

RECONTAMINATION AS A SOURCE OF PATHOGENS IN PROCESSED FOODS – A LITERATURE REVIEW



REPORT

Prepared under the responsibility of the
ILSI Europe Risk Analysis in Microbiology Task Force

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*By Martine Reij, Esther van Asselt,
Jean-Louis Cordier and Leon Gorris*

REPORT

**PREPARED UNDER THE RESPONSIBILITY OF THE
ILSI EUROPE RISK ANALYSIS IN MICROBIOLOGY TASK FORCE**

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For more information about ILSI / ILSI Europe, please contact

ILSI Press
One Thomas Circle, NW
Ninth Floor
Washington DC 20005-5802
USA
Phone: (+1) 202 659 0074
Fax: (+1) 202 659 3859
E-mail: ilsipress@ilsil.org
Website: <http://www.ilsil.org>

ILSI Europe a.i.s.b.l.
Avenue E. Mounier 83, Box 6
B-1200 Brussels
Belgium
Phone: (+32) 2 771 00 14
Fax: (+32) 2 762 00 44
E-mail: info@ilsieurope.be
Website: <http://europe.ilsil.org>

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Authors: Martine W. Reij, Wageningen University (NL), Esther van Asselt, Wageningen University (NL), Jean-Louis Cordier, Nestlé (CH) and Leon G.M. Gorris, Unilever (GB)
Scientific Reviewer: Dr. Don Schaffner, Rutgers University (US)
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ABSTRACT

Food products that have been submitted to an adequate heat treatment during processing are generally free of vegetative pathogens and, depending on the intensity of the heat treatment, of spore forming pathogens. Foods rendered pathogen free are generally regarded as safe. Processed products such as pâté, ice cream, infant formulae and others have nevertheless on occasion been responsible for outbreaks of food-borne illnesses. Typically, these products are ready-to-eat or may not receive an adequate lethal heat treatment at the point of consumption. Importantly, thorough epidemiological investigations of outbreaks related to several of such products have demonstrated that the presence of vegetative pathogens such as *Salmonella* spp. or *Listeria monocytogenes* in the consumed products was frequently due to post-process recontamination.

The investigation of disease outbreaks, including epidemiological studies and typing of strains, is very useful to trace the origin and source of the hazard. Published data demonstrate that the presence of pathogens in the vicinity of unprotected products in processing lines represents a significant risk of recontamination. In addition, cross-contamination between raw and heat treated food items in food service or consumer home kitchen settings can lead to hazardous food products.

The majority of studies on pathogens occurring incidentally in foods are devoted to investigations of their presence in raw materials or of their growth and behaviour in the finished products. Reference to recontamination is, however, only made in relatively few publications and very little is published on the sources and routes of these pathogens into products after they have passed through the final lethal processing step.

Microbiological Risk Assessment studies can be conducted as part of governmental activities determining the level of risk that a food product poses. Recontamination is often not considered in such studies. This report advocates that an effort should be made to develop our knowledge and information on recontamination further and start using it systematically in the exposure assessment part of Microbiological Risk Assessment studies.

INTRODUCTION

The production of safe foods is based on the implementation and application of general food safety management systems such as Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP). These measures represent the indispensable conditions required in facilities used for preparing or manufacturing foods (CAC, 1997). Significant specific hazards are addressed by applying the Hazard Analysis Critical Control Point (HACCP) system developed more than 30 years ago. Inadequate design or breakdown of such systems as well as abuse or improper preparation conditions can cause incidents and outbreaks with food-borne pathogens. Thorough knowledge of the epidemiology as well as of (historical) incidents and outbreaks is important to obtain a better understanding of problems and issues that can lead to food-borne disease outbreaks. This knowledge is essential and should be fed into the design of safe food products and manufacturing processes. Concrete insight in and use of experiences from incidents/outbreaks can help to correct or prevent errors occurring in production and, when necessary, to improve implemented preventive measures.

One important insight seems to be that post-process contamination, i.e. contamination of food products after a final lethal treatment, is frequently the cause of food-borne human disease incidents or outbreaks. This is rather an insight of practical experience and not one that is documented in a systematic way in the peer-reviewed literature. It is also an insight that is possibly quite unexpected as the food safety management systems mentioned above could adequately deal with post-process contamination provided it is identified as a factor to control.

Historical evidence is available to stress that recontamination is responsible for a large percentage of spoilage of, for instance, canned products (Stersky *et al.*, 1980). However, with specific food-borne pathogens it seems to be quite a rare event. For example, the occurrence of the spore forming pathogen *Clostridium botulinum* due to recontamination is only 1 in 260 billion cases (Anonymous, 1984). In other cases, e.g. pathogens transferred to the food product by contagious food handlers (Guzewich & Ross, 1999), it may be a frequent and important cause of outbreaks of food-borne disease. Unfortunately, scientific evidence and documentation in support of the importance of recontamination in food-borne disease events is scarce. There may be a number of reasons for this lack of supporting evidence, such as a failure to link outbreaks to illnesses possibly because of underreporting, the complexity of the investigations or a mere lack of scientific interest.

The aim of the work reported on here was to systematically assemble and present the currently available knowledge concerning the importance of recontamination as a cause of food-borne disease and to point out the extent to which recontamination has been incorporated or neglected in microbial risk assessment. A further aim was to identify the type of research and tools that will be required to include recontamination in microbiological risk assessments in a better way.

It should be noted that, while terms such as contamination, cross-contamination or post-process contamination are used in the different publications studied, the term recontamination will be used throughout the remainder of this review.

IMPORTANCE OF RECONTAMINATION

Although only a very small fraction of the total volume of food manufactured and prepared for consumption globally will be associated with an illness or disease event, absolute safety of the food supply is not possible and such events can occur in the form of sporadic or epidemic disease outbreaks.

Detailed information on the precise causes of food-borne disease outbreaks is frequently difficult to obtain for governments and industries alike and the available information is most often fragmentary. Partly, this is due to the many different institutions involved nationally, difficulties arising from the way in which public health policy and enforcement are organised in different countries as well as the differences between industries of varying sizes in their capability to investigate and communicate about root causes of food-borne disease.

Despite the incomplete insight in the root causes of food-borne diseases, indications on the importance and impact of recontamination regarding outbreaks of food-borne disease can be found in several surveys or surveillance reports. For instance, a recent survey performed in Europe by the World Health Organization indicated that recontamination is often recognised as a cause of food-borne disease outbreaks (Rocourt *et al.*, 2003). In the report, cross-contamination was mentioned as the most important factor relating to the presence of pathogens in prepared foods, as it was associated with 28.9% of the cases of outbreaks. Other causative factors identified were improper storage (25.3%), raw foods (18.4%), infected persons (9.7%), inadequate handling (9.2%), contaminated ingredients (4.8%) and contaminated equipment (3.0%). In a recent survey in Russia, it was found that in some 5-10% of the cases, food causing a disease outbreak was contaminated at the production stage, with contamination by an infected person and contamination by infective equipment being very prominent causes (Rocourt *et al.*, 2003).

In a compilation of disease outbreaks in the UK, recontamination accounted for 6.5% of the cases (Powell & Attwell, 1998). It should be noted that in this study, no contributing factors were apparent in 73% of the cases, while the causative agent was not even identified in 28% of the cases. The fact that, in many incidents, the responsible pathogen and/or the implicated food items remain unknown may not be surprising for older publications, but, surprisingly, this also holds true for many recent ones. Thus, a fairly recent survey of hospitalisations for gastroenteritis performed by Mounts *et al.* (1999), found that in 75% of the cases the specific aetiology was not identified.

Data from surveillance reports are not the only source of systematic and epidemiological information. Borgdorff and Motarjemi (1997) recommended to investigate outbreaks in great detail using epidemiological and microbiological methods, and to perform case-control studies of sporadic cases (for details see section “Techniques and tools for investigation of food-borne outbreaks” below). This would help to identify high-risk foods and practices, including the potential for recontamination.

The observation that food-borne outbreaks are not well documented in scientific, peer-reviewed papers may be due to the fact that such papers are not easy to publish. From a review of the existing literature it seems that, in order to make publication attractive, disease outbreaks need to fulfil certain criteria such as:

- a large number of patients affected or causing a long-lasting epidemic
- unusual or emerging pathogen
- occurrence of unusual types (e.g. serotypes or ribotypes) of the pathogen
- new or unusual food matrices.

A selection of publications on outbreaks involving recontamination mainly in food-processing establishments is compiled in Table 1. It can be seen that cross-contamination through processing equipment and from the factory environment both feature most frequently.

Despite the relative paucity of reports on disease outbreaks due to recontamination, it is apparent that there is an increase in publications that investigate the micro-flora of food-processing environments and food-processing lines. For example:

- *Staphylococcus aureus* in a whey powder plant (Kleiss *et al.*, 1994)
- *Bacillus cereus* in a whey processing plant (Pirttijärvi *et al.*, 1998)
- *Listeria* spp. in a meat processing plant (Senczek *et al.*, 2000)
- *Listeria monocytogenes* in fish processing (Fonnesbech-Vogel *et al.*, 2001; Rørvik *et al.*, 1995)
- *Salmonella* spp. in pork slaughtering and cutting plants (Giovannacci *et al.*, 2001; Olsen *et al.*, 2003).

These studies give a better understanding of sources and routes of pathogens and provide further support for the importance of recontamination in food-processing settings.

In comparison to the importance of recontamination as a cause of food-borne illness in processed foods during manufacture, rather more information is available about the importance of abuse, inadequate hygiene or insufficient final preparation close to or at the point of consumption. This can be in food service kitchens (e.g. restaurants, catering facilities) or in the consumer's home. Recontamination through unclean food surfaces, unhygienic behaviour of food handlers or, in particular, contact between unprocessed raw materials and ready-to-eat products are fairly well recognised routes leading to recontamination in such settings. Some publications provide a good insight into these routes (Barker *et al.*, 2003; Bloomfield, 2003; Hillers *et al.*, 2003; Redmond & Griffith, 2003; Kusumaningrum *et al.*, 2003). However, the present review focuses mainly on food-processing settings although parallels will be drawn between the two situations.

Table 1: Examples of outbreaks attributed to recontamination originating from various sources in the food chain.

Pathogen	Food implicated	Probable source and/or contamination route	Type(s) of investigation*	Reference
Raw materials				
<i>Clostridium botulinum</i>	Cheese	Contaminated onions added	M	Collins-Thompson and Wood, 1993
<i>Salmonella</i> serovars	Paprika potato chips	Contaminated paprika powder	M; T	Lehmacher et al., 1995
<i>Yersinia enterocolitica</i>	Chocolate milk	Probably contaminated chocolate syrup added to pasteurised milk	CC; M	Black et al., 1978
Processing equipment				
<i>Bacillus cereus</i>	Pasteurised milk	Filling equipment	M; T	Eneroeth et al., 2001
<i>E. coli</i> O157:H7	Different foods	Slicing and handling utensils in supermarket	CC; M; T	Banatvala et al., 1996
<i>Listeria monocytogenes</i>	Rillettes	Probably filling and packaging machine	CC; M; T	Goulet et al., 1998
<i>Salmonella agona</i>	Breakfast cereals	Environment of a processing line and equipment	CC; M; T	Breuer, 1999
<i>Salmonella berta</i>	Soft cheese	Buckets for cheese ripening previously used for chicken carcasses	M; T	Ellis et al., 1998
<i>Salmonella enteritidis</i>	Ice cream	Tanker used to transport ice cream previously used for raw eggs	Coh; M; T	Hennessy et al., 1996
<i>Salmonella enteritidis</i> PT4	Pastry	Mixing bowl, cream piping bags and nozzles not cleaned	CC; M; T	Evans et al., 1996
<i>Salmonella typhimurium</i>	Cooked sliced ham	Containers previously used for curing of raw pork	CC2	Llewellyn et al., 1998
<i>E. coli</i> O157:H7	Flavoured yoghurt	Pump previously used for unpasteurised milk; insufficient zoning?	CC; M; T	Morgan et al., 1993
Environment				
<i>Cryptosporidium parvum</i>	Water	Secondary contamination of potable water	CC; M	MacKenzie et al., 1994
<i>Listeria monocytogenes</i>	Butter	Processing environment	CC; M; T	Lyytikäinen et al., 2000a
<i>Listeria monocytogenes</i>	Hot dogs	Environment	CC; M; T	Anonymous, 1999
<i>Salmonella</i> serovars	Citrus products	Amphibians entering the production facility?	CC; M; T	Parish et al., 1998
<i>Salmonella ealing</i>	Infant formulae	Environment of processing lines and equipment	CC; M; T	Rowe et al., 1987
<i>Salmonella typhimurium</i> DT170	Kebab, yoghurt relish	Juice from carcasses dripping into open containers of yoghurt.	CC; M; T	Evans et al., 1999
<i>Yersinia enterocolitica</i> O:8	Pasteurised milk	Post-process contamination from the environment	CC; M; T	Ackers et al., 2000

Pathogen	Food implicated	Probable source and/or contamination route	Type(s) of investigation*	Reference
		Handling at or close to point of consumption		
<i>Cryptosporidium parvum</i>	Different foods	Infected food handler	CC; M; T	Quiroz <i>et al.</i> , 2000
<i>E. coli</i> O157:H7	Minced meat (hamburger)	No handwashing after handling raw beef	CC	Mead <i>et al.</i> , 1997
Norwalk-like virus	Lunch meals	Asymptomatic food handler who had taken care of sick child	CC; M	Daniels <i>et al.</i> , 2000
Norwalk-like virus	Salad items	Pre-symptomatic foodhandler who had taken care of sick child	Coh; CC	Lo <i>et al.</i> , 1994
Norwalk-like virus	Ham, chicken dish	Post-symptomatic food handler at conference	Coh	Patterson <i>et al.</i> , 1993
<i>Shigella sonnei</i>	Sandwiches	Food handlers	Coh; M; T	Hedberg <i>et al.</i> , 1992
<i>Yersinia enterocolitica</i>	Chitterlings (cooked pork intestines)	Unknown; patients too young to consume the product	CC; M; T	Jones <i>et al.</i> , 2003
		Unknown		
<i>Campylobacter jejuni</i> (O:33)	Tuna salad	Probably chicken handled in the same kitchen	CC; M; T	Roels <i>et al.</i> , 1998
<i>Enterobacter sakazakii</i>	Infant formula	Unknown	CC; M; T	Van Acker <i>et al.</i> , 2001
<i>Salmonella enterica</i> serovar Newport	Ham	Ham served at funeral meals; source of contamination unknown	Coh; M; T	Lyytikäinen <i>et al.</i> , 2000b

* M = Microbiological investigation of food and/or environment

T = Typing methods were applied to confirm strains

CC= Case-control study was performed

CC2= Two stages of case-control study were performed

Coh = Cohort study was performed

SIGNIFICANT RECONTAMINATION ROUTES AND SOURCES IN FOOD-PROCESSING ESTABLISHMENTS

With respect to the food-processing environment, vectors involved in the transfer or transmission of microorganisms have not received the same recognition and degree of attention as that given to recontamination close to or at the point of consumption. A concise overview of the various routes and sources of recontamination applying to food-processing environments is provided below.

Raw materials

Ready-to-eat products can be contaminated either due to inadequate hygiene in the processing environment, or by bacteria occurring on raw products that come into contact with prepared foods. Such contamination can occur in food-processing environments equally well as in food service kitchens, i.e. restaurants/catering, and household kitchens. Adequate precautions, such as physical separation of raw and processed products, shielding-off of product during processing from raw materials or handlers in contact with raw materials and specific training of operators of processing lines are required to minimise this cross-contamination route.

Another source of pathogens is the direct addition of contaminated raw materials or ingredients to a previously processed product. This route has been identified with several products manufactured on an industrial scale, e.g.

- paprika used to sprinkle onto potato-chips (*Salmonella*; Lehmacher *et al.*, 1995)
- chocolate syrup used to prepare chocolate milk (*Yersinia enterocolitica*; Black *et al.*, 1978)
- onions added to cheese (*Clostridium botulinum*; Collins-Thompson & Wood, 1993).

Food contact surfaces

Recontamination of otherwise sound products through contaminated surfaces has been observed in many cases and is a major issue in food manufacture. Table 1 shows examples in which unclean or insufficiently cleaned surfaces and pieces of equipment have been identified as the source of recontaminating pathogens. Such pathogens may be part of the micro-flora residing on food contact surfaces of processing equipment. The transfer of *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella* was demonstrated in meat and fish processing facilities (Adams & Mead, 1983; Olsen *et al.*, 2003; Rørvik *et al.*, 1995; Giovannacci *et al.*, 2001).

Occasionally, containers, pumps or tanks used for holding or transporting unprocessed raw materials, such as raw meat and poultry or unpasteurised liquid egg, have subsequently been used for processed products without any prior cleaning (Morgan *et al.*, 1993; Evans *et al.*, 1996; Hennessy *et al.*, 1996; Llewellyn *et al.*, 1998). This certainly represents a major deviation from good hygienic practices.

Ineffective or inadequate cleaning and disinfecting has also caused recontamination. The poor hygienic design of equipment is often the cause of such problems. In the study of Lundén *et al.* (2002), a dicing machine processing cooked meat was shown to harbour a very persistent strain

of *Listeria monocytogenes*. When this machine was transferred from one plant to the other, it carried the strain along, despite rigorous cleaning and disinfection. Thus, attention must be paid to the correct design of equipment and helpful recommendations in this respect, as well as guidelines for validating the ability of equipment to be kept clean, have been published (EHEDG, 1997). The correct hygienic design and proper maintenance of equipment is crucial to avoid recontamination through, for example, dripping condensation water or accumulating residues, cracks or micro-holes in heat exchangers or double-walled equipment, errors in the design or installation of the equipment allowing contact between unprocessed and processed product (Lecos, 1986). Modifications of the design of equipment and proper maintenance was, for example, found to decrease the occurrence of *Listeria monocytogenes* in cut meats and smoked fish (Tompkin *et al.*, 1999; Fonnesbech-Vogel *et al.*, 2001).

Airborne contamination

The impact of direct airborne recontamination of products is frequently overemphasised. It is probably limited to a few product categories where multiplication of the contaminant may occur. Such categories are for instance beverages, ice cream and semi-finished products for catering purposes. Dry powdered infant formulae, which require the viable counts level to be very low, are also susceptible to airborne contamination. Air filtration is usually performed in plants manufacturing products for which a high probability of airborne recontamination has been identified.

Airborne microorganisms are usually associated with dust particles, skin particles or water droplets (aerosols) and transmission occurs due to airflow. Distribution of pathogens through aerosols is probably more important than through dust. Aerosols were described to disseminate *L. monocytogenes* from drains in fish processing facilities (Rørvik *et al.*, 1997), from drip trays and cooling units (Goff & Slade, 1990) and through hosing under high pressure. Aerosol viable counts did not correlate with the contamination of cooked, frozen meat products during packaging (Helm-Archer *et al.*, 2004). Unfortunately, the latter study did not consider any other sources of contamination than aerosols, so the vector that did contaminate the frozen meat was not established.

A more systematic approach to investigating transmission via air, which is suitable for modelling, has been started (Den Aantrekker *et al.*, 2003b). The probability of (re-)contamination has been modelled by different authors and either linear or quadratic relationships between the number of microorganisms in the product and the air have been proposed (Radmore *et al.*, 1988). Dripping and splashing is of course also an important mode of spreading microorganisms. A mathematical model describing the distribution of microorganisms from falling drops has been developed (Pielaat, 2000).

Pests

Pests such as insects, birds and rodents have been recognised as important carriers of pathogens and other microorganisms (e.g. Olsen & Hammack, 2000; De Jesús *et al.*, 2004; Urban & Broce, 2000). In one interesting case, a *Salmonella* outbreak has been traced back to amphibians, which had accidentally entered the production facility (Parish, 1998). While massive direct recontamination can be excluded, sporadic cases may be attributed to these vectors. More important, however, is the transport and ingress of pathogens into food-processing environments and their possible establishment in suitable niches. Pest management is therefore an essential preventive measure and appropriate guidelines have been published (e.g. Marriot, 1997).

The food-processing environment

The food-processing environment is an important but still poorly recognised and understood source of recontamination. The fact is that certain pathogens can persist in food-processing or preparation environments and contaminate foods as they go through the manufacturing and preparation process (Tompkin 2002; 2004; ICMSF, 2002). Table 1 lists a number of outbreaks due to environmental recontamination of products such as infant formulae, dairy products, cooked meat products and cereals. Pathogens may access food-processing environments through pests, raw materials, personnel or mobile equipment such as forklifts, or through leaks and openings in buildings. Some pathogens may become established in the processing environment and find niches where they can survive for long periods of time. Cracks and crevices in floors and walls, interfaces between floor and equipment, hollow structures in the building or in equipment may form such niches. In dry environments of plants manufacturing products such as milk powder, chocolate or dehydrated soups, the levels of microorganisms in such niches will remain low. However, in wet environments or in dry environments that have become wet following wet cleaning procedures, pathogens may multiply to high levels.

A number of recent publications devoted to the occurrence of selected pathogenic, spoilage or indicator microorganisms in processing environments clearly demonstrate the importance of this source (Langfeldt *et al.*, 1988; Mead, 1992; Lawrence & Gilmour, 1995; Miettinen *et al.*, 1999; Fredriksson-Ahomaa *et al.*, 2000; Norton *et al.*, 2001; Thorberg & Engvall, 2001; Fries, 2002; Den Aantrekker *et al.*, 2003a).

Because the food-processing environment is only poorly recognised as a source of recontamination, it is frequently not considered during the establishment of preventive measures and sampling procedures to verify their efficacy. Detailed proof and facts concerning this issue have only been published occasionally (ICMSF, 2002). Fortunately, the number of publications on investigations of processing environments is increasing slowly, demonstrating an increased awareness (Tompkin, 2002 ; 2004; Yang *et al.*, 2002).

The regular surveillance of the processing environment for significant pathogens relevant to different types of products would be important in providing information on their incidence and thus on their potential presence in finished products. Analysis of samples from processing environments provides information on the efficiency of cleaning and disinfection procedures as well as on the efficacy of the preventive measures that have been implemented, such as zoning, limitations on movements of personnel and goods, cleaning and disinfection programs, etc. (Ingham *et al.*, 2000; Kleiss *et al.*, 1994; Lin *et al.*, 1998; Salvat *et al.*, 1995). This type of surveillance can serve as an early warning and can be complemented by monitoring hygiene indicators such as *Enterobacteriaceae* (Cox *et al.*, 1988; chapter 11 in ICMSF, 2002). However, as noted by Tompkin (2004), a key factor in the effectiveness of environmental monitoring programs, in addition to the availability of suitable sampling and detection methods, is the response by management when a problem is detected.

RECONTAMINATION DURING DISTRIBUTION AND HANDLING

Defective or soiled packaging material may also be responsible for recontamination. Defective seams and seals can cause, for instance, micro-leaks that may allow access of a variety of microorganisms, including pathogens. Drying the outside of pouches after retorting and storage of the pouches in a clean and dry environment remarkably reduced the spoilage rate (Michels & Schram, 1979).

Although probably underreported, recontamination at the retail level has been described on few occasions. Recontamination at the retail level is mainly due to poor hygienic practices of personnel and contaminated equipment, surfaces and utensils such as slicers, bowl choppers or knives (Banatvala *et al.*, 1996; Montville & Schaffner, 2004).

RECONTAMINATION CLOSE TO OR AT THE POINT OF CONSUMPTION

While the implementation of preventive measures is current practice in food factories, it poses problems in food service facilities such as restaurants, catering establishments and even more so in households. The occurrence of recontamination in the home has been demonstrated by epidemiological investigations (Mead *et al.*, 1997). An interesting example is the large-scale preparation of raw pork intestines in the home for a dish named chitterlings. Preparation of chitterlings was associated with yersiniosis in babies. These babies consumed neither the raw nor the cooked product, but they were nevertheless infected, probably via the environment, utensils, baby bottles or via the hands of their caregivers (Jones *et al.*, 2003).

According to Redmond and Griffith (2003) and Scott (1996), little attention is given to home hygiene because it is assumed that the home is not a likely place to acquire food poisoning. However, this assumption may often not be correct. Homes and in particular kitchens do provide a variety of opportunities for recontamination (Bloomfield, 2003; Redmond & Griffith, 2003). A recent study by WHO suggests that homes and restaurants are responsible for 31% and 32%, respectively, of the food-borne disease outbreaks in OECD countries (Rocourt *et al.*, 2003). As with food-processing establishments, pathogens may have become established in households for prolonged periods of time. An epidemiological study has demonstrated the spread of Salmonella in a household via an ill baby (Michanie *et al.*, 1987), and this is corroborated by the knowledge that children that have suffered from salmonellosis can excrete the pathogen for weeks (Van Schothorst *et al.*, 1978).

Only a few studies have addressed the fate of pathogens in kitchens and households in detail. A well-recognised example of a recontamination route in the home is contact of ready-to-eat food with raw materials through the use of cutting boards and utensils (such as transfer of pathogens to vegetables (Kususmaningrum *et al.*, 2003) or to cooked poultry (Gough & Dodd, 1998).

Transfer of pathogens by food handlers, especially from hands, is of particular importance in the home and in food service establishments (Bloomfield, 2003). In a literature review, Guzewich and Ross (1999) identified 81 food-borne outbreaks that were caused by food workers. In 93% (75) of those outbreaks the workers were reported to be infectious at or prior to the outbreak and in 89% (72) of the cases hand contact with the food was implicated. Deficient or absence of hand washing has been identified as the most frequent cause of transmission of pathogens with low infectious doses such as *Shigella*, viruses and pathogenic *E. coli* (Snyder, 1998; Hillers *et al.*, 2003). However, if the product and the storage conditions support the growth of microorganisms, then this mode of transmission and recontamination may become important for other pathogens as well (Guzewich & Ross, 1999).

Transfer of bacteria and viruses from hands to surfaces or food products, and vice versa, was recently quantified by a number of authors (Table 2). Unfortunately, these studies express the transfer efficiency in different units that cannot easily be compared. What is clear in each study, however, is that cross-contamination has a high variability. Transfer rates from cutting board to lettuce, for example, varied from 0.34 to 54%, with an average of 10% (Chen *et al.*, 2001). Both bacterial counts and transfer rates can be described by lognormal distributions (Den Aantrekker *et al.*, 2003b; Montville & Schaffner, 2004) that can be used as input in quantitative exposure assessments to form a scientific basis for the selection of effective preventive measures in both home and food service kitchens.

Table 2: Studies quantifying the transfer of micro-organisms in food preparation activities.

of	Transfer		Transfer expressed as	Reference
	from	to		
Calicivirus (surrogate for norovirus)	hand metal; ham; lettuce	metal; ham; lettuce hand	% virus recovered	Bidawit <i>et al.</i> , 2004
<i>Campylobacter jejuni</i>	stainless steel	dry and wet lettuce	log ¹⁰ CFU/item % transfer (CFU destination/ CFU total)	Moore <i>et al.</i> , 2003
<i>Enterobacter aerogenes</i>	chicken meat; cutting board	cutting board lettuce; cucumber	log ¹⁰ CFU/cm ² log ¹⁰ CFU/g	Zhao <i>et al.</i> , 1998
<i>Enterobacter aerogenes</i>	chicken meat; hand; cutting board; spigot	hand; spigot; lettuce	log ¹⁰ CFU/item transfer rate (%) (=CFU destination / CFUsource) statistical distributions	Chen <i>et al.</i> , 2001
<i>Enterobacter aerogenes</i>	chicken meat; hand; gloved hand	hand; gloved hand; lettuce	log ¹⁰ CFU/item transfer rate (%) (=CFU destination / CFUsource) statistical distributions	Montville <i>et al.</i> , 2001
<i>Escherichia coli</i> O157:H7	ground beef; cutting board; gloved fingers	gloved fingers; cutting board; lettuce	CFU / 10 fingers; CFU / cutting board; CFU/ g lettuce	Wachtel <i>et al.</i> , 2003
<i>Escherichia coli</i>	houseflies	surfaces	CFU / landing	De Jésus <i>et al.</i> , 2004
<i>Salmonella enteritidis</i> PT104	chicken	hand; kitchen utensils	frequency of counts (CFU)	Barker <i>et al.</i> , 2003
<i>Micrococcus luteus</i> ; <i>Serratia rubidea</i> ; phage PRD-1	dishcloth; sponge; faucet; carrot; hamburger	hand; lip	CFU/finger; CFU/lip; transfer efficiency [CFU/CFU]	Rusin <i>et al.</i> , 2002
<i>Salmonella enteritidis</i> ; <i>Campylobacter jejuni</i> ; <i>Bacillus cereus</i>	sponges; stainless steel surface	stainless steel; cucumber; chicken fillet	log CFU/cm ² ; transfer rate (%) (=CFU destination / CFUsource)	Kusumaningrum <i>et al.</i> , 2003

TECHNIQUES AND TOOLS FOR INVESTIGATION OF FOOD-BORNE OUTBREAKS

Epidemiological studies

In most of the disease outbreaks shown in Table 1, a case-control study (*see box*) was required to establish a link between the patients and a particular food item and to recognise whether recontamination was the cause of a food-borne disease outbreak. For instance, as described by Llewellyn *et al.* (1998), in the first stage of an investigation of a salmonellosis outbreak, the food implicated was identified as sliced ham. In the second phase, through a case-control study, the outbreak could be traced to a specific producer and a specific batch of ham that had not been properly processed.

Epidemiological Methods

A case-control study compares cases (i.e. patients) with controls regarding food intake, food preparation techniques and other possible risk factors. Controls should be representative for the population from which cases were drawn, but should not have had a food-borne disease in the period under study. The case-control study has to be performed quickly to be accurate. An outline of a case-control study for one risk factor is shown in Table 3.

The purpose of a case-control study is to estimate the relative risk or the odds ratio (OR). The controls can be either matched or unmatched. With unmatched controls, the odds ratio is calculated as the ratio of the odds of exposure in the group of cases to the odds of exposure in the group of controls [$OR = (a / c) / (b / d) = ad / bc$]. With matched controls each patient (case) is matched to a specific control person and each pair is considered. The odds ratio is then calculated as the number of exposed cases with matched non-exposed controls, divided by the number of exposed controls with unexposed cases. In either situation, a risk factor is considered to add significantly to the risk when the odds ratio exceeds 1.0 and the 95% confidence interval (CI) does not include 1.0.

In cohort studies the relative risk (RR) can be calculated as follows:

$$RR = \{ [a / (a + b)] / [c / (c + d)] \} \text{ (Jekel } et al., 1996).$$

Table 3: Schematic view to demonstrate the association between a risk factor and a disease in an unmatched case-control study with one risk factor (adapted from Jekel *et al.*, 1996).

		DISEASE STATUS		Total
		Present: case	Absent: control	
RISK FACTOR STATUS	Present	a	b	a + b
	Absent	c	d	c + d
	Total	a + c	b + d	a + b + c + d

Interpretation:

- a = subjects with both the risk factor and the disease (exposed cases, patients)
- b = subjects with the risk factor but not the disease (exposed controls)
- c = subjects with the disease, but not the risk factor (non-exposed cases, patients)
- d = subjects with neither the risk factor nor the disease (non-exposed controls)
- a + c = all subjects with the disease (cases)
- b + d = all subjects without the disease (controls)

Instead of a case-control study, a cohort study may be performed after an outbreak when the total population at risk can be easily identified and contacted, e.g. guests at a conference (Patterson *et al.*, 1993), airline passengers (Hedberg *et al.*, 1992), or when a detailed list of customers is available (Hennessy *et al.*, 1996). Cohort studies are less often performed than case-control studies, but they have the advantage that the attack rate can be measured, which is a measure of absolute risk. This is possible since in a cohort study the total population of patients and non-affected controls can be contacted, enabling exact data on the number of exposed and non-exposed individuals to be obtained. In case-control studies absolute numbers of patients are unknown, and the epidemiologist is limited to determining the odds ratio, which is a measure of relative risk (Borgdorff & Motarjemi, 1997).

Cohort studies have also been performed in groups of production facilities to find relationships between handling factors and the proportion of contaminated food. The 'risk' is then defined as the probability of finding a contaminated product, not a case of illness. Such studies have been performed for instance in the pork processing industry (Berends *et al.*, 1998), in facilities selling raw and cooked meat (Tebbutt, 1986) and in plants producing cold-smoked salmon (Rørvik *et al.*, 1997). Examples of risk factors identified by such an approach were job rotation of the workers between various departments, a bad state of repair of the equipment and occurrence of the pathogen in the drains (Rørvik *et al.*, 1997).

Source identification

Next to the proper identification of the implicated food and of the pathogen involved, there is also a need to address the origin and the route of access of that pathogen. Investigations of sources and routes of pathogens, in particular from food-processing environments, require specific techniques and tools. In situations where recontamination is a recognised issue, it is advisable to set up an environmental monitoring program that aims to verify the proper running of the production process but also will signal possible ingress of transient pathogens or the presence of persistent pathogens (ICMSF, 2002; Tompkin, 2002; 2004).

An important element in setting up adequate environmental monitoring is the sampling. The application of sampling plans and the actual sampling of finished and intermediate food products has been published (ICMSF, 1986; 2002). Sampling food-processing environments, however, is quite different from sampling products. It requires a particular knowledge of food-processing environments and their microbial ecology. The approach on how to develop sampling plans as well as the use of appropriate sampling tools and techniques has been described by ICMSF (2002). This publication has noted that the classical analytical methods represent a good base for testing but they may need to be modified to allow for an efficient detection of the pathogens of concern in the factory environment. Such modifications need to take into account the recovery of stressed cells (e.g. from disinfectants) or the presence of different and/or high levels of competitive micro-organisms.

While direct analysis for the pathogen of concern is useful and important, response times can be long and analytical costs high. Indicator groups such as *Enterobacteriaceae* or *Listeria* spp. represent interesting substitutes for direct analysis for particular pathogens. Indicators provide additional information on the micro-flora present in the environment or on changes due to, for example, the presence of water in a dry environment (Cox *et al.*, 1988; Eyles & Davey, 1989; Ingham *et al.*, 2000; ICMSF, 2002). Monitoring programs can also be set-up on the basis of a quick indicator for biological activity, such as ATP, which has been used for many years for rapid hygiene testing on processing lines and equipment (Flickinger, 1996).

Microbiological typing

Typing suspect micro-organisms has been applied in many investigations of disease outbreaks (Table 1). Numerous typing techniques and methods are available for epidemiological investigations of food-borne outbreaks, which have been developed or improved over recent years (Farber, 1996; Olive & Bean, 1999; Scott *et al.*, 2002). Strains isolated from patients and possibly involved foods or food environments can be typed using different techniques and then compared. This may allow the establishment of a firm link to a particular (batch of) food, production facility, supplier or selling point (e.g. Threlfall *et al.*, 1983; Powell & Attwell, 1998; Aguado *et al.*, 2001; Anonymous, 1999; Wagner *et al.*, 1999; Table 1, 4th column). Typing may also allow the exclusion of non-related cases from the investigation (Banatvala *et al.*, 1996).

These typing methods can help identify and trace sources of recontamination, but can also contribute to a better understanding of the specific micro-flora in food-processing facilities (Farber, 1996; Senczek *et al.*, 2000). For instance, typing of *B. cereus* strains isolated from pasteurised milk and processing lines indicated that in one case raw milk was the major source (Lin *et al.*, 1998) while in another case fillers were identified as the source of contamination (Eneroth *et al.*, 2001).

CONSIDERATION OF RECONTAMINATION IN MICROBIOLOGICAL RISK ASSESSMENT STUDIES

Internationally, there is a development to adopt the framework of Risk Analysis to manage the risk of food-borne pathogens in the context of public health protection and of fair trade (CAC, 2004). An important aspect of Risk Analysis is the so-called Microbiological Risk Assessment (CAC, 2001; Buchanan *et al.*, 2000; Lammerding & Fazil, 2000), abbreviated as MRA. MRA is a scientific and technical exercise to investigate a food-borne pathogen associated with a particular food or category of foods and to establish a measure of the magnitude of risk to the population posed by this pathogen through the food source. Importantly, such MRA studies are undertaken by governments or under the auspices of governments, although there may be specific applications for MRA techniques in other contexts as well (Brown & Stringer, 2002; Gorris, 2002; Van Gerwen & Gorris, 2004).

MRA studies can bring together a lot of detailed information relevant for risk managers in government to make decisions related to consumer protection. Part of that information can be relevant to food industries as well, in particular when factors are identified that contribute to or reduce the risk posed by a certain pathogen. Such factors can relate, for instance, to production and manufacturing practices or to consumer use and handling.

Microbiological Risk Assessment is composed of four activities: Hazard Identification, Hazard Characterisation, Exposure Assessment and Risk Characterisation. Several reviews of the concept and principles have been published (Van Schothorst, 1997; Buchanan, 1998; ICMSF, 1998; 2002; Brown & Stringer, 2002; ILSI Europe, 2001; 2004). Exposure assessment accumulates information and data on the actual exposure of consumers to a specific pathogen, i.e. it estimates the quantity of a pathogen ingested. Aspects related to presence, growth and inactivation of pathogens throughout the production process have been extensively studied and quantitative models have been designed (Van Gerwen & Zwietering, 1998; Lammerding & Fazil, 2000; EC, 2002). However, the possibility that hazardous microorganisms may gain access to ready-to-eat food products, e.g. to processed foods after the last lethal step in processing, is frequently not included in exposure assessment. To date, only a few quantitative models that can take account of recontamination have been developed (Den Aantrekker *et al.*, 2003b; c; Montville *et al.*, 2002; Schaffner, 2003; 2004). Although this may be due to a lack of data, it is more probable that the importance of including recontamination in exposure assessment is not broadly recognised. Recently published data on the prevalence, the statistical distributions (Chen *et al.*, 2001; Mattick *et al.*, 2003; Montville *et al.*, 2002, 2004) and the transfer (see Table 2) of microorganisms form a promising basis for further work in this area.

Taking the last lethal step for the target pathogen of concern as the starting point, recontamination could be included in exposure assessment models. These models can use explicit point estimate (often worst-case) values to describe sources of pathogens and routes of access to the product, where these have been established in appropriate studies. Alternatively, probabilistic models may be designed to include the variability of biological and physical events and uncertainty on the parameters relevant to recontamination. Such models may not yet be readily available to use as a risk assessment tool, but, as shown above, techniques and approaches have been developed.

An alternative to studying a specific production facility in detail, is a survey of products that are currently on the market (Buchanan *et al.*, 1997; Lindqvist & Westöö, 2000). The level of contamination of heat-treated products after treatment can be considered as a realistic estimate of the exposure to pathogens due to recontamination. This approach may be especially valuable for governmental risk assessors. A disadvantage of this approach is that it does not immediately give information on the sources and routes of recontamination. Furthermore, analysing the incidence of pathogens in food products on the market requires a massive amount of testing. But this effort might well be smaller than the enormous efforts that would be needed to describe all the sources, prevalence levels and contamination routes by full-blown exposure assessment studies.

A step towards obtaining more insight into recontamination sources and routes to complement studies on the incidence of recontamination in products on the market, might be a type of cohort study of production facilities. Such a study would compare a number of selected food production or food preparation establishments in order to identify factors contributing to recontamination. The selection could be based on information provided by the market study or by previous studies. On a conceptual basis, Havelaar *et al.* (2004), have compared traditional and modern slaughterhouses in terms of the risk of Shiga-toxin producing *Escherichia coli* O157 in steak tartare knowing that the level of recontamination in traditional facilities is higher than in modern facilities. In the case where such differences in the level of contamination are observed between comparable products produced by different establishments, the cohort could be composed of a number of establishments producing foods characterised by those different levels. Other selection criteria for the composition of the cohort could apply as well.

CONSIDERATIONS ON FUTURE NEEDS

Food manufacturers have gathered data on the prevalence of pathogens in their production environments over many years. Although these data are often not collected systematically and have a limited scope, specialists in industrial hygiene or industrial microbiology have developed efficient preventive measures based on these data. In general, however, these data are not publicly available and also not peer-reviewed. In addition, the knowledge of the microbial ecology in environments along the entire food chain (i.e. during processing, transport, retail and preparation) is not well developed in all cases and this hampers successful investigation of disease outbreaks. This knowledge needs urgently to be further expanded, because it is relevant for establishing effective HACCP schemes and for setting up meaningful environmental monitoring programs. This knowledge is also important for MRA studies, as it will help to properly identify pathogens and their possible sources as part of the "Hazard Identification" step. Where quantitative information describing the microbial ecology is available, this might be used as input for exposure assessment modelling.

However, it is clear that investments in infrastructure and human resources need to be made to assure adequate generation of recontamination-related data and use thereof in risk assessments. Only when better information and quantitative data on recontamination is compiled through systematic research, either in process lines or by sampling large numbers of products on the market, can the actual exposure of consumers be estimated with sufficient certainty. Apart from the investment in data generation and use, there is a need for investigating in much more detail than is current practice, the occurrence and incidence of pathogens in food-processing environments. Such investigations should consider both the vectors and routes of access for pathogens to processed foods and also include the role of preventive hygienic measures. Information from epidemiological studies of disease outbreaks as well as knowledge of the physiology and ecology of pathogens can help to identify those factors that contribute most significantly to consumer risk. Subsequent prevention or control programs can then focus on these factors.

Implementing systematic environmental monitoring will generate additional analytical information that will allow verification of the efficiency of the preventive measures and improvement of them when necessary. It will also provide new and better data enabling more precise and complete mathematical modelling of the dynamics of food-borne pathogens at various steps in the food chain and thus of the exposure of consumers to the hazard.

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