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Risk-analysis of human pathogen spread in the vegetable industry: a comparison between organic and conventional production chains

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Abstract

An overview is given of recent problems with food-borne enteric human pathogens originating from contaminated agricultural animals. The need for risk analysis is indicated, and the generally accepted procedure for risk assessment is outlined. Two main approaches to probability and risk calculations, namely the "frequentist" and Bayesian approaches, are described. Examples are given of microbial risk assessments in vegetable production that were mainly based on "frequentist" probability assessments. Finally, a Dutch-Russian collaborative project on risk assessment of enteric pathogens in organic and conventional vegetable production chains is outlined, and preliminary data are presented. We conclude that a Bayesian approach, using prior probabilities, is the most appropriate instrument for risk assessment of human-pathogen spread in the vegetable industry.

Introduction

Food can be a major source of human-pathogenic agents, both chemical (for example pesticides) and biological (prions, viruses, bacteria, fungi and protozoa). Of the approximately 1,500 million global cases of diarrhoea occurring annually, resulting in 3 million deaths among children under five (mainly in developing countries), 70% have been estimated to be caused by biologically contaminated food (*World Health Organisation* 1997). According to Tauxe (2001) in the United States alone, each year an estimated 76,000,000 persons experience a food-borne infection, 325,000 are hospitalized, and 5000 die. Among the established food-borne infections, bacterial infections account for an estimated 30% of cases, 63% of hospitalizations and 72% of deaths. In the United Kingdom the reported number of cases of food poisoning increased six-fold between 1982 and 1998 (Nicholson et al. 2000). So food-borne diseases are a persistent challenge to public health worldwide. In the last twenty years various pathogens have been either newly described (for example *Escherichia coli* O157:H7, *Vibrio vulnificus* and *Cyclospora cayetanensis*) or newly associated with food-borne illnesses (for example *Listeria monocytogenes* and *Campylobacter jejuni*) (Tauxe et al. 1997). Because of their prevalence in manure and the relation between enteric diseases and animal food products, it is generally accepted that almost

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all food-borne pathogens have a reservoir in (apparently healthy) animals from which they spread to the environment and humans. Known reservoirs are for example cattle (*Salmonella* spp., *L. monocytogenes, and E.coli O157*), pigs (particularly *Salmonella typhimurium* but also *L. monocytogenes, E.coli O157, Campylobacter* spp., and the protozoa *Cryptosporidium* spp. and *Giardia* spp.), poultry (particularly *Salmonella* spp. and *Campylobacter* spp.) and sheep (*Salmonella* spp., *E.coli O157, Campylobacter* spp. and *Cryptosporidium* spp.) (Nicholson et al. 2000; Tauxe et al. 1997).

Risk analysis

To assist (and to force) the food industry to produce foods that are safe to consume, the Hazard Analysis and Critical Control Point (HACCP) system has been developed (Bernard 2001). HACCP is a systematic approach to the identification, evaluation, and control of safety hazards. It can be considered a general risk analysis consisting of three parts: *risk assessment* (which evaluates the probability and severity of adverse health effects resulting from exposure to food-borne pathogens), *risk management* (to select and implement appropriate control options) and *risk communication* (to exchange information). The *risk assessment* procedure includes: 1) **hazard identification**, 2) **hazard characterization** (evaluation of the adverse health effects associated with the pathogen), 3) **exposure assessment** (the evaluation of the likely intake of the pathogen via food) and 4) **risk characterization** (probability of occurrence of quantities of pathogens in food leading to an adverse health effect). *Risk assessment* includes quantitative and/or qualitative expressions of risk. Qualitative assessments use categorical/descriptive representations of probability and risk, while quantitative assessments use numerical parameter measurements and result in a numerical expression of risk by the use of mathematical models.

An important aspect of the **hazard characterization** step is the dose-response relation. This is the relation between the number of ingested pathogen cells and the probability of illness. Currently, mathematical equations are used to describe dose-response relations empirically (see Buchanan, Smith and Long 2000 for an overview). Experimental data can be fit to one or more of these equations. The often-observed sigmoid relationship was interpreted as indicating that there is a threshold level of bacterial cells that must be ingested in order to get a disease response (the so-called minimum infectious dose). The very low infectious dose of for example *E. coli* 0157:H7, which is smaller than 50 cells (Tilden et al. 1996), led to the idea that if one considers a large enough cross section of the human population, the ingestion of a single cell has a possibility of causing disease and that this probability increases as the levels of the pathogen increase (Buchanan, Smith and Long 2000). The result was the development of sigmoid non-threshold dose-response equations. In general, dose-response models have limitations related to the availability of data representing the entire population, because often data are obtained from human-volunteer feeding studies. As a means of demonstrating how a mechanistic model might be developed, Buchanan, Smith and Long (2000) developed a simple three-compartment dose-response model. The first model compartment allows calculation of the number of viable cells surviving passage through the stomach, which is predominantly determined by the pH. The second model compartment examines the ability of the bacterial cells that have survived passage through the stomach to attach and colonize the intestinal epithelium. The third and final model compartment is the likelihood that an infection progresses to disease symptoms. The main determinant for this

compartment is thought to be the capability of the host defence system to thwart off the pathogen. Although this model requires additional work to increase its accuracy, the authors state that it provides an assessment that can more closely relate the effect of dose to the biological response being investigated relative to the classical dose-response approaches.

The identification of risk factors is an important step in the **exposure assessment** part of the risk analysis, because exposure depends on the occurrence of the pathogen in the starting materials (manure) and the development of the pathogen population during the production chain. A risk factor can be described as a particular component in the production chain where the pathogen is introduced or where there is a probability of increase or decrease in a pathogen population. Accurate knowledge about the dynamics of the pathogen as function of product quality is needed. The main problem in exposure assessment is often lack of relevant and accurate data (Zwietering and Van Gerwen 2000). Predictive models linking environmental factors to microbial kinetics are now commonly used to forecast the development of pathogens in foods, but these environmental factors are usually limited to abiotic factors such as temperature and pH. Also it is not common to take intraspecific variability into account (Delignette-Muller and Rosso 2000). In addition, it stands to reason that the microbial community surrounding the pathogen will influence its behaviour, but this aspect has not been included yet in any of the models.

The dose-response and exposure assessments are combined into a final risk estimation: the **risk characterization**. A qualitative estimation of risk is conducted by assigning terms like negligible, low, medium, high, etc. to risk factors. The outcome of a quantitative risk assessment is a numerical estimation of risk. Quantitative risk assessments can be divided into two categories: point-estimate (deterministic) and probabilistic (stochastic). The first uses single values as the average, worst-case or what-if as inputs to a risk assessment. The latter considers all of the data available and uses probability distributions, as opposed to single values, to describe the parameters that contribute to the risk. This probability distribution can be thought of as a frequency diagram of all the possible values of a variable in relation to the probability of each value occurring. A probability distribution of the final risk can be obtained by performing so called Monte Carlo simulations (see for example Cassin et al. 1998). This numerical technique is based on randomly selecting a single sample for every input factor from its own distribution parameter (for example the density of the pathogen in manure, reduction during manure storage, reduction in the soil after application and growth on a vegetable crop). These values are applied to a mathematical model that calculates, for example, the final density of the pathogen on the crop. This is repeated many times with different values, where values that are more likely to occur (according to the defined probability distributions of the input parameters) are selected more frequently. The outputs are combined into a probability distribution of the final risk (the density of the pathogen on the vegetable) reflecting the combined ranges and frequencies of the input parameters. Because risk is a chance of an effect to occur, stochastic modelling seems more appropriate to use in quantitative risk analysis. A drawback of this method is that one needs to know the stochastic distribution of all input factors, which may be difficult to obtain.

A probabilistic analysis may be conducted with a classical “frequentist” or a Bayesian statistical method. The differences between these are not only in the underlying philosophy, but also in the manner by which new evidence may be incorporated into an analysis. In the classical view, probability is regarded as the frequency by which an event occurs in a series of repeated observations. Probability

distributions specified for the inputs to a risk assessment are estimated from available data. Monte Carlo simulations can then be used to simulate from these distributions and to determine a predictive distribution of the final risk. On the other hand, in the Bayesian view a wide range of diverse subjective elements and uncertainties can be incorporated. The basis of Bayesian analysis is that it combines background information expressed as a prior distribution and likelihood functions of data (analogues to a probability distribution) to a joined posterior distribution that is normalized to a total risk probability of 1. This prior knowledge may be (incomplete) data from other experiments but can also include subjective elements like beliefs or expert judgments. Visually, a Bayesian network model is constructed by explicitly determining all the direct dependencies between random variables of the problem domain. In a Bayesian network each node represents one of the observable features of the problem domain, and the arcs between the nodes represent the direct dependencies between the corresponding variables. In addition, each node has to be provided with a table of conditional probabilities, where the variable in question is conditioned by its immediate predecessors in the network. As more data or knowledge become available these tables are updated, and the associated uncertainties are reduced. In fact, evidence accumulates in the Bayesian network because the “posterior” from one link becomes the “prior” for the next. Thus the final probabilities are expected to be of higher accuracy. Furthermore, once the posterior distribution has been derived it can be quickly updated as more information becomes available, without having to repeat the assessment from start. Thus Bayesian techniques can be used for the iterative development of quantitative risk assessment, whereby risk estimates are made and then continually updated with additional information.

Examples of microbial risk assessments

It is recognized that a large proportion of animal products can carry particular pathogens. Therefore, most attention has been paid to contaminated animal products to develop risk-assessment models. Traditionally, the food implicated in a food-borne outbreak was undercooked meat, poultry, seafood or unpasteurized milk.

An example of a quantitative risk assessment is the study done by the Food Safety and Inspection Service (*Salmonella enteritidis risk assessment, shell eggs and egg products - final report* 1998, not published). Available data were incorporated into a comprehensive quantitative model, which characterized the public-health effects associated with the consumption of whole eggs, and egg products infected with *Salmonella enterica* serotype *Enteritidis*. The model consists of five modules. The first module, the egg-production module, estimates the number of eggs produced that are infected. The shell-egg module, the egg-products module, and the preparation and consumption module estimate the increase or decrease in the numbers of the pathogen in eggs or egg products as they pass through storage, transportation, processing and transportation. The public-health module then calculates the incidence of illness. The distribution of disease incidence simulated by this model showed a substantial overlap with a distribution of disease incidence predicted from a national public-health surveillance, indicating that the model is reasonably accurate and representative in its description of the number of illness cases associated with this pathogen in these products.

In a Canadian study on *E. coli* O157:H7 in ground-beef hamburgers a new type of model was developed: the Process Risk Model (PRM) (Cassin et al. 1998). Conventional quantitative risk assessment uses the most direct available empirical

evidence to evaluate human exposure. A PRM attempts to predict exposure through the specification of food production, distribution and consumption processes (Cassin et al. 1998). Mathematical models are used to describe contamination sources, the likely numbers of a pathogen that might be introduced in the food and the influence of factors affecting growth, survival, distribution or inhibition of the pathogen. Experimental data in the form of probability distributions are incorporated. Also predictive microbiology is used to characterize pathogen dynamics. A representative distribution of the risk (probability of disease from a single hamburger meal) in the model was simulated many times with different values selected from the probability distributions of input parameters, using Monte Carlo techniques. In addition, the authors used Spearman rank correlation as a statistical tool to quantify the strength of the association between each of the model parameters and the predicted probability of disease. This provides an objective manner to rate the most important factors contributing to risk. Among the variables which show a high positive correlation with risk are the concentration of the pathogen in faeces, host susceptibility, retail storage temperature, growth during storage time and retail storage time. The main variables negatively correlating with risk are cooking preference and reduction due to decontamination. The authors also conducted simulations with the model: by changing values of the input parameters and observing the change in the risk estimate, hypothetical control strategies (like improved storage temperature) were evaluated. This kind of model experimentation provides information for decision-support strategies to reduce risk.

Other examples of quantitative risk assessments are for *Listeria monocytogenes* in salmon and rainbow trout in Sweden (Lindqvist and Westoo 2000), *Listeria monocytogenes* in soft cheese made from raw milk (Bemrah et al. 1998), *Listeria monocytogenes* in both pâté and soft cheese (Farber, Ross and Harwig 1996), *Salmonella enteritidis* in pasteurized liquid eggs (Whiting and Buchanan 1997) and *Bacillus cereus* in pasteurized milk (Notermans et al. 1997). Almost no risk assessments concerning food-borne pathogens using a Bayesian approach have been published. Barker, Talbot and Peck (2002) published a risk assessment for *Clostridium botulinum*, introducing the Bayesian Belief Network. This method integrates a graphical, flow-diagram like, representation of a hazard domain with a powerful technique for combining probabilities. Large quantities of complex information can be organized, disparate information sources can be combined and it provides a powerful opportunity for improved communication between mathematical modellers and subject experts (Barker, Talbot and Peck 2002).

Pathogens and ready-to-eat vegetables

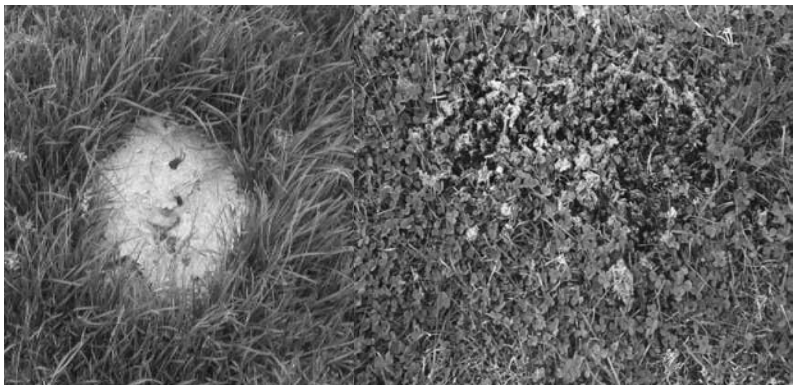
Recently, several outbreaks of intestinal infections have been associated with above-mentioned pathogens ingested with vegetables and fruit (Beuchat 1996; Beuchat 2002; Francis, Thomas and D 1999; Hilborn et al. 1999; De Roever 1998; Tauxe et al. 1997). Several reasons for the increase in produce-related human infections have been proposed. These include changes in dietary habits, including a higher per capita consumption of fresh or minimally processed fruits and vegetables, longer production chains (think about ready-to-eat packed salads for example) and increase in global trade (Tauxe et al. 1997; Beuchat 2002; De Roever 1998). Contrary to animal products, which are generally cooked, various fruits and vegetables are consumed raw. Such ready-to-eat products retain much of their indigenous microflora

after minimal processing. Pathogens may form part of this microflora, posing a potential health risk.

Several microbiologists have proposed that the increased use of raw manure for fertilization constitutes a higher risk of bacterial contamination of fresh vegetables and fruits. In several outbreaks the original source of these outbreaks has indeed been traced back to animal manure, used during the production of these fruits and vegetables (Nelson 1997; Guan and Holley 2003). Animal manure is generally used more frequently in organic than in conventional fruit and vegetable production farms. Therefore, the focus in the media has been on the safety of organically produced food, although it has not been proven that the risk of contamination would be higher in the organic than in the conventional production chain (e.g. Nicholson et al. 2000). As the application of most chemical treatments for controlling pathogen invasion is not allowed in organic farming, suppression of these pathogens from animal manure (when applied without composting) must rely on the buffering capacity of natural microflora near or within crop plants. Relationships between (root) disease severity, microbial diversity and/or microbial quantity have sometimes been found and it has been documented that soil-borne plant pathogens are generally better suppressed in organically managed soils thanks to the more diverse and active microflora and fauna (Van Bruggen 1995; Van Bruggen and Termorshuizen 2003). It is not known if and how human pathogens are influenced by the surrounding microbial community.

Pathogen content of manure varies depending on the type of animal, animal diet, stress and age (Cray et al. 1998). Manure as a fertilizer is used in different ways: as a slurry or dry manure, as pure manure or mixed with litter or straw, after a short or longer period of storage, composted or not, applied on the soil surface or injected into the soil, etc. Differences in these aspects of manure management may affect the behaviour of enteric pathogens (Gagliardi and Karns 2000; Jiang, Morgan and Doyle 2002; Kudva, Blanch and Hovde 1998; Nicholson et al. 2000). Survival of pathogens in manure after shedding and after land application depends on the interaction of biotic (microbial composition) and abiotic factors (temperature, moisture content, ammonia content, pH, nutrient availability, soil type, weather, time between applying and planting, etc.), which can be associated with management differences (Gagliardi and Karns 2000; Jiang, Morgan and Doyle 2002; Kudva, Blanch and Hovde 1998). Contamination of crops with pathogens from manure may take place via the roots, by splashing water from the ground onto leaf surfaces, by direct contact of the leaves with manure or by transmission by soil organisms. Enteric pathogens can reside on or inside the plants (Solomon, Yaron and Matthews 2002). The fate of pathogens on or in the produce is dependent on the interaction of the pathogens with the micro-organisms present, the characteristics of the plant (physiological state, leaf structure), the characteristics of the environment surrounding the plant tissue, and the effects of processing-practices (conditions during cutting, washing, storage, transport, packaging and finally display) (Takeuchi, Hassan and Frank 2001).

Of course, manure is not the only potential contamination source. Raw vegetables reach the consumer through long chains of industrial production, in which many opportunities for contamination exist. In principle, anything in the production chain that comes in contact with the plant has the potential to be a source of pathogens. Pre-harvest contamination of fresh produce may be the result of using contaminated water for irrigation or contamination from domestic animals or employees. After harvest contamination may occur as a result of using contaminated washing water or ice, improper handling, the presence of animals, the use of contaminated equipment, cross-contamination from other produce or directly from people.



Low-fibre, high-N feed High-fibre low-N feed

Figure 1. Differences in cow dung as affected by a cow's diet

A Dutch-Russian collaborative project on enteric pathogens in the vegetable production chain

Recently (2002), we initiated a project to assess the risks of occurrence and spread of enteric pathogens in organic and conventional vegetable production chains. The organic food production chain is defined as: an ecologically, economically, and socially responsible way of farming, food processing, and marketing, providing an enduring supply of safe and healthy food with the least possible nutrient and energy losses, the least possible negative environmental impacts, and respect for the integrity of plants, animals and life-sustaining soil, as regulated by organic certification agencies. This is a goal-oriented definition. The actual food production chain may not yet reach all intended goals even though it abides by the official certification rules. The conventional food production chain is the most widely used way of food production, processing and marketing with many regional variations. The most obvious differences between these two production methods are differences in the use of synthetic fertilizers and pesticides for crop production and the use of antibiotics and other medical drugs for animal production (see Box 1).

Conventional	Organic
- artificial fertilizer allowed	- no artificial fertilizer allowed
- high-N concentrates in feed	- limited organic low-N concentrates in feed
- low-fibre diet for cows	- high-fibre diet for cows
- sometimes antibiotics in feed	- no antibiotics in feed

Box 1. Major differences between conventional and organic dairy production

Different animal feeding strategies, with a high-N and low-fibre diet in conventional farms and a lower-N and higher-fibre diet in organic farms, result in different manure characteristics (Figure 1).

Low-N high-fibre manure decomposes faster and is less toxic to seedlings than high-N low-fibre manure (Bosker, Hoekstra and Lantinga 2002; Hoekstra, Bosker and

Lantinga 2002). These differences may also lead to differences in pathogen survival in manure. Moreover, organic farmers frequently compost solid manure, and use slurry less frequently than conventional farmers. Soil characteristics are known to differ, commonly with a greater microbial activity and diversity at organic farms than at conventional farms (Van Bruggen 1995; Van Bruggen and Termorshuizen 2003). The various ways of crop and manure management will probably lead to differences in risks of pathogen survival and spread in the organic and conventional vegetable production chains.

As mentioned before, raw vegetables reach the consumer through long production chains where many opportunities exist for an increase or decrease of the pathogen content of the product. Therefore it is important to consider the whole production chain, from the cow that produces the manure to the final buyers (Figure 2). Note that in the biological production chain also some conventional manure is used because of a shortage of biological manure.

To make the project manageable, *S. typhimurium* and *E. coli* O157:H7 have been chosen for this project, in combination with lettuce (*Lactuca sativa* var. *capitata*). Samples of manure and plants on organic and conventional farms, and samples of organic and conventional vegetable products will be analysed for human pathogens. In addition to this monitoring along the production chain, genetically marked pathogens are used in experiments that simulate parts of the production chain. In this way the fate of the pathogen in different stages of the production chain and during stage (niche) transitions can be determined. An example of such a preliminary experiment is described in Box 2. Other experiments published on survival of pathogens determined survival solely in manure (Himathongkham et al. 1999; Fukushima, Hoshina and Gomyoda 1999) or in freshly inoculated manure applied to soil (Jiang, Morgan and Doyle 2002). Our survival study analyses the niche transition of the pathogen when it is mixed with soil after having been in manure for forty days. This transition is characterized by a sharp decrease in pathogen survival (Box 2, Figure 3).

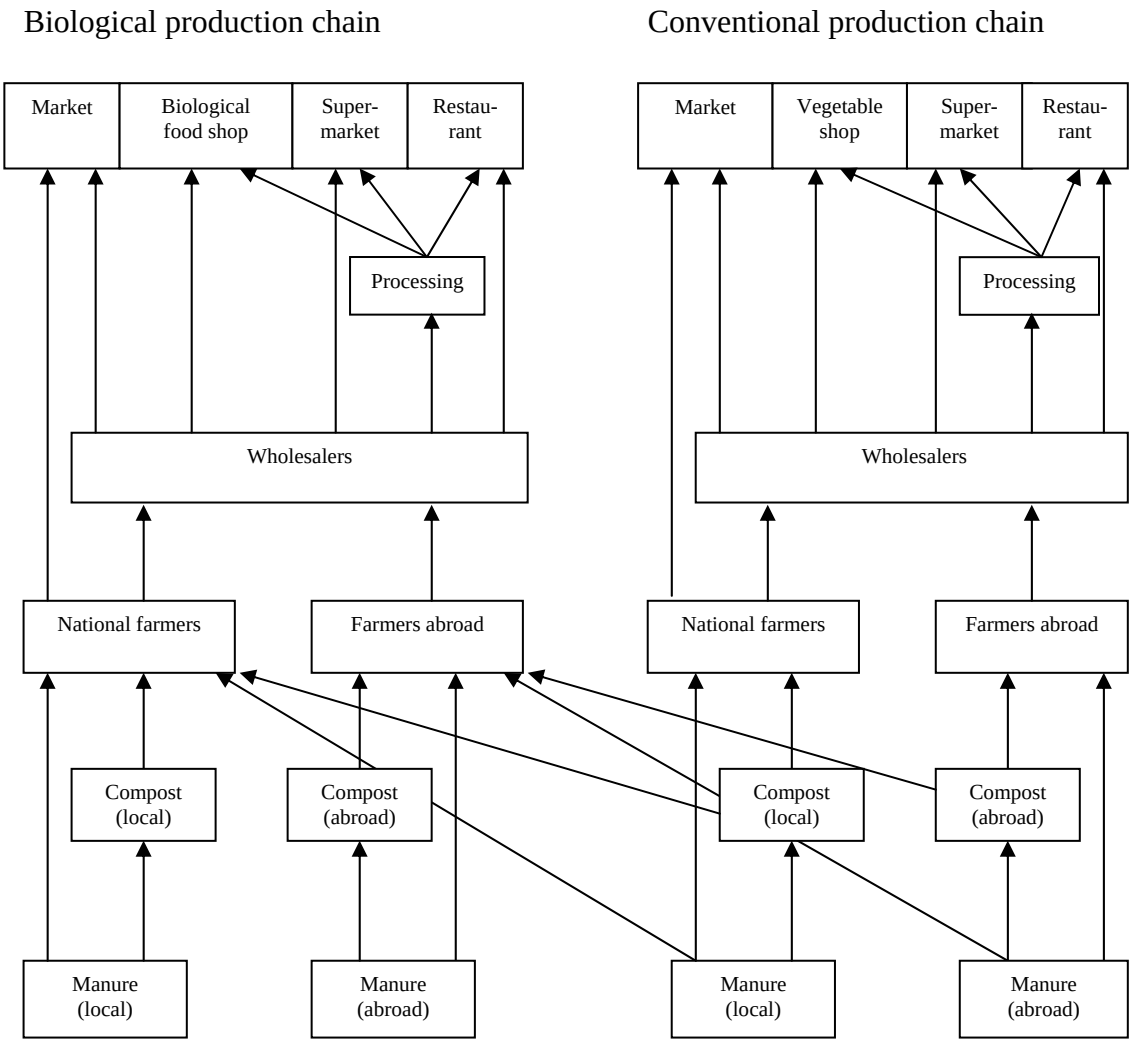


Figure 2. Schematic overview of the conventional and biological production chain of raw vegetables

Quantitative risk assessment will be carried out with the collected data. With this risk assessment we shall take the abiotic factors as well as the biotic factors (microbial composition and activity) into account. We intend to model the transmission of the pathogen through the lettuce production pathway, starting from the source: the cows that produce the manure used to fertilize the land on which the crops are grown. This model will follow probability distributions of the prevalence and the concentration of the hazard along the production chain, leading to a final risk estimation in the form of a probability distribution.

Conclusion

The potential presence of human pathogens in freshly consumed vegetables, grown in soils enriched by manure, is of major concern. This holds for both conventionally and organically produced vegetables, but the food-safety issue is at the centre of attention for organic produce, as manure is more commonly used to maintain soil fertility for organic production. However, different animal and plant production techniques and enhanced microbial diversity in the organic production chain may contribute to suppression of human pathogens in this chain. Very few quantitative risk

analyses have been carried out to estimate the risk of contamination of fresh vegetables by enteric pathogens, and the organic and conventional production chains have not been compared at all in this respect. Risk assessment includes hazard identification, hazard characterization, exposure assessment and risk characterization. Especially in the last two steps of risk assessment stochastic variability comes into play. Calculation of the probability of exposure and infection can be carried out using either the classical "frequentist" approach or Bayesian statistical methods, where prior probabilities are combined with experimental frequency distributions. We initiated a project to assess the risk of occurrence and spread of enteric pathogens in organic and conventional vegetable production chains. Besides monitoring along the production chain we will experimentally simulate different parts of the chain to determine the fate of the pathogen and to understand the mechanisms behind its behaviour. Bayesian models, using prior probabilities, seem to be the most appropriate instrument for a risk assessment because of the complexity of the system in combination with considerable uncertainty and variability at several levels. The implementation of both abiotic and biotic factors in this risk assessment is a unique approach and will hopefully lead to new insight in risks in the organic and conventional food production chains.

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A preliminary experiment on survival of *Salmonella typhimurium* MAE 110 *gfp* in manure and soil

Fresh manure was collected from a collective dairy farm near Moscow, Russia. The dairy cows were kept indoors (January 2003). The food ration per day for one cow consisted of: mixed dry hay (4 kg), corn silage (20 kg), residuals of sunflower seeds (1 kg), concentrate of grains (4 kg), dry straw (4 kg) and macro- and microelements. Initial pH of the manure was 6.6, and the moisture content ~ 90 %.

Salmonella typhimurium MAE 110 *gfp* (green fluorescent protein) was used to monitor surviving cells in manure and soil. Twenty ml of concentrated cell suspension was added to 500g of fresh manure in plastic pots, so that concentrations were approximately 10^{10} cells/g dry manure as determined by direct counting and plate counting. Each pot was covered by polyethylene film, which provided gas exchange but prevented water evaporation. Three pots with *S. typhimurium* MAE 110 *gfp* and three control pots were incubated at 25°C in darkness. Immediately after pathogen introduction and 1-2 times per week during the following weeks, 3 1-g samples were collected per pot for quantification of the *gfp* strain by dilution-plating and direct counting. Plates were incubated at 30°C during 3 – 4 days in a dark room. Fluorescent colony-forming units (CFU) were visualized directly under a dark-blue lamp (450-490 nm). For direct counting, 10µl suspension from the 10^{-3} or 10^{-2} dilution was spread on an object glass and covered by a cover slip. From 60 to 100 fields per slide were checked under an epifluorescent microscope at 1000x magnification. Densities of green fluorescing CFU and microscopically counted cells were calculated per gram of dry soil or dry manure.

To study the transition of *S. typhimurium* MAE 110 *gfp* from cow manure to soil, soil was collected from a fallow plot (0-15 cm deep) in the botanical garden of Moscow State University. No fertilizers were introduced into soil during the last two years. Sieved (2 mm), air-dried soil was stored in a plastic bag at room temperature. The soil was analysed for texture (12% clay, 32% sand and 56% silt), various macronutrients and pH. Half of the manure was transferred to soil 38 days after inoculation with *S. typhimurium* MAE 110 *gfp*; the other half was preserved to continue observations on survival of *S. typhimurium MAE 110 gfp* in manure. Mixing of manure and soil was done in proportion 2.5:1 of wet weight, corresponding to 21:1 of dry weight. Final moisture content of the soil-manure mixture was 30%. The soil-manure mixture in new pots was covered again by plastic film to prevent water evaporation. Samples were taken as described above.

Fifty days after mixing the manure in soil, 40 cress seeds (*Lepidum sativum* L.) were sown per pot. Shoots and roots (with rhizosphere soil) of 30 plants were inspected for the presence of *S. typhimurium* MAE 110 *gfp* after 4-5 days. Cell suspensions were obtained from shoots and roots separately in sterile water, and concentrated by centrifugation. Resuspended *gfp* bacteria were quantified by dilution-plating and direct microscopic counts.

The whole experiment was done with three replicas and repeated three times.

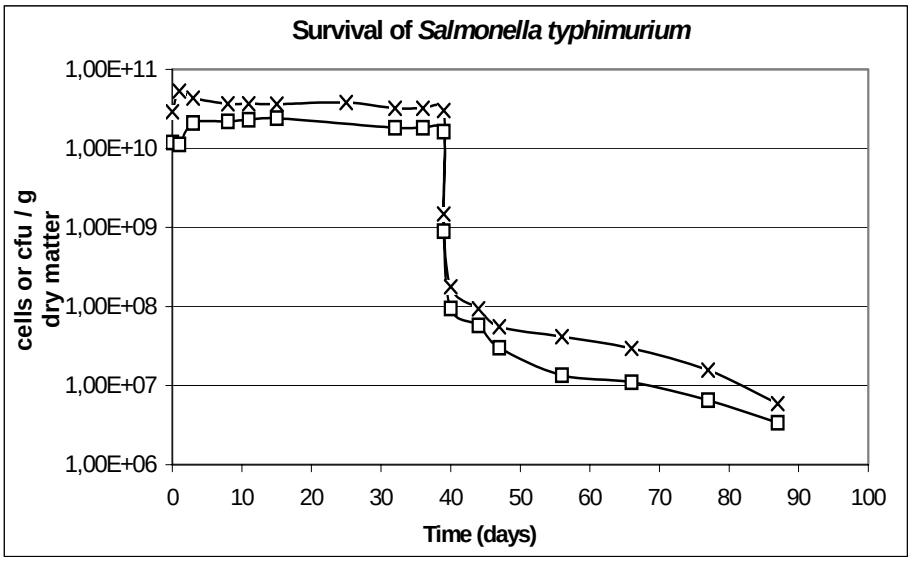

Figure 3. Survival of *Salmonella typhimurium* MAE 110 *gfp* introduced to manure and after the transition of the manure to soil, as determined by plating on selective medium (□) and by direct counting under the epifluorescent microscope (X)

Direct counts were about five times as high as plate counts throughout the experiment, indicating that there may be a substantial proportion of viable but non-culturable cells.The ability of these VBNC cells to be pathogenic needs to be determined. Concentrations of *S. typhimurium* MAE 110 *gfp* did not decline over a 40-day period in manure, but did decline significantly in soil. However, the decline in soil was insufficient to prevent infection of cress with this pathogen. Cells of *S. typhimurium MAE 110 gfp* were recovered from both roots and shoots of cress (about 10^4 per plant), despite the absence of overhead watering of the plants.

Box 2. A preliminary experiment

References

Barker, G.C., Talbot, N.L.C. and Peck, M.W., 2002. Risk assessment for *Clostridium botulinum*: a network approach. *International Biodeterioration and Biodegradation,* 50 (3/4), 167-175.

Bemrah, N., Sanaa, M., Cassin, M.H., et al., 1998. Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. *Preventive Veterinary Medicine,* 37 (1/4), 129-145.

Bernard, D., 2001. Hazard Analysis and Critical Control Point System: use in controlling microbiological hazards. *In:* Doyle, M. P. and Beuchat, L. R. eds. *Food microbiology: fundamentals and frontiers*. 2nd ed edn. ASM Press, Washington, D.C.

Beuchat, L.R., 1996. Pathogenic microorganisms associated with fresh produce. *Journal of Food Protection,* 59 (2), 204-216.

Beuchat, L.R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection,* 4 (4), 413-423.

Bosker, T., Hoekstra, N.J. and Lantinga, E.A., 2002. The influence of feeding strategy on growth and rejection of herbage around dung pats and their decomposition. *Journal of Agricultural Science,* 139 Part 2, 213-221.

Buchanan, R.L., Smith, J.L. and Long, W., 2000. Microbial risk assessment: dose-response relations and risk characterization. *International Journal of Food Microbiology,* 58 (3), 159-172.

Cassin, M.H., Lammerding, A.M., Todd, E.C.D., et al., 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *International Journal of Food Microbiology,* 41 (1), 21-44.

Cray, W.C., Casey, T.A., Bosworth, B.T., et al., 1998. Effect of dietary stress on fecal shedding of *Escherichia coli* 0157:H7 in calves. *Applied and Environmental Microbiology,* 64 (5), 1975-1979.

De Roever, C., 1998. Microbial safety evaluations and recommendations on fresh produce. *Food Control,* 9 (6), 321-347.

Delignette-Muller, M.L. and Rosso, L., 2000. Biological variability and exposure assessment. *International Journal of Food Microbiology,* 58 (3), 203-212.

Farber, J.M., Ross, W.H. and Harwig, J., 1996. Health risk assessment of *Listeria monocytogenes* in Canada. *International Journal of Food Microbiology,* 30 (1/2), 145-156.

Francis, G.A., Thomas, C. and D, O.B., 1999. The microbiological safety of minimally processed vegetables. *International Journal of Food Science and Technology,* 34 (1), 1-22.

Fukushima, H., Hoshina, K. and Gomyoda, M., 1999. Long-term survival of Shiga toxin-producing *Escherichia coli* O26, O113, and O157 in bovine feces. *Applied and Environmental Microbiology,* 65 (11), 5177-5181.

Gagliardi, J.V. and Karns, J.S., 2000. Leaching of *Escherichia coli* O157:H7 in diverse soils under various agricultural management practices. *Applied and Environmental Microbiology,* 66 (3), 877-883.

Guan, T.Y. and Holley, R.A., 2003. Pathogen survival in swine manure environments and transmission of human enteric illness: A review. *Journal of Environmental Quality,* 32 (2), 383-392.

Hilborn, E.D., Mermin, J.H., Mshar, P.A., et al., 1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. *Archives of Internal Medicine,* 159 (15), 1758-1764.

Himathongkham, S., Bahari, S., Riemann, H., et al., 1999. Survival of *Escherichia coli* O157:H7 and *Salmonella typhimurium* in cow manure and cow manure slurry. *Fems Microbiology Letters,* 178 (2), 251-257.

Hoekstra, N.J., Bosker, T. and Lantinga, E.A., 2002. Effects of cattle dung from farms with different feeding strategies on germination and initial root growth of cress (*Lepidium sativum* L.). *Agriculture Ecosystems and Environment,* 93 (1/3), 189-196.

Jiang, X.P., Morgan, J. and Doyle, M.P., 2002. Fate of *Escherichia coli* O157:H7 in manure-amended soil. *Applied and Environmental Microbiology,* 68 (5), 2605-2609.

Kudva, I.T., Blanch, K. and Hovde, C.J., 1998. Analysis of *Escherichia coli* 0157:H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology,* 64 (9), 3166-3174.

Lindqvist, R. and Westoo, A., 2000. Quantitative risk assessment for *Listeria monocytogenes* in smoked or gravad salmon and rainbow trout in Sweden. *International Journal of Food Microbiology,* 58 (3), 181-196.

Nelson, H., 1997. *The contamination of organic produce by human pathogens in animal manures*. Available: [http://www.eap.mcgill.ca/SFMC_1.htm] (29 Sep 2003).

Nicholson, F.A., Hutchison, M.L., Smith, K.A., et al., 2000. *A study on farm manure applications to agricultural land and assessment of the risks of pathogen transfer into the food chain*. Ministry of Agriculture, Fisheries and Food, London.

Notermans, S., Dufrenne, J., Teunis, P., et al., 1997. A risk assessment study of *Bacillus cereus* present in pasteurized milk. *Food Microbiology,* 14 (2), 143-151.

Salmonella enteritidis risk assessment, shell eggs and egg products - final report, 1998. The Food and Safety and Inspection Service (FSIS). [http://www.fsis.usda.gov/OPHS/risk/index.htm]

Solomon, E.B., Yaron, S. and Matthews, K.R., 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. *Applied and Environmental Microbiology,* 68 (1), 397-400.

Takeuchi, K., Hassan, A.N. and Frank, J.F., 2001. Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature. *Journal of Food Protection,* 64 (11), 1820-1823.

Tauxe, R., Kruse, H., Hedberg, C., et al., 1997. Microbial hazards and emerging issues associated with produce: a preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. *Journal of Food Protection,* 60 (11), 1400-1408.

Tauxe, R.V., 2001. Surveillance and investigation of food-borne diseases: roles for public health in meeting objectives for food safety. *Food Control,* 13 (6/7), 363-369.

Tilden, J., Young, W., McNamara, A.M., et al., 1996. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *American Journal of Public Health,* 86 (8) Part 1, 1142-1145.

Van Bruggen, A.H.C., 1995. Plant disease severity in high-input compared to reduced-input and organic farming systems. *Plant Disease,* 79 (10), 976-984.

Van Bruggen, A.H.C. and Termorshuizen, A.J., 2003. Integrated approaches to root disease management in organic farming systems. *Australasian Plant Pathology,* 32 (2), 141-156.

Whiting, R.C. and Buchanan, R.L., 1997. Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs. *International Journal of Food Microbiology,* 36 (2/3), 111-125.

World Health Organisation, 1997. Available: [http://www.who.int].

Zwietering, M.H. and Van Gerwen, S.J.C., 2000. Sensitivity analysis in quantitative microbial risk assessment. *International Journal of Food Microbiology,* 58 (3), 213-221.