Influences of sewage treatment plant effluents

on the occurrence of emerging waterborne pathogens in surface water

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July 2007

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Rijnwaterbedrijven

Table of Contents

1

Abstract

The river Rhine is an important source for drinking water production but at the same time it is used for the discharge of waste. Industrial discharge is mainly chemically polluted. However, domestic sewage contains important microbiological contaminants, as do discharges from hospitals and slaughterhouses. The goal of this study is to estimate the influence of treated and untreated sewage on the water quality of surface waters used for the production of drinking water. A short survey is given of purification processes in nature and application of these mechanisms in waste-water treatment plants. It is concluded that the removal of micro-organisms in these plants is not good enough to reach the high standards of surface water quality necessary for the production of safe drinking water, unless advanced treatment is applied.

A historic review of pathogens transmitted via drinking water is given to establish the emerging pathogens in the last hundred years. Some remarks are made on the methods of drinking water microbiology and especially on the new task for the Dutch drinking water companies to assure a maximum acceptable infection risk. This risk has to be 10^{-4} or less per year. It is possible to arrange the waterborne pathogens into two groups. Members of the first group are relatively easily related to the moment the client consumed the contaminated water. These illnesses are usually the classical waterborne diseases such as diarrhoea (cholera etc.). The second group of pathogens usually has longer incubation periods, and often other symptoms. In particular the longer time between infection and first symptoms makes it very difficult to relate illness and contaminated water (e.g. ulcer or even stomach cancer caused by *Helicobacter*). These types of illnesses are expected to be recognized as the new emerging pathogens in the near future. The most important waterborne pathogens derived form literature are listed in a table. It is stressed that there is no consensus among all researchers about pathogens classified as future waterborne pathogens.

The new Dutch approach in testing the raw waters on the presence of index pathogens and calculate possible concentrations in finished water gives a better protection compared to the classical techniques using faecal indicators.

It is recommended to study potential waterborne pathogens with respect to their properties of how they can be removed during purification processes. When certain types appear to be more persistent than the present index pathogens it is recommended to include the new, more persistent pathogen in the set of index-pathogens. It is concluded that sewage and effluents of sewage treatment plants have an impact on the microbial quality of surface waters. Modern biomembrane reactors may have an improved elimination capacity but data on the removal of pathogens in these plants are not yet available. The quantitative contribution of pathogens is not well known for many (classic) species. For new emerging pathogens presence and concentration is even less well known. It is reasoned that “new” waterborne pathogens show comparable physical properties to classical indicators or pathogens and occur in similar densities as those “classical species”. Therefore, the new ones probably do not pose a greater threat to the production of drinking water, provided modern types of treatment processes and technologies are being used. However, more persistent types or types occurring at much higher raw water concentrations demand further evaluation.

Samenvatting

De Rijn is een belangrijke bron voor de drinkwaterproductie maar dient tevens als mogelijkheid voor de afvoer van afval. Industrieel afvalwater betreft vooral chemische aspecten. Maar huishoudelijk afvalwater betreft ook voor een belangrijk deel microbiologische aspecten, in dit verband is ook het afvalwater van ziekenhuizen en slachterijen een belangrijke bron van microbiologische verontreinigingen. In deze studie wordt nagegaan of, al dan niet gezuiverd, rioolwater een bron kan zijn van nieuwe ziekteverwekkers die een mogelijke bedreiging vormen voor de productie van veilig drinkwater. Er wordt een kort overzicht van de zuiveringsmechanismen in oppervlaktewater en in rioolwater zuiveringsinstallaties (RWZI) gegeven. Geconcludeerd wordt dat RWZI´s onvoldoende verwijderingcapaciteit hebben voor microbiologische parameters. Moderne biomembraanreactioren zullen naar verwachting een betere verwijdering laten zien, hoewel kwantitatieve gegevens over de verwijdering van pathogenen nog ontbreken.

Verder wordt aan de hand van een grote hoeveelheid historische gegevens na gegaan welke via drinkwater overdraagbare pathogenen in de afgelopen bijna 100 jaar een rol hebben gespeeld. Ook wordt ingegaan op de manier waarop het microbiologische drinkwateronderzoek werd en wordt uitgevoerd en hoe Nederlandse drinkwaterbedrijven thans moeten zorgdragen voor een infectierisico voor de consument van maximaal 1 op 10.000.

Het is mogelijk de wateroverdraagbare ziekteverwekkers in te delen in twee groepen, de eerste groep is relatief eenvoudig te relateren aan het drinken van besmet water omdat de persoon na betrekkelijk korte tijd ziek wordt en vaak zijn de symptomen diarree (tyfus, cholera, dysenterie e.d.). De tweede groep is gekenmerkt door vaak een veel langere incubatietijd en veroorzaakt vaak andere ziektebeelden dan klassieke maagdarm stoornissen. Hierdoor is de relatie met het besmette water dat werd gedronken veelal niet meer eenduidig is vast te stellen (b.v. maagzweren of zelfs maagkanker veroorzaakt door *Helicobacter*). In deze studie wordt verondersteld dat het juist dit soort aandoeningen zijn die in de nabije toekomst herkend zullen worden als de “nieuwe Emerging Pathogens”. Er wordt een overzicht gegeven van water gerelateerde ziekteverwekkers, waarin zowel de meer klassieke als de huidige ”emerging pathogens” staan maar ook mogelijke toekomstige, bij deze laatste moet worden aangetekend dat niet alle onderzoekers overtuigd zijn dat dit daadwerkelijk de veronderstelde pathogene micro-organismen zijn.

De nieuwe Nederlandse benadering dat het ruwe water wordt getest op de aanwezigheid van index-pathogenen geeft een beter inzicht in de waterkwaliteit dan wanneer men uitsluitend met de klassieke indicator organismen (*E. coli* e.d.) werkt. Het wordt aanbevolen om nieuw gevonden wateroverdraagbare ziekteverwekkers te onderzoeken op hun eigenschappen t.a.v. het zuiveringsproces en wanneer blijkt dat een soort persistenter is dan de huidige index-pathogenen dan kan men overwegen deze pathogeen als aanvulling op de gangbare index-pathogenen te meten. Er is beredeneerd dat ongezuiverd rioolwater en het effluent van riool water zuiveringsnstallaties een merkbare invloed op de microbiologische water kwaliteit moeten hebben. De kwantitatieve bijdrage van pathogenen in deze effluenten is onvoldoende bekend. Voor de nieuwe “emerging pathogens” is aanwezigheid en het gehalte in deze effluenten meestal geheel onbekend. Het is te beredeneren dat nieuwe pathogenen die vergelijkbare eigenschappen hebben en in vergelijkbare concentraties voorkomen als de reeds bekende, geen extra bedreiging voor de productie van veilig drinkwater vormen. Maar soorten die duidelijk persistenter zijn of een ander bouw vertonen of in veel hogere concentraties voorkomen als de bekende pathogenen vereisen een nader onderzoek.

3 General intro

General introduction

3.1 Goal of this study

The river Rhine is an important source for drinking water production, serving around 4 million inhabitants in the Netherlands directly or indirectly. At the same time the river used for many purposes, among these the discharge of waste water. Industrial discharge is mainly chemically polluted (Van Beelen, 2007); however, domestic sewage contains important microbiological contaminations, as are discharges from hospitals and slaughterhouses.

The Association of Rhine Water Works, RIWA-Rhine is, therefore, interested in all aspects dealing with the river's water quality, both current state and developments, and potential threats thereof. A wide range of chemical (Van Beelen, 2007), biological, toxicological and hydrological studies have been made so far. The biological studies covered a number of pathogens. For instance on the occurrence of *Cryptosporidium* and *Giardia* (Medema *et al.*, 1996; Hoogenboezem *et al.*, 2001) and on the occurrence of human viruses (De Roda-Husman et al., 2005). These studies focus on the presence of certain well known species. In one occasion a survey of potential sources of pathogens in the River Meuse catchment area is given (Medema *et al.*, 1996). Another study aimed at the presence of *Cryptosporidium* oocysts and *Giardia* cysts in surface water, manure and the influents and effluents of sewage water treatment plants (Hoogenboezem *et al.*, 2001). From these studies and many others published in the literature it is clear that pathogens may contaminate surface waters in various ways. In general contamination sources can be divided in diffuse and point sources. With regard to pathogens diffuse sources are likely to occur in the agriculture, especially manure run-off into the river. Effluents of untreated or treated sewage and sewer overflows are regarded as point sources. Quite different types of pathogens (protozoa, bacteria and viruses) may be expected from these different sources.

In recent decennia a number of "new pathogens" have been identified, normally referred to as **Emerging Pathogens**. Sewage is without doubt an important source of waterborne pathogenic micro-organisms as it is often a flow of almost exclusively domestic wastewater. The aim of this literature study is to list and describe possible emerging waterborne pathogens that can be associated with sewage or sewage treatment plants and to assess their relevance for drinking water production from surface water.

Another goal of the study is to find clues why a certain pathogen becomes an emerging waterborne pathogen, since we may assume that many of these pathogens have been present in waste or surface water for a longer time. In this study pathogens affecting the human intestines or the urogenital system are regarded as pathogens potentially transmitted by raw sewage or poorly treated wastewater to the surface waters that may be used as a raw water source for drinking water production. Another way to facilitate new pathogens to emerge is to create environmental circumstances that allow these pathogens to grow. For instance the development of *Legionella* bacteria in artificial warm water systems.

3.2 Sewage and contamination of drinking water

The first investigator who showed a distinct relation between faecal contamination of drinking water and the occurrence of illness (cholera) was dr John Snow in Victorian London (Richardson & Frost, 1936). In the area of Broadstreet c. 500 people died of cholera within ten days. Snow plotted

all cases on a map and discovered that most of the victims lived near the Broadstreet pump. After tracing the contaminated drinking water pump he disabled the pump by removing the handle. This action prevented new cases of cholera in that district of the city. Afterwards he opened the well of the pump and concluded that the smell of the water was bad and a simple chemical test, using silver nitrate, showed a high content of organic matter. Microscopic investigation of the water from the well showed “very small swimming animals”. Snow further concluded that the ground around the borehole was very coarse, which he considered as a disadvantage. Although he did not know what the actual agent was he concluded that contamination with sewage was the most probable cause of cholera. In another study Snow concluded that an upstream intake for drinking water distribution is much safer than when the water is taken downstream of the city of London. The work of Snow is considered as the first epidemiological study. This study further clearly indicates the relation between faecal contamination as a transmission route for illness.

This happened in a time people did not know that micro-organisms (viruses, bacteria or parasitic protozoa) are the actual pathogens. A few decennia later the role of bacteria and illness was demonstrated by famous microbiologists such as Ro bert Koch and Louis Pasteur. Koch proposed to investigate the total number of bacteria in the water in order to evaluate the microbial water quality, now known as plate count. Later determination of *Escherichia coli*, as an example of a typical intestinal bacterium, was added to the standard water quality evaluation and soon the number of outbreaks started to decrease (fig. 1), although it should be stressed
that in the same period also other aspects have improved. Households were connected tot the distribution system of drinking water companies and refrained form drinking untreated surface water. Moreover, sewage systems were constructed reducing private well contamination etc.

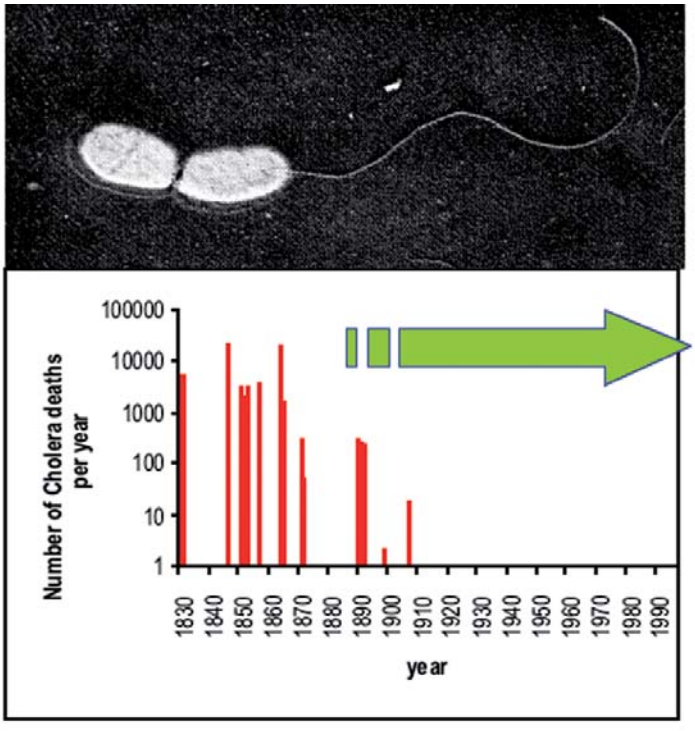


Fig. 1. Yearly number of cholera casualties in the Netherlands in the period 1830 – 1990. Inset electron micrograph of a Vibrio cholera bacterium. Note that in the same period a number of measures have been taken to improve the water quality, in the same period a large number of households were connected to public mains and sewage systems.

3.3 The role of indicator bacteria in drinking water quality control

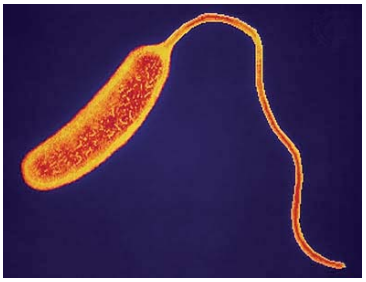
As mentioned above, Robert Koch advised to carry out microbiological investigations (colony count) to establish microbiological water quality. *Escherichia coli* was added later as an indicator for faecal contamination. Faecal contamination of drinking water should be identified as a potential threat for human health. Once proven that water is contaminated the water is regarded not to be suitable for human consumption. Later other indicators such as enterococci, sulphite reducing clostridia and *Clostridium perfringens* were added to the daily practice of the water laboratories. Good indicator bacteria for faecal contamination should be:

- easy and fast to determine
- abundant in faeces of humans or, more general, in mammals and birds, preferably in higher numbers than pathogens in faeces
- persistent in the environment at least as long as pathogens transmitted via faeces
- not able to multiply in the environment
- at least as persistent against treatment as pathogens that may be present in faeces

The use of indicators is important since it is impossible to test finished drinking water on every possible waterborne pathogen within a reasonable time.

A pathogen is defined as a micro-organism capable to cause illness. The main groups of pathogens are: viruses (Poliomyelitis, diarrhoea, SARS etc.), bacteria (cholera, typhoid fever etc.) and parasitic protozoa (Dysentery amoebae, *Cryptosporidium* etc.).

bacterie (Vibrio cholera)



In recent periods the current indicator bacteria have been shown not to be sufficient, since in a number of occasions indicator bacteria were not detectable in water containing detectable numbers of pathogens. For instance *Cryptosporidium* oocysts appeared to be far better resistant against disinfection with chlorine and (oo)cysts are much better “designed “ to persist over a long time in the environment compared to vegetative intestinal bacteria. At the time of the Milwaukee *Cryptosporidium* outbreak in 1993 none of the routine samples were *E. coli* positive, although there were some turbidity problems. However, no microbiological problems with indicator bacteria were encountered. Later, when the water company had been identified as the most probable source of the *Cryptosporidium* epidemic, investigators demonstrated the presence of the pathogen in “cooling ice” produced shortly before the outbreak was noted. During the outbreak in Milwaukee around 100 people died and over 400,000 became ill; a grim example for the failure of classical indicators.

Spores of *Clostridium perfringens* are doubtlessly better indicators for persistent (oo)cysts.

Scabler *et al.* (2003) studied both pathogens and indicators in river water and found that *E. coli*, due to a shorter survival rate in the environment is not a reliable indicator for pathogen viruses. It appeared that somatic coliphages are much better indicators for viruses.Payment (1998) concludes that *E. coli* is probably a very good indicator for the faecal load in surface water but certainly not for the evaluation of treated waters, due to different survival rates of different groups of organisms.

Bacteriophages are better indicators for viruses, but only species associated with intestinal bacteria should be studied as other types of phages may originate from environmental bacteria. In that case these phages are less reliable indicators.

Many more studies have been carried out on indicators for pathogens, for a more extensive survey on this subject the reader is referred to WHO/OECD (Fewtrell & Batram, 2002) and Medema and coworkers (2006).

3.4 Quantification of the infection risk

Dutch drinking water legislation (Anonymous, 2001[a]) sets certain demands on the maximum infection risk for consumers of drinking water. The level of acceptable risk is one infection in 10,000 consumers per year (10^{-4}-risk). Assessment of such a low risk is very difficult, as one should, in principle, demonstrate the absence of certain pathogens in very large amounts of water. The 10^{-4} risk for enterovirus in drinking water for instance is calculated on maximum of only one virus in nearly one million litres of drinking water, evidently a very impractical sample volume. The approach to assure the water meets the required safety level is to measure a number of index pathogen concentrations in the raw water source, and to determine the elimination capacity during various purifications steps of the plant and calculate the expected index pathogen concentration in the finished water. Index pathogens are enterovirus, *Campylobacter, Cryptosporidium* oocysts and *Giardia* cysts, representing examples of viruses, bacteria and parasitic protozoa.

3.5 Emerging pathogens

How can a survey of emerging pathogens be established?
First it is necessary to define the concept of "emerging pathogens". In the literature the concept of emerging pathogens is mentioned, a general definition of an emerging pathogen is given by Morse (1995):

"Infections that have recently appeared in the population or have existed but rapidly increasing in incidence or geographical range or have existed but are associated with a known pathogen with new features".

or:

Any new, re-emerging or drug resistant infection whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the future (Sharma et al. 2003).

The definition applied in this study is adapted to the problem:
"Emerging waterborne pathogens are those micro-organisms becoming gradually (or suddenly) more important as a cause of waterborne disease, in particular those which contaminate surface waters via sewage or sewage plant effluents."

There are different reasons why a certain micro-organism becomes an Emerging (Waterborne) Pathogen (EWP). In most cases it will not be because the pathogen is an entirely new species. Although new pathogens may evolve, for instance after genetic recombination between an animal virus and a common human one. An example of such a recombination is the possible transformation of a avian influenza virus into a new and virulent type of human flu virus. The SARS virus is an example of an animal virus which have become infectious to humans (Wang et al, 2005)

It will happen much more often that a certain pathogen may have been a problem over a much longer time, but it is only recently recognized for the first time as a new pathogen. Such a pathogen is not necessarily new or recently discovered. In the 1940, for instance, Kudo (1948) already mentions two species of *Cryptosporidium (C. muris* and *C. parvum*). However, the first case of human illness caused by this parasite is reported in 1976 (see Medema, 1999). But it was not before the 1990-ies, after an important outbreak, that the full importance of this kind of pathogens for drinking water production was completely understood. A species can become more important for instance by the presence of more sensitive persons in our modern community such as immunocompromised people (transplantation, or AIDS patients). Such persons may become readily ill from opportunistic patho-

gens, not posing any serious health risk for persons with a normal functioning immune system.
Also, global transportation of food, animals and persons facilitates the dispersion of species formerly restricted to certain areas. Changes in environment, e.g. man made environments such as: cooling towers (*Legionella*), dams, intensive farming etc. may also pose health threats.
Breaking down the public health systems me cause the re-emerging of certain disease. E. g. in some parts of the former Soviet Union typhoid fever and cholera are endemic again.
And last but not least new detection techniques play doubtlessly an important role in the recognition of pathogens. Once a new technique is available certain pathogens will be identified more often and that particular pathogen will become an Emerging Pathogen. New detection methods such as PCR enable the detection of non culturable pathogens. (Huffman et al, 2003)
Analysis of historical data will show what types of pathogens have emerged in certain periods, also decrease of certain illnesses can be seen in certain periods.

A differentiation among types of waterborne (emerging) pathogens is possible.

- Pathogens that cause gastro-entero illnesses, in a relatively short period after exposure to the pathogen (e.g. typhoid fever, cholera etc.)
- The second group are formed by those pathogens transmitted by the intestinal faecal route causing other types of illness and after a much longer incubation period (e.g. *Helicobacter pylori*)

This second type of disease is much more difficult to recognise as a waterborne illness. Although several "classical" waterborne pathogens can cause also illnesses after a longer incubation period (e.g. hepatitis), or more serious illnesses such as for instance the syndrome of Guillian-Barré: recently *Campylobacter* is recognised as a causative agent. Similarly, the haemolytic uraemic syndrome (HUS) has only recently been recognised as a complication of an infection with *E. coli* O157.

Many of future emerging pathogens are expected to belong to the second group. Due to the longer incubation period the aetiology of these illnesses is usually much more difficult to determine.

3.6 Resistance to antibiotics

Bacteria can develop resistance to certain antibiotics, these strains survive treatment with antibiotics and form an increasing problem in hospitals. The so called hospital acquired infections are often difficult to treat, as certain strains appear to be multiple resistant e.g. (almost to every antibiotic) "multi resistant *Staphylococcus aureus*" (MRSA). The behaviour of resistant bacteria was studied in sewage treatment plants (Bendt et al., 2002). These authors observed variying concentrations of resistant bacteria , the highest number were obtained form hospital sewage. Removal in the treatment process is estimated to be 99.9% (3 log) and no increase of multiresistancy has been observed in this study (Bendt,et al., 2002). It is assumed that the application of antibiotics also influences the intestinal flora, since the average E. coli bacterium contains 1.26 resistance factors. (Bendt et al., 2002).
Resistant bacteria are able to transmit their (genetically acquired) resistance to other members of their species and even to bacteria of other genera (horizontal transmission). It has been demonstrated that hospital wastewater contains a higher number of resistant bacteria, also strains possessing resistance against antibiotics only recently developed (Schwartz et al. 2003; Stieber et al. 2004). Presently there is no evidence for the existence of waterborne pathogens becoming emerging pathogens as a result of acquired resistance against certain antibiotics. The physiological condition for pathogenic bacteria in the environment is usually poor; therefore exchange of genetic material under these conditions is less likely.
Some monitoring on this type of problems is recommended.

3.7 Climate change and extreme low- and high-flow conditions

The climate change may result in more extreme periods of drought followed by episodes of heavy rain and considerably larger amounts of water in shorter periods of time. In the dry periods the concentrations of sewage in the river may increase considerably, in extreme situations this will pose an extra demand on the drinking water treatment plants, designed for lower concentrations of pathogens. The wet periods may pose the risk of inundation of water recharge installations with contaminated water. No studies on the microbiological effects of extreme low water conditions in Rhine or Meuse have been found. Overflow of stormwater sewers in the direct vicinity of an intake point for drinking water production, may contribute to the pathogen load of surface water. Increasing temperatures may facilitate pathogens form warmer areas into temperate areas as, for example, the toxic cyanobacterium (blue green algae) *Cilindrospermopsis* spec. (Anonymous 2001[b], Mooij et al. 2005).

Higher watertemperatures are expected to further favour and stabilize cyanobacterial growth in phytoplankton communities (Mooij et al. 2005).

Burkholderia pseudomallei is the causative agent of Melioidosis, a sometimes life threatening septic infection (Guillot & Loret, 2006). These authors mention the greatest concentration of cases from tropical and subutropical (Southeast Asia – Northern Australia) areas. A recent study in Italy (Zaneti et al., 2000) showed the occurrence of both *B. pseudomallei* and *Burkholderia cepacia* in drinking water samples from the province of Bologna. *B. pseudomallei* was found in 7% of the drinking water samples, with a mean concentration 578 cfu per 100 ml, there is a positive correlation with colony counts (Zeneti et al. 2000). Since there is no historic information on these species in Italian drinking water, the presence of these bacteria cannot be related to global warming processes with certainty. The protozoon Isospora is considered to have a tropical and subtropical distribution. When the annual mean temperature increases this parasite may become of more importance in temperate regions.

3.8 Microbial aspects of surface water influenced by sewage.

3.8.1 Self purification of surface waters

It has been known for quite some time that river systems are able to eliminate organic or biological pollution from surface water. Once the load of impurities becomes too large this self purification process decreases significantly, due to oxygen depletion. In the 1960-ies and 70-ies it became clear that the organic load of the Dutch larger surface waters was much higher than the self purification capacity of the water, resulting in anoxic stinking waters. In the 17 and 18th century such situations were only known from much smaller water bodies within the cities.

What are the mechanisms of this self purification process? According to Rheinheimer (1991) the most important processes are sedimentation, oxidative processes, conducted by a variety of micro-organisms (bacteria, moulds, protozoa etc.), but also fish and even birds and mammals may play a role in the process. Solar radiation is also an aspect of inactivation of micro-organisms in water (Lonnen *et al.*, 2005). Complete mineralization would be ideal but is rarely achieved. Proteins, sugars and starch are easily consumed; fat and larger carbohydrates like cellulose and wood (lignin) are decomposed at a much slower rate. The composition of the bacterial flora has been demonstrated to change with the types of organic components present in the river. Fast flowing shallow rivers possess the highest self purification capacity, due to sufficient gas exchange and dilution of the pollutant. An overdose of nutrients causes oxygen depletion and anoxic sediments which diminishes the benthic community.

Human pathogens (viruses, bacteria or parasitic protozoa) are usually unable to multiply in sewage

or river water, they will inactivate or decompose fairly rapidly over time. Also consumption by protozoan or invertebrate grazers is probably an important reduction factor. The problem is that the need for reuse of the water is earlier than the time needed for the inactivation of human pathogens, such as *Salmonella typhi, S. paratyphi, Shigella, Vibrio cholerae, Mycobacterium, Clostridium perfringens, E. coli O157, Campylobacter.* As well as different viruses: polio, coxsackie echo and hepatitis etc. and parasites as *Cryptosporidium, Giardia* parasitic amoebae. The survival of pathogens depends also on the composition of the microbial community. The survival rate of pathogens is smaller at a greater autochthonous microbial community. The longest pathogen survival has been shown to occur in relatively clean environments (Rheinheimer, 1991).
Rheinheimer concludes his section on self purification with the remark that sewage treatment processes are based on the same mechanisms, but concentrated and controlled on oxygen influx and mixing.

3.8.2 Sewage treatment plants

Mudack & Kunst (2003) summarised the history of sewage treatment over the last 100 years. The first goal of sewage treatment was to maintain a sufficient oxygen regime, in order to keep the oxidation processes going. Then the eutrophication became important, the reduction of nitrogen (N) and phosphorus (P) became an issue. Due to the fact that surface waters are increasingly used for the production of drinking water, the subject of persistent compounds became a target of improvement. The present state of the art is that BOD is removed fairly well, biological nitrogen elimination is well improved, the elimination of phosphorus is reasonable, but needs further improvement. According to these authors the removal of persistent compounds is still poor. It is remarkable that removal or inactivation of pathogenic organisms in sewage treatment processes is not even mentioned.
An extensive book on water and wastewater microbiology (Mara & Horan, 2003) includes some papers on the removal of micro-organisms and some chapters on the various wastewater treatment processes.

Oragui (2003) highlights some virological aspects in wastewater treatment; the most important conclusion is that wastewater treatment plants are not designed for the removal of viruses. A removal of 94% is reported for rotavirus from sewage. Oragui (2003) concludes that viruses detection in raw sewage is very difficult since only small amounts of sewage can be processed (25 – 200 µl) resulting in poor detection characteristics. Moreover, concentration techniques cannot be applied as toxic compounds in the concentrate cause toxic effects on the cell-line used for virus cultivation. This is perhaps the main reason that only limited numbers of quantitative virus data in wastewater (or sewage) are available in the literature. Many kinds of viruses have been detected in sewage and wastewater (e.g. rota-, astro-, calici-, corona-, hepatitis-A, Adenoviruses, Enteroviruses, etc.). Lodder et al. (1999) demonstrated identical RT-PCR sequences in stools of patients and sewage. More different types of viruses were detected in Dutch sewage (Lodder et al. 2005).
It is still unclear whether certain species of animal viruses are able to affect humans or not. For instance certain corona viruses causing severe diarrhoea in calves and pigs may also affect humans (zoonosis). Norovirus genes were found in farm animals (Van der Poel, et al. 2003[a]) and actual norvirus infections in cattle were described subsequently (Van der Poel et al., 2003[b]).
Bofil-Mas (2005) found polyomaviruses in almost every type of sewage (51 out of 52 samples) studied in Europe, Africa and USA); in a River near Barcelona a concentration of 33/l of these viruses was found.
Enteroviruses were detected in raw sewage at two sampling sites. The geometric means of the

detected concentrations were 34 and 190/L, the removal efficiency at both plants was 2.1 and 2.l log units, respectively. The same samples were tested for the presence of reoviruses, these viruses were found in larger numbers (69 and 370/L). The removal efficiency of these viruses was estimated at 1,4 and 1,6 log units respectively. Bacteriophages were removed with efficiencies of 1.8 – 2.6 log units (Tab. 1). Kimmig and Fleischer (2001) mention an even smaller reduction (15 – 30%) for viruses during the treatment of wastewater. In periods of a higher load of enterovirus in raw sewage, a capacity of 2 – 3 log-units is necessary. During rain storms the overflow of sewage will increase the concentration in surface water even more. Extra measures are proposed by Kimmig and Fleischer (2001) to ensure sufficient disinfection of viruses.

The fate of pathogenic bacteria in wastewater depends strongly on the treatment principles applied (aerobic and/or anaerobic) and the duration of the process, longer treatment times resulting in better pathogen elimination. Most treatment plants are designed to eliminate organic matter, the fact that pathogens are eliminated at the same time is a lucky coincidence (Curtis, 2003). Primary sedimentation removes 50 -60% of the pathogenic bacteria, activated sludge eliminates 90 – 99% of the bacteria. The actual mechanism of removal here is still unknown but adsorption is most likely. *E. coli* is a relatively good indicator for most of the pathogenic bacteria, except perhaps for Campylobacter which is slightly different for it is more sensitive for the presence of oxygen (Curtis, 2003).

Some data on pathogenic bacteria in raw sewage: *Campylobacter 70* – 1600/100 ml; enterohaemoragic *E. coli* is widespread but there are no quantitative data; *Helicobacter* (no quantitative data); *Salmonella typhi*; *Shigella* (no quantitative data); *Salmonella enteriticus* 20 – 1800/100 ml; *Vibrio cholerae* (O_1, O_{130}) 1 – 10^7/100ml (Curtis, 2003). This author also refers to a poor elimination capacity for pathogens during wastewater treatment.

With regard to parasites more or less similar conclusions were drawn by Stott (2003); there is only a limited number of data on parasites in sewage and wastewater especially on possible emerging pathogens such as *Isospora*, *Microsporidia* and *Cyclospora*. The elimination capacity for parasites is poorly known, although Stott (2003) mentioned that (oo)cysts or helminth eggs can be found in all types of wastewater treatment plants effluents. The removal of parasitic protozoa was measured at two sewage treatment plants in the Netherlands. The geometric mean of the removal efficiency during sewage treatment for *Cryptosporodium* (oocysts) varied between 1,3 and 1,5 log units. Giardia cysts are removed slightly better with an geometric mean from 19, - 2,0 log units (Hoogenboezem *et al.*, 2001) Reuse criteria for effluents are set by 0.1 – 1 helminth eggs per litre and 1 cyst per 40 litres. According to this author 50% or even more of the world population is infected with one or more helminth species.

Stott (2003) states that longer treatment processes result in better removal.

The occurrence of parasites is different between developing countries and developed countries, in the latter less people are infected with helminths, therefore wastewaters in the developing countries usually contain more parasite eggs.

Some concentrations in raw wastewater:

In faeces up to 102 - 104 helminth eggs/g and 10^5 – 10^7 (oo)cysts/g may occur; such concentrations impose a considerable load on the concentrations in sewage.

Sewage treatment plants are very beneficial for a number of ecological aspects (N, P solid matter and oxygen) but for the removal of pathogens is the contribution only limited. Leaving a distinct discrepancy between sewage treatment plants and the high standard demands for raw water quality necessary for drinking water production. Modern biomembrane reactors may have an improved elimination capacity but data on removal of pathogens in these plants are not yet availab

Table 1 Geometric mean values of (oo)cysts and other parameters in the influent and effluent of the Sewage treatment plants Kralingseveer near Rotterdam and Amsterdam Westpoort and the relevant purification efficiency for the period from June 1997 to June 1998 (After Hoogenboezem et al. 2001).

	RWZI Kralingseveer			RWZI Amsterdam Westpoort Municipal		
Geometric mean	Untreated sewage water	effluent from SWTP	Purification efficiency	untreated sewage water	effluent from SWTP	Purification efficiency
microorganisms (number/l)						
Cryptosporidium	540	17	1.5 log (96.8%)	4650	250	1.3 log (94.7%)
Giardia	1220	13	2.0 log (99.0%)	21300	250	1.9 log (98.8%)
SSRC	6.2×10^5	1.7×10^4	1.6 log (97.2%)	7.9×10^5	3.8×10^4	1.3 log (95.1%)
SCP	6.0×10^5	1.5×10^4	1.6 log (97.4%)	5.4×10^5	2.1×10^4	1.4 log (96.2%)
THCOL	9.4×10^7	1.1×10^6	1.9 log (98.8%)	1.6×10^8	6.9×10^5	2.4 log (99.6%)
FSTREP	3.6×10^6	5.7×10^4	1.8 log (98.4%)	1.6×10^7	1.1×10^5	2.1 log (99.3%)
FRNAPH	2.2×10^6	5.7×10^3	2.6 log (99.7%)	4.3×10^6	3.1×10^4	2.1 log (99.3%)
Enterovirus	34	0.27	2.1 log (99.2%)	190	0.53	2.6 log (99.7%)
Reovirus	69	2.7	1.4 log (96.1%)	370	8.4	1.6 log (97.7%)
general parameters (mg/l)						
BOD	87	3.1	96%	310	2.3	99%
COD	270	37	86%	570	33	94%
Suspended matter	96	< 10	> 90%	230	14	94%
Chloride	140	120	14%	190	210	0%

Elimination of indicator bacteria E. coli, fecal enterocci and spores of sulphite reducing clostridia varied at two Dutch treatment plants from 1.3 – 2.4 log-units (Table 1).

Sewage treatment plants are very beneficial for a number of ecological aspects (N, P solid matter and oxygen) but for the removal of pathogens is the contribution only limited. Leaving a distinct discrepancy between sewage treatment plants and the high standard demands for raw water quality necessary for drinking water production. Modern biomembrane reactors may have an improved elimination capacity but data on removal of pathogens in these plants are not yet available.

Although quantitative information on pathogens in sewage is only limited, the US Environmental Protection Agency (EPA) gives an overview of pathogens detected in treated sewage sludge bacteria (tab. 2), viruses (tab. 3) and parasites (tab. 4). The fact that these species are mentioned as 'commonly found' indicates a considerable density.

Table 2. Bacterial pathogens commonly found in treated sewage sludge.
(source: http://members.aol.com/wwanglia/frame_pathogen.htm)

Bacteria	Diseases
Vibro cholera	Cholera (not applicable in the UK)
Salmonella typhi	Typhoid and other enteric fevers
Salmonella other species	Food poisoning.
Shigella species	Bacterial dysentery.
Campylobacter	Gastro-enteritis
Proteus species	Diarrhoea
Coliform species	Diarrhoea
E coli o157	Gastro-enteritis, renal failure
Clostridium species	Botulism
Pseudomonas species	Local infection
Tuberclebacilli	Tuberculosis
Leptospira	Leptospirosis (Weil's disease)
Yersinia enterocolitica	Gastro-enteritis

Table 3. Viral pathogens commonly found in treated sewage sludge.
(source: http://members.aol.com/wwanglia/frame_pathogen.htm)

Viruses	Diseases
Infectious Hepatitis	Inflammation of the liver
ECHO viruses	Enteric diseases and the causative
Coxsackie virus	Agents of aseptic Meningitis
Polio virus	Poliomyelitis
Epidemic gastroenteritis virus	Gastro-enteritis
Small round viruses (norovirus)	Gastro-enteritis

Table 4. Parasites commonly found in treated sewage sludge.
(source: http://members.aol.com/wwanglia/frame_pathogen.htm),
Parasitic worms were ommited in this table

Parasites	Diseases
Entamoeba histolyticad	Amoebic dysentery
Balantidium coli	Balantidial dysentery
Isospora hominis & others	Coccidiosis
Giardia lamblia	Diarrgoea
Crytosporidium	Epidemic diarrhoea

4

Waterborne disease outbreaks, an historic overview

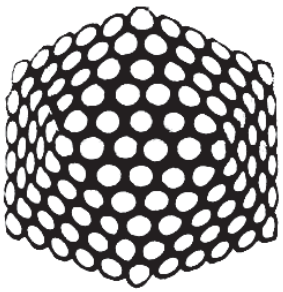
Craun & Calderon (1999) report 1,884 waterborne outbreaks that have caused 882,144 illness cases and 1,169 deaths in the USA over the period 1920 – 1996. This may seem high compared to only three outbreaks in Dutch distributions systems in the last 30 years.

There are over 20 million private wells in northern America (Van Der Laan, personal comm.) and many community water supply systems are small. Thi is an important difference between for instance the Netherlands where only a limited number of larger drinking water companies exist. The quality control in large production plants is probably more extensive than for a relatively small private well. The treatment of water by many of these smaller American water companies is often limited to filtration or disinfection, even untreated water is distributed (Craun & Calderon, 1999). There is also a considerable difference in outbreak reporting. This explains largely the remarkable difference between the number of outbreaks in USA and The Netherlands. Correlated to population size there are ten times more outbreak reports in the USA compared to the Netherlands. Nevertheless it is important to look at the American data as they show what types of organisms are present in the environment and are able to cause outbreaks through drinking water.

4.1 Viruses

Outbreaks associated with viruses are fairly frequently reported (Tab. 5). The virus causing poliomyelitis was reported from only one outbreak in the 40-50-ies. In that period vaccination campaigns started in many countries, which may explain the fact that no more waterborne outbreaks have been observed since that period. The shift from hepatitis to hepatitis-A is the result of the recognition of more types among these viruses in that time. The hepatitis from the first period belongs almost certainly also to the hepatitis-A type. In more recent times small round structured viruses and Norwalk viruses (norovirus) were detected using Reverse-transcriptase (RT)-Polymerase Chain Reaction (PCR) techniques. Over the whole period a relatively large part of the outbreaks was attributed to viral pathogens (tab. 5). As many of these viruses were not identified it is likely that among these there are future (viral) emerging pathogens.

virus (schematic image)



Tab. 5. Drinking water related viral outbreaks in the USA over the period 1910 – 2000. (data after, Hunter, 1997; extended with Barwick et al. 2000 and Lee et al., 2002)

Disease	Outbreaks 1910 -1940	Outbreaks 1941 -1960	Outbreaks 1961 -1970	Outbreaks 1971 -1980	Outbreaks 1981 -1990	Outbreaks 1991 - 2000
Viruses						
Hepatitis	1	23	30			
Hepatitis A				16	11	2
Polyomyelitis		1				
Small round stuctered viruses (norovirus)						2
Norwalk virus (norovirus)						3
Viral gastroenteritis				12	15	
Gastroenteritis (chronic)	144	265	39	181	128	61

4.2 Bacteria

Bacteria as the cause of waterborne outbreaks also have been important during the last 80 years, but not always at the same level of health burden. Typhoid fever outbreaks were most numerous in the first period (1910-1930) but their numbers gradually decreased to a neglectable level in the last period (tab. 6). Also outbreaks by other *Salmonella* bacteria tend to decrease as a cause of waterborne outbreaks.

Tab. 6. Drinking water related bacterial outbreaks in the USA over the period 1910 – 2000. (data after Hunter, 1997 extended with Barwick et al. 2000 and Lee et al., 2002)

Disease	Outbreaks 1910-1940	Outbreaks 1941-1960	Outbreaks 1961-1970	Outbreaks 1971-1980	Outbreaks 1981-1990	Outbreaks 1991-2000
Bacteria						
Typhoid	372	94	14	4	1	
Paratyphoid		3				
Salmonella		4	9	8	4	3
Shigella	10	25	19	24	22	8
Cholera					1	1
E.coli O157/H7 and Toxigenic			4	1	1	8
Campy-lobacteriosis				3	10	5
E. coli O157/H7 and *Campylobacter*						1
Leptospirose		1				
Yersiniosis					2	
Tularaeremia		2				

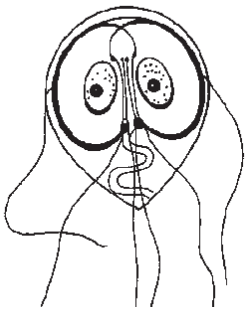
Remarkably, outbreaks caused by *Shigella* bacteria remain at a more or less similar level over the entire period, no explanation for this phenomenon has been found. From this survey *Campylobacter* and toxigenic *Escherichia coli* bacteria can be regarded as emerging (waterborne) pathogens. A few outbreaks associated with *Leptospora*, *Yersinia* and *Tulaeremia* are regarded merely as incidents, not as emerging pathogens in the periods of occurrence. A single outbreak of *Plesiomonas shigelloides* may be regarded as an accidental outbreak since this pathogen is relatively easy to disinfect.

4.3 Protozoa

Parasitic protozoa have been known as waterborne pathogens for a long time, for instance as the causative agent of amoebic dysentery (*Entamoeba histolytica*). Amoebiasis has decreased in outbreak numbers and giardiasis and cryptosporidiosis have emerged as diseases related to waterborne pathogens in the last 30 years (tab. 7). In the 80-ies the first outbreak of *Cyclospora* has been reported from the USA. In Canada a drinking water related outbreak of toxoplasmosis has been recorded (anonymous, 1995)

Parasitic protozoa form persistent (oo)cysts as a protection against unfavourable environmental circumstances on the parasite's way to the next host. These (oo)cysts appear to be very insensitive to disinfection procedures at drinking water treatment plants. It is remarkable that *Cryptosporidium* and *Giardia* have not been identified as important water borne pathogens much earlier for it is unlikely that the first recognised outbreaks were actually the first ones ever. Especially because of their resistance for disinfection parasitic protozoa are considered to be important possible new emerging pathogens.

parasitaire protozoa (Giardia)



Cyclospora are obligate intracellular parasites, belonging to the Coccidia. According to Ortega (1999) *Cyclospora* were first observed in 1979 in Papua New Guinea, but Kudo (1948: 475) mentions this genus already isolated from the intestine of the mole (*Talpa europaea*), the parasite infests the nucleus of epithelial cells. The name *Cyclospora* is derived form the spherical oocysts excreted in the stool of patients. *Cyclospora* infection is less severe in children, 12% of the young (1.5 – 5 years old) children in Nepal, often even asymptomatic (Ortega, 1999).

The symptoms of cyclosporidiasis are a watery diarrhoea, abdominal cramping and a low grade fever, usually weight loss.

The illness has been observed world wide (North and South America, Europe, Asia and Australia). In the USA and Canada c. 850 cases of cyclosporidiasis have been reported in 1996, these cases were epidemiologically associated with the consumption of strawberries and raspberries. The next year again outbreaks were associated with (Guatemalan) raspberries. In 1998 import of raspberries from Guatamala was not permitted and no outbreak has been reported in the USA. A waterborne outbreak has been recorded in Nepal where 12 of 14 infected British soldiers developed a *Cyclospora* diarrhoea after drinking chlorinated drinking water, the oocysts were actually detected in the drinking water. Detection techniques are microscopical investigations of stool and PCR (18 s ribosomal RNA).

A single case of a Cyclospora infection in The Netherlands was observed in a travelar returning from Sri Lanka (Bänffler et al. 1996).

A waterborne outbreak of intestinal microsporidiosis in persons with and without HIV-infection has been studied in France 1993-1996 (Cotte et al., 1999).

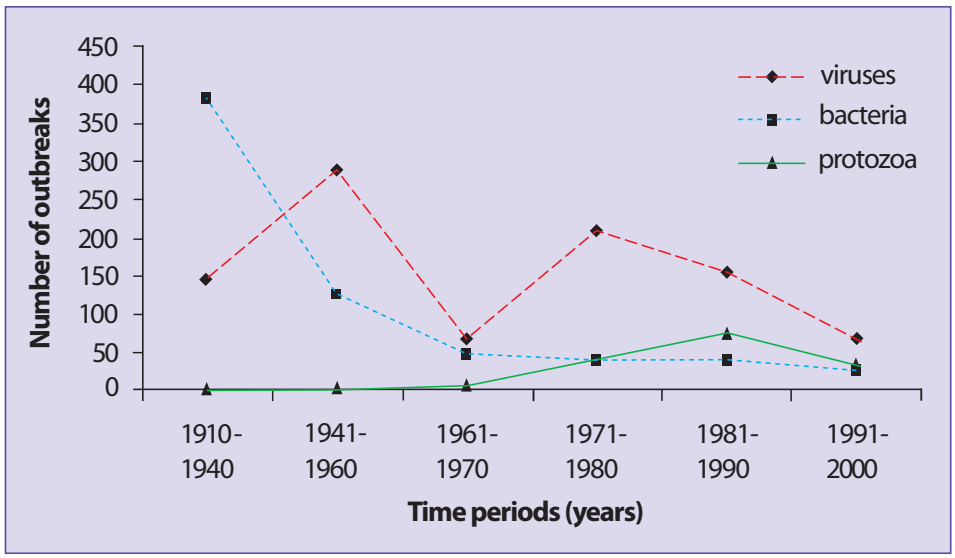
Parasitic metazoa such as helminths and other parasitic worms are not treated in this survey as this type of pathogens are not considered as important drinking water related pathogens in our region.

Tab. 7. Drinking water related outbreaks caused by parasitic protozoa in the USA over the period 1910 – 2000. (data after, Hunter, 1997; extended with Barwick et al. 2000 and Lee et al., 2002).

Disease	Outbreaks 1910 -1940	Outbreaks 1941 -1960	Outbreaks 1961 -1970	Outbreaks 1971 -1980	Outbreaks 1981 -1990	Outbreaks 1991 -2000
Protozoa						
Giardiosis			3	39	71	21
Cryptosporidiosis					2	11
Cyclosporidiosis					1	
Amoebiasis	2	2	3		1	

By comparing the numbers of outbreaks the three groups of pathogens show different trends. Outbreaks associated with viruses are over the whole period most numerous, those caused by bacteria are in general decreasing and those caused by protozoa are increasing in numbers (Fig. 2). The number of outbreaks is the most simple approach. A more detailed survey can be obtained by inclusion of the number of cases in the individual outbreaks and even better is to include the burden of the disease. However, the main goal of this study is to identify what types of pathogens may be of importance as future waterborne pathogens. Since viruses were discovered as pathogens later than bacteria and protozoa, less information on viruses in the first period (1910 – 1940) is presumed. This may explain the lower number of outbreaks in this period. On the other hand some overestimation may have influenced these data as physicians may have attributed a certain outbreak as viral when no causative bacteria or protozoa could be detected, thus without actual proof of a viral nature of the outbreak. Bacterial related outbreaks have decreased considerably and outbreaks caused by parasitic protozoa increase since the 1970-ies and are considered as important emerging pathogens.

Fig. 2. Number of waterborne disease outbreaks in the USA over a period of 90 years (data derived form Hunter, 1997, extended with Barwick et al. 2000 and Lee et al., 2002).



The most evident changes during the last 80 years in the USA (Fig. 2) in the number of outbreaks is the distinct decrease of the number of outbreaks caused by bacteria. Gastroenteritis from which no pathogen is identified remains a large group of the total outbreaks (44.2% over the whole period). This number has not changed much, since over the period 1990 – 2000 this ratio was 40,7%.
It is remarkable that outbreaks caused by *Shigella*, remain at a similar level over the whole period. Typhoid fever is significantly decreased. From this survey *Campylobacter*, *Giardia* and *Cryptosporidium* can be considered as emerging pathogens over a certain period of time.

4.4 Outbreaks in other countries

In Europe various outbreaks have been reported and a systematic review of these incidents (tab, 8) over a longer period has been written recently (Risebro et al, in press). A survey of drinking water outbreaks in thirteen European countries (Tab. 8; 1990 – 2004) , showed most outbreaks occurred in Finnish groundwater (14%) and English companies using surface water for drinking water production (8%), *Campylobacter* and norovirus outbreaks were observed mainly in northern countries (Finland and Sweden).

Table 8 Waterborne outbreaks in EU (13 countries), After Risebro et al, in press.

Pathogen	No. Outbreaks	no. cases	outbreaks/year	cases/year
Norovirus	8	11408	0,53	761
Virus (indet)	1	2500	0,07	167
Campylobacter	9	16222	0,60	1081
Shigella	3	531	0,20	35
Cryptosporidium	46	7772	3,07	518
Giardia	2	232	0,13	15
Mixed	5	2511	0,33	167
Gastro-enteritis	12	31370	0,80	2091
Total	**86**	**72546**	**5,73**	**4836**

The EU data show that 89% of the outbreaks associated with companies using surface water for drinking water production are caused by protozoa. In groundwater related outbreaks c. 50% are caused by protozoa, the remainder are caused by bacteria and viruses. Only in 45% of the outbreaks the pathogens were detected in drinking water during the outbreak. The indicator organisms were detected in 53% of samples taken during the outbreak. In raw water the figures are only slightly higher 53% of the samples contained the pathogen during the outbreak and in 71% of these sample indicator bacteria were detected (Risebro et al, in press).

Also the EU-Project MICRORISK (Medema et al. 2006) has focused on this subject. From individual reports it is clear that in Europe the same pathogens are involved in waterborne outbreaks.
A well-known outbreak is the Zermatt outbreak of typhoid fever in 1963 with over 400 cases and three persons have died from this epidemic. Two possible causes have been suggested: upstream contamination of one of the small rivers providing Zermatt with raw water and an insufficient chlorination in relation to the production due to the tourist season. Later a broken sewage pipe was found in the neighbourhood of the raw water reservoir, tests with fluorescein proved that sewage water was transported to the nearby reservoir.
Another outbreak was La Neuville (CH): due to a defect pump sewage infiltrated ground water and an outbreak of Shigella and Campylobacter took place (1600 cases).
Anderson and Bohan (2002) report a number of Swedish drinking water related outbreaks caused

by *Campylobacter, Giardia* and *Entamoeba* and unknown causative agent. These authors also report 710 outbreaks across Europe associated with recreational and drinking water with over 40,000 cases. Most identified pathogens in these outbreaks are amoebic and bacterial dysentery, *Cryptosporidium, Giardia, norovirus, Campylobacter*, typhoid fever, *salmonella*, Hepatitis A virus and a number of cholera outbreaks in Albania and Romania.
In Walkerton, Canada some 2000 persons became ill from *E. coli* O157:H7 when cattle manure contaminated drinking water .
In the Netherlands only three outbreaks have been recorded in the period after the second world war. In Rotterdam the sewage effluent pipe of a foreign ship was connected to a drinking water bunker point. This cross connection contaminated the local drinking water distribution network and c. 600 persons became ill. In Amsterdam a small outbreak occurred where only 10 persons became ill. More recently (2002) 200 people became ill (norovirus) after being exposed to water not completely purified due to cross connection with a technical water system.

An extensive survey of the current knowledge on waterborne pathogens is given by Guillot and Loret (2006).

4.5 Two types of pathogens

By evaluating outbreaks and literature on possible pathogens transmitted via the sewage wastewater route, it becomes clear that a distinction between various types of pathogens is needed. The first and best known group are those causing gastro intestinal illness (diarrhoea etc.) within a short incubation period. This type was first recognised as waterborne pathogens.
Pathogens of the second group need a much longer period to induce illness. Due to the much longer incubation period it is difficult to determine the link between the moment of infection and the first signs of illness. This makes the recognition of the actual source of the pathogen very difficult. The role of *Helicobacter pylori* and the development of ulcers and certain types of stomach cancer are only from a recent date. Recently these bacteria have been detected in USA drinking water from private wells. A statistically significant correlation between people using drinking water containing *H. pylori* and the number of ulcers has been observed (Baker, 1999).

Polyoma viruses are small DNA viruses infectious to a wide range of hosts including humans. These viruses seem to affect especially immunodepressed persons, it is lethal in 4% of the AIDS patients. Bofill-Mass cs. (2001) associate these viruses with the development of a brain illness (Dimyelinating disease) and colon-rectal tumours, Also urinary illness has been described concerning this type of virus. This virus is excreted via urine and faeces. Sewage samples have been investigated and concentrations up to thousands per litre have been found in sewage samples in Europe, Africa and the USA. The contamination route via sewage has not been demonstrated yet but seems to be quite likely (Mass-Bos et al., 2003).

As shown in figure 2 the number of outbreaks of parasitic protozoa is increasing, Giardia and Cryptosporidium are already well known, and a USA outbreak of Cyclospora has been mentioned already. However there are more types that may become more important in the future. For instance Microsporidia, this group of intra cellular parasites form a group (not a taxonomic unity) of more than 1000 species infecting various animals throughout the animal kingdom, varying from insects to mammals. In humans twelve pathogenic species have been recognised, different tissues such as brain, lung, intestine muscles may become affected by microsporidia. Illnesses induced by microsporidia are probably less often recognised because of difficult diagnosis and poor detection

techniques. These parasites are assumed to be a risk for immunecompromized persons. The oocysts are persistent. The evaluation of environmental samples on microsporidia is difficult since several oocyst from species affecting only animals may cause false positive results (Cali, 1999). Spores of some microsporidia are excreted via urine and faeces. Spores of microsporida have been detected in raw sewage and sewage effluent, surface water and swimming water. No quantitatvive data are available (Percival et al. 2004). One outbreak has been recorded in the literature so far (Cotte et al. 1999).

Another poorly known species is *Tropheryma whippelii*, this bacterium belonging to the Actinomycetes infects the wall of the human intestine, slowly diminishing the uptake of food, this illness is sometimes fatal. It is assumed that many persons are infected and only a few develop this remarkable illness (Herbay, 2005). As it is an intestinal illness it may contribute to the contaminants of wastewater.

Another group of organisms may become important when more evidence has been collected on the role nanobacteria may play as possible pathogens. Nanobacteria (0.2 µm) are much smaller than normal bacteria. Some investigators consider nanobacteria as the cause of kidney stones (Kajander & Ciftocioglu, 1998). Some nanobacteria form a cover of minerals around their cells (carbonate or phosphate based). An enormous conglomerate of these mineral covered cells is considered to be the kidney stone. Some species form a carbonate covering while other species form a phosphate based cover. According to the researchers this may be an explanation for the existence of different types of kidney stones. Drancourt and coworkers (2003) observed scanning electronmicroscopically nanobacteria like particles in four of four kidneystones. This investigation however, failed to isolate nanobacteria using cell culture techniques. Some 30 kidney stones were investigated, all contained antibodies against nanobacteria, which is considered as evidence favouring the idea of nanobacteria as the causative agent for kidney stones. However, other scientists have doubt on this interpretation: they claim that DNA sequences occurring in (presumed) nanobacteria resemble too much to DNA sequences found in organisms used for the production of the reagents for PRC-determination (Cranton, 2005).
It may be a bit too early to consider these nanobacteria as emerging waterborne pathogens, but when these bacteria are indeed the cause of kidney stones, then a connection between sewage wastewater and drinking water cannot be ruled out. Other illnesses are also considered to be a result of infections with nanobacteria such as hearth disease and even Alzheimer. If nanobacteria appear to be the agent for the development of kidney stones it will show us the usual way of identifying a new waterborne pathogen, since in The Netherlands c. 1% of the population suffers from kidney stones, probably causing a certain load on sewage effluents. These peculiar bacteria probably have a better resistance against disinfection due to the solid mineral coating of their cells. When more evidence of these pathogens has been collected attention should be paid to their removal during water purification processes.

From the collected data it is clear that not much wastewater related information is available. It is often not known whether a certain species is present in raw or treated sewage. The abundance of these organisms in terms of concentrations is therefore not known for most of them. Quantitative information for indicator organisms is often available.

4.6 Algal toxins

Cyanobacteria (blue greens algae) are able to produce toxins such as microcystin anatoxin and other types. Although not every strain of a potential toxic species may produce toxins, the trigger for toxin production is not understood. Wastewater containing nutrients may induce growth of the cyanobacteria, high concentrations of these blue greens are of concern for the drinking water companies (Falconer, 2005; Hoogenboezem et al 2004). The influence of sewage treatment plants is only indirect by increasing the nutrient level (phosphorus and nitrate) of the water facilitating algal growth. When climate change proceeds and water temperatures further increase more blooms of toxic cyanobacteria are expected to occur. Moreover very toxic species, as Cylindrospermopsis raciborskii may become dominant in Dutch surface waters (Mooij et al. 2005).

4.7 Prions

Another type of illness is caused by so-called prion, which are in fact pathogenic proteins causing mad cow disease (Bovine spongiforme Encephalopathy; BSE) and Creutzfeldt-Jacob disease. These proteins have not yet been found in wastewater, but they are known to decompose poorly (MacMahon & Benson, 2004) In New Guinea where people were known to eat brain material, this illness is known as Kuru (laughing death). It is not yet known weather prions form a risk for drinking water production or not. Gale and coworkers (1998) carried out a risk analysis for (BSE) prions, they concluded that prion protein is very sticky and will, consequently, stick readily to particles. Once attached to particles they are relatively easily removed in treatment processes. The level of disinfection during drinking water production would have little effect on (BSE) prions (Gale et al., 1998). The overall estimation of the infection risk via drinking water is regarded as extremely small (Gale et al 1998).

4.8 Survey of waterborne pathogens

A number of recent papers dealing with waterborne pathogens and mentioning Emerging waterborne pathogens were screened for the species mentioned and listed in tables 9 and 10. Some species were not found in these articles but were still included in the present list (Tab. 9 and 10) as they are considered to be potential waterborne pathogens. Astro, hepatis-B and Reo virus are apparently not considered as important waterborne pathogens, though they may be of importance to the drinking water industry. The same holds for Plesiomonas, Francisella and Leptosira although not many outbreaks of these species heave been reported they may be of interest. Also Three species of parasitic protozoa (Balantidium, Blastocystis and Isospora) have been included in this survey. Nanobacteria and trophyrema were included as presumed waterborne pathogens which may become of importance in the (near) future.

Table 9. Viruses and Bacteria referred to as emerging pathogen in literature ([1] Nwachcucu & Gerba, 2004; [2] Gannon et al. 2004; [3] Oldfield, 2001; [4] Köster et al, 2002; [5] Friedman-Huffman & Rose, 1998; [6] LeChevallier et al. 1999[a]; [7] LeChevallier et al. 1999[b] ; [8] Sharma et al 2003; [9] Howard & Inglis, 2005; [10] Anonymous, 2004; [11] Guillot & Loret 2006)

Waterborne pathogen	Disease	Reference
Viruses		
Calicivirus	Gastroenteritis	8
Hepatis-A virus	Hepatitis (liver infection)	4, 5, 7, 10
Norovirus	Diarrhoea	2, 4, 7, 10
Picornavirus		
Enterovirus	Various symptoms	7, 10
Polio	Poliomyelitis	7
Coxackievirus	Meningitis and other symptoms	7
ECHOvirus	Various diseases	7
Hepatitis-E-virus	Hepatitis (liver infection)	2, 5, 8, 10
Astrovirus	Diarrhoea	
Corona virus (SARS)	SARS	1, 2
Reovirus	Respiratory and gastrointestinal	
Rota virus	Diarrhoea	2, 4, 10
Hepatitis_B virus	Hepatitis	
Adenovirus	Various clinical syndromes	10
Parvo virus	Gastroenteritis	1
Circovirus	human disease still unknown (hepatitis like syndrome)	1
Polyoma virus	Demyelinating disease	1
Bacteria		
Vibrio cholera (type O139)	Cholera	2, 5, 8, 10
Campylobacter	Campylobacteriosis	2, 4, 6, 8, 10
E. coli (O157)	Haemorrhagic colitis	3, 6, 8, 10
Helicobacter pylori	Gastroenteritis and ulcer	2, 5, 6
Salmonella	Typhoid and Paratyphoid fever	3, 10
Shigella	Bacillary dysentery	10
Yersinia	Yersinia infections	4, 8, 10
Aeromonas	Aeromonas infections	8
Plesiomonas	Plesiomonas infections	
Burkholdia *pseudomallei*	Melioidosis	9, 10
Multiresitent Mycobacterium tuberculosis	Tuberculosis	2
Mycobacterium (avium intracellulare)	Various symptoms	1, 5, 6, 8, 10
Lysteria	Listeriosis	3
Legionella	Pneumonia	8, 10
Francisella tularensis	Tularaemia	
Nanobacteria	kidney stones (?)	
Pseudomnas auruginosa	Various symptoms	
Tropheryma whippelii (Actinomyceteae)	Intestinal disease	
Leptospira	Weils disease (liver, kidney)	

Table 10. Waterborne pathogens referred to as possible emerging pathogens in literature (protozoans and miscellaneous biological threats). ([1] Nwachcucu & Gerba, 2004; [2] Gannon et al. 2004; [3] Oldfield, 2001; [4] Köster et al, 2002; [5] Friedman-Huffman & Rose, 1998; [6] LeChevallier et al. 1999a; [7] LeChevallier et al. 1999b ; [8] Sharma et al 2003; [9] Howard & Inglis, 2005; [10] Anonymous, 2004; [11] Guillot & Loret 2006).

Waterborne pathogen	Disease	References
Parasitic protozoa		
Entamoeba histolytica / E. dispar	Amoebic dysentery	10
Naegleria fowlery	PAM (primary amoebic meningoencephalitis)	10
Acanthamoeba spp	GAE (Granulmatous amoebic Encphalitis)	10
Balantidium coli (Ciliophora)	Dysentery	
Blastocystis ghominis (Flagellata)	Diarrhoea	
Cryptosporidium	Cryptosporidiosis (diarrhoea)	2, 5, 8, 10
Giardia	Giardiasis (diarrhoea)	4, 10
Cyclospora cayetanesis	Cyclosporiasis (diarrhoea)	2, 3, 5, 7, 8, 10
Isospora		
Microsporidia (12 human pathogenic spec.)	Diarrhea (including other symptoms)	1, 5, 7, 8
Toxoplasma gondii	Congenital infection in children	2, 4, 7, 10
Miscellaneous		
Prion (pathogenic protein)	Bovine spongiforme Encephalopathy (BSE)	2
Cyanobacteria (toxins)	Hepathotoxin (liver), Liver cancer (?)	6

5 Discussion

Discussion

By building wastewater treatment plants along the river Rhine (and branches as IJssel, Lek, Lekkannaal and Amsterdam Rijnkanaal) the river water quality has improved much. Especially the organic load has decreased very much leading to a better oxygen content of the water. However, due to the fact that these treatment plants are not designed to eliminate pathogens efficiently the decrease of pathogens is not proportional to the organic substances. There is an elimination capacity of maximal 1 – 2 log units (90 -99%). Therefore the load of pathogens may be higher than one may expect on the basis of other improvements of water quality. Previous RIWA studies confirmed this, a considerable load of pathogens has been observed at Lobith (Hoogenboezem et al. 2001; De Roda-Husman et al. 2005)

The production of safe drinking water in the Netherlands has to meet certain safety standards. New legislation (Anonymous, 2001[a]) demands a maximum risk of infection of 10^{-4} thus a maximum of only one infection per 10,000 consumers per year. Consequently, drinking water production using surface water with a high load of water borne pathogens needs a high removal capacity.
As certain persistent pathogens survive longer in surface water, water from the delta is expected to contain more pathogens compared to the more pristine water quality at the source area of the river. Moreover a much larger input of wastewater cause a larger pathogen load in the lower parts of the river compared to the relatively unaffected waters in the upper parts of the catchment area.

Microbial investigations of raw, process and finished water have been based on indicator organisms already for many years. Removal of bacterial pathogens is well indicated by the elimination behaviour of faecal indicator bacteria. In the USA the number of outbreaks caused by bacteria have been reduced considerably (Fig. 2). Dutch legislation now requires also the investigation of entero-viruses and some persistent parasitic protozoa's (*Cryptosporidium* oocysts and *Giardia* cysts). Monitoring the elimination behaviour of these index pathogens will also ensure the removal of other viruses and persistent protozoa. This monitoring program will provide a more reliable drinking water quality. But when certain types possess quite other more persistent properties extra attention is needed, e.g. the waxy cell wall of mycobacterium (Nwachcuka & Gerba, 2004) or the mineral covered cell walls of nanobacteria may have much more resistance to disinfection and need therefore other removal characteristics. Species of extreme small size and those possessing a low iso-electric point are considered as potential risk organisms. A low hydrophobicity allow them to pass through treatment plants more easy than hydrophobic species (Nwachcuka & Gerba, 2004). Especially species possessing one or more of these characteristics are probably less susceptible for water treatment and may be recognized as emerging pathogens in the (near) future.
Another important aspect is the concentration of the pathogen, most drinking water plants can efficiently cope with concentrations of 1 – 10 entero-viruses per litre. However, when a new pathogen is found to occur in considerably higher densities special attention should be paid and further evaluation is required.

The analysis of the surveys of outbreaks is useful for the identification of waterborne pathogenic species that can be relevant to the Dutch situation. But a considerable difference between both counties is that drinking water in the USA is supplied by a vast number of relatively small drinking

water suppliers, including many private wells, with only limited treatment, the water is distributed with residual chlorine during transport. In the Dutch situation a small number of larger companies serving almost all Dutch inhabitants, hardly any private wells are used. These large companies distribute this water usually after a multi barrier treatment and the water is distributed without transport disinfection.
The surveillance of waterborne outbreaks in the USA and the reports written on this subject show us a gradual change over time: there is a distinct decrease in outbreaks caused by bacteria (Fig. 2).
From the evaluation of historic data it is expected that new emerging pathogens will belong to the group of viruses, although parasitic protozoa may also be important. In the list of Nwachcuka and Gerba (2004) a relatively large number of viruses is included, this agrees with the indication obtained form the historic survey in this study: it was concluded that especially viruses are suspected to become future emerging pathogens. These authors listed a number of viruses which have persistent characteristics. More in general they emphasize the risks of persistent types of bacteria viruses and protozoa as potential candidates for emerging pathogens.
The relation between the effluent of wastewater treatment plants and the microbial quality of surface water is rather difficult to determine.
Beside new viruses new emerging pathogens will probably belong to the second group of pathogens (longer incubation periods).
As we do not have quantitative information on the occurrence and densities on the “new” or emerging pathogens. It is therefore difficult to estimate possible risks of these pathogens, in relation to the production of safe drinking water from surface water.

New water borne pathogens and Emerging waterborne pathogens are found by using new detection techniques, especially PCR enables researchers to detect for instance none culturable species which was formerly not possible. The occurrence of bacteria with resistance against antibiotics need attention. The increase of people with sub optimal immune systems may increase the number of cases caused by pathogens not posing any serious threat to persons with a normal functioning immune system.

From the present evaluation it is clear that the Decimal Elimination Capacity (DEC) in wastewater treatment is only 1 – 2 log units. Both raw sewage and treated sewage contain high numbers of indicator organisms and (known) pathogens. The presence or densities of the new or emerging pathogens is usually unknown from sewage, treated sewage or surface waters. In this study it is assumed that micro-organisms affecting the gastro-intestinal tract or renal system are brought into the environment via sewage. Due tot the limited removal of indicators and known pathogens during sewage treatment it is assumed that removal of the new pathogens is similarly low. Some of the presented species are regarded as presumptive waterborne pathogens, their actual role as waterborne pathogen and the transmission route via water have to be demonstrated in more detail for some of these forms (e.g. nanobacteria and *Tropheryma*).
Due to the lack of knowledge on concentrations and physical properties of the new waterborne pathogens it is difficult to estimate the actual risk from these new emerging pathogens.

Which of the mentioned waterborne pathogens (tab. 9 and 10) will become an Emerging pathogen depends on many aspects and is hardly predictable. Important is to keep a close watch on developments within the medical research and the field of water technology.

6 Conclusions and recommendations

- Sewage is a potential risk for the transmission of pathogens via drinking water.
- Sewage treatment plants do not remove pathogens efficiently from wastewater.
- Due to the large population size in our part of the world the natural system of self purification in surface water is not reliable.
- Historic data can show us new types of pathogens being determined for the first time, in relation to drinking water.
- In this study is concluded that two types of potential emerging pathogens can be recognised:
  - Those with only a short incubation period and causing "classical" waterborne illness (Diarrhoea etc.).
  - Those pathogens causing other types of illness, after a much longer incubation period (*Helicobacter* and perhaps illness caused by nanobacteria).
- It is likely that new emerging pathogens (of the "first type") will be found in the group of viruses and parasitic protozoa.
- For the second type of pathogens it is much more difficult to predict future emerging pathogens, but unexpected forms as nanobacteria or other types may appear to have a transmission route via drinking water.
- For drinking water companies it will be of great importance to focus on those pathogens that are more persistent than better known pathogens. It is therefore important to find indicators matching the characteristics of the most persistent pathogens. After all, a treatment system shown to be reliable in the elimination of highly persistent pathogens will most likely also eliminate unknown but less persistent ones.
- It is for the time being difficult to evaluate the risks of raw sewage and treated sewage in surface water used for drinking water production. A better insight in the behaviour of micro-organism removal during sewage treatment, may be obtained by selection and study of some kind of persistent indicator during the process. In that way an impression of the behaviour of persistent pathogens can be obtained.

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Acknowledgements

Dr. Gertjan Medema (Kiwa water research) and dr. Peter G. Stoks (RIWA) are kindly acknowledged for the critical review and helpful comments on an earlier version of this report, their suggestions have improved the final version considerably.

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Colofon

Author: W. Hoogenboezem

Publisher: Association of River Waterworks – RIWA

Design: Meyson Communicatie, Amsterdam

Print: ATP Digitale Media

ISBN: 978-90-6683-125-4

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