*, 2008).

Predictive modeling

Crucial is the conversion of molecular physiological 'analogue' data at the population level to data at the level of single cells relevant to the prediction of the behavior of low-numbers exposed to stressful environments (discussed in McMeekin *et al.*,, 2007; see for original research amongst others Den Besten *et al*., 2007). This provides insight into the link between the genome, gene-expression, protein and metabolic functional cellular units (see for the original physiology data Balaban *et al.*, 2004).). Koutsoumanis (2008) provides a clear example of the variability in growth limits of individual *Salmonella* Enteritidis cells subjected to NaCl stress. Another highly relevant example is the quantification of the germination and outgrowth processes operative in bacterial endospores (Stringer *et al*., 2005; Smelt *et al*., 2008). Both examples do not yet include a mechanistic analysis e.g. at the level of inclusion of genome-wide expression data. The initial challenge for future research is to do just that i.e.

study at single cell / spore level the molecular physiology in order to enable the generation of mechanistic models that describe the cellular heterogeneity in genetically homogeneous microbial populations. Phenotypic heterogeneity in microbial populations mediated by bi-stable signaling networks is a much discussed topic in general microbiology (e.g. Veening et al., 2008, Locke and Elowitz, 2009). To be of relevance to (predictive) food microbiology this will require performing experiments under the relevant physiological (food) conditions and as much as possible with the relevant food isolates.

Taxonomy

Regulators in the US use the GRAS approach (generally recognized as safe) to assess the safety of microbes used in food production, while the EFSA employs the QPS (qualified presumption of safety) system (Anonymous, 2007d). Basically it is assumed that a micro-organism that has been used for a considerable time in food manufacturing without causing problems can be considered "safe". The difficulty in establishing these regulatory systems is the breadth of the taxonomic unit for which QPS or GRAS status can be conferred. If it only can be conferred at strain level, a full risk assessment will still have to be performed for any other strain, even those that are closely related to the ones having GRAS or QPS status. If it is applied at an overly high level, e.g. genus, it can happen that pathogenic cousins of a safe strain are wrongly considered harmless. The taxonomic units concerned are defined in the QPS list and the list is reviewed every year to take into account changes in the taxonomy and other considerations. If the history of safe use and the body of knowledge concerns only one strain, this property cannot be generalized to apply to the entire species, (an example is the *Enterococcus* spp). That is, if there is a risk that closely related strains of the safe strains are pathogenic, the taxonomic unit, species or genus, is not given QPS status, unless the pathogenic strains can be specifically identified, for instance by the presence of virulence

839 factors. In the latter case the unit can be QPS, but with additional qualification. This is, for
840 instance, the case for some *Bacillus* species.
841 Guidelines for the selection of "safe" cultures for biopreservation exist in the area of feed, but
842 not for food (apart from the probiotics area). For general biopreservation, screening of
843 cultures for virulence factors or other genes coding for undesirable properties would be
844 relevant as well as studies of cultures possibly acquiring resistance.
845 For the approval of specific biopreservation agents (e.g. bacteriocins such as nisin)
846 governmental as well as academic and industry views on the criteria to be applied differ
847 around the world. Inconsistencies can cause problems, especially where criteria for safety
848 evaluation or for an agent's effectiveness are too lenient or because they do not take sufficient
849 account of ill-informed use of such agents. The inverse, when a harmless strain is considered
850 pathogenic is less problematic as the only consequence is that the strain undergoes an
851 unnecessary safety assessment. Safe and robust use would need to follow general guidelines
852 on the steps to be taken, as defined in the safe design of the product or the process.

853 **8. Risk communication and education**

854 *Collaboration/communication between scientists*

855 Historically, food systems used to be fairly simple; most foods were produced and eaten
856 locally. Nowadays, a large share of our diet is produced in another country, and not
857 uncommonly, several food ingredients come from different parts of the world (Käferstein *et*
858 *al*., 1997). Furthermore, we prefer to eat the food as fresh as possible (Doyle and Erickson,
859 2008). This trend increases the need for worldwide food safety systems and thus collaboration
860 and communication between all players in the food chain. As food chains extend or expand
861 from local chains into worldwide chains, more and different factors may affect food safety.
862 This implies that for food safety management knowledge or information from different

scientific disciplines needs to be combined. Furthermore, the format in which information is made available needs to be standardized.

Food safety starts at primary production. To reduce the risk of foodborne gastroenteritis, especially for foods to be eaten raw, such as fresh produce and shellfish, knowledge about contamination routes and preventive measures is of great importance. In certain cases interventions could be effective early in the primary production phase, including the production environment. For fresh produce, grown in the open field, *Escherichia coli* (in particular the verocytotoxin producing strains VTEC) is, amongst others, a food safety hazard. This is underlined by a massive foodborne infection outbreak in the USA in 2006 (Anonymous, 2007e) caused by baby spinach eaten raw. The probable source of the VTEC in this outbreak was either irrigation water, or feces from cattle or wild boar. Cattle are a known source of this pathogenic bacterium (Hussein and Sakuma, 2005), yet cow manure continues to be used as a main soil fertilizer in organic farming (Anonymous, 2000b). Preventive measures could be a change of feeding diet in order either to reduce numbers of VTEC shed by cattle (Diez-Gonzalez *et al.*, 1998; Synge, 2000) or to reduce their survival in manure-amended soils (Franz *et al.*, 2005). Thus, produce safety can be increased in this case by combining agricultural science and (food) microbiology.

In other outbreaks, preventive measures are straighter foreword. In 2008, a *C. jejuni* outbreak in Alaska was linked to the consumption of raw peas contaminated on the field by Sandhill crane feces. The outbreak investigation identified a lack of chlorine residual in pea-processing water, which could have been easily prevented (Gardner and McLaughlin, 2008). Introducing buffer zones, set-back distances and fences to restrict wildlife access to the production environment of produce such as leafy greens may prevent problems with feral swine or deer (Atwill, 2008). Although these solutions seem sometimes fairly simple, they could only be

887 taken due to a proper outbreak investigation that elucidated the (probable) source of the food
888 contamination.
889 In order to stop an ongoing outbreak, such outbreak investigations should be carried out
890 quickly, which relies on close collaboration between different (scientific) disciplines such as
891 microbiologists, epidemiologists, wildlife control specialists, risk communicators, etc, often
892 represented by different organisations or institutes, which may hamper the investigation.
893 Although not standing on its own, the lack of proper communication between different
894 scientific disciplines and/or institutes/departments during an outbreak investigation is shown
895 in the 2009 U.S.A. *Salmonella* Saintpaul outbreak caused by raw jalapeño and serrano pepper
896 in which more than 1400 cases were registered. This outbreak continued to spread due to
897 malfunctioning at the level of policy, the public-health system's organization and outbreak
898 response, and its communications with the media and the public as concluded by the post-
899 mortem investigation into this outbreak (Anonymous, 2008). This clearly shows that
900 collaboration of scientific disciplines is eminent to increase the safety of our food system.
901 Other examples that demonstrate the added value of combining different expertises to limit
902 the risk of foodborne illness are for instance *Vibrio* spp. in shellfish and mycotoxins in grain
903 (products). The quality of shellfish depends on the quality of the water they are grown in.
904 Pathogenic bacteria of importance in these types of products are *Vibrio* spp. The number of
905 vibrios present in the water is positively related to the water temperature (Motes *et al.*, 1998)
906 and models that predict ocean water temperatures can be used to predict the level of these
907 pathogens in shellfish, thus combining (food) microbiology and oceanography, Ford et al.,
908 2009). This knowledge was used by Californian lawmakers in order to ban the sale of raw
909 oysters from certain waters during the warmer months of the year (Anonymous, 2003). The
910 presence of mycotoxins on grain products is affected by weather conditions during growth
911 and harvest. As humidity increases, growth increases of the moulds that produce mycotoxins

on these products. Thus meteorological data are a reliable indicator of the risk of the concentration of mycotoxins on grain products (Schaafsma and Hooker, 2007; Van der Fels-Klerx *et al.*, 2008).

To determine which intervention strategy is the best or most cost effective to be taken to reduce risk of foodborne illness, a detailed risk assessment needs to be conducted and combining with a proper economic analysis may prove necessary. This requires a highly multidisciplinary approach, involving microbiologists, epidemiologists, risk modelers, economists and social scientists (Havelaar *et al.*, 2007a). An example of this approach is the CARMA project, carried out in the Netherlands, to evaluate options to reduce the risk of campylobacteriosis due to consumption of chicken meat (Havelaar *et al.*, 2007b).

For risk assessment studies, many data are needed like prevalence and numbers of foodborne pathogens in food. However, these data are not always available or are not in the correct format. For instance, data on the occurrence of *Salmonella* in food is mainly available as "presence or absence in 25 g of product" as legislation requires testing based on this criterion (Anonymous, 2005a). For risk assessment studies, however, the exact level of contamination is important (Malorny *et al.*, 2008). Even when data are available in the preferred format, problems may arise with nomenclature of foods or lack of other necessary details. For instance, a meatball can be either made from beef or pork or a combination of both. And problems increase with multiple-ingredient products, such as in lasagna. Uniformity in nomenclature of foods is, therefore, of great importance and a tool such as LanguaL, a food description thesaurus (www.langual.org), can be very useful.

In many food consumption surveys foods are categorized as, for example "beef with or without sauce", whereas the degree of cooking of the meat is of greater microbiological relevance. To improve the use of data generated in food consumption surveys, closer collaboration between risk assessors, food microbiologists and nutritionists is needed.

In the Netherlands, the Dutch Food and Consumer Product Safety Authority (VWA) works closely together with the National Institute for Public Health and the Environment (RIVM) to improve the quality and usefulness of the data obtained from routine monitoring programs of the microbiological quality of foods. Some recent studies focused on the relative microbiological risk to consumers associated with the consumption of fresh vegetables (Pielaat and Wijnands, 2008) and prevalence of potentially pathogenic *Bacillus cereus* in food (Wijnands *et al.*, 2006). A simple, spreadsheet-based tool is being developed to assess consumer risks associated with such products using available data (Evers and Chardon, 2008).

These examples clearly show the benefits of inter- and intra- scientific collaboration. Close personal collaboration may not always be necessary when data can be shared by other means. In the scientific literature, many data are published that can be used by others. However, translation of these data to a uniform data set is time consuming. In order to improve sharing of data on microbial growth and inactivation, the ComBase Initiative was established, a collaboration between the Food Standards Agency and the Institute of Food Research from the United Kingdom, the USDA Agricultural Research Service and its Eastern Regional Research Center from the United States and the Australian Food Safety Centre of Excellence (Combase Consortium, 2008). ComBase is a combined database of microbial responses to food environments and data can be used for predictive modelling. Recently, Combase started a collaboration with the Journal of Food Protection, which request authors to submit their data to the Combase database. A clearinghouse of interdisciplinary data is offered by Foodrisk.org, an initiative of the Joint Institute for Food Safety and Applied Nutrition (JIFSAN), and which is a collaboration between the University of Maryland (UM) and the Food and Drug Administration (FDA). The clearinghouse provides data and methodology on food safety risk analysis offered by the private sector, trade associations, federal and state agencies, and international sources (www.foodrisk.org).

In conclusion, although interdisciplinary collaboration sounds very promising, it must be noted that it is generally not straightforward as, for instance, the data thus made available may be limited or be in the wrong format. Despite this, with some extra effort more progress may be achieved than would otherwise be the case.

Education

New trends in food consumption patterns, e.g. the consumption of raw foods, and the introduction of a wide variety of newly developed food products with each their specific way of preparation (e.g. ready-to-eat; ready-to-heat), in combination with an increase in the number of vulnerable people, require clear communication about food safety aspects, communication between industry, consumer and government.

Risk managers should be aware and understand public concerns about food safety as this must be the basis of a risk management strategy (Frewer, 2004). Whether such a strategy will be judged as effective by the public, will depend on the expertise of food risk managers (Van Kleef *et al*, 2007) and cultural variation: what is effective in one country, is not always as effective in another (Van Dijk *et al*, 2008).

Industry

When introducing new food products or new food preparation techniques, it is crucial to provide pertinent information concerning safe food handling and preparation. The food industry can contribute to food safety in several ways. Labeling, providing information about correct storage conditions and ways of preparation, can contribute to food safety, although the addition of more information is at odds with providing clear food labels (Mills *et al*, 2004). Icons can be used. The food industry can further contribute to food safety by educating professionals working along the food production chain. As an example, the efforts of the

public-private partnership formed by the Industry Council for Development with FAO and WHO may be noteworthy (Motarjemi, 2006).

Consumers

Presence of pathogenic bacteria on raw food materials, such as meat and fresh produce is in most cases not totally avoidable, therefore intervention strategies are also needed in subsequent steps of the food chain to reduce the risk of foodborne illness for the consumer. At the other side of the farm-to fork continuum the consumers also play an important role in maintaining food safety. Different information campaigns therefore focus on improving home hygiene (Anonymous, 2008d, 2008e). However, the impact of such campaigns is often not evaluated. A combined research project undertaken by social scientists, food microbiologists and risk assessors showed the limited effect of such campaigns on reducing the actual level of bacteria present in a meal, and on the associated risk of human illness (Nauta *et al.*, 2008).

Slovic (1987) developed a psychometric paradigm, which demonstrated that psychological factors determine a person's response to different hazards, including those in the area of food safety. Do consumers know what they should do in order to prepare a safe meal, in particular how to avoid cross-contamination and proper heating of reused food (Fischer *et al*, 2007)? According to Nauta *et al* (2008), consumers already possess the necessary knowledge regarding hygiene practices; this knowledge only needs to be activated. How to achieve this? What can we learn in this respect from other education campaigns, (e.g. smoking, alcohol, and fat), and from communication of medical product risks (Goldman, 2004), or from programs focusing on disease prevention and control (O'Loughlin *et al.*, 1995; Sarraf-Zadegan *et al.*, 2003).

Health related behaviors are often differentially distributed across socioeconomic groups. Close collaboration with social scientists therefore seems logical. Communication with

1009 different consumer groups requires different approaches and media. Information should be
1010 targeted to specific groups at risk, like single households, pregnant women or elderly people.
1011 This is because different groups have different food preparation and cooking habits and
1012 therefore are exposed to different levels of risk. Kornelis *et al.* (2007) showed that different
1013 consumers prefer different information sources when posing questions about food safety.
1014 Two-thirds of all consumers prefer information from either institutional or social sources.
1015 *Your life*, a free magazine containing articles about fashion, lifestyle and entertainment
1016 published by the UK National Health Service, is an illustrative example of such a social
1017 source. Members of the lower socioeconomic groups are more likely to respond to
1018 information from their direct social environment (Weenig and Midden, 1997). Apparently,
1019 communication with different consumer groups requires different approaches and media. The
1020 importance of educating children through the school system cannot be emphasized enough.

1021 *Government*

1022 Food preparation and cooking practices are based on habits. This goes for consumers and
1023 often also for food professionals in small food establishments, such as food services and
1024 restaurants. Since such behavior is difficult to change, education should be a life long learning
1025 process on general aspects of food safety as hygiene and contamination routes, starting at a
1026 young age, making use of all types of media, including video-gaming. Education in food
1027 safety aspects could be combined with nutritional information. Concurrent with the
1028 development of education programs, strategies should be developed to measure the impact of
1029 such programs (Nauta *et al.*, 2008). In addition, governmental organizations should consider
1030 introducing and supporting specific education programs for consumers and professionals in
1031 small food preparation enterprises as well as for producers, especially for producers of fresh
1032 produce, as all have their responsibility with regard to food safety.

Finally, while education might be expected to improve food safety in the developed world, in developing countries, economic growth rather than education might be the best way to minimalize food-related mortality and morbidity amongst children.

Acknowledgements

The authors wish to thank all participants of the conference for their willingness to share their insights in plenary and group discussions. Special thanks are due to the plenary speakers (see contributions elsewhere in this Special Issue), the chairs and rapporteurs of breakout groups and the members of the Scientific Committee. These include, apart from the authors, Andrea Ammon, József Baranyi, Bob Buchanan, Patrice Buche, John D. Collins, Paul Cook, Mike Doyle, Aamir Fazil, Leon Gorris, Colin Hill, Marta Hugas, Marion Koopmans, Hilde Kruse, Hein van Lieverloo, Pia Makelä, Tom McMeekin, Diane Newell, Wim Ooms, Tom Quested, Peter Raspor, Tom Ross, Moez Sanaa, Don Schaffner, Claudia Stein, Mark Tamplin, Rob Tauxe, Mieke Uyttendaele, Yvonne Van Duynhoven, Didier Verloo, and Martin Wagner.

References

Adak, G.K., Meakins, S.M., Yip, H., Lopman, B.A., O'Brien, S.J., 2005. Disease risks from foods, England and Wales, 1996-2000. Emerging Infectious Diseases 11, 365-372.

Amar, C. F., East, C. L., Gray, J., Iturriza-Gomara, M., Maclure, E. A., and McLauchlin, J. (2007). Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993-1996). European Journal of Clinical Microbiology and Infectious Diseases 26, 311-23.

Anonymous, 2000a. Dorland's Illustrated Medical Dictionary. 29th ed. WB Saunders Co, Philadelphia, PA.

Anonymous, 2000b. National Organic Program. American Society for Microbiology. Available at http,//www.asm.org/Policy/index.asp?bid=3585. Accessed 9 December 2008.

Anonymous, 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. Official Journal of the European Communities, L31/1.

Anonymous, 2003. Raw gulf oysters, labeling, written warnings and additional requirements. California Department of Public Health. CCR, Title 17, Section 13675,. Available at http,//www.cdph.ca.gov/services/Documents/fdb%20Raw%20Gulf%20Oyst%20Regs.pdf. Accessed 9 December 2008.

Anonymous, 2005a. Commission regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Communities, L 338/1.

Anonymous, 2006. Development of practical risk management strategies based on microbiological risk assessment outputs. FAO and WHO, Rome and Geneva.

Anonymous, 2007a. The Community Summary Report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. The EFSA Journal 130, 1-352.

Anonymous, 2007b. Opinion of the Scientific Panel on Biological Hazards on microbiological criteria and targets based on risk analysis. The EFSA Journal 462, 1-29.

1074 Anonymous, 2007c. Multistate outbreak of *Salmonella* serotype Tennessee infections associated with peanut
1075 butter--United States, 2006-2007. MMWR Morbidity Mortality Weekly Report 56, 521-524.

1076 Anonymous, 2007d. EFSA Scientific Committee, Introduction of a Qualified Presumption of Safety (QPS)
1077 approach for assessment of selected microorganisms referred to EFSA. The EFSA Journal 587, 1-16.

1078 Anonymous, 2007e. Investigation of an *Escherichia coli* O157,H7 outbreak associated with Dole pre-packaged
1079 spinach. California Food Emergency Response Team Sacramento/Alameda, CA, USA. Available at
1080 http,//www.marlerclark.com/2006_Spinach_Report_Final_01.pdf. Accessed 22 December 2008.

1081 Anonymous, 2007f. Scientific Opinion of the Panel on Biological Hazards on a request from the European
1082 Commission on public health risks involved in the human consumption of reptile meat. The EFSA Journal
1083 578, 1-55.

1084 Anonymous, 2008a. Outbreak of *Salmonella* serotype Saintpaul infections associated with multiple raw produce
1085 items--United States, 2008. MMWR Morbidity Mortality Weekly Report 57, 929-934.

1086 Anonymous, 2008b. Climate change, Implications for food safety. Food and Agriculture Organization of the
1087 United Nations, Rome.

1088 Anonymous, 2008c. Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline
1089 survey on the prevalence of *Salmonella* in slaughter pigs, in the EU, 2006-2007[1] - Part A, *Salmonella*
1090 prevalence estimates. The EFSA Journal 135, 1-11.

1091 Anonymous, 2008d. Fight Bac! Educating consumers about safe food handling. Partnership for Food Safety
1092 Education. Available at http,//www.fightbac.org/. Accessed 9 December 2008.

1093 Anonymous, 2008e. Hygiëne. Voedingscentrum. Available at
1094 http,//www.voedingscentrum.nl/EtenEnVeiligheid/Hygiëne/. Accessed 9 December 2008.

1095 Anonymous, 2009. Multistate outbreak of Salmonella infections associated with peanut butter and peanut butter-
1096 containing products--United States, 2008-2009. Morbidity Mortality Weekly Report 58, 85-90.

1097 Ara, K., Ozaki, K., Nakamura, K., Yamane, K., Sekiguchi, J. Ogasawara, N., 2007. *Bacillus* minimum genome
1098 factory, effective utilization of microbial genome information. Biotechnology and Applied Biochemistry. 46,
1099 169-178.

1100 Arvanitoyannis, I.S. Traikou, A., 2005. A comprehensive review of the implementation of hazard analysis
1101 critical control point (HACCP) to the production of flour and flour-based products. Critical Reviews in Food

1102 Science and Nutrition 45, 327-370.

1103 Balaban, N.Q., Merrin, J., Chait, R., Kowalik, L., Leibler, S., 2004. Bacterial persistence as a phenotypic switch.
1104 Science 305, 1622-1625.

1105 Batz, M.B., Doyle, M.P., Morris, G., Painter, J., Singh, R., Tauxe, R.V., Taylor, M.R., Lo Fo Wong, D.M.A.,
1106 2005. Attributing Illness to Food . Emerging Infectious Diseases 11, 993-999.

1107 Beckmann, M., Enot, D.P., Overy, D.P. and Draper, J., 2007. Representation, comparison, and interpretation of
1108 metabolome fingerprint data for total composition analysis and quality trait investigation in potato cultivars.
1109 Journal of Agricultural and Food Chemistry. 55, 3444-3451.

1110 Boschi-Pinto, C., Velebit, L., Shibuya, K., 2008. Estimating child mortality due to diarrhoea in developing
1111 countries. Bulletin of the World Health Organization 86, 710-717.

1112 Boyd, A., Philbin, V.J., Smith, A.L., 2007. Conserved and distinct aspects of the avian Toll-like receptor (TLR)
1113 system, implications for transmission and control of bird-borne zoonoses. Biochemical Society Transactions
1114 35, 1504-1507.

1115 Boyle, R.J., Robins-Browne, R.M., Tang, M.L., 2006. Probiotic use in clinical practice: what are the risks?
1116 American Journal of Clinical Nutrition 83, 1256-64.

1117 Brzuszkiewicz , E., Bruggemann, H., Liesegang, H., Emmerth, M., Olschlager, T., Nagy, G., Albermann, K.,
1118 Wagner, C., Buchrieser, C., Emody, L., Gottschalk, G., Hacker, J., Dobrindt, U., 2006. How to become a
1119 uropathogen, comparative genomic analysis of extraintestinal pathogenic *Escherichia coli* strains.
1120 Proceedings of the National Acadamy of Sciences USA 103, 12879–12884.

1121 Callaway, T.R., Edrington, T.S., Anderson, R.C., Byrd, J.A., Nisbet, D.J., 2008. Gastrointestinal microbial
1122 ecology and the safety of our food supply as related to *Salmonella*. Journal of Animal Science 86, E 163-
1123 172.

1124 Combase Consortium, 2008. ComBase. Available at http,//www.combase.cc/, Accessed 9 December 2008.

1125 Cretikos, M., Telfer, B., McAnulty, J., 2008. Enteric disease outbreak reporting, New South Wales, Australia,
1126 2000 to 2005. New South Wales Public Health Bulletin 19, 3-7.

1127 Dalton, C.B., Austin, C.C., Sobel, J., Hayes, P.S., Bibb, W.F., Graves, L.M., Swaminathan, B., Proctor, M.E.,
1128 Griffin, P.M., 1997. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. New
1129 England Journal of Medicine 336, 100-105.

1130 Den Besten, H.M.W., Ingham, C.J., Van Hylckama Vlieg, J.E.T., Beerthuyzen, M.M., Zwietering, M.H,. Abee,
1131 T., 2007. Quantitative analysis of population heterogeneity of the adaptive salt stress response and growth
1132 capacity of *Bacillus cereus* ATCC 14579. Applied and Environmental Microbiology 73, 4797-4804.

1133 De Wit, M.A.S., Koopmans, M.P.G., Kortbeek, L.M., van Leeuwen, N.J., Bartelds, A.I.M., van Duynhoven,
1134 Y.T.H.P., 2001a. Gastroenteritis in sentinel general practices, The Netherlands. Emerging Infectious
1135 Diseases 7, 82-91.

1136 De Wit, M.A.S., Koopmans, M.P.G., Kortbeek, L.M., Van Leeuwen, N.J., Vinjé, J., Van Duynhoven, Y.T.H.P.,
1137 2001b. Etiology of gastroenteritis in sentinel general practices in the Netherlands. Clinical Infectious
1138 Diseases 33, 280-288.

1139 De Wit, M.A.S., Koopmans, M.P.G., Kortbeek, L.M. , Wannet, W.J., Vinjé, J., Van Leusden, F., Bartelds,
1140 A.I.M., Van Duynhoven, Y.T.H.P., 2001c. Sensor, a population-based cohort study on gastroenteritis in the
1141 Netherlands, incidence and etiology. American Journal of Epidemiology 154, 666-674.

1142 Diez-Gonzalez, F., Callaway, T.R., Kizoulis, M.G., Russell J.B., 1998. Grain feeding and the dissemination of
1143 acid-resistant *Escherichia coli* from cattle. Science 281, 1666-1668.

1144 Doyle, M.P., Erickson, M.C., 2008. Summer meeting 2007 - the problems with fresh produce: an overview.
1145 Journal of Applied Microbiology105, 317-330.

1146 Earl, A.M., Losick, R., Kolter, R., 2008. Ecology and genomics of *Bacillus subtilis*. Trends in Microbiology 16,
1147 269-275.

1148 Edwards, R.A., Olsen, G.J., Malov, S.R., 2002. Comparative genomics of closely related salmonellae. Trends in
1149 Microbiology 10, 94-99.

1150 Ellis, D.I., Broadhurst, D., Rowland, J.J. and Goodacre, R., 2007. Rapid detection method for microbial spoilage
1151 using FT-IR and machine learning. In: Van Amerongen, A., Barug, D and Lauwaars, M. (eds.). Rapid
1152 Methods (for Food and Feed Quality Determination). Wageningen Academic Publishers, Wageningen, the
1153 Netherlands, pp. 73-84.

1154 Erickson, M.C., Doyle, M.P., 2007. Food as a vehicle for transmission of Shiga toxin-producing *Escherichia*
1155 *coli*. Journal of Food Protection 70, 2426-2449.

1156 Evers. E.G., Chardon, J.E., 2008. A swift quantitative microbiological risk assessment (sQMRA) tool. Abstract
1157 K7, Food Micro 2008, 1-4 September, Aberdeen, Scotland.

1158 Fearnley, C., Manning, G., Bagnal, M., Javed, M.A., Wassenaar, T.M., Newell, D.G., 2008. Identification of
1159 hyperinvasive *Campylobacter jejunii* strains isolated from poultry and human clinical sources. Journal of
1160 Medical Microbiology 57, 570-580.

1161 Fischer, A.R., de Jong, A.E., de Jonge, R., Frewer, L.J., Nauta, M.J., 2005. Improving food safety in the
1162 domestic environment, the need for a transdisciplinary approach. Risk Analysis 25 ,503-517.

1163 Fischer, A.R., De Jong, A.E., Van Asselt, E.D., De Jonge, R., Frewer, L.J., Nauta, M.J., 2007. Food safety in the
1164 domestic environment, an interdisciplinary investigation of microbial hazards during food preparation. Risk
1165 Analysis 27, 1065-1082.

1166 Flint, J.A., Van Duynhoven, Y.T., Angulo, F.J., DeLong, S.M., Braun, P., Kirk, M., Scallan, E., Fitzgerald, M.,
1167 Adak, G.K., Sockett, P., Ellis, A., Hall, G., Gargouri, N., Walke, H., Braam, P., 2005. Estimating the burden
1168 of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food, an international
1169 review. Clinical Infectious Diseases 41, 698-704.

1170 Ford, T.E., Colwell, R.R., Rose, J.B., Morse, S.S., Rogers, D.J., Yates, T.L., 2009. Using Satellite Images of
1171 Environmental Changes to Predict Infectious Disease Outbreaks. Emerging Infectious Diseases 15, 1341-
1172 1345.

1173 Franz, E., van Diepeningen, A.D., De Vos, O.J., van Bruggen A.H., 2005. Effects of cattle feeding regimen and
1174 soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enterica* serovar
1175 Typhimurium in manure, manure-amended soil, and lettuce. Applied and Environmental Microbiology 71,
1176 6165-6174.

1177 Fraser-Liggett, C. M., 2005. Insights on biology and evolution from microbial genome sequencing. Genome
1178 Research 15, 1603–1610.

1179 Frewer, L., 2004. The public and effective risk communication. Toxicology Letters 149, 391-397.

1180 Gebreyes, W.A., Bahnson, P.B., Funk, J.A., McKean, J., Patchanee, P. 2008. Seroprevalence of Trichinella,
1181 Toxoplasma, and Salmonella in antimicrobial-free and conventional production systems. Foodborne
1182 Pathogens and Disease 5, 199-203.

1183 Gerner-Smidt, P., Hise, K., Kincaid, J., Hunter, S., Rolando, S., Hyytia-Trees, E., Ribot, E.M., Swaminathan, B.,
1184 2006. PulseNet USA, a five-year update. Foodborne Pathogens and Disease 3, 9-19.

1185 Ginsberg, M.M., Granzow, L., Teclaw, R.F., Gaul, L.K., Bagdure, S., Cole, A., Drumgoole, R., Barzilay, E.J.,

1186 Biggerstaff, M.S., Lynch, M.F., Maslanka, S.E., Williams, I.T., Juliao, P.C., Barton Behravesh, C., Olson,
1187 C.K., 2007. Botulism associated with commercially canned chili sauce--Texas and Indiana, July 2007.
1188 MMWR Morbidity Mortality Weekly Report 56, 767-769.

1189 Goldman, S.A., 2004. Communication of medical product risk, how effective is effective enough? Drug Safety
1190 27, 519-534.

1191 Hall, G., Kirk, M.D., Becker, N., Gregory, J.E., Unicomb, L., Millard, G., Stafford, R., Lalor, K., 2005.
1192 Estimating foodborne gastroenteritis, Australia. Emerging Infectious Diseases 11, 1257-1264.

1193 Hald, T., Vose, D., Wegener, H.C., Koupeev, T., 2004. A Bayesian approach to quantify the contribution of
1194 animal-food sources to human salmonellosis. Risk Analysis 24, 255-269.

1195 Hansen, J., Holm, L., Frewer, L., Robinson, P., Sandøe, P., 2003. Beyond the knowledge deficit, recent research
1196 into lay and expert attitudes to food risks. Appetite 41, 111-121.

1197 Havelaar, A.H., Mangen, M.J., de Koeijer, A.A., Bogaardt, M.J., Evers, E.G., Jacobs-Reitsma, W.F., van Pelt,
1198 W., Wagenaar, J.A., de Wit, G.A., van der Zee, H., Nauta, M.J., 2007a. Effectiveness and efficiency of
1199 controlling *Campylobacter* on broiler chicken meat. Risk Analysis 27: 831-844.

1200 Havelaar, A.H., Bräunig, J., Christiansen, K., Cornu, M., Hald, T., Mangen, M.J., Molbak, K., Pielaat, A., Snary,
1201 E., van Pelt, W., Velthuis, A., Wahlstrom, H., 2007b. Towards an integrated approach in supporting
1202 microbiological food safety decisions. Zoonoses and Public Health 54: 103-117.

1203 Havelaar, A.H., van Duynhoven, Y.T.H.P., van Pelt, W., 2008. Microbiologische ziekteverwekkers in voedsel.
1204 Omvang van het probleem . Hoe vaak komt ziekte als gevolg van microbiologische ziekteverwekkers in
1205 voedsel voor? In: Volksgezondheid Toekomst Verkenning, Nationaal Kompas Volksgezondheid. Bilthoven,
1206 RIVM. Available at http://www.rivm.nl/vtv/object_document/o3617n22451.html. Accessed 12 September
1207 2008.

1208 Herwaldt, B.L., 2000. *Cyclospora cayetanensis*, a review, focusing on the outbreaks of cyclosporiasis in the
1209 1990s. Clinical Infectious Diseases 31, 1040-57.

1210 Hiller, N. L. *et al.* 2007. Comparative genomic analyses of seventeen *Streptococcus pneumoniae* strains, insights
1211 into the pneumococcal supragenome. Journal of Bacteriology 189, 8186–8195.

1212 Hoogenboom, L. A.; Bokhorst, J. G.; Northolt, M. D.; van de Vijver, L. P.; Broex, N. J.; Mevius, D. J.; Meijs, J.
1213 A., and Van der Roest, J., 2008. Contaminants and microorganisms in Dutch organic food products: a

1214 comparison with conventional products. Food Additives & Contaminants. Part A: Chemistry, Analysis,
1215 Control, Exposure & Risk Assessment. 25:1195-1207.

1216 Hounsome, N., Hounsome, B., Tomos, D., Edwards-Jones, G., 2008. Plant metabolites and nutritional quality of
1217 vegetables. Journal of Food Science 73, R48-65.

1218 Huck, J.R., Sonnen, M., Boor, J.K., 2008. Tracking heat resistant, cold-thriving fluid milk spoilage bacteria from
1219 farm to packaged product. Journal of Dairy Science 91, 1218-1228.

1220 Hussein, H.S., Sakuma. T., 2005. Prevalence of shiga toxin-producing *Escherichia coli* in dairy cattle and their
1221 products. Journal of Dairy Science 88, pp. 450-465.

1222 Jones, T.F., Scallan, E., and Angulo, F.J., 2007. FoodNet, overview of a decade of achievement. Foodborne
1223 Pathogens and Disease 4, 60-66.

1224 Käferstein, F. K., Motarjemi, Y., Bettcher, D. W., 1997. Foodborne disease control: a transnational challenge.
1225 Emerging Infectious Diseases 31, 503-510.

1226 Keijser B.J.F., Ter Beek, A., Rauwerda, H., Schuren, F., Montijn, R., van der Spek, H., Brul, S. 2007. Analysis
1227 of temporal gene expression during *Bacillus subtilis* spore germination and outgrowth. Journal of
1228 Bacteriology 189, 3624-3634.

1229 Kirk, M.D., Little, C.L., Lem, M., Fyfe, M., Genobile, D., Tan, A., Threlfall, J., Paccagnella, A., Lightfoot, D.,
1230 Lyi, H., McIntyre, L., Ward, L., Brown, D.J., Surnam, S., Fisher, I.S., 2004. An outbreak due to peanuts in
1231 their shell caused by *Salmonella enterica* serotypes Stanley and Newport--sharing molecular information to
1232 solve international outbreaks. Epidemiology and Infection 132, 571-517.

1233 Kirk, M.D., McKay, I., Hall, G.V., Dalton, C.B., Stafford, R., Unicomb, L., Gregory, J., 2008. Food safety,
1234 foodborne disease in Australia, the OzFoodNet experience. Clinical Infectious Diseases 47, 392-400.

1235 Kornalijnslijper, J.E., Rahamat-Langendoen, J.C., Van Duynhoven, Y.T.H.P., 2008. Volksgezondheidsaspecten
1236 van veehouderijmegabedrijven in Nederland: zoönosen en antibioticumresistentie. Bilthoven, the
1237 Netherlands: RIVM. Briefrapportnr. 215011002. Available at
1238 http://www.rivm.nl/bibliotheek/rapporten/215011002.pdf.

1239 Kornelis, M., de Jonge J., Frewer, L., Dagevos, H., 2007. Consumer selection of food-safety information
1240 sources. Risk. Analysis 27, 327-335.

1241 Kort, R., Keijser, B.J., Caspers, M.P.M., Schuren, F., Montijn, R., 2008. Transcriptional activity around bacterial

1242 cell death reveals molecular biomarkers for cell viability. BMC Genomics 9, 590 (Published ahead of print).

1243 Koutsoumanis, K., 2008. A study on the variability in the growth limits of individual cells and its effect on the
1244 behavior of microbial populations. International Journal of Food Microbiology 128, 116-121.

1245

1246 Kroneman, A.; Verhoef, L.; Harris, J.; Vennema, H.; Duizer, E.; van Duynhoven, Y.; Gray, J.; Iturriza, M.;
1247 Bottiger, B.; Falkenhorst, G.; Johnsen, C.; von Bonsdorff, C. H.; Maunula, L.; Kuusi, M.; Pothier, P.;
1248 Gallay, A.; Schreier, E.; Hohne, M.; Koch, J.; Szucs, G.; Reuter, G.; Krisztalovics, K.; Lynch, M.;
1249 McKeown, P.; Foley, B.; Coughlan, S.; Ruggeri, F. M.; Di Bartolo, I.; Vainio, K.; Isakbaeva, E.; Poljsak-
1250 Prijatelj, M.; Grom, A. H.; Mijovski, J. Z.; Bosch, A.; Buesa, J.; Fauquier, A. S.; Hernandez-Pezzi, G.;
1251 Hedlund, K. O., Koopmans, M., 2008 Analysis of integrated virological and epidemiological reports of
1252 norovirus outbreaks collected within the foodborne viruses in Europe Network from 1 July 2001 to 30 June
1253 2006. Journal of Clinical Microbiology 46,2959-2965.

1254 Kunst, F., Ogasawara, N., Moszer, I., Albertini, A.M., Alloni, G., Azevedo, V., Bertero, M.G., Bessieres, P.,
1255 Bolotin, A., Borchert, S., Borriss, R., Boursier, L., Brans, A., Braun, M., Brignell, S.C., Bron, S., Brouillet,
1256 S., Bruschi, C.V., Caldwell, B., Capuano, V., Carter, N.M., Choi, S.K., Codani, J.J., Connerton, I.F.,
1257 Danchin, A., *et al*., 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*.
1258 Nature 390, 249-256.

1259 Lindsay, D., Brözel, V.S., Von Holy, A., 2006. Biofilm-spore response in *Bacillus cereus* and *Bacillus subtilis*
1260 during nutrient limitation. Journal of Food Protection 69, 1168-1172.

1261 Locke, J.C. and Elowitz, M.B. 2009. Using movies to analyse gene circuit dynamics in single cells. Nature
1262 Reviews Microbiology 7, 383-392.

1263 Magnino S., Colin P., Dei-Cas E., Madsen M., McLauchlin J., Nockler K., Maradona, M. P., Tsigarida, E.,
1264 Vanopdenbosch, E., Van Peteghem, C., 2009. Biological risks associated with consumption of reptile
1265 products. International Journal of Food Microbiology 134,163-175.

1266 Majowicz, S.E., Edge, V.L., Fazil, A,. McNab, W. B., Dore, K. A., Sockett, P. N., Flint, J. A., Middleton, D.,
1267 McEwen, S. A., Wilson, J. B, 2005. Estimating the under-reporting rate for infectious gastrointestinal illness
1268 in Ontario. Canadian Journal of Public Health 96, 178-181.

1269 Malorny, B., Löfström, C., Wagner, M., Krämer, N., Hoorfar, J., 2008. Enumeration of *Salmonella* bacteria in

food and feed samples by real-time PCR for quantitative microbial risk assessment. Applied and Environmental Microbiology 74, 1299-1304.

McMeekin, T.A., Baranyi, J., Bowman, J., Dalgaard, P., Kirk, M., Ross, T., Schmid, S., Zwietering, M.H., 2006. Information systems in food safety management. International Journal of Food Microbiology 112, 181-194.

McMeekin, T.A., Mellefont, L.A., Ross, T., 2007. Predictive microbiology, past, present and future. In, Modelling microorganisms in food Brul, S., van Gerwen, S., Zwietering, M. (eds.), Woodhead, Cambridge (UK). pp. 7-21.

McMeekin, T.A., Ross, T., 2002. Predictive microbiology, providing a knowledge-based framework for change management. International Journal of Food Microbiology 78, 133-153.

Medini, D., Serruto, D., Parkhill, J., Relman, D.A., Donati, C., Moxon, R., Falkow, S., Rappuoli, R., 2008. Microbiology in the post-genomic era. Nature Reviews Microbiology 6, 419-430.

Mevius D.J., Van Pelt, W. (editors), 2006. Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2005. Lelystad, the Netherlands: Central Institute for Animal Disease Control. Available on-line: http://www.cvi.wur.nl/UK/publications/otherpublications/maran/.

Michel, P., Wilson, J.B., Martin, S.W., Clarke, R.C., McEwen, S.A., Gyles, C.L., 2000. Estimation of the under-reporting rate for the surveillance of *Escherichia coli* O157:H7 cases in Ontario, Canada. Epidemiology and Infection 125, 35-45.

Mills, E.N.C., Valovirta, E., Madsen, C., Taylor, S.L., Vieths, S., Anklam, E., Baumgartner, S., Koch, P., Crevel, R.W.R., Frewer, L., 2004. Information provision for allergic consumers-where are we going with food allergen labeling? Allergy 59, 1262-1268.

Mollapour, M., Shepherd, A., Piper, P.W., 2008. Novel stress responses facilitate *Saccharomyces cerevisiae* growth in the presence of the monocarboxylate preservatives. Yeast 25, 169-177.

Monday, S.R., Beisaw, A. and Feng, P.C.H. 2007. Identification of Shiga toxigenic *Escherichia coli* seropathotypes A and B by multiplex PCR. Molecular and Cellular Probes 21, 308-311.

Motarjemi, Y., 2006. ICD in perspective: putting social responsibility into practice. Food Control 17, 1018-1022.

Motes, M.L., DePaola, A., Cook, D.W., Veazey, J.E., Hunsucker, J.C., Garthright, W.E., Blodgett, R.J., Chirtel, S.J., 1998. Influence of water temperature and salinity on *Vibrio vulnificus* in Northern Gulf and Atlantic

1297 Coast oysters (*Crassostrea virginica*). Applied and Environmental Microbiology 64, 1459-1465.

1298 Nauta, M., Fischer, A., van Asselt, E., de Jong, A., Frewer, L., de Jonge, R., 2008. Food safety in the domestic
1299 environment, the effect of consumer risk information on human disease risk. Risk Analysis 28, 179-192.

1300 Noteborn, H.P.J.M., Ooms, W., De Prado, M., 2005. Emerging risks identification in food and feed for human
1301 health. Food and Consumer Product Safety Authority, The Hague.

1302 O'Brien, S.J., Gillespie, I.A., Sivanesan, M.A., Elson, R., Hughes, C., Adak, G.K., 2006. Publication bias in
1303 foodborne outbreaks of infectious intestinal disease and its implications for evidence-based food policy.
1304 England and Wales 1992-2003. Epidemiology and Infection 134, 667-74.

1305 O'Loughlin, J., Paradis, G., Kishchuk, N., Gray-Donald, K., Renaud, L., Fines, P., Barnett, T., 1995. Coeur en
1306 santé St-Henri- a heart health promotion programme in Montreal, Canada, design and methods for
1307 evaluation. Journal of Epidemiology and Community Health 49, 495-502.

1308 Oomes, S.J., van Zuijlen, A.C., Hehenkamp, J.O., Witsenboer, H., van der Vossen, J.M. and Brul, S., 2007. The
1309 characterisation of *Bacillus* spores occurring in the manufacturing of (low acid) canned products.
1310 International Journal of Food Microbiology 120, 85-94.

1311 Pielaat, A., Wijnands, L., 2008. Survey analysis of microbiological contamination in the fresh vegetables food
1312 chain and the associated relative risk to consumers. Food Micro 2008, Aberdeen, Scotland, p. 85.

1313 Pires, S.M., Evers, E.G., Van Pelt, W., Ayers, T., Scallan, E., Angulo, F.J., Havelaar, A.H., Hald, T., 2009.
1314 Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathogens and
1315 Disease 6, 417-423.

1316 Poly, F., Guerry, P., 2008. Pathogenesis of *Campylobacter*. Current Opinion in Gastroenterology 24, 27-31.

1317 Perna, N.T., Plunkett, G., 3rd, Burland, V., Mau, B., Glasner, J.D., Rose, D.J., Mayhew, G.F., Evans, P.S.,
1318 Gregor, J., Kirkpatrick, H.A., Posfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck,
1319 E.J., Davis, N.W., Lim, A., Dimalanta, E.T., Potamousis, K.D., Apodaca, J., Anantharaman, T.S., Lin, J.,
1320 Yen, G., Schwartz, D.C., Welch, R.A., Blattner, F.R., 2001. Genome sequence of enterohaemorrhagic
1321 *Escherichia coli* O157:H7. Nature 409, 529–533.

1322 Pontrelli, G., Boccia, D., Di Renzi, M., Massari, M., Giugliano, F., Celentano, L.P., Taffon, S., Genovese, D., Di
1323 Pasquale, S., Scalise, F., Rapicetta, M., Croci, L., Salmaso, S., 2008. Epidemiological and virological
1324 characterization of a large community-wide outbreak of hepatitis A in southern Italy. Epidemiology and
1325 Infection 136, 1027-1034.

1326 Roy, S.L., Scallan, E., Beach, M.J., 2006. The rate of acute gastrointestinal illness in developed countries.
1327 Journal of Water Health 4, Supplement 2, 31-69.

1328 Ropers, D., de Jong, H., Page, M., Schneider, D., Geiselmann, J., 2006. Qualitative simulation of the carbon
1329 starvation response in *Escherichia coli*. BioSystems 84, 124–152.

1330 Rossmanith, P., Süss, B., Wagner, M., Hein, I., 2007. Development of a matrix lysis for concentration of gram
1331 positive bacteria from food and blood. Journal of Microbiological Methods 69, 504-511.

1332 Sarraf-Zadegan, N., Sadri, G., Malek Afzali, H., Baghaei, M., Mohammadi Fard, N., Shahrokhi, S., Tolooie, H.,
1333 Poormoghaddas, M., Sadeghi, M., Tavassoli, A., Rafiei, M., Kelishadi, R., Rabiei, K., Bashardoost, N.,
1334 Boshtam, M., Asgary, S., Naderi, G., Changiz, T., and Yousefie, A., 2003. Isfahan healthy heart program, a
1335 comprehensive community-based programme for cardiovascular disease prevention and control. Design,
1336 methods and initial experience. Acta Cardiologica 58, 309-320.

1337 Scaria, J., Palaniappan, R.U., Chiu, D., Phan, J.A., Ponnala, L., McDonough, P., Grohn Y.T., Porwollik, S.,
1338 McClelland, M., Chioa, C.S., Chu, C., Chang, Y.F., 2008. Microarray for molecular typing of *Salmonella*
1339 *enterica* serovars. Molecular and Cellular Probes. 22, 238-243.

1340 Schaafsma, A.W., Hooker, D.C., 2007. Climatic models to predict occurrence of *Fusarium* toxins in wheat and
1341 maize. International Journal of Food Microbiology 119, 116-125.

1342 Schilling, C.H., Edwards, J.S., Letscher, D., Palsson B.O., 2000. Combining pathway analysis with flux balance
1343 analysis for the comprehensive study of metabolic systems, Biotechnology and Bioengineering 71, 286–306.

1344 Schmidt, H., Hachler, H., Stephan, R., Baumgartner, A. and Boubaker, K., 2008. Outbreak of *Salmonella*
1345 *enterica* serovar Typhimurium in Switzerland, May-June 2008, implications for production and control of
1346 meat preparations. Eurosurveillance 13, 44, 19020.

1347 Senhaji, A.F., 1977. The protective effect of fat on the heat resistance of bacteria (II). International Journal of
1348 Food Science and Technology 12: 217-230.

1349 Setlow, P., 2006. Spores of *Bacillus subtilis*, their resistance to radiation, heat and chemicals. Journal of Applied
1350 Microbiology 101, 514–525.

1351 Siguier, P., Filee, J., Chandler, M., 2006. Insertion sequences in prokaryotic genomes. Current Opinion in
1352 Microbiology 9, 526-31.

1353 Simonsen, J., Strid, M.A., Molbak, K., Krogfelt, K.A., Linneberg, A., Teunis, P., 2008. Sero-epidemiology as a

tool to study the incidence of *Salmonella* infections in humans. Epidemiology and Infection 136, 895-902.

Slovic, P., 1987. Perception of risk. Science 236, 280-285.

Smelt, J.P.P.M., Bos, A.P., Kort, R. and Brul, S., 2008. Modelling the effect of sub(lethal) heat treatment of *Bacillus subtilis* spores on germination rate and outgrowth to exponentially growing vegetative cells. International Journal of Food Microbiology 128, 34-40.

Stein, C., Kuchenmuller, T., Hendrickx, S., Pruss-Ustun, A., Wolfson, L., Engels, D., Schlundt, J., 2007. The Global Burden of Disease Assessments-WHO Is Responsible? PLoS Neglected Tropical Diseases 1, e161.

Stenfors Arnesen, L.P., Fagerlund, A., Granum, P.E., 2008. From soil to gut, *Bacillus cereus* and its food poisoning toxins. FEMS Microbiological Reviews 32, 579-606.

Sternberg, S., Johnsson, A., Aspan, A., Bergström, K., Kallay, T.B., Szanto, E., 2008. Outbreak of *Salmonella* Thompson infection in a Swedish dairy herd. Veterinary Record 163, 596-599.

Stringer, S.C., Webb, M.D., George, S.M., Pin, C., Peck, M.W., 2005. Heterogeneity of times required for germination and outgrowth from single spores of nonproteolytic *Clostridium botulinum*. Applied and Environmental Microbiology 71, 4998-5003.

Synge, B.A., 2000. Verocytotoxin-producing *Escherichia coli*, a veterinary view. Journal of Applied Microbiology 88, p. 31S-37S.

Tait, J., Meagher, L., Lyall, C., Suk, J., 2006. Foresight. Infectious Diseases: preparing for the future. T2: Risk Analysis. Office of Science and Innovation, London.

Taylor, E., Taylor, J. Z., 2004. Using qualitative psychology to investigate HACCP implementation barriers. International Journal of Environmental Health Research 14, 53-63.

Ter Beek, A., Keijser, B.J.F., Boorsma, A., Zakrzewska, A., Orij, R., Smits, G.J., Brul, S., 2008. Transcriptome analysis of sorbic acid stressed *Bacillus subtilis* reveals a nutrient limitation response and indicates cell membrane remodeling Journal of Bacteriology 190, 1751-1761.

Teunis, P.F., Ogden, I.D., Strachan, N.J., 2008. Hierarchical dose response of *E. coli* O157:H7 from human outbreaks incorporating heterogeneity in exposure. Epidemiology and Infection 136, 761-770.

Thomas, M.K., Majowicz, S.E., Sockett, P.N., Fazil, A., Pollari, F., Dore, K., Flint, J.A., Edge, V.L., 2006. Estimated numbers of community cases of illness due to *Salmonella*, *Campylobacter* and verotoxigenic

1381 *Escherichia coli*, Pathogen-specific community rates. Infectious Diseases and Medical Microbiology 17,
1382 229-234.

1383 Tompkins, D.S., Hudson, M.J., Smith, H.R., Eglin, R.P., Wheeler, J.G., Brett, M.M., Owen, R.J., Brazier, J.S.,
1384 Cumberland, P., King, V., Cook, P.E., 1999. A study of infectious intestinal disease in England,
1385 microbiological findings in cases and controls. Communicable Diseases and Public Health 2, 108-113.

1386 Van Der Fels-Klerx, H.J., Kandhai, M.C., Booij, C.J.H., 2008. A conceptual model for identification of
1387 emerging risks, applied to mycotoxins in wheat-based supply chains. World Mycotoxin Journal 1, 13-23.

1388 Van Der Giessen, J., Fonville, M., Bouwknegt, M., Langelaar, M., Vollema, A., 2007. Seroprevalence of
1389 *Trichinella spiralis* and *Toxoplasma gondii* in pigs from different housing systems in The Netherlands. Vet
1390 Parasitol 148:371-374.

1391 Van Der Spiegel, M., Luning, P.A., Ziggers, G.W., Jongen, W.M., 2004. Evaluation of performance
1392 measurement instruments on their use for food quality systems. Critical Reviews in Food Science and
1393 Nutrition 44, 501-512.

1394 Van Dijk, H., Houghton, J., van Kleef, E., van der Lans, I., Rowe, G., Frewer, L.J., 2008. Consumer responses to
1395 communication about food risk management. Appetite 50, 340-352.

1396 Van Kleef, E., Houghton, J.R., Krystallis, A., Pfenning, U., Rowe, G., van Dijk, H., van de Lans, I.A., Frewer,
1397 L.J., 2007. Consumer evaluation of food risk management quality in Europe. Risk Analysis 27, 1565-1580.

1398 Van Kreijl, C.F., Knaap, A.G.A.C., Van Raaij, J.M.A., Busch, M.C.M., Havelaar, A.H., Kramers, P.G.N.,
1399 Kromhout, D., Van Leeuwen, F.X.R., Van Leent-Loenen, H.M.J.A., Ocké, M.C., Verkleij, H., 2006. Our
1400 food, our health - healthy diet and safe food in the Netherlands. National Institute for Public Health and the
1401 Environment, Bilthoven.

1402 Van Pelt, W., Van De Giessen, A.W., Van Leeuwen, W.J., Wannet, W., Henken, A.M., Evers, E.G., De Wit,
1403 M.A.S., Van Duynhoven, Y.T.H.P., 1999. Oorsprong, omvang en kosten van humane salmonellose. Deel 1.
1404 Oorsprong van humane salmonellose met betrekking tot varken, rund, kip, ei en overige bronnen.
1405 Infectieziekten Bulletin 10, 240-243.

1406 Veening, J.W., Kuipers, O.P., Brul, S., Hellingwerf, K.J., Kort, R. 2006. Effects of phosphorelay perturbations
1407 on architecture, sporulation, and spore resistance in biofilms of *Bacillus subtilis*. Journal of Bacteriology
1408 188, 3099-3109.

1409 Veening, J.W., Smits, W.K. and Kuipers, O.P. 2008. Biostability, epigenetics and bet-hedging in bacteria.
1410 Annual Reviews in Microbiology 62, 193-210.

1411 Verhoef, L. P.; Kroneman, A.; van Duynhoven, Y.; Boshuizen, H.; van Pelt, W., Koopmans, M., 2009. Selection
1412 tool for foodborne norovirus outbreaks. Emerging Infectious Diseases 15,31-38.

1413 Voetsch, A.C., Van Gilder, T.J., Angulo, F.J., Farley, M. M., Shallow, S., Marcus, R., Cieslak, P. R., Deneen,
1414 V.C., Tauxe, R. V., 2004. FoodNet estimate of the burden of illness caused by nontyphoidal *Salmonella*
1415 infections in the United States. Clinical Infectious Diseases 38, S127-S134.

1416 Wagner, M., Dahl, A., 2008. Direct molecular quantification of food-borne pathogens. In: Proceedings of the
1417 'Future challenges to microbial food safety' symposium, Wolfheze, The Netherlands.

1418 Wang, S., Duan, H., Zhang, W., Li, J.W., 2007. Analysis of bacterial foodborne disease outbreaks in China
1419 between 1994 and 2005. FEMS Immunology and Medical Microbiology 51, 8-13.

1420 Wattiau, P., Weijers, T., Andreoli, P., Schliker, C., Veken, H.V., Maas, H.M., Verbruggen, A.J., Heck, M.E.,
1421 Wannet, W.J., Imberechts, H., Vos, P., 2008. Evaluation of the Premi Test *Salmonella*, a commercial low
1422 density DNA microarray system intended for routine identification and typing of *Salmonella enterica*.
1423 International Journal of Food Microbiology 123, 293-298.

1424 Weenig, M.W.H., Midden, C.J.H., 1997. Mass-media information campaigns and knowledge-gaps effects.
1425 Journal of Applied Social Psychology 27, 945-958.

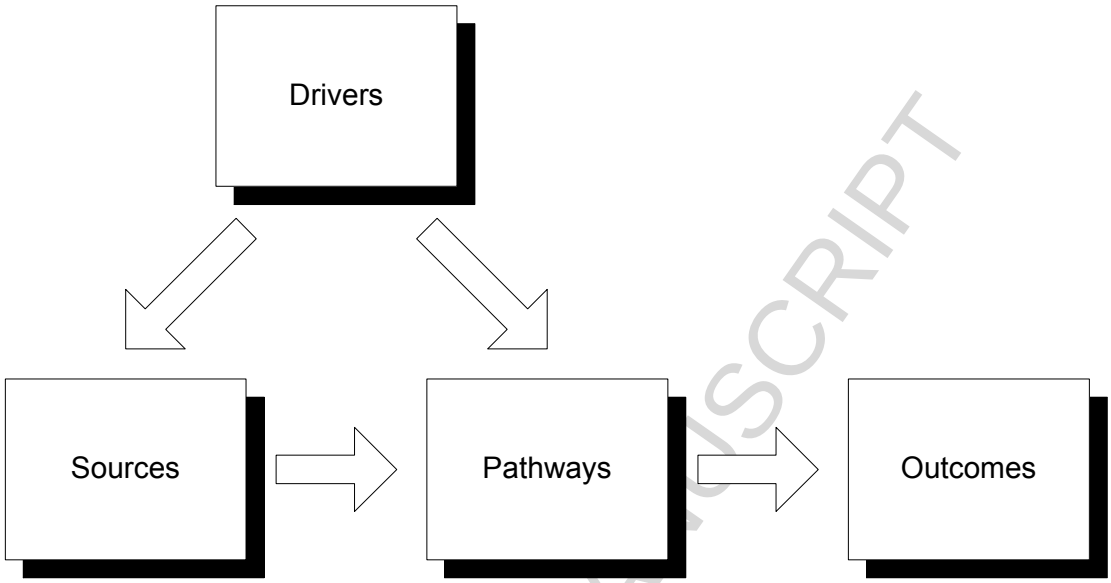
1426 Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, M.J., Roderick,
1427 P.J., 1999. Study of infectious intestinal disease in England, rates in the community, presenting to general
1428 practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. British
1429 Medical Journal 318, 1046-1050.

1430 Wijnands, L.M., 2008. *Bacillus cereus* associated foodborne disease. Quantitative aspects of exposure
1431 assessment and hazard characterization. PhD thesis, Wageningen University.

1432 Wijnands, L.M., Dufrenne, J.B., Rombouts, F.M., In 't Veld, P.H., van Leusden, F.M., 2006. Prevalence of
1433 potentially pathogenic *Bacillus cereus* in food commodities in The Netherlands. Journal of Food Protection
1434 69, 2587-2594.

1435 Wilson, D.J., Gabriel, E., Leatherbarrow, A.J., Cheesbrough, J., Gee, S., Bolton, E., Fox, A., Fearnhead, P., Hart,
1436 C.A., Diggle, P.J., 2008. Tracing the source of campylobacteriosis. PLoS Genetics 4, e1000203.

1438 Figure 1. Basic risk framework for infectious diseases (from Tait *et al.*, 2006)



1439

1440

1441 **Table 1. Different aggregation levels for evaluation of food safety.**

MICROORGANISM-RELATED FACTORS	HUMAN-RELATED FACTORS
Ecosystems	Global systems
Food chains	Regions
Food products	Countries
Food products	Consumer
Populations of micro-organisms	Human populations
Individual cells of micro-organisms	Human individuals
Cellular and molecular processes	Cellular and molecular processes

Table 2. A systems approach to food safety.

Bold red font: Source increases risk to food safety;

Blue, normal font: Effect on risk to food safety unclear or neutral;

Green italics font: Source reduces risk on food safety.

DRIVERS	**SOURCES**	**PATHWAYS**			**OUTCOMES**
	Pathogens	*Farm*	*Processing/distribution*	*Preparation/Consumption*	*Public health*
Economy					
Globalization	**Reduced geographical barriers to spread (of new variants)**	**Inadequate sanitation: higher pathogen loads** **Global sourcing** **Intensified contact structures**	**Long and complex supply chains** **Varying hygiene levels**		**Increased risk**
Food price / income level		**Less profit margins; decreased investment in food safety**		Preference for cheaper alternatives (e.g. less meat and butter; discounters; home brands)	Risk not clear
Science and technology and industry					
Minimal processing	**Adaptation**		Less kill steps		**Increased risk if not well controlled**
Innovation		**New food animal species**	Step change food innovation *Smart packaging* *Bacteriophages*	*Smart labels*	Risk not clear

DRIVERS	SOURCES	PATHWAYS			OUTCOMES
	Pathogens	*Farm*	*Processing/distribution*	*Preparation/Consumption*	*Public health*
Laboratory methods	**Discovery of new pathogens or variants** **Omics approaches**				**Increased observed risk**
Culture and demography					
Population growth		Polluted environments		**Increased demand**	**Increased risk**
Migration				New food habits	
Age structure				**Increase in elderly** **More premature babies**	**Increased risk**
Nature and environment					
Climate change and regional differences	Changing ecology	Droughts, floods Competition for land resources Movement of farms to new areas		**Population displacement** **Increased difficulties to maintain cold chain**	Changing spatial patterns of risk
Water, waste and energy		**Irrigation water quality** **Waste recycling**	Water/energy savings cleaning, process and ingredient water quality		**Increased risk**
Evolution	**Emergence and transfer of virulence factors** **Antimicrobial resistance**	**New reservoirs**	**Increased survival**	**Increased infectivity**	**Increased risk**
Population contact structures	**Species jumps (spill-over from epizootics or exploitation of new**	**Contact zoonoses (MRSA, Q-fever)**			**Increased risk**

DRIVERS	**SOURCES**	**PATHWAYS**			**OUTCOMES**
	Pathogens	*Farm*	*Processing/distribution*	*Preparation/Consumption*	*Public health*
	agricultural areas)				
Consumer behavior					
Food choice	**Psychrotrophs** **Re-emerging pathogens**	Exotic/ethnic foods Regional products	**No or mild processing, less heat treatment** Increased pre-processing and -packaging	**Convenience foods** **Year round availability** Healthy foods (fish, vegetables & fruits) Less fat/salt/sugar Eating outside home	**Increased risk**
Food handling Technologies Attitudes/education			**No acceptance of irradiation**	**Storage: inadequate time/temp control**	**Increased risk**
Information					
Surveillance	**Identification of new pathogens** **Detection of unexpected events**		*Effectiveness of current controls*	Changes in consumption patterns: who, what, where, why?	**Increase in observed risk**
Education		*Professional education*		Hygiene campaigns Attitude changes to accept safe technologies	*Reduced risk*
Government and policies					
Regulations		*Standardisation* *Ban of antibiotic growth promoters*	*GHP/HACCP* **Fraudulent behavior**		Reduced risk

DRIVERS	**SOURCES**	**PATHWAYS**			**OUTCOMES**
	Pathogens	*Farm*	*Processing/distribution*	*Preparation/Consumption*	*Public health*
Risk (-benefit) assessment		*Targets for pathogen reduction*	*Risk-based targets*		*Reduced risk*
Food defense		Agro/bioterrorism			Increased risk
Agriculture					
Animal friendly and organic production	*Reduced AMR*	**Re-emergence (Trichinella, Toxoplasma)** **Higher (*Campylobacter*)** or *lower prevalence (Salmonella)*			Risk not clear
Aquaculture		More farmed fish			Risk not clear
Antimicrobial use	**Resistance development**	**Increased therapeutic use**			**Increased risk**