MICRORISK

Microbiological risk assessment: a scientific basis for managing drinking water safety from source to tap

Pathogens in source water

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1. RATIONALE

Sources: [Medema *et al.*, 2003; Pond *et al.*, 2004]

Assessment of source water pathogen contamination in baseline and peak conditions is the first step to quantitative microbial risk assessment of drinking water. In many cases, outbreaks of disease through drinking water have occurred as a result of hazardous events, such as heavy rainfall, which lead to high loads of pathogens in the source water. It is therefore important to incorporate hazardous events along with the variable baseline contamination in the QMRA. Furthermore, understanding of the contributing factors within the catchment is essential to assess and manage these risks. It should be based on:

- Knowledge of the different sources of contamination in the catchment and of their contribution to the contamination of the source water;
- Identification of hazardous (peak) events;
- Assessment of the levels of baseline and peak pathogen contamination of water sources.

After a review of pathogens in sources waters, this chapter proposes a framework for performing a catchment survey and designing an effective monitoring program for baseline and peak event contamination assessment. Finally, results from the Microrisk project are presented and discussed in a risk assessment context.

Pathogens in source waters

During the last 20 years, the reliability of the faecal indicators as a mean to assure the safety of water has been increasingly challenged by water quality and public health microbiologists. In support of this contention, many publications report the limited correlation between the presence and concentration of faecal indicators and the presence and concentration of waterborne pathogens. They demonstrate in particular that faecal indicator bacteria such as *E. coli* are poor surrogates for protozoa and viral pathogens. Furthermore, several authors have shown that outbreaks of waterborne disease have occurred despite the absence of faecal indicators in source water [Barrell *et al.,* 2000]. These limitations have led several groups of workers to advocate the routine testing of water for specific pathogens. Indeed, during the recent revision of the WHO Guidelines for Drinking Water Quality, the WHO working committees suggested a list of reference pathogens that could be used as part of a water quality monitoring and assessment program.

This review is focussed on a selection of pathogens considered to be of high risk to human health and which are considered to be of concern in source water used for drinking water supply. These are (see Table 1):

- Protozoa: Cryptosporidium and Giardia
- Bacteria: Campylobacter and E. coli 0157:H7
- Viruses: Enterovirus and Norovirus

Table 1: Waterborne pathogens and their significance in water supplies after [WHO, 2004]

Pathogen	Infectious dose*	Persistence in water supplies	Resistance to chlorine	Relative infectivity	Important animal source
Campylobacter jejuni, C. coli	Low	Moderate	Low	Moderate	Yes
E. coli 0157:H7	Low	Moderate	Low	High	Yes
Enterovirus	Low	Long	Moderate	High	No
Cryptosporidium	Low	Long	High	High	Yes
Giardia intestinalis	Low	Moderate	High	High	Yes
Norovirus	Low	Long	Moderate	High	Potentially

* A detailed description of the dose-response relationship is given Chapter 7.

Protozoa

Cryptosporidium and *Giardia*

Cryptosporidium is a significant cause of waterborne outbreaks of diarrhoeal diseases. *Giardia* has been reported as the most common cause of protozoan diarrhoeal illness worldwide [Farthing, 1989; Adam, 1991]. Between 1971 and 1994, more than 25,000 cases of giardiasis were recorded in the USA [Craun, 1986; Anon, 1993, 1996]. The Centre for Disease Control and Prevention in Atlanta, USA, attributed 71% of waterborne disease outbreaks in 1993 and 1994 to *Cryptosporidium parvum* and *Giardia lamblia*, which respectively cause cryptosporidiosis and giardiasis [Gostin *et al.,* 2000]. Attack rates of cryptosporidiosis in these outbreaks are about 40% for the population at risk, as compared to 5-10% for giardiasis [Smith and Rose, 1990].

Bacteria

Campylobacter

Campylobacter is considered the most important bacterial agent in waterborne diseases in many European countries [Stenström *et al.,* 1994; Furtado *et al.,* 1998]. A large number of outbreaks of Campylobacter have been reported in Sweden for example, involving over 6,000 individuals [Furtado *et al.,* 1998].

E. coli 0157:H7

E. coli is an enteric organism and comprises the majority of the normal flora of the gut. E. coli 0157:H7 is the most widely recognised verocytotoxin-producing E. coli (VTEC) serotype and is now recognised as an important cause of food and waterborne illness in developed and some developing countries. High incidence of VTEC infections has been reported from regions of Canada, Scotland, and Argentina. In most European countries, the annual incidence may range from 1 to 4 infections per 100,000 inhabitants.

Virus

Enterovirus

Enteroviruses are one of the most common causes of human infections. They are ubiquitous, enterically transmitted viruses that have been estimated to cause about 30 million infections in the USA each year [WHO, 2004].

Norovirus

Noroviruses are a group of related, single-stranded RNA, non-enveloped viruses. Noroviruses are considered the most common viral etiologic agent of epidemic waterborne viral gastroenteritis [Brugha *et al.,* 1999].

A number of studies has been undertaken to investigate the occurrence of Campylobacter, *Cryptosporidium* and *Giardia* in source waters (Table 2). Fewer studies have been published on the levels of viruses and E. coli 0157:H7. In all cases presented below, it should be kept in mind that the sampling and testing methods varied and such variations can influence the numbers of pathogens detected. Methods differ in their sensitivity and selectivity, and in vitro culturing techniques do not isolate all the organisms present in samples due to the differences in metabolic condition of individual cells.

Table 2: Summary of concentrations of selected pathogens in water bodies

Pathogen	Water body	Concentrations	Country	Reference
Cryptosporidium	Surface water	0.006-2.5 oocysts/L	UK	Badenoch,1995
	Surface water	0-252.7 oocysts/L	11 countries	Smith & Grimason, 2003
	River water	4.1-12 oocysts/L	The Netherlands	Medema *et al.*, 1996
	Spring fed lake	0.24 oocysts/L	Ireland	Garvey *et al.*, 2002
	Surface water	3.8-21oocysts/L	Honduras	Solo-Gabriele *et al.,* 1998
	River	<5 oocysts/L	France	Rouquet *et al.,* 2000
Giardia	River	2.3 cysts/L	Canada	Ong *et al.,* 1996
	Surface water	5 cysts/L	8 countries	Smith & Grimason, 2003
	River	10-100/L	The Netherlands	Medema *et al.,* 1996
	Streams	0.1-5.2 cysts/L	USA	Ongerth *et al.,* 1989
	Surface water	0.02 cysts/L	Russian region	Ergov *et al.,* 2002
Campylobacter	Surface water	109,000 MPN/L	Germany	Feuerpfiel *et al.,* 1997
	River water	100-360/L	UK	Bolton *et al.,* 1982
	River	<100-2400 CFU/L		Stelzer *et al.,* 1989
	River	<2-93 MPN/L	Australia	Ashbolt *et al.,* 2002
	River	<1.2-110 MPN/L	Australia	Savill *et al.,* 2001
E. coli 0157	River and lake	>2000/L	Germany	Schindler, 2001
Enterovirus	Drinking WTT	0.0006 MPN/L	USA	Payment *et al.,* 1985
	River	0.3-4/L up to 13/L	The Netherlands	Theunissen *et al.*, 1998
	Dune filtrate	<0.003-13/L	The Netherlands	Theunissen *et al.*, 1998
	River	0.0033-0.46 PFU/L	Germany	DeRoda Husman *et al.*, 2004
	River	0.66-29/L	Worldwide	Gerba *et al.*, 1996
	Surface water	0.0033-0.46 PFU/L	Finland	Horman *et al.*, 2004

There are a number of limitations and sources of uncertainty in these data due to the sensitivity of analytical techniques, particularly for viruses and protozoa, and to the lack of knowledge about the viability and human infectivity of *Cryptosporidium* oocysts, *Giardia* cysts and viruses detected in the different studies.

Concentrations in Table 2 vary greatly (zeros are not included):

- *Cryptosporidium* 0.006-250 oocysts / L
- *Giardia* 0.02-100 cysts / L
- Campylobacter 1.2-109,000 MPN / L
- Enterovirus 0.003-29 / L

These variations are greatly dependant on the sampling conditions and principally on the local context and hydrology. Wet weather conditions may provoke peak events with extreme values of concentrations. Monitoring is a valuable tool for identifying baseline and peak event contamination in local contexts.

Sources and routes of contamination

The relative significance of the different pathogens sources at a specific water site is determined by a combination of factors: (1) the contamination level of these sources, (2) the magnitude of these sources, (3) the persistence of the pathogen, (4) their transport behaviour from the source to the specific site and finally, (5) their resistance against treatment processes. Knowledge of these characteristics and about the health outcome after infection allows the appraisal of the health significance of the pathogen. The pathogens of particular interest in this project have been selected because they are considered of high health significance.

Overview on the potential sources of contamination

Source waters are vulnerable to contamination from many origins. Humans, livestock and wild animals are all sources of faecal contamination. It has been shown that many rivers in Europe are significantly contaminated with microbes arising from municipal wastewater and/or livestock [EEA, 2003]. Furthermore, source waters, and particularly surface waters, are often used for purposes such as irrigation, recreation, transport which may also affect water quality. Groundwater contamination may be induced by different practices in management of domestic wastewater and livestock manure. Precipitation events can lead to higher pathogen loads in source waters.

Waste water treatment plants are an obvious high risk source of pathogens both in terms of number and strain of pathogens (see Table 3). During periods of high rainfall or plant failure, WWTP may release significant amounts of poorly treated effluent. Moreover, pathogens may be dispersed in the environment through the use of sewage sludge as fertiliser.

Table 3: Typical concentrations of pathogens in raw and treated domestic wastewater [Medema et al., 2003]

	Raw waste water	Secondary effluent
Cryptosporidium	1,000-10,000 n/L	10-1,000 n/L
Giardia	5,000-50,000 n/L	50-500 n/L
Enterovirus	10-100 n/L	1-100 n/L

Agricultural practices are an important source of contamination especially from *Cryptosporidium* oocysts, *Giardia* cysts, and Campylobacter [Carey *et al.,* 2004;

Lack, 1999; Monis and Thompson, 2003]. As well as direct runoff into surface waters, animal waste is often collected in impoundments from which effluent may infiltrate groundwater.

Other sources of faecal contamination that may be a threat to water sources are stormwater discharges, accumulation of pathogens in sediment, swimming pool water?, water treatment plant discharges and wild animals.

Advances in source tracking techniques (for review of techniques see [Meays et al., 2004; Pond et al., 2004]) which differentiate animal and human sources of faecal pollution will allow more precise information on the contamination sources and will assist managers in developing strategies to protect source waters. More information on the sources and health implications of the pathogens selected in this study can be found in [Pond *et al.*, 2004].

Persistence of pathogens in the environment

After leaving the body of their host, most pathogens gradually lose viability and the ability to infect new hosts. The waterborne pathogens and parasites of greatest concern are those that have high infectivity and that can either proliferate in water or possess high resistance to decay outside the body.

The ability of pathogens to survive in surface water is variable. In general, survival is prolonged when water temperature is low. Other factors that influence survival include sunlight intensity and the presence of aquatic microorganisms that may use the pathogens as a food source or cause pathogen disintegration. Adsorption to particles facilitates survival. A summary of the major influencing factors on pathogen survivals are listed in Table.4. Table 5 outlines the disappearance rate and time for a 50% reduction in concentration of pathogens in surface water using examples of published data.

Table.4: Major factors influencing pathogen inactivation in surface water [Pond et al., 2004]

	Solar radiation	Temperature	Salinity	Predation
Cryptosporidium	Medium (+)	High (+)	Medium (+)	Low (+)
Giardia	Medium (+)	High (+)	Medium (+)	Low (+)
Campylobacter	High (+)	High (+)	Medium (+)	Low (+)
E. coli 0157:H7	High (+)	High (none)	Medium (+)	Low (+)
Enterovirus	High (+)	High (+)	Medium (+)	Low (+)
Norovirus	Likely High (+)	Likely High (+)	Unknown – likely Medium (+)	Low(+)

It is possible that in nutrient rich sediments, micro-organisms survive for extended periods of time [Davies *et al.,* 1995]. In the case of oocysts, it has been shown that they may remain infective up to 12 weeks in water at 25°C and survive for several months in water at 4°C [Carey *et al*., 2004].

Table 5: Disappearance of selected pathogens in surface water [Medema et al., 2003]

	Disappearance rate (per day)	Time for 50% reduction of concentration (days)
Cryptosporidium	$5.7 \cdot 10^{-3}$-$4.6 \cdot 10^{-2}$	15-150
Giardia	0.023-0.23	3-30
Enterovirus	0.01-0.2	3-70

Disappearance rates are lower in groundwater than in surface water. Pathogens may be removed during soil transfer by adsorption and inactivation. Inactivation is influenced by many factors such as soil temperature, moisture, pH, microflora and organic carbon content. International literature reveals that viruses survive longer than faecal bacteria. No data on the survival of protozoa in groundwater are available yet, but it can be assumed that these pathogens are able to survive longer than viruses [Medema *et al.*, 2003].

Transport of pathogens

Most pathogens have no means of transport in the aquatic environment other than being transported with the water flow. Pathogens can therefore be regarded as biological particles that are transported by advection. Sedimentation of viruses and parasites is very slow and probably not significant. However, many pathogens readily attach to particles in water [Gerba *et al.*, 1984] which largely determine the transport characteristics. Sedimentation may then become significant.

Sediments may contain important numbers of faecal indicators and pathogens. For example, virus levels are generally 10-fold higher in sediments than in overlying waters. Since pathogens remain viable in the sediments for variable lengths of time, it is important to consider the importance of their resuspension and subsequent redistribution. Rain events and activities such as shipping or dredging may give rise to resuspension.

Several factors affect the hydrodynamic distribution of pathogens in lakes and reservoirs. In temperate climates, lakes may be stratified in summer, with warm water at the top and colder contaminated water at the bottom of the lake. Destratification (due to temperature decrease or storms) will cause water layers to mix and particles to return to the surface layer. Inflow characteristics are also important factors: inflow speed, entrainment of lake water and resulting dilution, insertion depth [Brookes *et al.,* 2004; Hipsey *et al.*, 2005].

Rain events not only affect water quality because of runoff and stormwater discharges but also because of water flow increase. This may result in faster transport of pathogens from the contamination source to the abstraction site. Concentration of *Giardia* oocysts has been shown to be positively correlated to water flow and turbidity levels [Atherholt *et al.*, 1998].

The most important factors in the transport of microorganisms through the subsurface are water flow (the driving force) and attachment [Schijven *et al.*, 2000]. Adsorption is affected both by the characteristics of soil (texture, pH, composition) and pathogens. Bacteria and parasites are more readily removed than viruses because of

their size (1-20 μm versus 20-80 nm); differences in isoelectric points and surface composition determine the pathogen adsorption rates. The unsaturated flow zone can play an important role in retarding or even eliminating pathogens and must be considered when assessing aquifer vulnerability. Increased water flow may remobilize adsorbed microorganisms.

NB: Highly fractured aquifers
Highly fractured and karstic aquifers represent a particular problem. Groundwater flow through fractured systems may be very rapid. The potential for microorganisms to be attenuated by interaction with the aquifer matrix is reduced but not entirely absent.

Conclusions

The six pathogens reviewed in this document all have high health significance. It is clear that source waters are contaminated to varying degrees with these pathogens. Their presence and persistence in water is due to a number of different factors such as survival, transport, type of water source or aquifer characteristics in the case of groundwater. There sometimes is a strong seasonal effect in the occurrence of these pathogens in surface waters with periods of rainfall contributing to higher source water contamination.

To understand the dynamics of source water pathogen contamination, it is important to determine the sources of pathogens in a catchment and to quantify their environmental loadings, especially under conditions that may favor high pathogen concentrations (hazardous events). The natural variability of potentially pathogenic microorganisms from anthropogenic, natural, and livestock sources is large and difficult to quantify. It is complex to rank the various sources and transport routes in terms of relative importance to human disease. Risks depend much on the specific case and need to be considered in the local context. This is of course a big challenge for water and/or health managers.

If a monitoring program is to be planned, it is essential to identify the main sources of contamination and potential causes of peak events in the local context.

The following should be considered:

- Magnitude of contamination;
- Frequency of the discharge (continuous versus event related);
- Type of contamination (animal or human);
- Distance from the water source and travel time during events;
- Transport and survival properties of potential pathogens.
-

In the following paragraphs, a protocol for assessing contamination sources and events in a catchment is given (2). How this can be used to guide pathogen monitoring is developed further (3).

2. CATCHMENT SURVEY

The purpose of this step is to develop a broad overview and basic understanding of the catchment. It is not intended to be an extensive data collection exercise but rather the characterisation of the system at an appropriate level of detail to provide useful information [Nadebaum *et al.*, 2004]. The following conclusions should be drawn from this survey:

- Vulnerability of the source water;
- Importance and location of pathogen sources;
- Peak events leading to high contamination risks (type, intensity, frequency, duration).

This type of survey has been conducted on 12 different Catchment to Tap Systems (CTSs) throughout Europe plus one in Australia (see Table 6). They vary in size, occupation, protection, climate, etc.

Table 6: List of the 12 Catchment to Tap Systems (CTSs)

CT	Country	Source water	Protectio	Climate	Catchment
1	United Kingdom	River	No	Humid oceanic	12,917
2	The Netherlands	River	No	Humid oceanic	198,735
3	France	River	No	Humid oceanic	10,050
4	France	River	No	Mediterranean	522
5	Sweden	River with controlled input	No	Sub-arctic	50,180
6	Sweden	Reservoir	No	Sub-arctic	50,180
7	Germany	Groundwater & river bank filtrate	No	Humid oceanic	145
8	Australia	Reservoir	No	Mediterranean	140
9	The Netherlands	Reservoir	No	Humid oceanic	198,735
10	France	Reservoir	No	Humid oceanic	30
11	Germany	Reservoir	Yes	Humid oceanic	300
12	France	Aquifer	Yes	Humid oceanic	100

In this project, source water quality is assessed at the intake of the treatment plant. This implies that reservoir and river bank water filtrate are regarded as source waters. Another point of view may be to consider reservoirs and river bank filtration as the first treatment step and therefore sample source water upstream.

Guidelines for performing catchment survey

The proposed outline for performing a catchment survey is detailed in Table 7. Recommendations include description of the water abstraction, key catchment characteristics (morphology, hydrology, hydrogeology and climate) plus description and location of potential sources of faecal contamination.

Table 7: Outline for catchment survey

SURFACE WATER	GROUNDWATER
Description of water abstraction	
Water intake description	• Number of wells • Depth • Wellhead
Type of source • River • River with reservoirs upstream • Artificial reservoir (dam) • Natural reservoir (lake)	Type of source • River-aquifer connection (e.g. karstic aquifer) • Shallow hole • Lowland river gravel abstraction • Shallow water table • Confined aquifer
Catchment description	
Size of the catchment, length of river, main tributaries, maximum and minimum height, dimension of reservoir	• Total catchment • 50-days catchment • Surface water catchment (if connected)
Uses of water • Agriculture • Urban • Industry • Other	Uses of water • Agriculture • Urban • Industry • Other
Hydrology & Hydrogeology	
• Average flow • Monthly average flow • Sorted Flows • High flows (1-year, 10-year, 50-year) • Main soils • Slopes	• Description of catchment geology and hydrogeology • Average water pumped (yearly and monthly) • Maximum water pumped (yearly and monthly)
Climate	
Description of the climate including • Temperature (monthly average, minimum and maximum) • Rainfall (monthly average, minimum and maximum) • Snowmelt	
Location and description of potential sources of faecal contamination	
• Human – Waste Water Treatment Plants – Combined Sewers Overflows – Biosolids (storage and use in agriculture) • Animal – Animal breeding (manure storage, manure used as fertiliser, grazing) – Roosting birds – Slaughterhouses or livestock markets – Wildlife	• Human – Septic tanks – Biosolids (storage and use in agriculture) • Animal – Animal breeding (manure storage, manure used as fertiliser, grazing) • Other – Wellhead or borehole liable to flooding • If connected to surface water – See potential sources for surface water

Example of CTS surveys

Figure 2 and Figure 1 show map examples of potential contamination sources for two CTSs. Table 8 summarises a fulfilled generic catchment description.

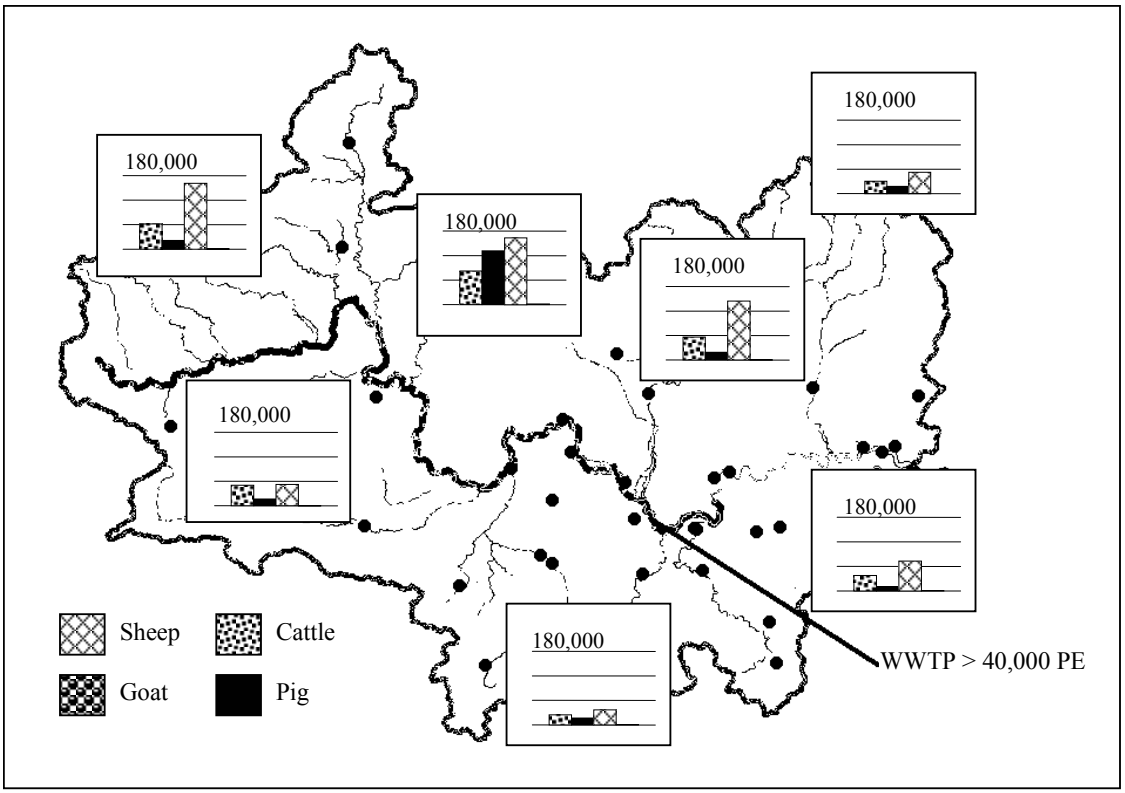

Figure 1: Animal breeding & waste water treatment plants in CTS 1 (UK)

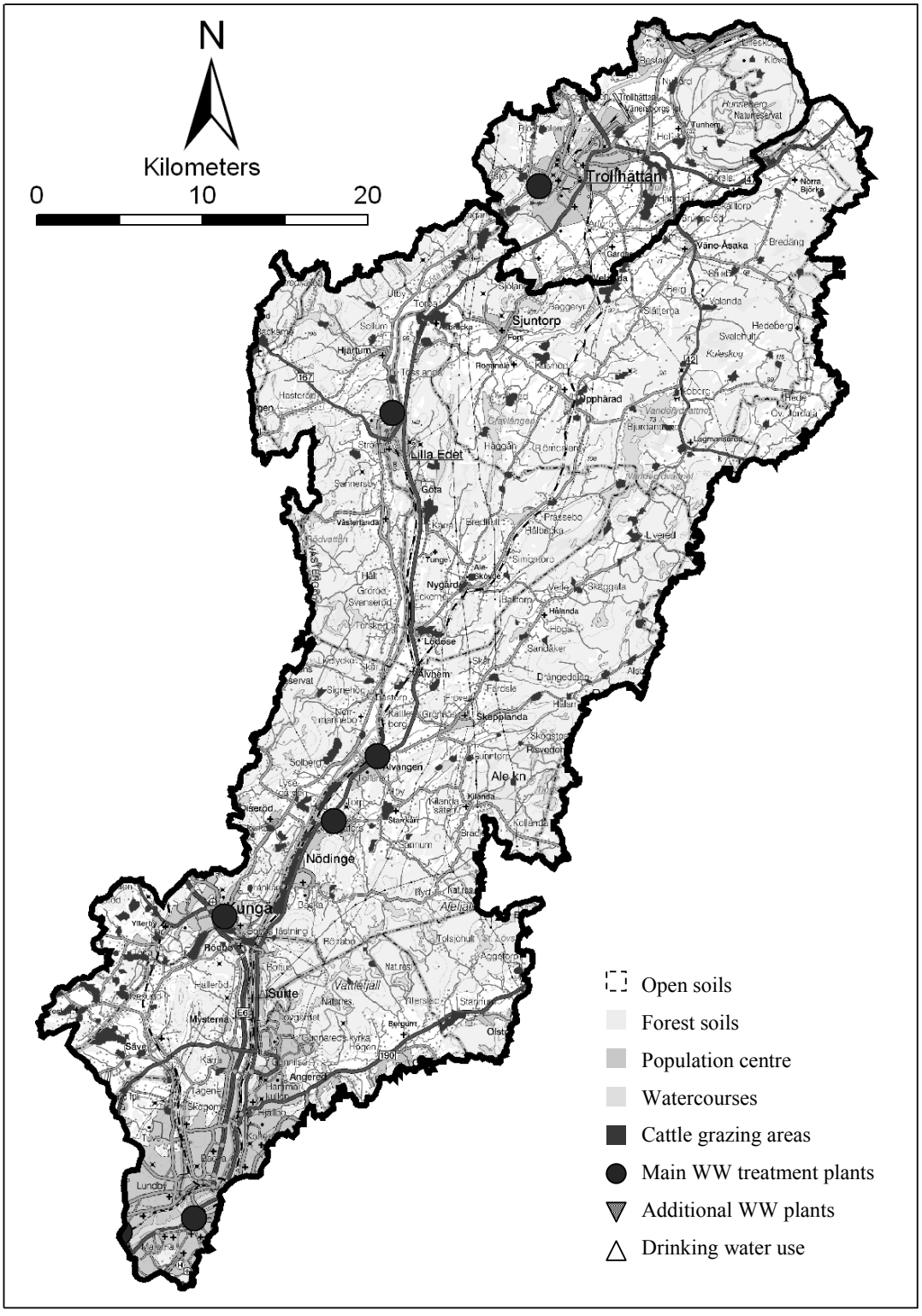

Figure 2: Lower catchment area of CTS 5 & 6 (Sweden)

Table 8: Catchment survey for CTS 8 (Australia)

CTS Name and number	**xxxxxxx**	**8**
Country	Australia	
Catchment size (km^2)	Surface water Population supplied = 50,000 Catchment area = 140 km^2	Groundwater Total: 50-days: Surface water catchment (if connected):
Potential sources of faecal contamination	Human ☒ Waste Water Treatment Plants ☐ Combined Sewers Overflows ☒ Biosolids (storage and use in agriculture) Animal ☒ Animal breeding ☒ Roosting birds ☐ Slaughterhouses or livestock markets	
Type of source (location of intake)	Surface water ☐ River ☐ River with reservoirs upstream (farm dams) ☒ Artificial reservoir (dam) ☐ Natural reservoir (lake) Groundwater ☐ River-aquifer connection ☐ Swallow hole ☐ Lowland river gravel abstraction ☐ Shallow water table ☐ Confined aquifer	

Hazard identification and peak events

Understanding the reasons for variations in source water quality is important, as it will influence the requirements for treatment, treatment efficiency and the resulting health risk associated with the finished water. Raw water quality is influenced by both natural and human use factors. Human use factors include point sources (municipal wastewater discharges) and non-point sources (urban and agricultural runoff).

Whether water is drawn from surface or underground sources, it is important that the characteristics of the local catchment or aquifer are understood and that the scenarios that could lead to water pollution are identified and managed. Groundwater from deep and confined aquifers is usually microbiologically safe; however, shallow or unconfined aquifers can be subject to contamination from discharges or seepages, on-site sanitation and sewerage. Hazardous, peak events that may have an impact on the catchments and that should be taken into consideration as part of a hazardous events assessment include:

- Upstream events (waste water and stormwater discharges, waste disposal sites);
- Human access (recreational activity);
- Cleaning of the river course;
- Land use (animal husbandry, agriculture, forestry) and changes in land use;

- Unconfined and shallow aquifer, including groundwater under influence of surface water and karstic aquifers;
- Inadequate wellhead protection and unhygienic practices;
- Climatic and seasonal variations (rainfall, thaw, snowmelt, droughts) [WHO, 2004].

Other situations may be important to consider locally, such as:

- Farming practices, such as in CTS 9;
- Different farming practices may yield peak events. For example, in the late winter and spring, farm animals and their young are put back on the fields. CTS 9's catchment survey identified that young animals may shed higher concentrations of pathogens.

Example: high bird loads in CTS 2
River water is abstracted, pre-treated and transported to the dunes along the North Sea coast where it remains from 60 to 400 days. It is then abstracted in an open canal system, collected in a reservoir and treated once more before distribution. During frost periods, water in the reservoir usually remains unfrozen longer than in the surrounding water bodies due to the constant temperature of the abstracted water and the flow in the basin. As a consequence, geese, ducks and swans tend to assemble on the reservoir, leading to very high bird loads. This causes high loads of pathogenic microorganisms, especially Campylobacter.

NB: The normal presence of birds on the reservoir is not necessarily a peak event; it can be considered as a baseline situation for this particular source water.

Historical data analysis

Historical data analysis is an essential first step for proper identification of local peak events. This analysis is necessary to define appropriate peak event sampling strategies adapted to the local context (type, propitious periods of the year, availability of real time data...).

Heavy rainfall remains the major cause of peak events and most CTSs focused on sampling this type of peak event. They are associated with high surface runoff and discharge of untreated wastewater. The difficulty lies in starting the sampling program as soon as possible after the beginning of an event. Some examples of sampling strategies are given hereafter and investigation on other potential peaks is given in 0.

Historical analysis of rain events

Unprotected surface water reservoir: CTS 8 (Australia)
The outcomes of CTS 8 historical data analysis are as follows:

- Event size and complexity are highly variable and hard to predict;
- Rainfall is not a good predictor of event occurrence by itself but it can be considered as a precursor when the hydraulic characteristics of the catchment are known;
- Events are recognisable by a rapid rise in river level;

- Response time to runoff is of the order of 4 to 6 hours after rainfall;
- The hydrographs evolve (rising limb > peak > falling limb) over a similar time frame which could be used to develop sampling protocols;
- Initial peak rise and fall lasts approximately 24 hours.

This led to the development of the following event based sampling strategy:

- Use of automated samplers to ensure capture of rising limb samples;
- Activation of samplers on warning of storm from weather forecasts and radar checks;
- Triggering of collection based on rate of change and magnitude of water level rise;
- Collection of excess samples to ensure 3 main stages of the hydrograph;
- Collection at increasing intervals to allow for the hydrograph skew;
- Where resources are limited, collection of first peak runoff as a priority.

Detailed pathogen data were collected for 3 small events (Figure 3).

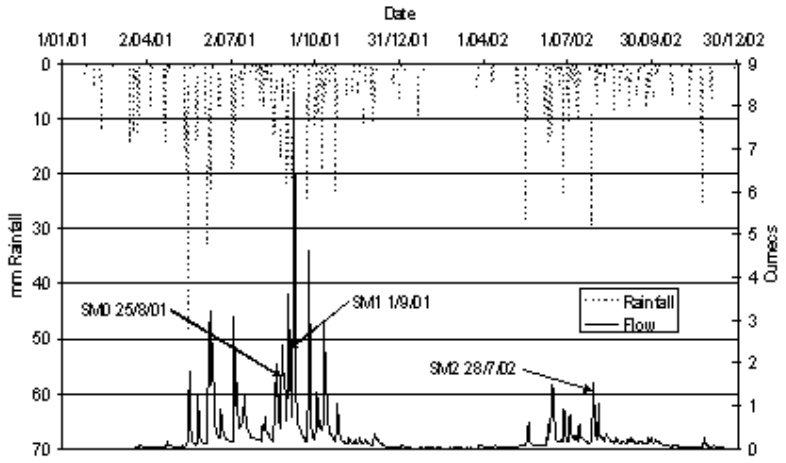

Figure 3: CTS 8 - Daily flow and rainfall in 2001-2002; 3 events (SM0-SM2)

Unprotected river water: CTS 3 (France)

Sampling was focused on rare, significant events. The following definition was set: a rainfall peak is a reasonably rare event and it should thus have "rare" turbidity and flow rise. Comparison of the time series for a 2-year period gives a linear relation between flow and turbidity: $r^2 = 0.73$ for daily data and $r^2 = 0.89$ for monthly averages. To avoid small variations due to minor runoff events, the sampling strategy is based on threshold values both for flow and turbidity. They are derived from the analysis of the sorted flow and turbidity curves (Figure 4).

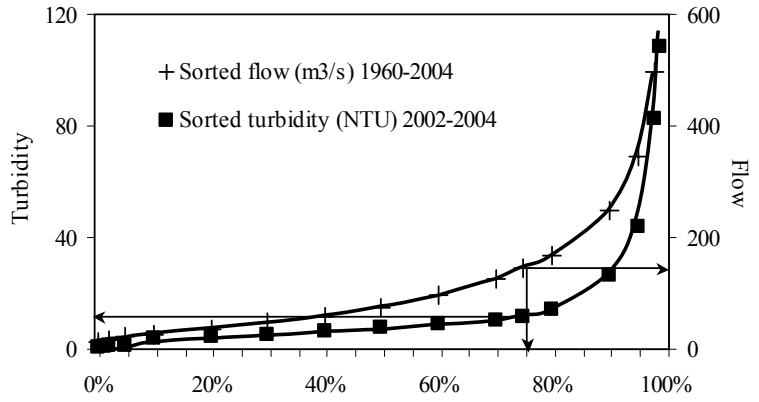

Figure 4: CTS 3 – Sorted flow and turbidity curves

The 75% occurrence is selected from the shape of the sorted turbidity curve. This yields thresholds of 150 m^3/s and 12 NTU. A peak starts with an increase of flow and turbidity. Unfortunately, only turbidity was measured in real time at the water treatment plant. Sampling was based on this parameter alone but peak relevance was confirmed later with flow data.

Based on historical data, rainfall peaks have the following characteristics for CTS 3:

- 9 peaks per year;
- Average duration of 8 days (minimum 2, maximum 14);
- Peak maximum reached after 3,3 days (minimum 1, maximum 9);
- Months when peaks are most frequent are November through January.

Unprotected groundwater: CTS 7 (Germany)

The CTS 7 water treatment plant uses bank filtration as treatment. About 65% of the source water is abstracted from the river after bank filtration and the rest comes from groundwater.

The events leading to high risk of contamination of the wells are fast rising water levels in the river up to very high water levels. There are several aspects to this. Firstly, fast rising water levels (3 meters or more within five days) after long dry periods cause much faster groundwater flow in the direction of the wells, thus reducing bank filtration efficiency for removing pathogens. Secondly, high river water levels lead to increased groundwater levels. Distance between the soil surface and the groundwater level becomes very small and removal of pathogens in the unsaturated zone is reduced. With falling water levels in the river, contaminated groundwater will reach the wells and lead to contamination when contact of groundwater with faecal contaminants is made possible by removal of the protecting soil layers, manure storage in garden plots, etc.

Figure 5 shows the changes in river water level within five days over a period of 50 years, information that can be used to determine criteria for peak events. Increase of water level of 3 meters or more within five days happened in 1.1% of time or 3.9 days per year on average. However, in the last decade, average is of 4.6 days per year.

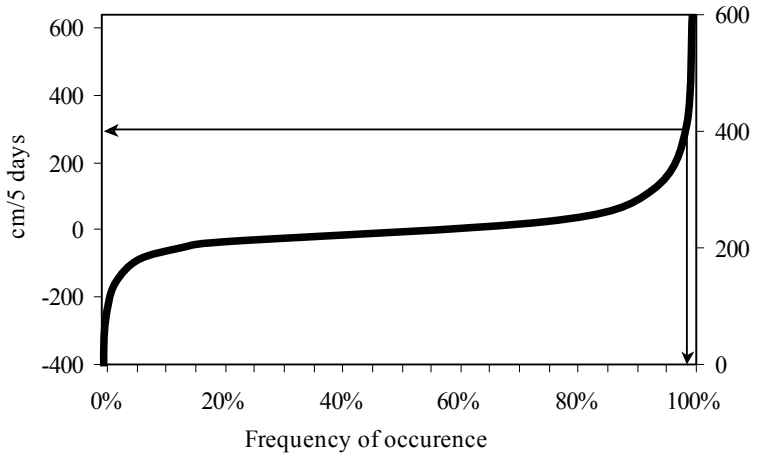

Figure 5: CTS 7 – Changes of river water level within 5 days (1953 –2003)

Other peak events

The following examples illustrate other types of peak events, analysed with historical data or water quality monitoring.

Historical data analysis: CTS 5 (Sweden)
Incidents leading to peak contaminations and source water intake closures are identified by microbiological source water monitoring at the intake, upstream monitoring stations and incident reports (telephone and fax). During the 2001-2005 period, 260 closures occurred. Incidents registered at the source water intake were most of the time related to high bacteria counts. In 2003 and 2004, discharge of untreated wastewater was the most common microbial incident and happened mainly in connection to heavy rain/snow. The high bacteria counts were also related to technical failures, such as the breakage of a high-pressure sewage pipe.

Water quality monitoring: CTS 9 (The Netherlands)
Water quality monitoring can demonstrate the occurrence of peak events and give information about their frequency, magnitude and duration.

In CTS 9, water is abstracted from a polder, flows through an open transport canal to a flocculation pretreatment and remains in an open lake reservoir for 89 days. Under conditions of high demand, water can also be abstracted from the nearby canal. Water from the lake is filtered and sent to the treatment plant or into an open buffer reservoir (closed in 2003 due to waterfowl contamination, as in CTS 2).

Multiyear E. coli and Coli 44 data show that peak contaminations do occur in the canal, polder, after flocculation and at the reservoir intake. A short, high peak occurred in winter 1995-1996 and a broader peak occurred in summer 1999. Several smaller peaks are visible in 1998. The peaks observed at the reservoir intake in 1998 and in summer 1999 coincide with peak E. coli concentrations from polder water. This suggests that peak contamination in the polder may travel to the water treatment plant intake much faster than the average residence time of the reservoir would suggest. None of these peaks corresponded to periods of heavy rainfall.

3. PATHOGEN MONITORING

The purpose of the pathogen monitoring programs is to evaluate the levels of pathogen contamination for specific sites, in baseline and peak conditions, so as to provide a strong, quantitative basis for risk assessment and QMRA. In the MicroRisk context, the monitoring programs were also valuable for assessing the levels of pathogen contamination in a representative set of EU catchment situations.

Design of monitoring program

The monitoring program includes the selected pathogens (*Cryptosporidium*, *Giardia*, Campylobacter, E. coli 0157:H7, Enterovirus and Norovirus) as well as faecal indicators (E. coli, Clostridia, Total Coliforms, Enterococci) and physico-chemical characteristics of the source water (turbidity, conductivity, temperature, pH). When possible, water flow is also evaluated in order to distinguish baseline from rain event contamination.

Standard methods of sampling, sample processing and analysis are recommended to ensure comparable results. Since source water pathogen concentrations may be very low, concentration/enrichment of large volumes of water, sometimes thousands of litres, may be necessary for detection. Consequently, it is important to collect proper sample volumes.

MicroRisk samples are collected at the intake of the water treatment plant. This implies that reservoir or bank filtration is not considered as a treatment but as a water source. The reader is of course free to reconsider this in his local context.

Baseline contamination

Baseline contamination assessment requires a full year of monthly samples. Samples should be collected:

- During dry weather conditions as rain events may lead to peak events;
- Each month so as to have an idea of the seasonal variations due to hydrological or hydrogeological conditions and/or to abstraction of groundwater due to increasing (seasonal) demand.

Peak contamination due to rain events

The objective is to sample at least two peak rain events during the year of sampling. Forecasting the rain event period is necessary in order to be ready for sampling and analysis. The proposed approach distinguishes surface water, protected groundwater and groundwater influenced by surface water.

- Surface water

Rain event indicators usually available in real time are turbidity and water flow (or water level). Turbidity and/or water flow increase indicate that runoff is ongoing. Historical data analysis is valuable for estimating which values of turbidity and water flow correspond to averages and which correspond to rain events. Rain event

thresholds can be fixed locally and used to set simple rules for starting the sampling period.

Example of a simple sampling strategy
Turbidity and/or flow are increasing. This indicates that water is running off. When flow reaches twice the yearly average flow, we can consider it is a rain event (Figure 6). Start sampling once a day for five days and continue if the peak flow is not reached (flow did not start to decrease).

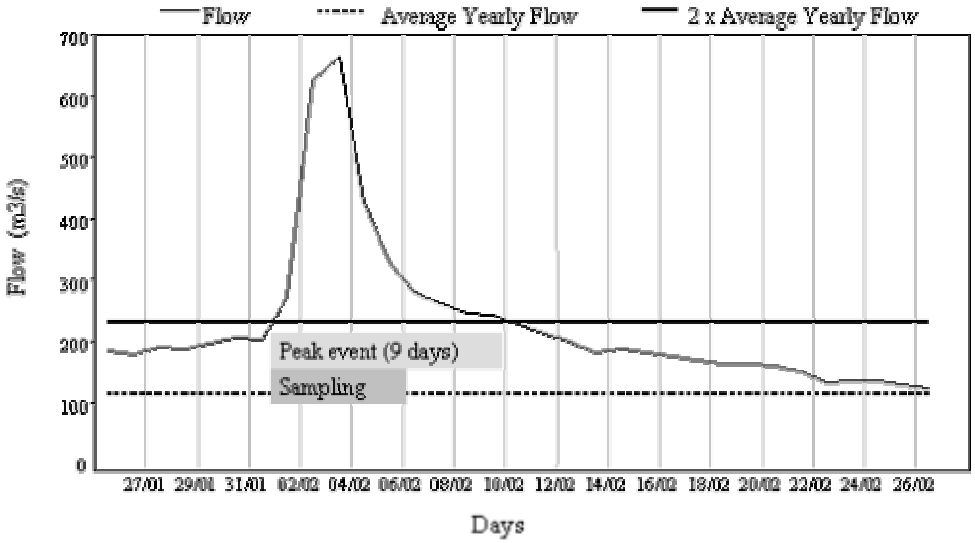

Figure 6: Example for water flow threshold in rain event conditions (CTS 3)

- Groundwater influenced by surface water

Such events are dependant on hydraulic conditions of surface water (see Figure 5) and abstraction rates. Historical data analysis is necessary to understand when influence from surface water is the highest (e.g. high abstraction rate, high surface water level). The same methodology as for surface water may be applied.

- Protected groundwater

By definition, contamination due to rain events should not occur, unless there are specific local conditions.

Detection methods

Assessment of the risk of infection from waterborne pathogens requires accurate determinations of microbial occurrence, concentration, viability, infectivity and human dose response data [LeChevallier *et al.,* 2003]. Existing methods have limitations in one or more of the criteria; for example, nucleic acid and antibody-based methods do not readily provide information about the concentration, viability and infectivity of the pathogen, whereas culture methods can be used only for the relatively small group of pathogens that are capable of growth in culture. Furthermore, the recovery rates of many culture methods may be very low, leading to a significant underestimate of pathogen numbers. It is important when selecting the

method of analysis to balance the advantages and disadvantages of each in terms of the required output. [Source: Pond *et al.*, 2004]

An important consideration for any project is that the methods of analysis are sufficiently detailed in their scope to ensure comparable results. Therefore, whenever possible, international standard methods of analysis should be used. Standard methods are published by several organisations (for example, ISO, CEN, APHA) and there are many supporting standards for the validation of methods and monitoring of laboratory performance. Laboratories should provide their Quality Assurance/Quality Control data on the method performance characteristics so it can be included in (statistical) interpretation of the results.

There are different ways to evaluate analytical performance and it is common for each laboratory to apply its own methodology. Recovery is evaluated from source water and/or ultra-pure water samples. It can be calculated for each sample or for a whole data set, using an average value. Controls were only available for *Cryptosporidium* and *Giardia* (Table 9).

Table 9: Example of Quality Assurance/Quality Control data for Cryptosporidium and Giardia

	Cryptosporidium		*Giardia*		Design of recovery experiments
	Mean recovery	σ	Mean recovery	σ	
CTS 2	12%	16%	6%	5.4%	Determination from 3 source water samples
CTS 3 & 4	30-40%		30-40%		Recovery is tested 6 times a year on spiked ultra-pure water samples
CTS 5	12%	7%	8%	7%	Determination from 4 source water samples
CTS 7	19.2%	5.7%	14.9%	4.5%	500 L water spiked with oocysts/cysts in concentrations of 10^2 to 10^4 / 500 L
CTS 8	50%	13%	47%	17%	Determination for each source water sample
CTS 10	26%	21%	30%	29%	Results are issued from spiked source water samples + spiked ultra-pure water samples
CTS 11	12%	3.1%	10.7%	7.3%	500 L water spiked with oocysts/cysts in concentrations of 10^2 to 10^4 / 500 L; 5 mL *Cryptosporidium*/*Giardia*-free sediment were added to simulate source water

Some laboratories encountered detection problems with standard methods of analysis:

Example: CTS 10 (France)
High values of turbidity were found to interfere with *Cryptosporidium* and *Giardia* analysis. No oocysts/cysts could be recovered from spiked samples for turbidity values higher than 8 NTU in the case of *Cryptosporidium*, or 3.5 NTU in the case of *Giardia*.

Laboratory performance in analysing pathogens is still highly variable and the quality of data produced by a laboratory cannot be taken for granted. Pathogen concentrations

may otherwise be greatly underestimated. Quality control data and details of the confirmation methods should be provided along with the count results [Roser *et al*., 2002.]

Lacks in data

Despite all precautions, lacks in data always seem to emerge once datasets are acquired. The reader's attention is brought to the following possible deficiencies:

- Quality Assurance/Quality Control data

Laboratories do not easily provide relevant quality data or full methodology (number of samples, number of spikes). The MicroRisk project could only gather very heterogeneous recovery data for *Cryptosporidium* and *Giardia* (Table 9). Campylobacter, E. coli 0157:H7, Enterovirus and Norovirus quality data was not available.

- Turbidity and water flow

These two parameters represent valuable characteristics of the sampling conditions. They are particularly important for a proper assessment of peak events. Daily water flow measurements are interesting for situating samples in the course of a hydrological event.

- Precipitation data

Heavy rainfall is generally the most common peak event. Precipitation data may be useful to quantify the significance of such events.

4. DATA ANALYSIS

The MicroRisk partners monitored source water quality for nine European water sources and one Australian. The monitoring programs provide information on source water baseline and peak contamination in pathogens (*Cryptosporidium*, *Giardia*, Campylobacter, E. coli O157, Enterovirus and Norovirus) and faecal indicators. The objectives are to:

- Draw a picture of source water pathogen contamination in European countries,
- Assess significance of peak event contamination,
- Analyse correlation between commonly monitored faecal indicators and/or turbidity and pathogens.

NB: Laboratory determination of QA/QC[1] data not being consistent for all CTSs and all parameters, raw results are presented directly.

Full results per CTS

Baseline contamination results are given in Table 10 and in Table 11 for rain events. Total number of samples, number of positive samples and average, minimum and

[1] Quality Assurance/Quality Control

maximum calculated on the positive samples are given. If results come as a range of values, for example 10-100, they are given as 10-100(3). This means that the 10-100 range was encountered in 3 samples.

Baseline contamination

Table 10: Baseline contamination in the CTSs

CTS 1 - UK	***River***			***Catchment: 46,830***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	11	2	0.35	0.3	0.4
Giardia	n/L	11	0	-	-	-
Campylobacter	CFU/L	11	0	-	-	-
E. coli O157:H7	CFU/L	11	0	-	-	-
Enterovirus	PFU/L	11	4	1.55	0.4	3.4
E. coli	MPN/L	11	11	14,191	5,300	22,000
Clostridia	CFU/L	11	11	2,871	80	8,000
Total Coliforms	MPN/L	11	11	63,927	20,500	112,000
Enterococci	CFU/L	11	10	1,710	100	6,000

CTS 2 - The Netherlands	***River***			***Catchment: 198,735***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	11	5	0.093	0.05	0.2
Giardia	n/L	11	3	0.015	0.003	0.023
Campylobacter	MPN/L	69	57	1,703	0.4	15,000
Enterovirus	PFU/L	3	2	0.015	0.005	0.024

CTS 3 - France	***River***			***Catchment: 10,050***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	11	5	0.09	0.05	0.2
Giardia	n/L	11	10	1.16	0.05	4.7
Campylobacter	n/L	11	0	-	-	-
E. coli O157:H7	CFU/L	11	10	10-100 (9)	>1000 (1)	-
Enterovirus	PFU/L	11	0	-	-	-
E. coli	n/L	11	10	1,900	700	7,000
Clostridia	n/L	11	11	2,909	500	4,350
Total Coliforms	n/L	10	10	5,692	750	20,000
Enterococci	n/L	10	10	617	50	2,800

CTS 4 - France	***River***			***Catchment: 522***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	12	3	0.12	0.05	0.2
Giardia	n/L	12	11	0.36	0.05	0.75
Campylobacter	n/L	11	0	-	-	-
E. coli O157:H7	CFU/L	12	8	10-100 (3)	100-1,000 (2)	>1,000 (3)
Enterovirus	FPU/L	12	0	-	-	-
E. coli	n/L	12	12	8,551	10	80,000

Clostridia	n/L	12	12	3,392	800	17,500
Total Coliforms	n/L	12	12	34,660	220	270,000
Enterococci	n/L	12	11	1,503	30	6,600

CTS 5 - Sweden	***River & lake***			***Catchment:***	***50,180***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium[2]	n/L	13	3	0.09	0.08	0.1
Giardia[1]	n/L	12	2	0.09	0.016	0.16
Campylobacter	n/L	13	1	10	-	-
E. coli O157:H7	n/L	13	0	-	-	-
Enterovirus[1]	n/L	12	0	-	-	-
Norovirus[1]	n/L	12	0	-	-	-
E. coli	n/L	14	14	927	310	2,200
Clostridia	n/L	15	15	157	60	350
Total Coliforms	n/L	14	14	22,650	2,600	82,000
Enterococci	n/L	15	15	519	70	1,800

CTS 7- Germany	***Groundwater and river bank filtration***			***Catchment:***	***145***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	11	0	-	-	-
Giardia	n/L	11	0	-	-	-
E. coli	MPN/L	11	1	10	-	-
Clostridia	CFU/L	11	0	-	-	-
Total Coliforms	MPN/L	11	6	29	10	42
Enterococci	CFU/L	11	0	-	-	-

CTS 8 - Australia	***Reservoir***			***Catchment:***	***140***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	51	2	0.1	0.1	0.1
Giardia	n/L	51	1	0.1	-	-
E. coli	n/L	78	55	125	10	1,200
Total Coliforms	n/L	124	118	2,620	10	24,000

CTS 9 - The Netherlands	***Reservoir***			***Catchment:***	***198,735***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	25	25	0.33	0.01	4.6
Giardia	n/L	25	25	2.94	0.01	41.3
Campylobacter	n/L	37	32	72.3	0.4	500
Enterovirus	PFU/L	12	0	-	-	-

[2] On concentrate

CTS 10 - France	***Reservoir***			***Catchment:***	***30***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	9	5	0.54	0.1	1
Giardia	n/L	9	6	0.73	0.1	3
Campylobacter	MPN/L	9	2	10-100 (2)	-	-
E. coli O157:H7	MPN/L	9	3	10-100 (3)	-	-
Enterovirus	PFU/L	9	0	-	-	-
E. coli	MPN/L	9	9	340	60	1,080
Total Coliforms	MPN/L	9	9	2,200	1,180	4,350
Enterococci	MPN/L	9	8	246	10	1,300

CTS 11 - Germany	***Reservoir***			***Catchment:***	***300***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	11	11	0.039	0.019	0.06
Giardia	n/L	11	1	0.004	-	-
Campylobacter	CFU/L	9	0	-	-	-
E. coli	MPN/L	11	8	25.6	10	53
Clostridia	CFU/L	11	5	48	20	80
Total Coliforms	MPN/L	11	11	124	20	504
Enterococci	CFU/L	11	4	12.5	10	20

CTS 12 - France	***Groundwater***			***Catchment:***	***100***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	10	0	-	-	-
Giardia	n/L	10	0	-	-	-
Campylobacter	MPN/L	10	0	-	-	-
E. coli O157:H7	MPN/L	10	0	-	-	-
Enterovirus	PFU/L	10	0	-	-	-
E. coli	MPN/mL	10	0	-	-	-
Total Coliforms	MPN/mL	10	1	10	-	-
Enterococci	MPN/mL	10	0	-	-	-

Rain event contamination

Rain events should be the object of a second sampling program. However, some may be sampled inadvertently during the baseline contamination program. Turbidity and/or water flow should always be checked to determine the sampling conditions.

Figure 7 shows an example taken from the CTS 3 baseline contamination program. The January sample was collected during a rain event: water flow is 425 m^3/s (2004 average is 98 m^3/s) and turbidity is 25 NTU (2004 average is 8.5 NTU). Such samples should be added to rain event results.

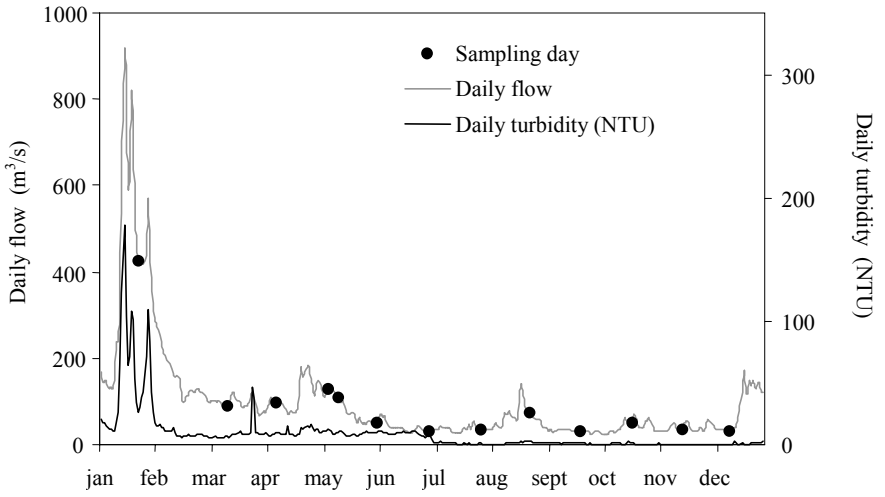

Figure 7: Rain event sample during the 2004 baseline contamination program (CTS 3)

Other samples transferred from the baseline contamination program are

- CTS 1: turbidity of the January sample is 36 NTU (year 2004 average is 2 NTU)
- CTS 11: turbidity of the February sample is 5 NTU (year 2004 average is 0.2 NTU)

Table 11: Rainfall contamination in the CTSs

CTS 1 - UK	***River***		***Catchment:***	***46,830***	***km²***
Parameter	Unit	Samples	Positive samples	Concentration	
Cryptosporidium	n/L	1	1	0.4	
Giardia	n/L	1	0	-	
Campylobacter	CFU/L	1	0	-	
E. coli O157:H7	CFU/L	1	0	-	
Enterovirus	PFU/L	1	0	-	
E. coli	MPN/L	1	1	111,000	
Clostridia	CFU/L	1	1	>10,000	
Total Coliforms	MPN/L	1	1	517,000	
Enterococci	CFU/L	1	1	35,000	

CTS 3 - France	***River***			***Catchment:***	***10,050***	***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	2	1	0.5	-	-
Giardia	n/L	2	2	3.05	1.6	4.5
E. coli O157:H7	CFU/L	2	2	10-100 (1)	>1,000 (1)	-
Enterovirus	PFU/L	1	0	-	-	-
E. coli	n/L	1	1	300	-	-
Clostridia	n/L	2	2	5,750	5,500	6,000
Total Coliforms	n/L	2	2	66,000	22,000	110,000
Enterococci	n/L	2	2	4,700	300	9,100

CTS 5 - Sweden	***River & lake***	***Catchment:***	***50,180***	***km²***

Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium[3]	n/L	10	5	0.16	0.1	0.2
Giardia[1]	n/L	10	4	0.18	0.1	0.3
Campylobacter	n/L	10	0	-	-	-
E. coli O157:H7	n/L	10	0	-	-	-
Enterovirus[1]	n/L	7	3	330	250	370
Norovirus[1]	n/L	7	3	148	111	167
E. coli	n/L	13	13	2,635	20	8,300
Clostridia	n/L	12	12	280	80	500
Total Coliforms	n/L	13	13	34,131	3,200	130,000
Enterococci	n/L	13	13	1,318	60	4,300

CTS 7 - Germany	***Groundwater and river bank filtration***			***Catchment: 145***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	10	0	-	-	-
Giardia	n/L	10	0	-	-	-
E. coli	MPN/L	10	7	34.7	10	87
Clostridia	CFU/L	9	0	-	-	-
Total Coliforms	MPN/L	10	10	126	10	406
Enterococci	CFU/L	10	3	10	10	10

CTS 10 - France	***Reservoir***			***Catchment: 30***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	4	1	1.9	-	-
Giardia	n/L	4	3	0.37	0.2	0.6
Campylobacter	MPN/L	4	1	10-100	-	-
E. coli O157:H7	MPN/L	4	4	10-100 (2)	>1,000 (2)	-
Enterovirus	PFU/L	2	0	-	-	-
E. coli	MPN/L	4	4	19,160	550	54,800
Total Coliforms	MPN/L	4	4	83,838	4,790	242,000
Enterococci	MPN/L	4	4	5,028	100	15,800

CTS 11 - Germany	***Reservoir***			***Catchment: 300***		***km²***
Parameter	Unit	Samples	Positive samples	Average	Min	Max
Cryptosporidium	n/L	10	10	0.053	0.031	0.132
Giardia	n/L	10	2	0.006	0.004	0.008
E. coli	MPN/L	9	9	134	42	254
Clostridia	CFU/L	9	9	113	60	210
Total Coliforms	MPN/L	9	9	357	178	504
Enterococci	CFU/L	9	7	28.6	20	60

[3] On concentrate

Results per pathogen

Protozoa

Table 12 and Table 0.13 show average, minimum and maximum concentrations for *Cryptosporidium* and *Giardia*. These two pathogens are frequently detected at relatively low concentrations. MicroRisk levels vary around:

- *Cryptosporidium*: 0.01-0.5 n/L and up to 4.6 n/L (literature review 0.006-250 n/L),
- *Giardia*: 0.01-1 n/L and over 40 n/L in one case (literature review 0.2-100 n/L).

CTS 1, CTS 9 and CTS 10 have the highest concentrations of *Cryptosporidium*. CTS 3, CTS 9 and CTS 10 have the highest concentrations of *Giardia*. Results are variable and concentrations are not clearly higher during runoff events. However, one must keep in mind that rain events were scarce and that there are many more baseline concentrations available than rain event concentrations. The rain event population may not be fully representative of such concentrations.

Table 12: Baseline and rainfall contamination in Cryptosporidium

CTS	Event	Unit	Samples	Positive samples	Average	Min	Max	Source
CTS 1	Baseline	n/L	11	2	0.35	0.3	0.4	River
CTS 1	Rain	n/L	1	1	0.4	-	-	River
CTS 2	Baseline	n/L	11	3	0.005	0.001	0.012	River
CTS 3	Baseline	n/L	11	5	0.09	0.05	0.2	River
CTS 3	Rain	n/L	2	1	0.5	-	-	River
CTS 4	Baseline	n/L	12	3	0.12	0.05	0.2	River
CTS 5[4]	Baseline	n/L	13	3	0.09	0.08	0.1	River & lake
CTS 5[1]	Rain	n/L	10	5	0.16	0.1	0.2	River & lake
CTS 7	Baseline	n/L	11	0	-	-	-	Groundwater & river bank filtration
CTS 7	Rain	n/L	10	0	-	-	-	Groundwater & river bank filtration
CTS 8	Baseline	n/L	51	2	0.1	0.1	0.1	Reservoir
CTS 9	Baseline	n/L	25	25	0.33	0.01	4.6	Reservoir
CTS 10	Baseline	n/L	9	5	0.54	0.1	1	Reservoir
CTS 10	Rain	n/L	4	1	1.9	-	-	Reservoir
CTS 11	Baseline	n/L	11	11	0.039	0.019	0.06	Reservoir
CTS 11	Rain	n/L	10	10	0.053	0.031	0.132	Reservoir
CTS 12	Baseline	n/L	10	0	-	-	-	Groundwater

[4] On concentrate

Table 0.13: Baseline and rainfall contamination in Giardia

CTS	Event	Unit	Samples	Positive samples	Average	Min	Max	Source
CTS 1	Baseline	n/L	11	0	-	-	-	River
CTS 1	Rain	n/L	1	0	-	-	-	River
CTS 2	Baseline	n/L	11	3	0.02	0.003	0.023	River
CTS 3	Baseline	n/L	11	10	1.16	0.05	4.7	River
CTS 3	Rain	n/L	2	2	3.05	1.6	4.5	River
CTS 4	Baseline	n/L	12	11	0.36	0.05	0.75	River
CTS 5[5]	Baseline	n/L	12	2	0.09	0.016	0.16	River & lake
CTS 5[1]	Rain	n/L	10	4	0.18	0.1	0.3	River & lake
CTS 7	Baseline	n/L	11	0	-	-	-	Groundwater & river bank filtration
CTS 7	Rain	n/L	10	0	-	-	-	Groundwater & river bank filtration
CTS 8	Baseline	n/L	51	1	0.1	-	-	Reservoir
CTS 9	Baseline	n/L	25	25	2.94	0.01	41.3	Reservoir
CTS 10	Baseline	n/L	9	6	0.73	0.1	3	Reservoir
CTS 10	Rain	n/L	4	3	0.37	0.2	0.6	Reservoir
CTS 11	Baseline	n/L	11	1	0.004	-	-	Reservoir
CTS 11	Rain	n/L	10	2	0.006	0.004	0.008	Reservoir
CTS 12	Baseline	n/L	10	0	-	-	-	Groundwater

Bacteria

Campylobacter is not always detected in source water. It was found in 4 out of 9 CTSs (Table 14). The sample volumes may have been too small. Concentrations sometimes do reach high levels (15,000 MPN/L in CTS 2). Rain event concentrations are not necessarily higher. Literature review referenced 1-109,000 MPN/L.

E. coli 0157:H7 is more commonly encountered but usually at low concentrations. However, CTS 3, CTS 4 and CTS 10 show higher concentrations in some cases and particularly during rain events (
Table ***15***).

Table 14: Baseline and rainfall contamination in Campylobacter

CTS	Event	Unit	Samples	Positive samples	Average	Min	Max	Source
CTS 1	Baseline	CFU/L	11	0	-	-	-	River
CTS 1	Rain	CFU/L	1	0	-	-	-	River
CTS 11	Baseline	CFU/L	9	0	-	-	-	Reservoir
CTS 2	Baseline	MPN/L	69	57	1,703	0.4	15,000	River
CTS 10	Baseline	MPN/L	9	2	10-100 (2)	-	-	Reservoir
CTS 10	Rain	MPN/L	4	1	10-100	-	-	Reservoir
CTS 12	Baseline	MPN/L	10	0	-	-	-	Groundwater
CTS 3	Baseline	n/L	11	0	-	-	-	River
CTS 4	Baseline	n/L	11	0	-	-	-	River
CTS 5	Baseline	n/L	13	1	10	-	-	River & lake
CTS 5	Rain	n/L	10	0	-	-	-	River & lake
CTS 9	Baseline	n/L	37	32	72.3	0.4	500	Reservoir

[5] On concentrate

Table 15: Baseline and rainfall contamination in E. coli 0157:H7

CTS	Event	Unit	Samples	Positive samples	Average	Min	Max	Source
CTS 1	Baseline	CFU/L	11	0	-	-	-	River
CTS 1	Rain	CFU/L	1	0	-	-	-	River
CTS 3	Baseline	CFU/L	11	10	10-100 (9)	>1000 (1)	-	River
CTS 3	Rain	CFU/L	2	2	10-100 (1)	>1,000 (1)	-	River
CTS 4	Baseline	CFU/L	12	8	10-100 (3)	100-1,000 (2)	>1,000 (3)	River
CTS 10	Baseline	MPN/L	9	3	10-100 (3)	-	-	Reservoir
CTS 10	Rain	MPN/L	4	4	10-100 (2)	>1,000 (2)	-	Reservoir
CTS 12	Baseline	MPN/L	10	0	-	-	-	Groundwater
CTS 5	Baseline	n/L	13	0	-	-	-	River & lake
CTS 5	Rain	n/L	10	0	-	-	-	River & lake

Virus

Enteroviruses are rarely detected (Table 16). In CTS 5, concentrations go up as high as 370 n/L during rain events while they are undetected in baseline conditions. Literature review referenced 0.003-29 n/L.

Noroviruses were investigated in CTS 5 only. Once again, concentrations are clearly higher during rain events (Table.17).

Table 16: Baseline and rainfall contamination in Enterovirus

CTS	Event	Unit	Samples	Positive samples	Average	Min	Max	Source
CTS 5[6]	Baseline	n/L	12	0	-	-	-	River & lake
CTS 5[1]	Rain	n/L	7	3	330	250	370	River & lake
CTS 1	Baseline	PFU/L	11	4	1.55	0.4	3.4	River
CTS 1	Rain	PFU/L	1	0	-	-	-	River
CTS 2	Baseline	PFU/L	3	2	0.015	0.005	0.024	River
CTS 3	Baseline	PFU/L	11	0	-	-	-	River
CTS 3	Rain	PFU/L	1	0	-	-	-	River
CTS 4	Baseline	PFU/L	12	0	-	-	-	River
CTS 9	Baseline	PFU/L	12	0	-	-	-	Reservoir
CTS 10	Baseline	PFU/L	9	0	-	-	-	Reservoir
CTS 10	Rain	PFU/L	2	0	-	-	-	Reservoir
CTS 12	Baseline	PFU/L	10	0	-	-	-	Groundwater

Table.17: Baseline and rainfall contamination in Norovirus

CTS	Event	Unit	Samples	Positive samples	Average	Min	Max	Source
CTS 5[1]	Baseline	n/L	12	0	-	-	-	River & lake
CTS 5[1]	Rain	n/L	7	3	148	111	167	River & lake

[6] On concentrate

Source water quality

Levels of contamination

Levels of contamination for baseline and rain events are given in Table 18. They represent surface water quality (river and reservoir). Groundwater[7] concentrations are usually very low or below detection limits and are not included.

Table 18: Summary of faecal indicators and pathogen concentrations in surface water

	Baseline contamination	Rain event contamination
Faecal indicators		
E. coli	10^2-10^4 MPN/L	10^3-10^4 MPN/L and up to 50,000 MPN/L
Clostridia	≈ 3000 n/L and up to 17,500 n/L	5,000-6,000 n/L
Enterococci	10^2-10^3 n/L	> 10^3 n/L
Total Coliforms	10^3-10^5 MPN/L	30,000-130,000 MPN/L
Pathogens		
Cryptosporidium	0.05-0.5 n/L and up to 4.6 n/L	Concentrations not clearly higher
Giardia	0.01-1 n/L and over 40 n/L in one case	Concentrations not clearly higher
Campylobacter	0-100 MPN/L but up to 15,000 in one case	Concentrations not clearly higher
E. coli 0157:H7	10-100 CFU/L and up to >1,000 CFU/L	Concentrations not clearly higher
Enterovirus	Rarely detected	≈ 300 n/L in one CTS
Norovirus	Not detected (one CTS tested)	150 n/L in one CTS

These results do not account for the recovery of analytical methods. This means that pathogen contamination may be underestimated. Corrections are discussed and developed in Chapter 8.

Rain events undoubtedly yield higher faecal indicators concentrations. However, results are not as clear for pathogens. Three reasons are considered:

- Scarcity of hydrological events for most CTSs; there were many more baseline concentrations available than rain event concentrations. The rain event population may not be fully representative.
- Higher turbidity during rain events; as seen in Paragraph 0, this may affect the performance of analytical methods and concentrations may be underestimated.
- Dilution effect of rain events on concentrations but not on pollution loads.

NB: Although the MicroRisk dataset does not provide clear evidence of higher pathogen concentrations and loads during peak events, this has been largely shown in the international literature [Stelzer and Jacob, 1991; Atherholt *et al.*, 1998; O'Connor, 2002; Signor *et al.*, 2005].

Reservoir[8] water quality is often better than river[9] water quality. Concentrations are in the low range of Table 18. For example, in the case of E. coli, reservoir water

[7] CTS 7 & CTS 12

concentrations vary around 100 MPN/L in baseline conditions. But it is not always the case. *Giardia* was an exception with highest concentrations encountered in a reservoir during baseline conditions. In rain event conditions, reservoir and river water microbial quality are generally of the same order.

Improvement of water quality

River bank filtration, selective intake, dilution and storage are ways to improve river water quality. The MicroRisk project confirmed the performance of these methods.

River bank filtration: CTS 7 (Germany)
CTS 7 uses source water from river bank filtrate (65%) and groundwater (35%). Filtration, sorption and biological processes in the river banks plus dilution with groundwater greatly improve water quality, in baseline or rain event conditions. Concentrations before and after bank filtration are presented Table 19 in baseline conditions.

Table 19: Microbial concentrations before and after bank filtration in baseline conditions (CTS 7)

Parameter	Unit	River average	River max	Groundwater average	Groundwater max
Cryptosporidium	n/L	0.051	0.112	<0.001	<0.001
Giardia	n/L	0.014	0.024	<0.001	<0.001
E. coli	MPN/L	7,300	22,200	<10	10
Clostridia	CFU/L	645	1300	<10	<10
Total Coliforms	MPN/L	26,100	78,200	29	42
Enterococci	CFU/L	1,700	4800	<10	<10

Reservoir: CTS 8 (Australia)
Source water is pumped from a large surface reservoir. Incoming river quality is greatly improved by dilution, particle settling and physico-chemical and biological processes in the reservoir (
Table. ***20***).

Table. 20: Microbial concentrations before and in a water reservoir (CTS 8)

Parameter	Unit	River average	River max	Reservoir average	Reservoir max
Cryptosporidium	n/L	0.78	5.4	<0.1	0.1
Giardia	n/L	0.22	1.7	<0.1	0.1
E. coli	MPN/L	107,000	560,000	125	1,200

Data variability

Current QMRA techniques are reliant on the understanding of the overall tendencies and variations in microbiological quality of the source water [Teunis & Havelaar 1999]. Possible variations are due to the specificities of the catchment, seasons, peak events etc. If a parameter, such as pathogen concentration, is known to be a variable and not a constant, it can be quantified in different ways.

[8] CTSs 8, 9, 10 & 11
[9] CTSs 1, 2, 3, 4 & 5

A first approach is presented here with the triangular distribution. The triangular distribution is defined by a minimum, average and maximum value. This is of course a starting point because source water quality cannot be expected to be triangularly distributed, but it is a useful representation of a parameter's variation. It also can be used to assess the sensitivity of the experimental data.

More generally, variability in QMRA is accounted for by describing parameters using a Probability Density Function (PDF). When described by a PDF, the variable may take a range of values, each with a known probability of occurrence. Monte Carlo simulations are then used for risk assessment (see Chapter 7).

Correlation analysis

The following figures illustrate the presence or absence of correlations in the MicroRisk dataset.

Figure 8 represents Total Coliforms, Clostridia, Enterococci concentrations and turbidity as a function of E. coli concentrations for the complete dataset. It shows that faecal indicators are generally well correlated together and with turbidity to a lesser extent.

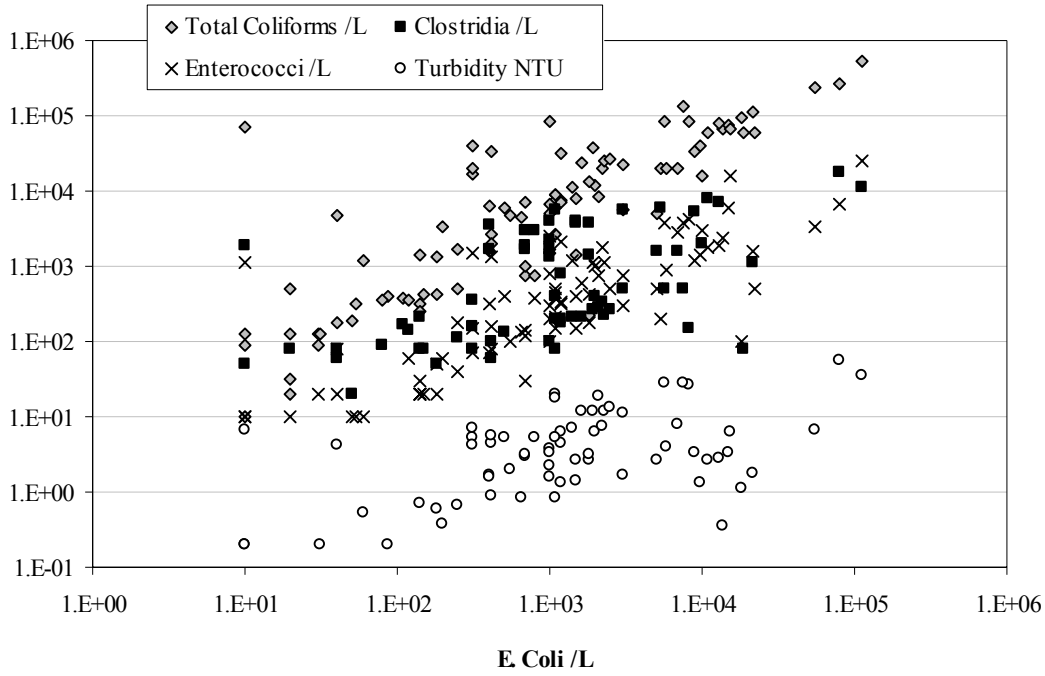

Figure 8: Faecal indicators concentrations and turbidity versus E. coli concentrations for all CTSs

When it comes to faecal indicator and pathogen correlations, results are not as clear. For example, E. coli and Total Coliforms concentrations vary together in CTS 11 but *Cryptosporidium* concentrations remain in the same range of values (2-7 n/100 L), independently of faecal indicators (Figure 9). Samples with E. coli concentrations

over 10 MPNL/100 mL were all collected during rain events. They correlate with Total Coliforms concentrations but not to *Cryptosporidium* concentrations.

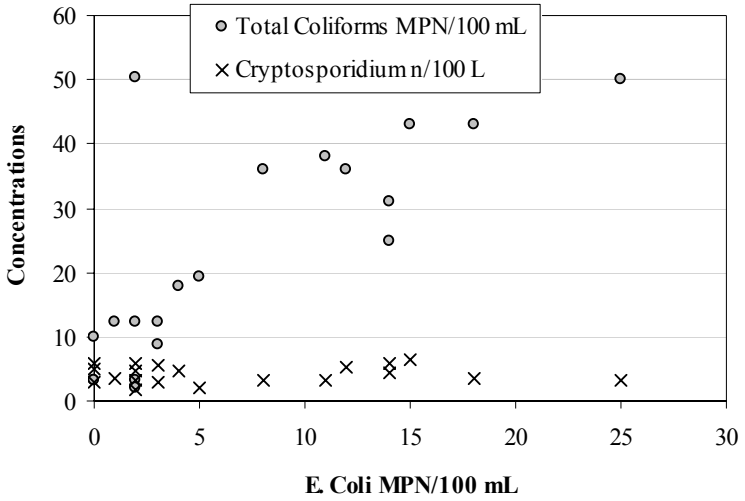

Figure 9: Total Coliforms and *Cryptosporidium* versus E. coli concentrations in CTS 11 (Germany)

Cryptosporidium and *Giardia* concentrations are respectively represented versus E. coli concentrations in the case of CTS 5 (Figure 10) and CTS 10 (Figure 11). These figures show that protozoa and E. coli concentrations are not correlated in these two cases. Rain event concentrations are not necessarily higher than baseline concentrations although high E. coli concentrations during the 24-25/10/2004 rain event are associated with higher *Cryptosporidium* concentrations.

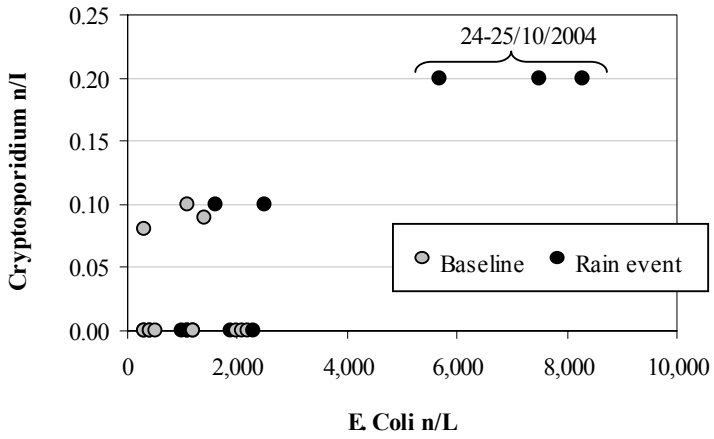

Figure 10: *Cryptosporidium* versus E. coli concentrations in CTS 5 (Sweden)

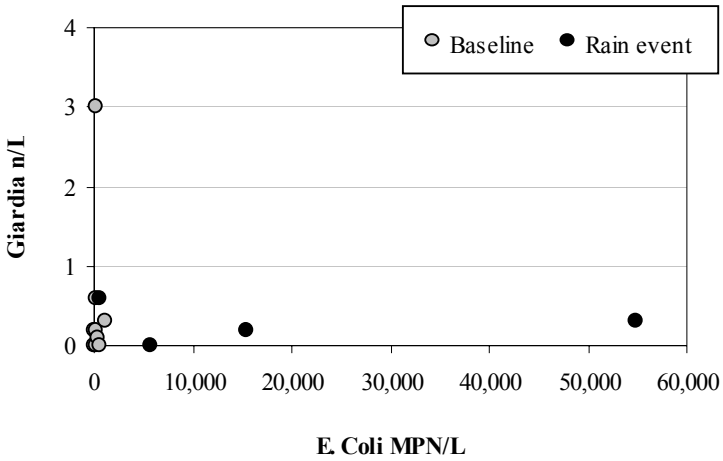

Figure 11: *Giardia* versus E. coli concentrations in CTS 10 (France)

Pathogen correlation is different in each case and generalisation is impossible. There is no recurring evidence of pathogens correlated together, correlated with faecal indicators and/or correlated with turbidity. Each CTS has its own behaviour, thus showing that source water quality and links between microbial parameters are site specific.

NB: The link between turbidity and analytical performance was previously discussed (see CTS 10 example in 0.). A logarithmic relationship between turbidity and recovery of protozoa analytical methods was established in CTS 10. This shows that data adjustment may be necessary to improve correlation investigation.

5. CONCLUSION

The MicroRisk project focused on a selection of pathogens of high risk to human health and of concern in source water used for drinking water supply:

- Protozoa: Cryptosporidium and Giardia
- Bacteria: Campylobacter and E. coli 0157:H7
- Viruses: Enterovirus and Norovirus

Source waters are contaminated to varying degrees with these pathogens. Their presence and persistence in water is due to different factors such as survival, transport and control of inputs, depending on the type of surface water and/or aquifer characteristics. Periods of rainfall usually contribute to higher source contamination. The natural variability of potentially pathogenic microorganisms in the environment from anthropogenic, natural, and livestock sources is large and difficult to quantify. It is complex to rank the various sources and transport routes in terms of relative importance to human disease. Risks depend much on the specific case and need to be considered in the local context. This is of course a considerable challenge for water and/or health managers although more and more water utilities do have pathogen data available.

As part of the MicroRisk project, a framework was set to review possible sources of pathogens in catchment areas and to assess of baseline and peak contamination in source waters. It includes catchment survey and monitoring programs in baseline and peak hydrological conditions. This methodology was applied to nine European and one Australian source waters.

Main outcomes

Levels of pathogen contamination

The following table gives the levels of pathogen contamination encountered in the MicroRisk surface source waters. The results are consistent with those found in the literature (Table 2); in addition, they differentiate baseline and rain event contamination.

	Baseline contamination	Rain event contamination
Cryptosporidium	0.05-0.5 n/L and up to 4.6 n/L	Concentrations not clearly higher
Giardia	0.01-1 n/L and over 40 n/L in one case	Concentrations not clearly higher
Campylobacter	0-100 MPN/L but up to 15,000 in one case	Concentrations not clearly higher
E. coli 0157:H7	10-100 CFU/L and up to >1,000 CFU/L	Concentrations not clearly higher
Enterovirus	Rarely detected	≈ 300 n/L in one CTS
Norovirus	Not detected (one CTS tested)	150 n/L in one CTS

Surface reservoir water quality is often better than river water quality. Reservoir concentrations are usually in the low range of the above values in baseline conditions. Groundwater concentrations are either very low and/or below detection limits.

Significance of rain events

Hydrological peak events yield higher faecal indicators concentrations in surface waters. Groundwater seems unaffected. Results are not as clear for pathogens. Three reasons are suggested: non-representative rain event population, performance of analytical methods hindered by high turbidity or the dilution effect of a hydrological peak event. Anyhow, even if concentrations do not appear greater in rain event conditions, pollution flows certainly are.

On the question of faecal indicators and pathogens correlation

In most cases, faecal indicators are well correlated among them and with turbidity. However, pathogen correlation is different. There is no recurring evidence of pathogen correlated together, correlated with faecal indicators and/or turbidity. Faecal indicators and turbidity are generally poor surrogates for pathogens presence and concentrations, as reported in the international literature (see 0). Links between microbial parameters appear to be site specific. All this shows that for a proper assessment of pathogen contamination, baseline and peak event concentrations need to be evaluated in a local context with a specific monitoring program.

Analytical methods

At present, pathogen detection methods are not optimal. There are a number of limitations and sources of uncertainty due to the sensitivity of analytical techniques, particularly for viruses and protozoa, and to the lack of knowledge about the viability

and human infectivity of *Cryptosporidium* oocysts, *Giardia* cysts and viruses. Recovery rates of analytical methods may be very low, as seen in the case of *Cryptosporidium* and *Giardia*, and are not always available. Conditions of high turbidity seem to interfere with detection, making it more difficult to assess peak event concentrations. All this may lead to significant underestimation of pathogen loads. Concentrations should be corrected in regard to recovery rates and turbidity/analytical performance relationships should be investigated.

Recommendations for QMRA

Determination of the occurrence of pathogens in source water should be based on:

Catchment survey

The purpose of this step is to develop a broad overview and basic understanding of the catchment, i.e. source water vulnerability, importance and location of pathogen sources, peak events leading to high contamination risks (type, intensity, frequency, duration).

Levels of contamination

Pathogen monitoring of source water should be carried out using the information of the catchment survey. It is particularly important to assess peak event contamination as it usually yields the highest risks. Specific sampling strategies should be designed for baseline and peak event contamination.

Quality of the data

The pathogen detection methods are ideally targeted to viable and infectious pathogens. The performance of the detection methods can have implications for the applicability of the data in risk assessment. These should be identified and evaluated in the early stages of the process.

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