Epidemiology and Public Health Significance of *Norovirus* in Switzerland

INAUGURALDISSERTATION

zur

Erlangung der Würde eines Doktors in Philosophie

vorgelegt der

Philosophisch-Naturwissenschaftlichen Fakultät

der Universität Basel

von

Rainer Fretz-Männel

aus

Opfikon ZH

Basel, 2004

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag von

Herrn Prof. Dr. Marcel Tanner, Herrn Prof. Dr. Alfred Metzler, Herrn Dr. Paul Svoboda, Herrn Dr. med. Dominik Schorr

Basel, den 6. April 2004

Prof. Dr. Marcel Tanner

Dekan

For Eveline

Table of Contents

Acknowledgement

I gratefully acknowledge the framework of cooperation between the Swiss Tropical Institute (STI) in Basel and the Cantonal Laboratory Basel-Landschaft in Liestal which allowed me to undertake the present thesis.

My sincerest thanks are addressed to my supervisor at the STI, Prof. Marcel Tanner (Director) and to Dr. Paul Svoboda (Head of the Division Microbiology of the Cantonal Laboratory Basel-Landschaft), who was in charge for the guidance trough my doctoral dissertation. I am very thankful for their constant providing of strong support and for giving me the opportunity to develop this thesis. I want to thank heartily Dr. Paul Svoboda for his coaching, mentoring and friendship.

I would like to thank cordially Dr. Andreas Baumgartner (Swiss Federal Office of Public Health, Division of Food Science) for his confidence in my work and for the many fruitful and stimulating discussions. Mr. Thomas Lüthi (Head of the Institute for Quality Management and Food Safety, University of Applied Sciences Wädenswil) should be thanked sincerely for his great support and advise in field epidemiology, especially during the conducting of the *Norovirus* outbreak investigations.

I am grateful to Dr. med. Dominik Schorr (Cantonal Surgeon Basel-Landschaft) for his enthusiasm and support and especially for his medical coordination within the frame of this thesis.

Furthermore, I would like to thank Mr. Louis Herrmann (Viollier AG, Department of Bacteriology) for his precious cooperation during the studies. Dr. med. Olivier Dubuis (Viollier AG, Department Head of Bacteriology) should be thanked for his confidence and for agreeing to build up this superb inter-laboratory cooperation. Many thanks are also addressed to Dr. med. Urs Schibli (Head of the Bakteriologisches Institut Olten AG) for the constructive and very straightforward cooperation.

In addition, I want to express my gratitude to Dr. Hans Schmid (Swiss Federal Office of Public Health, Division of Communicable Diseases) for his brilliant support and cooperation. Also, I want to thank Dr. Christian Beuret (Spiez Laboratory) for providing the RT-PCR methodology used and for his assistance in implementing the method in the Cantonal Laboratory Basel-Landschaft.

I am thankful to Dr. Niklaus Jäggi (Cantonal Chemist Basel-Landschaft) for harbouring my thesis at the Cantonal Laboratory Basel-Landschaft. A very warm thanks goes to the complete staff of the Cantonal Laboratory, especially to the persons from the microbiology department, for taking me into their middle. Especially Mr. Jürg Grimbichler must be further thanked for his laboratory support and teaching.

Many thanks are sent to Mr. Andri Christen for his friendship and for his excellent cooperation within his MSc thesis.

Furthermore, I am highly indebted to the many persons and institutions that are not mentioned here by name for their broad support during the different studies, namely within the numerous *Norovirus* outbreak investigations. Only with their goodwill and assistance it was possible to reach the defined goals within this thesis !

Most important: many thanks to my family, especially for their help and understanding during all these years. Finally, I want to thank my wife Eveline. Her precious love, understanding, coaching and patience made it possible to overcome the hard times during my thesis and to finalize this dissertation in such a successful way.

Ethic Commissions

All undertaken epidemiological studies were approved by the Ethikkommission beider Basel (project 42/01) and by the Ethikkommission des Kantons Solothurn (project EKO-0109).

Financial Support

This thesis was financed by the Swiss Federal Office of Public Health (project no 00.001332) and by the Cantonal Laboratory Basel-Landschaft. Further financial support was granted by the Swiss Tropical Institute (STI) in Basel, by the Swiss Society of Food Hygiene (SGLH) in Zurich, by the Basler Stiftung für experimentelle Zoologie in Basel and by the Josef und Olga Tomcsik Stiftung in Basel.

Summary

Epidemic and sporadic gastroenteritis is an important public health problem in both high-income and low-income countries. In the last 30 years, several viruses have been identified as etiological agents of gastroenteritis in humans. Outbreaks of gastroenteritis may be caused by rotaviruses, astroviruses, adenoviruses and the human caliciviruses. The human caliciviruses are assigned to two genera, the *Norovirus* (NV) and *Sapovirus* (SV). The NV cause illness in people of all age groups, whereas the SV predominantly cause illness in children. Epidemic viral gastroenteritis or "winter vomiting disease" was described as early as 1929 but it took over 40 years to the discovery of the Norwalk virus using immune electron microscopy (IEM) in faecal samples in 1972. These specimens were collected during an outbreak of acute gastroenteritis which occurred in 1968 in an elementary school in Norwalk, Ohio, USA.

Following an incubation period of approximately 1-2 days, persons infected with NV develop the main symptoms of projectile vomiting and diarrhoea, accompanied by rather unspecific symptoms like abdominal cramps, muscle pain, headache and in some cases low-grade fever. The illness generally is considered mild and self-limiting, with symptoms lasting in the mean 2-3 days. The potential of the NV to rise outbreaks with attack rates ranging between 30-90% is massive. This can be explained mainly by the high infectivity and environmental stability and by the facilitated spread of NV either by contaminated fomites (such as food and water) and environment, or directly from person-to-person. The faecal-oral route is described to be the most common route of transmission. Recent international studies have shown that NV infections are the most frequent cause of gastroenteritis in the community regarding the endemic and the epidemic situation. These viruses account for an estimated 6% and 11% of all infectious intestinal diseases in England and The Netherlands, respectively, and for an estimated 23 million cases in the United States each year. In the past ten years, NV-outbreaks were increasingly recognised in Switzerland. However, reliable epidemiological data were missing due to the fact that NV are not routinely searched for in diagnostic laboratories and there is no obligation to report known cases.

For this reason, the Swiss Federal Office of Public Health (SFOPH) launched a series of studies for a first epidemiological assessment of the situation of the NV in Switzerland. Within this program, several studies (also within the frame of this thesis) were conducted.

Three main study designs were used during this thesis: firstly, a NV screening of bacteriological-negative tested patient stool samples, secondly, a general practitioner (GP) based case-control study on sporadic NV infections and thirdly, a systematic compilation of epidemiological information on NV outbreaks from the whole country and the conducting of separate outbreak investigations.

The screening for the presence of NV in previously analysed human stool samples at least negative for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. from July 2001 to July 2003 revealed that 17.9% (125) of totally 699 stool samples tested positive for NV by RT-PCR. Additionally, a winter seasonality could be observed within both years under study. The highest rate of NV-positives (38.3%) was detected in the first quarter 2002. The time trend of the positivity-rate has to be seen in the context of a newly emerged variant of NV thought to possess certain characteristics like a higher virulence and/or a higher environmental stability than the previous circulating NV. Parallel to the mentioned study, a second screening was carried out to assess the importance of NV mix-infections. Only in one specimen of totally 132 bacteriologically-positive stool samples from gastroenteritis patients NV were detected.

The GP-based case-control study was performed between July 2001 and July 2003 in the German speaking part of Switzerland in order to identify risk factors for sporadic NV infections. Different transmission modes under study, e.g. the consumption of certain foodstuff and mineral waters, displayed no measurable risk association. These findings are consistent with person-to-person transmission as the most important route of transmission for community-acquired, sporadic NV infection, in that 39% of all patients reported they had had contact with ill persons before their illness. The fact that 33% reported contact with ill persons, mainly within family groups, after their own illness suggested that a substantial proportion of patients were part of family mini-outbreaks.

Between 2001 and 2003, a study was launched to compile actively and systematically NV outbreak information, mostly from the German speaking part of the country. In total, 73 NV-outbreaks were registered. Most affected were closed settings, like nursing homes (34% of all outbreaks) and hospitals (25%). Transmission pathways were identified in 74% of the outbreaks. In 81% of these cases person-to-person transmission was the primary route of infection and on seven occasions (13%), a foodborne transmission was the possible cause.

Finally, a broad phylogenetic analysis of the human NV sequences solely and in comparison with NV sequences obtained from a recent mineral water study and from an oyster screening in Switzerland was conducted. 63 of the 74 (85%) human NV sequences belonged to NV Genogroup II and a temporal clustering was observed within the NV sequences, corresponding to the described emergence of a new NV Genogroup II variant. The phylogenetic comparison revealed that the NV sequences derived from mineral waters were highly related and clustered predominantly separate to the human NV sequences. However, single human NV sequences were also found within the mineral water clusters. Additionally, a temporal correlation between the dates of the stool specimen with the period of bottling of the mineral waters was observed. The oyster sequences displayed a far greater variability and no specific clustering with either mineral water or human NV sequences was found.

The results from the present studies – together with the findings from earlier Swiss studies in the field of the NV – allowed for the first time the generation of an overview on the current epidemiological situation of the NV in Switzerland.

Zusammenfassung

Das weltweite Auftreten von sporadischen und auch epidemischen Gastroenteritis-Erkrankungen in den Industrie- und Entwicklungsländer stellt ein ernsthaftes Problem für die Öffentliche Gesundheit dar. In den letzten 30 Jahren konnten mehrere virale Gastroenteritis-Erreger identifiziert werden. So können Ausbrüche durch Rotaviren, Astroviren, Adenoviren und durch die humanen Caliciviren verursacht werden. Die letztere Virusgruppe wird in zwei Genera unterteilt: Noroviren (*Norovirus*, NV) und Sapoviren (*Sapovirus*, SV). An einer NV-Infektion können Personen jeglichen Alters erkranken, hingegen werden SV-Infektionen vor allem bei Kindern beobachtet.

Die in Epidemien auftretende virale Gastroenteritis, auch „Winter Vomiting Disease" genannt, wurde erstmals 1929 beschrieben. Jedoch mussten noch über 40 Jahre verstreichen, bis im Jahre 1972 der Norwalk Virus mittels der Immun-Elektronenmikroskopie (IEM) dargestellt werden konnte. Jene untersuchten Viren stammten ursprünglich aus einem Gastroenteritisausbruch in einer Grundschule in Norwalk (Ohio), USA, aus dem Jahre 1968.

Die ersten Krankheitssymptome einer NV-Infektion treten nach einer Inkubationszeit von ca. 1-2 Tagen in Erscheinung. Die Hauptsymptome sind explosionsartiges Erbrechen und Diarrhö. Diese Leitsymptome werden häufig begleitet durch unspezifische Symptome, wie Bauchkrämpfe, Muskel- und Kopfschmerzen und manchmal leicht erhöhte Temperatur. Die Erkrankung an sich ist selbstlimitierend und kann als mild eingestuft werden. Nach etwa 2-3 Tagen lassen die Symptome in den meisten Fällen nach und verschwinden gänzlich. Jedoch ist das epidemische Potential der NV massiv und es können Erkrankungsraten von 30-90% innerhalb eines Epidemienkollektives verzeichnet werden. Dieser Umstand kann einerseits durch die hohe Infektiosität und andererseits durch die grosse Umweltstabilität der NV erklärt werden. NV können durch kontaminierte Vektoren, wie Nahrungsmittel und Wasser, via kontaminierter Umwelt und vor allem von Person zu Person übertragen werden. Dabei spielt die fäkal-orale Übertragungsweise die bedeutendste Rolle. Internationale Studien konnten aufzeigen, dass die NV epidemisch, wie auch endemisch, den wichtigsten Gastroenterits-Erreger darstellen. Schätzungen ergaben, dass etwa 6% aller infektiösen Magen-Darmerkrankungen in England, respektive etwa 11% in den Niederlanden, den NV zugeschrieben werden müssen. In den USA wird vermutet, dass jährlich etwa 23 Mio. Personen an einer NV-Infektion leiden. In den letzten 10 Jahren wurde zwar eine steigende

Tendenz im Auftreten von NV-Ausbrüchen in der Schweiz festgestellt, jedoch erwies sich die epidemiologische Datenlage als ungenügend verlässlich, da einerseits die NV nicht routinemässig nachgewiesen werden und andererseits auch keine NV-Meldepflicht in der Schweiz besteht.

Um eine erste epidemiologische Einschätzung der Lage der NV in der Schweiz zu ermöglichen, wurden mehrere Studien vom Bundesamt für Gesundheit (BAG) lanciert. Im Rahmen dieses Programms wurden ebenfalls die vorliegenden epidemiologischen Studien durchgeführt, welche hauptsächlich aus den folgenden drei Studientypen bestanden: ein NV-Screening von bakteriologisch-negativen Patientenstuhlproben, eine auf Allgemeinpraktiker basierende Fall-Kontrollstudie mit sporadischen NV-Patienten und einer schweizweiten systematischen Erfassung von NV-Ausbruchsdaten mit vereinzelten Abklärungen von NV-Epidemien.

Das zwischen Juli 2001 und Juli 2003 durchgeführte NV-Screening von Patientenstuhlproben, welche zuvor negativ auf *Campylobacter* spp., *Shigella* spp. und *Salmonella* spp. getestet wurden, ergab eine NV-Positivitätsrate mittels RT-PCR von 17.9% (125 NV-positive von total 699 Stuhlproben). Zusätzlich konnte eine Wintersaisonalität in der Häufigkeit der positiven Befunde verzeichnet werden. Die höchste NV-Positivitätsrate (38.3%) wurde im ersten Quartal 2002 festgestellt. Der zeitliche Trend in der Häufigkeitsrate entspricht dem Auftreten eines neuen Stammes der NV Genogruppe II in Europa. Es wird vermutet, dass dieser Stamm im Vergleich zu älteren zirkulierenden NV eine höhere Virulenz und/oder eine grössere Umweltstabilität aufweist. Um die Relevanz von möglichen NV Mischinfektionen abzuschätzen, wurde ein zweites Screening mit bakteriologisch-positiven Stuhlproben von Gastroenteritis-Patienten durchgeführt. NV konnten lediglich in einer von total 132 bakteriologisch-positiven Patientenstuhlproben NV nachgewiesen werden.

Die auf Allgemeinpraktiker basierende Fall-Kontrollstudie, welche in der Zeit von Juli 2001 und Juli 2003 in der Deutschschweiz durchgeführt wurde, hatte zum Ziel, Risikofaktoren für die Erkrankung an einer sporadischen NV-Gastroenteritis zu identifizieren. Verschiedenen untersuchten Übertragungswegen, u.a. die Konsumation gewisser Nahrungsmittel und Mineralwasser, konnten keine Risikoassoziation zugeschrieben werden. Diese Resultate sind konsistent mit der postulierten Hauptübertragungsroute der Person-zu-Person Übertragung bei sporadischen und community-acquired NV-Infektionen. Dies wird gestützt durch die

Ergebnisse, dass 39% der NV-Patienten Kontakt zu zuvor erkrankten Personen und 33% der Patienten Kontakt zu danach erkrankten Personen, hauptsächlich innerhalb der Familie, aufwiesen. Die letzte Patientengruppe weist darauf hin, dass wahrscheinlich ein beträchtlicher Teil der NV-Patienten zu familiären Mini-Ausbrüchen gehörten.

Zwischen 2001 und 2003 wurden systematisch Daten über NV-Ausbrüche, vor allem aus der Deutschschweiz, gesammelt und ausgewertet. Insgesamt konnten Informationen von 73 Ausbrüchen zusammengetragen werden. Die meisten Epidemien fanden in geschlossenen Settings statt. So wurden die häufigsten Ausbrüche in Altersheimen (34%) und in Spitälern (25%) verzeichnet. Der Übertragungsweg konnte in 74% der NV-Ausbrüche identifiziert werden. In 81% jener Fälle konnte die Person-zu-Person Übertragung als hauptsächlicher Infektionsweg ermittelt werden. Lediglich bei 7 Ausbrüchen (13%) war eine Übertragung via mit NV kontaminierte Lebensmittel möglich.

Letztlich wurden die zusammengetragenen NV-Stämme aus den humanen Stuhlproben phylogenetisch ausgewertet und ebenfalls mit den NV-Sequenzen, welche aus der kürzlich durchgeführten Mineralwasserstudie und dem Austern-Screening stammen, verglichen. 63 der 74 (85%) humanen NV-Sequenzen konnten der NV Genogruppe II zugeschrieben und zeitliche Häufungen der NV-Sequenzen konnte festgestellt werden. Der phylogenetische Vergleich der NV-Sequenzen aus Humanproben mit jenen aus den Mineralwässern ergab, dass die Mineralwasser-Sequenzen einen hohen Verwandtschaftsgrad aufwiesen und sich überwiegend gesondert zu den humanen NV-Sequenzen clustern. Dennoch konnten vereinzelte humane NV-Sequenzen in den Mineralwässer-Cluster gefunden werden. Zusätzlich wurde eine temporale Korrelation zwischen den Stuhlprobeentnahmedaten mit der Abfüllperiode der Mineralwässer gefunden. Die NV-Sequenzen der Austernproben wiesen hingegen eine grosse Variabilität auf und zeigten kein Clustering; weder mit den NV-Sequenzen aus den Mineralwässern, noch mit jenen aus den Humanproben.

Die Ergebnisse dieser Studien – in Kombination mit den Resultaten früherer Untersuchungen in der Schweiz – erlaubten es nun zum ersten Mal einen Überblick über die epidemiologische Situation der NV in der Schweiz zu generieren.

1. Introduction

1.1 Background

Gastroenteritis is one of the most common and, in public health terms, most important diseases in man. During the first 5 years of life, every child will contract diarrhoeal disease, and with it comes the risk of dehydration and nutritional deficiency (1). Gastroenteritis in children in low-income countries is one of the main reasons for child mortality. In high-income countries, mortality is rare, but it is nonetheless an important cause of morbidity and economic cost (1-2). In England and Wales, one out of every five people has a case of infectious intestinal disease (IID) annually (3). In The Netherlands, the incidence of gastrointestinal diseases was also found to be high, with 283 episodes per 1000 person-years (4). The burden of illness is highest in the young and elderly (3). In the last years, *Norovirus* (NV) outbreaks have regularly occurred in Switzerland, in Europe and in the US (5-6). Nowadays, NV are considered to be the most common cause for human viral gastroenteritis (2).

1.2 Taxonomy and Genetic Classification

Noroviruses (NV) belong to the family of Caliciviridae and include human and animal pathogens. They are non-enveloped, positive-sensed, single stranded RNA viruses and the virion is about 28-35nm in diameter (7). The family Caliciviridae was recently divided into four designated genus: *Lagovirus*, *Vesivirus*, *Norovirus* and *Sapovirus* (8-9). Unlike the NV and sapoviruses (SV), members of the lagoviruses and vesiviruses are principally of veterinary importance. Based both on morphology and genome sequence and organisation, NV and SV are grouped as separate genera (1). The NV genus branches into at least three distinct Genogroups (GGI, GGII and GGIII) based on genetic divergence of the RNA polymerase and in the capsid region (Figure 1). GI and GII infect humans and each Genogroup includes several genetic clusters, whereas GIII infects pigs and cows (10-11). GGI comprises approximately seven clusters including the prototype Norwalk, Southampton and Desert Shield reference stains and GGII comprises approximately ten genotypes including the Snow Mountain, Toronto, Bristol and Hawaii reference strains (1,10).

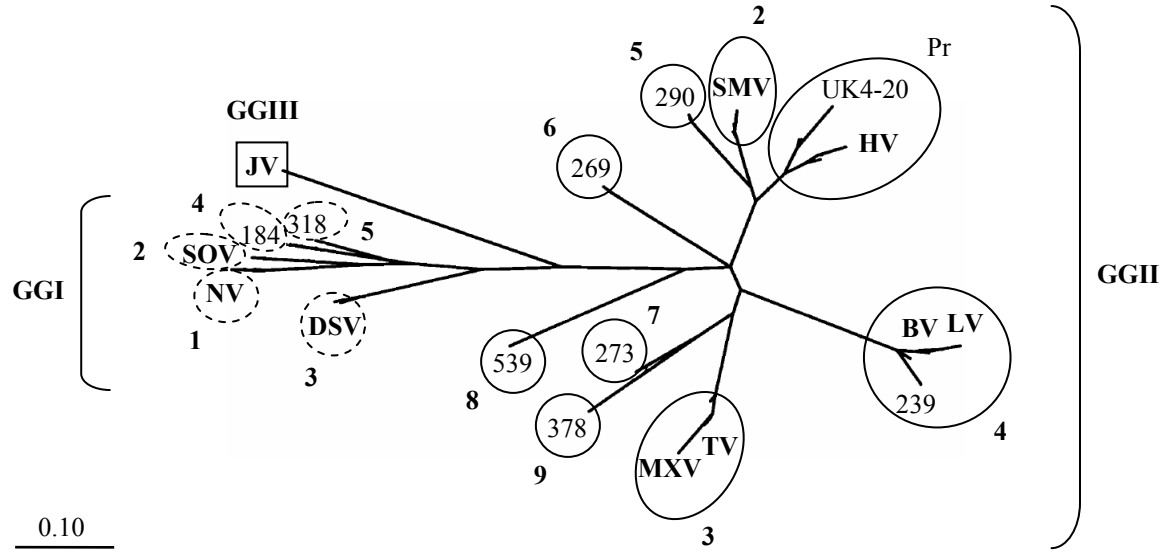


Figure 1: Unrooted phylogenetic tree of NV Genogroups I to III (GGI-III), modified after Ando et al., 2000 (10). Genetic clusters are enclosed by circles or a square. Human pathogenic clusters belonging to GGI are enclosed by a dashed circle and clusters belonging to GGII are surrounded by closed circles. The GGIII cluster is enclosed by a square. Not all described clusters are plotted in the phylogram. The following prototype strains are included: SMV = Snow Mountain virus, HV = Hawaii virus, JV = Jena virus, SOV = Southampton virus, NV = Norwalk virus, DSV = Desert Shield virus, BV = Bristol virus, LV = Lordsdale virus, TV = Toronto virus, MXV = Mexico virus. Dimension of circles and square were randomly chosen.

1.3 Clinical Picture

In the absence of other factors, infections in immunocompetent patients with NV are typically mild and self-limiting (1). The onset of illness is abrupt, usually within 12–48 h after exposure and the duration of illness is with 12–72 h relatively short (6,12). The main symptoms are projectile vomiting and diarrhoea, accompanied by abdominal cramps, nausea, muscle pain, headache and sporadic low-grade fever. But it is the high frequency and intensity of projectile vomiting that distinguishes NV from other viral and common bacterial enteric pathogens (1,6). On very rare occasions, NV infections may be lethal in persons belonging to risk groups (e.g. the elderly) due to serious dehydration (2). Further, the underlying health condition of NV patients seem to have no influence on the course of disease (13). The shedding of infectious virions may occur at least 2-3 days (up to two weeks) subsequent to the ending of clinical symptoms (6,11). Patients suffering from NV infection can only be treated supportive, mainly by compensation of the fluid loss (2). It is important to note that besides the typical NV symptoms, further atypical symptoms (e.g. prolonged or interrupted courses of disease) and asymptomatic disease may occur (1,6,11).

NV are contracted by humans via the oral route. As acid-stable viruses they pass through the stomach; replication is thought to occur in the small intestine (1,11). It was shown that individuals with clinical illness exhibit lesions on the small intestinal mucosa. The mucosa lining becomes inflamed and absorptive epithelial cells develop an abnormal appearance. Within two weeks, however, the small intestine returns to a normal histological appearance (1). Although some degree of short-term immunity appears to be present, long-term immunity seems not to exist. This circumstance is demonstrated by the high incidence of NV antibodies in otherwise healthy adults even though most of them would have been previously infected in childhood (1). It has been observed that some persons involved in NV outbreaks did not establish the NV illness even if they were exposed heavily to the agent. An explanation for this can be found in differences of genetic susceptibility. Variations in the local immune response of the intestinal mucosa or a genetic characteristic (e.g. specific receptors, AB0 histo-blood group type) may explain why some individuals can develop NV illness and others cannot (1,11,14). A recent study revealed that antibody is broadly cross-reactive across GGI stains, whereas genetically similar GGII strains were shown to be antigenically distinct (15). The overall reason for the lack of sustained immunity gained after NV infections could be associated with the high diversity within NV strains and Genogroups as a result of the great mutation rate of those viruses (6,7,11).

1.4 Diagnosis of *Norovirus* Infection

Although the syndrome associated with caliciviral gastroenteritis was described in the medical literature over 70 years ago (1), the Norwalk virus prototype was discovered in 1972, four years after an outbreak of gastroenteritis in an elementary school in the US (Norwalk, Ohio) (16). In the following years, NV (besides rota-, astro- and adenoviruses) were increasingly recognised as causes of acute gastroenteritis. But the real medical relevance of the NV was not acknowledged until the 1990ies (6). An explanation for this can be found in the inability to cultivate NV until to date. Further, NV were mostly detected by electron microscopy (EM) or by immune electron microscopy (IEM) previous to the establishment of molecular detection methods like the polymerase chain reaction (PCR). Under the electron microscope, NV can be identified by their characteristic morphology. Approximately 10^6–10^7 virus particles per ml stool is required for visualisation by EM; therefore, this technique is useful only for specimens collected during the early stages of illness when substantial quantities of virus are shed (1,11). Thus, the virus can be found by EM in only 10%–20% of faecal

specimens collected on days 2 or 3 of illness. IEM can improve the sensitivity of EM by 10- to 100-fold. However, the success of the IEM detection is highly dependant on the skill and expertise of the microscopist. Furthermore, the virus might be totally masked if a large excess of antibody is present, resulting in a false-negative test (11). Immunoassays, like enzyme-linked immunosorbent assays (ELISA), are showing an increased sensitivity compared to the IEM detection methodology, but their use in diagnostic laboratories has been limited by their narrow specificity and failure to detect the majority of these genetically diverse viruses (1). A recent study could demonstrate that even an improved ELISA system is still not appropriate for the detection of NV on a larger scale (17). Therefore, the PCR detection methodology (particularly the reverse transcription-polymerase chain reaction, RT-PCR) must still be seen as the golden standard (6,17). RT-PCR is able to detect NV up to two weeks after infection and possibly longer (1). The sequencing of the RT-PCR amplicons, while expensive and labour-intensive, offers the most virological information. Sequences from various strains (e.g. human origin and environmental origin) can be compared and may provide an indication about possible chains of infection (6).

In Switzerland, NV are still not routinely searched for in diagnostic laboratories. Therefore, the ability to detect NV is limited to a few medical diagnostic and cantonal laboratories (6). However, NV infections – especially within outbreaks – can be confirmed with a high probability by epidemiological profiling. This profiling is based upon the following syndrome of the NV-infection and further epidemiological characteristics (6,12,18): The incubation period varies between 1-2 days (range: 12-48 hours); main symptoms are vomiting (frequently explosive) and diarrhoea (sometimes profuse), partially accompanied by nausea, abdominal pain and cramps, muscle pain, headache and sporadic low-grade fever; in analysed patient stool samples, pathogenic bacterial and parasitic agents of gastroenteritis are typically not detected; secondary cases are typical within NV-outbreaks; more than 50% of patients are suffering from vomiting; there are more patients with vomiting than with fever and adolescent patients are suffering predominately from vomiting whereas adult patients are suffering predominately from diarrhoea. However, this method of profiling cannot be used to confirm a certain viral aetiology (e.g. differentiation between NV and SV is not possible), but is a strong tool to conduct a fast and first assessment of a NV-suspicious outbreak scenario (6). Table 1 summarises the key characteristics used for epidemiological profiling in Switzerland.

Table 1: Characteristics used for epidemiological profiling of NV-outbreaks in Switzerland.

Symptoms of NV infection	
Main symptoms	**Possible accompanying symptoms**
• Diarrhoea and/or • Vomiting	• Abdominal cramps • Muscle pain • Nausea • Headache • (Low-grade) fever
Epidemiological characteristics of NV infections	
• No detection of bacterial and parasitic pathogens	
• Description of vomiting:	Frequently projectile, noncontrollable, >50% of patients with vomiting
• Secondary cases:	Often, typical
• Incubation period:	Approximately 1-2 days (12-48h)
• Duration of illness:	Approximately 2-3 days (12-72h), may be prolonged
• Ratio vomiting vs. diarrhoea:	Children / adolescents: more vomiting than diarrhoea Adults: more diarrhoea than vomiting

1.5 Occurrence of Noroviruses in Europe

Community-Based Studies

A recent study in The Netherlands found that the incidence of infectious gastroenteritis was 283 cases per 1000 person-years. In the case-control component of the study, viral agents accounted for 34% of all cases, with NV the most common viral pathogen, accounting for 11% of cases. SV were found in 6% of all cases (4). Similar, results from the England's IID study revealed an overall rate of 194 cases per 1000 person-years (3). The rate of NV infection was 13 cases per 1000 person-years (6% of all cases) and the rate of SV was 2.2 cases per 1000 person-years (0.01% of all cases) (1,3). In Finland, a study revealed that in toddlers (2 months to 2 years of age) NV were responsible for 20% of cases and SV for 9%. Together, NV and SV were found with similar frequency as rotaviruses (19).

General Practice-Based Studies

England's IID study also showed that NV and SV rates, when measured by presentation to a general practitioner (GP), correspond to approximately one sixth of all community cases. However, there may be a substantial under-representation of community cases since institutions where outbreaks may be disproportionately frequent (e.g. residential homes) were excluded from the study population (1,3). Nevertheless, 6.5% of the cases presenting to a GP tested positive for NV and 1.5% tested positive for SV (20). A comparable GP-based study in The Netherlands found NV slightly less frequently (5.0% of cases) and SV were detected in 2.0% of cases (21).

Surveillance

A system of general outbreak surveillance for IID in England and Wales has been operated from the Communicable Disease Surveillance Centre (CDSC) since 1992. From the year 1992 to the year 2000, 5'421 general outbreaks of gastroenteritis were registered. Laboratory confirmation of NV was recorded for 36% of these outbreaks. Another 14% of all outbreaks were suspected of being caused by viral agents (13). Germany has introduced a NV-specific reporting system in 2001 (22). In the year 2002, 47'000 cases of NV infection were registered. The NV incidence for the year 2002 in Germany was calculated to be 57 cases per 100'000 persons (23). In England and Wales, Germany, and in The Netherlands, a striking increase in NV outbreaks occurred in 2002. This coincided with the emergence of a new predominant NV GGII/4 variant (24). A very similar situation could be observed in the US regarding an increase in the number of outbreaks and in the occurrence of a new dominant GGII/4 strain (25). Overall, the systems of NV surveillance differ substantially within the different European countries (26) and therefore overview data is hardly available from these countries. In Switzerland, NV are not routinely searched for in diagnostic laboratories and there is no obligation to report known cases to date (6), apart from the obligatory reporting of outbreaks registered by the cantonal health authorities (Epidemiengesetz SR 818.101).

Seasonality

Between 1992 and 2000, in England and Wales, NV outbreaks began increasing in September and peaked in the months of January, February, and March. Outbreaks in hospitals and residential facilities occur more frequently in the 6 months from November to April than the rest of the year, but NV outbreaks in other settings displayed no winter peak (13). This finding is consistent with the results of a study from the US which examined the pattern of

NV disease occurrence in several countries and found that the low point for disease reports for both sporadic cases and outbreaks occurred in the summer months (27). The winter seasonality was confirmed also by other studies (2,11).

Age Distribution Within Patients

NV and SV infections can occur at any age. The highest incidence of these infection can be found in children under 5 years and, among children, the most common cause of gastroenteritis is viral, with NV being at least as frequent as rotavirus (4,19-20). GP data from The Netherlands and England suggests that the odds of seeing a doctor because of NV infection generally decreases with increasing age (21,28). Unlike SV, NV infection is common among adults (4,28). It should be remembered that GP-based studies essentially measure consultation rates, not infection rates. Since NV and SV typically cause a fairly mild infection, rates derived from GP settings may be inaccurate and biased towards children, who may be more likely to consult a doctor (1). Frequenting a doctor is generally associated with more severe illness (29). Further, it is well known that nursing homes are considered to be settings with a high risk for NV outbreaks, consequently a large number of outbreaks were recorded (13,23,25). However, attack rates are only slightly lower among staff than among elderly residents (1). In a broad English survey published in 1993, more than 70% of over 3000 analysed serum specimens were tested positive for recombinant Norwalk virus antibody particles (rNV). Antibody prevalence was highest among the middle age and elderly. In the age groups older than 30 years, antibody prevalence was >80%. Prevalence was also high among infants (up to 6 months) at 75%. This is likely a measure of maternal antibodies, reflecting the high seroprevalence among adults. In the 6–11 months age, antibody prevalence was 25%, then rising through adolescence and young adulthood (1,30). A very similar pattern was found in 1995 in Sweden (antibodies to Norwalk virus) with overall prevalence at approximately 80% (1,31). Viruses closely related to the original Norwalk virus are rarely found in molecular studies, yet seroprevalence of antibody to Norwalk virus is high (1) Antibodies to Southampton virus (another GGI virus), which was in the late 1990ies more commonly recognised was found to have a much lower seroprevalence of only 30% (1,32). To obtain an up-to-date overview, parallel studies using harmonised assays are needed to compare seroprevalence rates across countries (1).

1.6 Transmission Routes and Settings

NV exhibit a high stability against various environmental influences and conditions. NV are stable at temperatures from -20°C to 60°C and survive relatively high concentration of chlorine (up to 10 ppm) and variations in pH-values (11). There is little information about the length of environmental stability. Results from an English study revealed that NV may survive up to 12 days on a contaminated carpet (33-34). In general, NV transmission occurs via the faecal-oral route, following contamination of fomites by stool and vomitus and subsequent ingestion of virus particles. NV are highly infective, mainly due to the low infectious dose of 10-100 virus particles and their high environmental stability (11). NV cause illness and outbreaks through a number of transmission routes including person-to-person, foodborne and waterborne routes and environmental contamination. Person-to-person transmission has been documented by two routes, faecal–oral and aerosol formation following projectile vomiting. (1,6). Because of the aerosolisation, NV particles can be transmitted over distances easily exceeding the range of person-to-person transmission (1,6). NV outbreaks were often the result of a mixture of more than one mode of transmission (1).

Person-to-Person Transmission

Overall, the person-to-person spread is the most common mode of transmission in outbreaks (1,6,13). The investigation of 1877 NV-outbreaks between 1992 and 2000 in England and Wales showed clearly the domination of the person-to-person transmission: in 85% of all outbreaks (1’599) this infection route was the cause of outbreak (13). The airborne transmission route plays a key role within the person-to-person spread because of the high frequency of vomiting and the resultant aerosolisation of particles associated with NV illness (1,6). Since respiratory infection has not been found, aerosolised virus must presumably be swallowed after inhalation to cause infection (1).

Foodborne Transmission

Estimates of the relative importance of foodborne transmission of NV vary greatly from country to country. In Sweden, 16% of NV outbreaks from 1994 to 1998 were associated with food- or waterborne transmission; in the UK food was implicated in 5% of outbreaks from 1992 to 1999 as were 17% of outbreaks reported in The Netherlands (35). Newer data from the examination of 1877 NV-outbreaks between 1992 and 2000 in England and Wales showed that the foodborne transmission pathway was effective in 93 outbreaks (5%) and that in

further 91 outbreaks (5%) foodborne followed by person-to-person spread in (13). In contrast, a study from the US, postulated that 39% of the 348 registered NV outbreaks between 1996 and 2000 happened due to foodborne infection and only 12% due to person-to-person spread (11). Biases in different surveillance systems partly explain the wide variation in estimates of the levels of foodborne transmission in NV outbreaks, e.g. in the US, foodborne outbreaks were more likely to be reported because surveillance may be focused on detecting foodborne outbreaks (1,36). Foodborne vehicles of NV infection are typically one of three forms: contaminated shellfish, items contaminated by infected food handlers or fruits/vegetables contaminated through irrigation or washing. (1,37). Since NV can probably be destroyed by adequate cooking at 90°C for a short time, vehicles contaminated by infected food handlers are typically products eaten raw (e.g. salads) or not cooked after handling (e.g. sandwich fillings) (1). In many foodborne outbreaks, a food-handler who was ill prior to or during preparation of the implicated food can be identified. Furthermore, fruit and vegetables can become contaminated by irrigation waters or by washing prior to freezing or by infected food handlers involved in harvesting (1). Generally, a wide range of food types were reported as vehicles of infection, including oysters, salad vegetables, poultry, red meat, fruit, soups, desserts (13).

Waterborne Transmission

Drinking water can provide a source for outbreaks of viral gastroenteritis in nearly any setting. Outbreaks have occurred as a result of contamination of private wells, public wells as well as small and large-scale community water systems (1). Waterborne outbreaks with NV were shown to be associated with contaminated septic tanks, industrial water system and swimming water as well as drinking water worldwide (38). Two waterborne outbreaks occurred in 1998 and in 1999 in Switzerland. The first outbreak with 3500 patients was a result of a pump failure producing a spill of sewage into the groundwater (39), the second outbreak with 1400 cases occurred due to the usage of contaminated and accidentally untreated surface water (6). But there is a strong tendency that such outbreaks, at least in Switzerland, are most often the result of deficiencies in the infrastructure or in the water treatment process (40). Commercial distribution and production of ice was found also to be a vector (1,41). Two Swiss studies detected NV sequences in commercially available mineral waters, though no cases of NV illness were linked (42-43).

Environmental Contamination

Environmental contamination with NV is a logical consequence regarding the projectile vomiting and diarrhoea. Several studies showed that environmental contamination played a key role in NV outbreaks. These studies had clearly shown that NV-particles may keep their infectivity a long time (33-34,44). On carpets for example, NV particles stay infectious up to 12 days (33).

Settings of NV Outbreaks

The cited investigation of 1877 NV-outbreaks between 1992 and 2000 in England and Wales showed the following situation: 40% of the outbreaks occurred in hospitals, 39% in residential-care facilities, 8% in hotels, 4% in schools, 6% were linked to food outlets and the remaining 4% occurred in other settings (13). These proportions were confirmed in a further study in the year 2002 for England and Wales (45). Interestingly, one of the most affected setting, the hospitals, did not appear in the study from the Centers for Disease Control and Prevention (11). As mentioned, this study is probably biased due to over-representation of foodborne outbreaks (1,36).

Zoonoses

NV (and caliciviruses in general) are also important pathogens in animals. However, numerous unsuccessful attempts to infect animals as well as cell lines with NV suggests that NV and SV are highly species-specific pathogens (1,46). Up to date, no zoonotic transmission of NV were found (47). Additional, it could be revealed that the NV strains in animals (calf and pig) were genetically distinct to any NV found in people (47). Interspecies transmission, if it does occur, is likely to be a very rare event (1).

1.7 References

1. Lopman BA, Brown DW, Koopmans M (2002) Human caliciviruses in Europe. J Clin Virol 24:137-160
2. Künkel U and Schreier E (2002) Caliciviren, virale Auslöser akuter Gastroenteritiden. Bundesgesundheitsbl – Gesundheitsforsch – Gesundheitsschutz 45:534–542
3. Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, Hudson MJ, Roderick PJ (1999) Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. BMJ 318:1046-1050

4. de Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J, van Leusden F, Bartelds AI, van Duynhoven YT (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol 154:666-674

5. Wilhelmi I, Roman E, Sanchez-Fauquier A (2003 Viruses causing gastroenteritis. Clin Microbiol Infect 9:247-262

6. Fretz R, Svoboda P, Schmid H. Baumgartner A (2003) Durch Noroviren verursachte Gastroenteritis – eine Übersicht. Bulletin SFOPH 46:828-833

7. Koopmans M, von Bonsdorff CH, Vinje J, de Medici D, Monroe S (2002) Foodborne viruses. FEMS Microbiol Rev 26:187-205

8. van Regenmortel MH, Fauquet CM, Bishop DHL et al. (eds.) (2000) Virus taxonomy: Seventh report on the international committee on taxonomy of viruses. Academic Press, San Diego, Wien, New York

9. Virus taxonomy: Reports of the International Committee on Taxonomy of Viruses (ICTV) (2002) Internet: http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fr-fst-a.htm, last updated: 2002-06-28, downloaded: 2004-03-02

10. Ando T, Noel JS, Fankhauser RL (2000) Genetic classification of Norwalk-like viruses. J Infect Dis 181(Suppl 2):S336-S348

11. CDC (2001) "Norwalk-Like Viruses": public health consequences and outbreak management. MMWR Recomm Rep 50(RR-9):1-18

12. Lüthi TM (1998) Ermittlung eines möglichen Ursprungs gastrointestinaler Gruppenerkrankungen durch die Kombination klinischer, bakteriologischer und epidemischer Kriterien. Mitt Lebensm Hyg 89:196-218

13. Lopman BA, Adak GK, Reacher MH, Brown DW (2003) Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. Emerg Infect Dis 9:71-77

14. Hutson AM, Atmar RL, Graham DY, Estes MK (2002) Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis 185:1335-1337

15. Noel JS, Ando T, Leite JP, Green KY, Dingle KE, Estes MK, Seto Y, Monroe SS, Glass RI (1997) Correlation of patient immune responses with genetically characterized small round-structured viruses involved in outbreaks of nonbacterial acute gastroenteritis in the United States, 1990 to 1995. J Med Virol 53:372-383

16. Kapikian AZ (2000) The discovery of the 27-nm Norwalk virus: an historic perspective. J Infect Dis 181(Suppl 2):295-302

17. Christen A. Fretz R. Tanner M. Svoboda P (2003) Evaluation of a commercial ELISA kit for the detection of Norovirus antigens in human stool specimens. Mitt Lebensm Hyg 94:594-602

18. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW (1982) The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am J Public Health 72:1329-1332

19. Pang XL, Honma S, Nakata S, Vesikari T (2000) Human caliciviruses in acute gastroenteritis of young children in the community. J Infect Dis 181(Suppl 2):S288-S294

20. Tompkins DS, Hudson MJ, Smith HR, Eglin RP, Wheeler JG, Brett MM, Owen RJ, Brazier JS, Cumberland P, King V, Cook PE (1999) A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun Dis Public Health 2:108-113

21. de Wit MA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Bartelds AI, van Duynhoven YT (2001) Gastroenteritis in sentinel general practices, The Netherlands. Emerg Infect Dis 7:82-91

22. Höhne M, Schreier E (2004) Detection and characterization of norovirus outbreaks in Germany: application of a one-tube RT-PCR using a fluorogenic real-time detection system. J Med Virol 72:312-319

23. RKI (2003) Erkrankungen durch Norwalk-ähnliche Viren (Noroviren). Epidemiologisches Bulletin 6:39-43

24. Lopman B, Vennema H, Kohli E, Pothier P, Sanchez A, Negredo A, Buesa J, Schreier E, Reacher M, Brown D, Gray J, Iturriza M et al. (2004) Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet 363:682-688

25. CDC (2003) Norovirus Activity – United States, 2002. MMWR 52:41-45

26. Lopman BA, Reacher MH, Van Duijnhoven Y, Hanon FX, Brown D, Koopmans M (2003) Viral gastroenteritis outbreaks in Europe, 1995-2000. Emerg Infect Dis 9:90-96

27. Mounts AW, Ando T, Koopmans M, Bresee JS, Noel J, Glass RI (2000) Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J Infect Dis 181(Suppl 2):S284-S287

28. IID Study Executive (2000). A report of the study of infectious intestinal disease in England. The Stationery Office Books, UK

29. Tam CC, Rodrigues LC, O'Brien SJ (2003) The study of infectious intestinal disease in England: what risk factors for presentation to general practice tell us about potential for selection bias in case-control studies of reported cases of diarrhoea. Int J Epidemiol 32:99-105

30. Gray JJ, Jiang X, Morgan-Capner P, Desselberger U, Estes MK (1993) Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. J Clin Microbiol 31:1022-1025

31. Hinkula J, Ball JM, Lofgren S, Estes MK, Svensson L (1995) Antibody prevalence and immunoglobulin IgG subclass pattern to Norwalk virus in Sweden. J Med Virol 47:52-57

32. Pelosi E, Lambden PR, Caul EO, Liu B, Dingle K, Deng Y, Clarke IN (1999) The seroepidemiology of genogroup 1 and genogroup 2 Norwalk-like viruses in Italy. J Med Virol 58:93-99

33. Cheesbrough JS, Green J, Gallimore CI, Wright PA, Brown DW (2000) Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. Epidemiol Infect 125:93-98

34. Evans MR, Meldrum R, Lane W, Gardner D, Ribeiro CD, Gallimore CI, Westmoreland D (2002) An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiol Infect 129:355-360

35. Koopmans M, Vinje J, de Wit M, Leenen I, van der Poel W, van Duynhoven Y (2000) Molecular epidemiology of human enteric caliciviruses in The Netherlands. J Infect Dis 181(Suppl 2):S262-S269

36. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) Food-related illness and death in the United States. Emerg Infect Dis 5:607-625

37. Beuret C, Baumgartner A, Schluep J (2003) Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland. Appl Environ Microbiol 69:2292-2297

38. Nygard K, Torven M, Ancker C, Knauth SB, Hedlund KO, Giesecke J, Andersson Y, Svensson L. Emerging genotype (GGIIb) of norovirus in drinking water, Sweden (2003) Emerg Infect Dis 9:1548-1552

39. Maurer AM, Stürchler D (2000) A waterborne outbreak of small round structured virus, campylobacter and shigella co-infections in La Neuveville, Switzerland, 1998. Epidemiol Infect 125:325-332

40. Baumgartner A. (2001) «Norwalk-like»-Viren (NLV) und Lebensmittel – eine Situationsanalyse für die Schweiz. Bulletin SFOPH 46:909-916

41. Pedalino B, Feely E, McKeown P, Foley B, Smyth B, Moren A (2003) An outbreak of Norwalk-like viral gastroenteritis in holidaymakers travelling to Andorra, January-February 2002. Euro Surveill 8:1-8

42. Beuret C, Kohler D, Lüthi T (2000) Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction in mineral waters imported into or bottled in Switzerland. J Food Prot 63:1576-1582

43. Beuret C, Kohler D, Baumgartner A, Lüthi TM (2002) Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl Environ Microbiol 68:1925-1931

44. Kuusi M, Nuorti JP, Maunula L, Minh NN, Ratia M, Karlsson J, von Bonsdorff CH (2002) A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. Epidemiol Infect 129:133-138

45. PHLS (2003) Norovirus outbreaks peak in 2002: England and Wales. J Public Health Med 25:179-180

46. Clarke IN, Lambden PR (1997) Viral zoonoses and food of animal origin: caliciviruses and human disease. Arch Virol Suppl 13:141-152

47. Koopmans M, Duizer E (2004) Foodborne viruses: an emerging problem. Int J Food Microbiol 90:23-41

2. Goal and Objectives

2.1 Epidemiological Starting Position

This thesis was set-up in the second half of the year 2000 parallel to the nascent awareness for the relevance of NV especially in Great Britain, The Netherlands and in the US. In this period, key publications like “Food-related Illness and Death in the US” by Mead et al. in 1999 (1) and others from the British “Study of Infectious Intestinal Disease in England”, conducted between 1992 and 1996 (2) and from the “International Workshop on Human Calicivirus” organised in 1999 (3) were available for the public and for the scientific community. Nested within this period, the Cantonal Laboratories Solothurn and Basel-Landschaft, together with the Swiss Federal Office of Public Health (SFOPH), realised the gap in the epidemiological data concerning the NV situation in Switzerland. Prior to 2001, only sporadic NV outbreak information was available. In March 2001, a pilot study was carried out by the Cantonal Laboratories Solothurn and Basel-Landschaft. 100 stool samples previously analysed negative for enteric bacterial and parasitic pathogens were analysed for the presence of NV and in 44 specimens (44%) NV were detected (4). As a consequence to the international data on NV and in response to the results of the pilot study, several studies were launched by the Cantonal Laboratories Basel-Landschaft and Solothurn in mandate of the SFOPH. The aims of these studies were firstly the generating of a NV detection methodology and the subsequent application of this method on objectives relevant in terms of food safety (5-8) and secondly, the gaining of general epidemiological information on the NV situation in Switzerland.

2.2 Goal and Objectives

Based on the use of the newly designed NV RT-PCR detection method (7), which is also the recommended method for NV in water samples by the SFOPH in Switzerland (9), the following objectives were realised with the overall goal to obtain information for a first-time epidemiological assessment of the NV situation in Switzerland.

2.2.1 Objectives

I. To determine the frequency of NV-positive RT-PCR results on patient stool samples previously tested negative for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. and optionally diagnosed negative for other gastroenteric pathogens.

II. To accumulate risk information concerning sporadic NV illness.

III. To compile systematically information in respect to the main transmission modes and settings within NV-outbreaks.

IV. To perform phylogenetic analyses on obtained human NV strains and to carry out phylogenetic comparisons of NV sequence information, derived from human stool samples, from mineral waters and from oysters.

2.2.2 Realisation of Objectives

- From July 2001 to July 2003:
  Carrying out of a NV screening of previously bacteriological-negative tested patient stool samples in cooperation with the medical diagnostic laboratory Viollier AG. The screening was conducted parallel and was partially integrated within the following mentioned case-control study. Study area: German speaking part of Switzerland.

- From July 2001 to July 2003:
  Conducting of a general practitioner based case-control study on sporadic NV infections in cooperation with the medical diagnostic laboratories Viollier AG and Bakteriologisches Institut Olten AG. Study area: German speaking part of Switzerland.

- From January 2001 and December 2003:
  Systematic compilation of epidemiological information on NV outbreaks from the whole country and conducting of outbreak investigations within the German speaking part of Switzerland. All investigations were performed in cooperation with the SFOPH and the specific cantonal health authorities.

2.3 References

1. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) Food-related illness and death in the United States. Emerg Infect Dis 5:607-625
2. IID Study Executive (2000) A report of the study of infectious intestinal disease in England. The Stationery Office Books, UK
3. Monroe SS, Ando T, Glass RI (eds.) (2000) International Workshop on Human Calicivirus. J Infect Dis 181(Suppl 2):S249-S391
4. Baumgartner A. (2001) «Norwalk-like»-Viren (NLV) und Lebensmittel – eine Situationsanalyse für die Schweiz. Bulletin SFOPH 46:909-916
5. Beuret C, Kohler D, Lüthi T (2000) Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction in mineral waters imported into or bottled in Switzerland. J Food Prot 63:1576-1582
6. Beuret C, Kohler D, Baumgartner A, Lüthi TM (2002) Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl Environ Microbiol 68:1925-1931
7. Beuret C (2003) A simple method for isolation of enteric viruses (noroviruses and enteroviruses) in water. J Virol Methods 107:1-8
8. Beuret C, Baumgartner A, Schluep J (2003) Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland. Appl Environ Microbiol 69:2292-2297
9. Beuret C, Baumgartner A (2002b) Empfohlenes Verfahren für den Nachweis von “Norwalk-like” Viren (NLV) und Enteroviren in Wasser. Mitt Lebensm Hyg 93:91-103

3. Frequency of *Norovirus* in Stool Samples from Patients with Gastrointestinal Symptoms in Switzerland

R. Fretz[1,2], L. Herrmann[3], A. Christen[2], P. Svoboda[1], O. Dubuis[3], E.H. Viollier[3], M. Tanner[2], A. Baumgartner[4*]

[1] *Cantonal Laboratory Basel-Landschaft, 4410 Liestal, Switzerland*

[2] *Swiss Tropical Institute, 4002 Basel, Switzerland*

[3] *Viollier AG, 4002 Basel, Switzerland*

[4] *Swiss Federal Office of Public Health, 3003 Bern, Switzerland*

* Corresponding author:
Dr. Andreas Baumgartner, Swiss Federal Office of Public Health, Division of Food Science, 3003 Bern, Switzerland.
Phone: ++41 31 322 95 82; E-mail: andreas.baumgartner@bag.admin.ch

Paper 1:

Frequency of *Norovirus* in stool samples from patients with gastrointestinal symptoms in Switzerland.

Published in: *Eur J Clin Microbiol Infect Dis (2005) 24:214–216*

3.1 Abstract

To determine the frequency of sporadic community-acquired *Norovirus* (NV) infection in the German-speaking part of Switzerland, an evaluation of gastroenteritis cases seen in physicians' practices between July 2001 and July 2003 was conducted. A total of 699 stool specimens documented to be free of common bacterial pathogens was screened for the presence of NV by RT-PCR. NV was detected in 125 (17.9%) of these specimens. In the seasonal analysis, the highest rate of NV-positive samples (38.3%) was found between January and March 2002. After July 2002, the study was expanded to additionally test for NV in stool samples containing a known bacterial pathogen. Among 132 such specimens, NV was detected in only one. This suggests that NV mixed-infections are playing a marginal role in Switzerland.

3.2 Introduction

Recent international studies have shown that viral infections, especially with noroviruses (NV; formerly known as "Norwalk-like viruses"), are the most frequent cause of community-acquired gastroenteritis in endemic and epidemic situations. These viruses have been reported to account for an estimated 6% and 11% of all infectious intestinal diseases in England and the Netherlands, respectively [1, 2]. NV are also the most common cause of infectious intestinal disease outbreaks in Western Europe and in the USA [1, 3]. Illness is characterized by the acute onset of vomiting and diarrhea after an average incubation period of 12–48 h. The fecal-oral route is described as the most common route of transmission. NV are transmitted either by contaminated vectors (such as food and water) and contaminated fomites, or directly from person to person [4].

In the past 10 years, NV outbreaks have been recognized increasingly in Switzerland. However, solid epidemiological data are lacking due to the fact that NV are not routinely searched for in diagnostic laboratories and there is no obligation to report known cases. For this reason, the Swiss Federal Office of Public Health launched a series of studies to elucidate the epidemiology of NV in Switzerland [5]. In the context of this program, the present study was conducted between July 2001 and January 2003.

3.3 Material and Methods

All stool samples analyzed in this study were obtained from patients in 17 cantons located in the German-speaking part of Switzerland. Between July 2001 and July 2003, a total of 699 stool samples with negative bacteriological test results were screened for the presence of NV. In order for stool samples to be included in the analysis, the following criteria had to be met. (i) Results of compulsory testing for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. And any optional tests to detect other gastroenteric pathogens had to be negative. (ii) The subject had to be living within the defined study area. (iii) No other family member could be included in the study. (iv) The subject had to be between 6 months and 75 years of age. (v) The subject could not be suspected of being part of an NV outbreak or of having a nosocomial history or having traveled.

Between July 2002 and January 2003, stool samples that tested positive for at least one bacterial enteric agent were also screened for the presence of NV. For this part of the study, valid stool samples had to meet the following criteria. (i) Each stool sample had to have been found positive for at least one enteropathogenic bacterial agent such as *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Aeromonas* spp. or *Yersinia* spp. (ii) The subject had to live within the defined study area. (iii) The subject had to be aged 2 years or older. (iv) The subject could not have been hospitalized or in a hospital setting as an outpatient at the time of sample collection.

The routine analyses for gastrointestinal bacterial pathogens (and optional viral and/or parasitic agents) were conducted at the medical diagnostic laboratory Viollier AG, Switzerland. The RT-PCR assay used to screen for NV was based on degenerated primers located in regions of the RNA polymerase, and the assay was performed at the Cantonal Laboratory Basel-Landschaft as described elsewhere [6]. Since the detection method used in this study is common in Switzerland, general sequencing of NV-positive RT-PCR products was not performed. For quality control, randomly chosen RT-PCR products were sequenced and confirmed by comparison with NCBI GenBank.

3.4 Results and Discussion

A total of 699 stool samples negative for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. were tested for the presence of NV from July 2001 to July 2003. Among these specimens 125 (17.9%) were found to be positive for NV by RT-PCR. This rate of positivity is clearly higher than the 6% and 11% rates previously reported for NV among cases of infectious intestinal diseases in England and the Netherlands, respectively [1, 2]. The high rate of NV positivity we found can most likely be attributed to presence of the recently emerged and previously described NV variant, which is probably more virulent or of a higher environmental stability than previous NV strains: The emergence of this strain coincided with an increase in the incidence of NV outbreaks registered in 2002 in parts of Europe and in the USA [7, 8].

The rates of NV-positive stool samples we detected per canton are presented in Table 1 and ranged from 11.4% to 40.7%. This diversity may be explained by the variation in the number of stool samples analyzed per canton.

Table 1: Overall rate of NV-positive stool samples per canton.

Cantons	**Total number of NV-analyses**	**Number of NV-positives**	
Argovia (AG)	168	26	(15.5%)
Berne (BE)	128	18	(14.1%)
Basle-Landschaft (BL)	140	20	(14.3%)
Basle-Town (BS)	65	13	(20.0%)
Grisons (GR)	35	4	(11.4%)
Lucerne (LU)	27	11	(40.7%)
Solothurn (SO)	39	6	(15.4%)
Schwyz (SZ)	12	4	(33.3%)
Uri (UR)	21	5	(23.8%)
Zurich (ZH)	30	4	(13.3%)
7 other cantons	34	14	-
Total	**699**	**125**	**(17.9%)**

Figure 1 shows the seasonal distribution of the NV-positive findings. A winter seasonality can be observed for both years studied. Starting from the assumption that most cases of NV infection detected in this study were sporadic due to the inclusion criteria, the described winter seasonality accounts for non-outbreak cases. This finding is consistent with the results of a study conducted by US-based researchers in which the incidence of NV disease was examined in several countries and the low point for disease attributable to both sporadic cases and outbreaks was found to occur in the summer months [9]. In our study, a high rate of NV detection (38.3%) was recorded between January and March 2002. Increased incidence (14.7%) was also noted between October 2002 and March 2003. But this second period of augmented activity was relatively moderate compared to the first one.

In the first screening year, from July 2001 to July 2002, 84 of 346 (24.3%) stool samples were found to be NV-positive. In the second year, from July 2002 to July 2003, only 41 of 353 (11.6%) of the samples were NV-positive. This accounts for a remarkable 52.3% drop in the rate of NV-positive findings within 2 years. This decrease may indicate that the novel NV strain that emerged in 2002 has started to decline. The highest rate of NV-positive results we observed was in the first quarter of the year 2002, which corresponds with the previously described first appearance of the new NV variant in Europe [7].

Interestingly, the age structures of the two patient populations (i.e., NV-positive and NV-negative patients) were not significantly different (p-value 0.186 with the chi-square test). The mean age of the NV-positive patients was 32.8 years (SD, 18.8 years; range, 1.0–74.4 years) and that of the NV-negative patients was 33.4 years (SD, 18.7 years; range, 1.1–74.3 years). The NV-positive group consisted of 64 (51.2%) men and 61 (48.8%) women, and the NV-negative group included 257 (44.8%) men and 317 (55.2%) women. In accordance with the inclusion criteria, certain parts of the population considered at risk for NV infection (e.g., babies <6 months and elderly subjects >75 years of age) [10] were not included in the screening. Since it is well known that nursing homes and hospitals are considered to be settings with a high risk for NV outbreaks and our study excluded subjects older than 75 years and those who had been hospitalized, it is likely that the actual incidence of NV in the cantons studied is higher than our findings indicate.

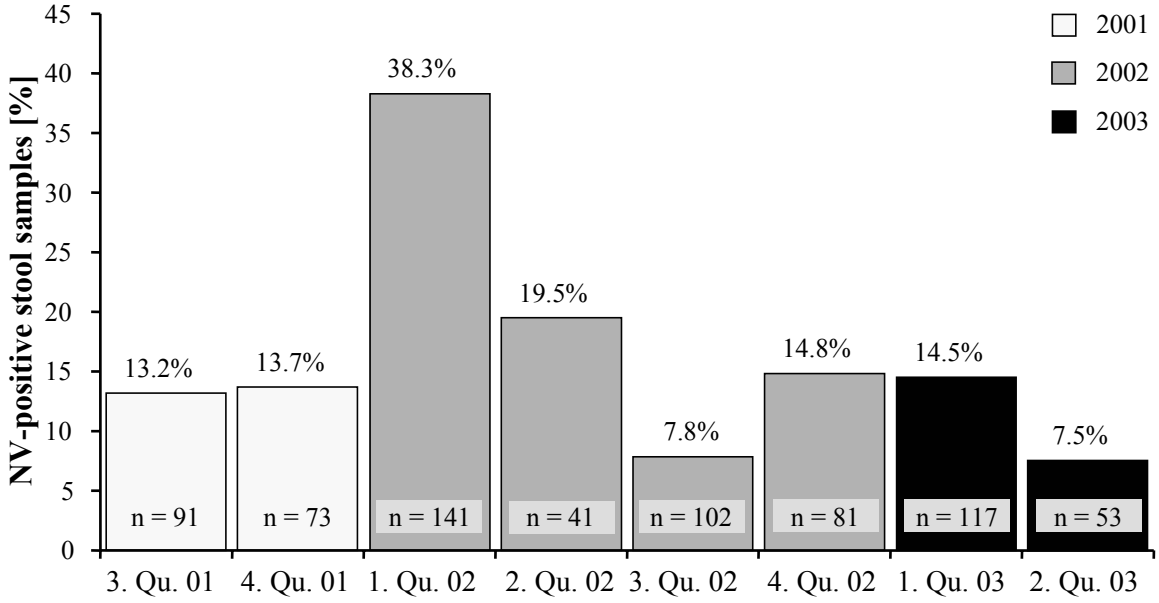


Figure 1: Seasonal distribution of the 125 (17.9%) NV-positive stool samples detected among 699 human stool samples negative for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp.

Among a total of 132 stool samples that tested positive for at least one bacterial pathogen (*Aeromonas* spp., 7; *Campylobacter* spp., 104; *Salmonella* spp., 20; *Yersinia enterocolitica*, 1) and were additionally tested for NV, only one was found to be NV positive. Of these 132 patients, 23 had a history of travel before the first symptoms of disease appeared: 14 (60.9%) had traveled within Europe, 8 (34.8%) had traveled outside of Europe, and for one person the destination was not provided. One stool sample tested positive for both NV and *Salmonella enteritidis*, and it was obtained from a patient who had traveled to Greece. The mean age of these 132 patients was 35.0 years (SD, 20.5 years; range, 1.1–78.8 years) and the gender distribution was 69 men and 63 women.

Only a few European studies on gastroenteritis have been conducted in which a broad panel of intestinal pathogens was tested using recently developed techniques (e.g., molecular methods, ELISA). One population-based prospective study on gastroenteritis in the Netherlands found more than one pathogen in 8% of all stool samples studied [2]. Compared to our results, the Dutch study showed a higher number of NV infections with multiple pathogens. However, discrepancies with our findings may be explained, in part, by the fact that the relevant part of our study only included stool samples that were positive for a bacteriological pathogen and children under 2 years of age, who are often infected by *Cryptosporidium* spp. [11] and rotaviruses [12], were excluded.

Our results suggest that NV are playing a marginal role in sporadic cases of multi-pathogen gastrointestinal infections in the German-speaking part of Switzerland. In patients with gastrointestinal symptoms in whom no other pathogen was found, NV was detected in a sizable percentage (17.9%) of infections, and the incidence was highest during the winter months. However, with 24.3% of infections being found from July 2001 to July 2002 and 11.6% from July 2002 to July 2003, we suspect the relatively high overall incidence was attributable to the emergence of a variant NV strain in 2002.

3.5 Acknowledgement

The authors would like to thank J. Grimbichler, Cantonal Laboratory Basel-Landschaft, for his support with the NV analysis. The study was fully financed by the Swiss Federal Office of Public Health and the Cantonal Laboratory Basel-Landschaft.

3.6 References

1. Lopman BA, Adak GK, Reacher MH, Brown DW (2003) Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. Emerg Infect Dis 9:71–77
2. de Wit MAS, Koopmans MPG, Kortbeek LM, Wannet WJB, Vinje J, van Leusden F, Bartelds AI, van Duynhoven YT (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands, incidence and etiology. Am J Epidemiol 154:666–674
3. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) Food-related illness and death in the United States. Emerg Infect Dis 5:607–625
4. Centers for Disease Control and Prevention (2001) "Norwalk-like viruses": public health consequences and outbreak management. MMWR Recomm Rep 50(RR-9):1–18
5. Fretz R, Svoboda P, Schmid H, Baumgartner A (2003) Durch Noroviren verursachte Gastroenteritis—eine ¨ Ubersicht. Bulletin SFOPH 46:828–833
6. Beuret C (2003) A simple method for isolation of enteric viruses (noroviruses and enteroviruses) in water. J Virol Methods 107:1–8
7. Lopman B, Vennema H, Kohli E, Pothier P, Sanchez A, Negredo A, Buesa J, Schreier E, Reacher M, Brown D, Gray J, Iturriza M et al (2004) Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet 363:682–688
8. Centers for Disease Control and Prevention (2003) Norovirus activity—United States, 2002. MMWR 52:41–45
9. Mounts AW, Ando T, Koopmans M, Bresee JS, Noel J, Glass RI (2000) Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J Infect Dis 181(Suppl 2):S284–S287

10. Robert Koch Institute (2003) Erkrankungen durch Norwalk-ähnliche Viren (Noroviren). Epidemiologisches Bulletin 6:39–43

11. Fretz R, Svoboda P, Ryan UM, Thompson RCA, Tanner M, Baumgartner A (2003) Genotyping of Cryptosporidium spp. isolated from human stool samples in Switzerland. Epidemiol Infect 131:663–667

12. Lopman BA, Brown DW, Koopmans M (2002) Human caliciviruses in Europe. J Clin Virol 24:137–160

4. Risk Factors for Infections with *Norovirus* Gastrointestinal Illness in Switzerland

R. Fretz[1,2], P. Svoboda[1], D. Schorr[3], M. Tanner[2], A. Baumgartner[4*]

[1] *Cantonal Laboratory Basel-Landschaft, 4410 Liestal, Switzerland*

[2] *Swiss Tropical Institute, 4002 Basel, Switzerland*

[3] *Cantonal Surgeon Basel-Landschaft, 4410 Liestal, Switzerland*

[4] *Swiss Federal Office of Public Health, 3003 Bern, Switzerland*

* Corresponding author:
Dr. Andreas Baumgartner, Swiss Federal Office of Public Health, Division of Food Science, 3003 Bern, Switzerland.
Phone: ++41 31 322 95 82; E-mail: andreas.baumgartner@bag.admin.ch

Paper 2:

Risk factors for infections with *Norovirus* gastrointestinal illness in Switzerland.

Published in: *Eur J Clin Microbiol Infect Dis. In press (E-Pub 2005 Apr 16)*

4.1 Abstract

Viral infections, particularly those caused by noroviruses (NV, genus *Norovirus*), are the most common cause of community-acquired gastroenteritis in Europe, with respect to both endemic and epidemic occurrence. For the first time, a general practitioner-based case-control study was performed between July 2001 and July 2003 in the German-speaking part of Switzerland in order to identify risk factors for sporadic NV infections. The consumption of different foodstuffs and of bottled mineral water did not show any significant association with the risk of NV gastroenteritis, nor was there any significant effect of individual ABO histo-blood group or household size on the incidence of NV gastroenteritis. The findings are consistent with person-to-person transmission as the most important route of transmission for community-acquired, sporadic NV infection, in that 39% of all patients reported they had had contact with ill persons before their illness. The fact that 33% reported contact with ill persons, mainly within family groups, after their own illness suggested that a substantial proportion of patients were part of family mini-outbreaks.

4.2 Introduction

Recent studies have shown that viral infections, especially those with noroviruses (NV) (genus name, *Norovirus*; former name, Norwalk-like viruses), are the most frequently identified cause of infectious intestinal diseases in Western European communities [1, 2]. NV account for an estimated 6% and 11% of all infectious intestinal diseases in England and the Netherlands [1, 2], respectively, and for an estimated 23 million cases of NV infection in the USA each year [3]. The illness is characterised by acute onset of vomiting and diarrhoea, after an average incubation period of 12–48 h. The faecal-oral route seems to be the most common route of transmission. NV are transmitted either by contaminated fomites (such as food and water) or by direct person-to-person contact [4]. In Switzerland, NV are not routinely searched for in clinical microbiology laboratories, nor is there an obligation to report infections caused by this viral agent within the system of infectious disease surveillance. Therefore, a series of studies was launched by the Swiss Federal Office of Public Health (SFOPH) in 2001 in order to get an overall view of NV epidemiology in Switzerland [5].

In this context, a case-control study was performed in the German-speaking part of the country with the objective of identifying for the first time risk factors for sporadic NV disease

in Switzerland. The potential risk factors investigated were the consumption of different foodstuffs, travel history, contact with ill persons, and ABO histo-blood group type.

4.3 Materials and Methods

Study-Area and Study-Population

The study included patients suffering from gastroenteritis who consulted a medical practitioner and subsequently provided stool samples that tested negative for *Campylobacter* spp., *Shigella* spp., and *Salmonella* spp. and in which NV were detected. Patients and controls were recruited in the following cantons: Appenzell Inner-Rhodes, Appenzell Outer-Rhodes, Argovia, Basle-Landschaft, Basle-Town, Berne, Glarus, Grisons, Lucerne, Nidwalden, Obwalden, Schaffhausen, Schwyz, Solothurn, St. Gall, Thurgovia, Uri, Zug, and Zurich, all of which belong to the German-speaking part of Switzerland.

Study Design and Sampling

A general-practitioner-based, matched case-control study was performed in the period between July 2001 and July 2003. Cases were identified by collaboration with the medical laboratories Viollier AG and Bakteriologisches Institut Olten AG. Cases had to fulfil the following case definition: (i) episode of diarrhoea and/or vomiting in patients consulting a medical practitioner in the study area; (ii) stool sample negative for *Campylobacter* spp., *Shigella* spp., and *Salmonella* spp. and optionally diagnosed negative for other gastroenteric pathogens; (iii) stool sample positive for NV genogroup I (GGI) or genogroup II (GGII) by reverse transcriptase-polymerase chain reaction (RT-PCR); and (iv) residence of patient within the defined study area during the period of illness and medical treatment. Only one case per family was included in the study.

The following exclusion criteria were applied: (i) babies under the age of 6 months and elderly patients above 75 years; (ii) patients with a history of possible nosocomial disease; and (iii) patients known to be a part of an NV outbreak.

One control per case was selected through the assistance of each patient (friend/relative control). The control had to be someone who had not consulted a medical practitioner for a gastrointestinal illness and had not reported any gastrointestinal symptoms in the 1 month before completion of the questionnaire. Furthermore, the control had to be of the same sex

and had to belong to the same age group as the corresponding case. The age groups were defined as 5-year intervals over the range 5–20 years (i.e., 5–10, 11–15, 16–20) and as 10-year intervals over the range 20–60 years (i.e., 20–29, 30–39, etc.). In addition, the control had to live within 10 km of the case. Questionnaires were sent to the cases and controls after they had given consent to participate in the study. The self-administered questionnaires for cases elicited demographic information and contained questions on the date of onset, the nature of symptoms, the date of inquiry, and further factors considered relevant for the disease under study, such as consumption of different food items and beverages, travel history, contact with ill persons, and ABO histo-blood group type. Copies of questionnaires are available upon request.

Data Collection and Analysis

Returned questionnaires were coded and double-entered into EpiData 3.0 [6]. Descriptive analysis was performed with MS Excel 97. Univariate analysis was carried out with the statistical software Intercooled Stata 7.0 [7]. Two logistic regression procedures were performed: a conditional logistic regression (clogit) for the standard analysis of the matched case-control pairs and a random effects logistic regression (xtlogit) as described elsewhere [8]. The latter procedure permits the extension of the analysis beyond matched case-control pairs and can analyse all the available data, taking into account the unequal chance of selection for the subjects included in the study. Power calculations for the analysis of the matched case-control pairs were carried out with the statistical software package NCSS 2000/PASS 2000 [9].

Microbiological Analysis of Patient Stool Samples

Analyses of bacteriological pathogens (and of optional viral and/or parasitic agents) were conducted by the medical laboratories Viollier AG and the Bakteriologisches Institut Olten AG. The NV RT-PCR diagnosis was performed at the Cantonal Laboratory Basel-Landschaft. The method used consisted of a genogroup-specific RT-PCR system for the detection of NV GG II based on degenerate primers, which are located in highly conserved regions of the RNA polymerase and of a second generic RT-PCR system, also based on degenerate primers. This combined system was designed for the detection of NV from human stool specimens and water samples [10].

Single NV RT-PCR products were chosen randomly, sequenced, and analysed in the frame of a broader phylogenetic study (manuscript in preparation).

4.4 Results

Descriptive Epidemiology

Within the study period from July 2001 to July 2003, 143 patients with stools positive for NV and negative for *Campylobacter* spp., *Shigella* spp., and *Salmonella* spp. were identified. Of these 143 patients, 127 (89%) returned a completed questionnaire and 126 patients met the study inclusion criteria. However, an appropriate control person could be found only for 76 (60%) study subjects. For the matched case-control study (conditional logistic regression), 73 properly matched case-control pairs (34 male and 39 female pairs) were obtained. Three case-control pairs had to be excluded because the distance between the places of residence exceeded the 10-km radius. These three pairs were only included in the broader analysis with the random effects logistic regression. The distribution of nationalities was very similar for cases and controls: 92% of the cases and 95% of the controls were Swiss; moreover, the age structure did not differ between the two groups. The mean age of cases was 32.7 years (median, 34.0 years), with a range of 1.1–69.3 years. The mean age of controls was 33.2 years (median, 37.1 years), with a range of 1.3–70.1 years. Likewise, the distribution of these characteristics among all 126 cases who participated in the study corresponded well with the matched pairs. The mean age of all cases was 32.9 years (median, 33.8 years), with a range of 1.0–69.3 years. Finally, 90% of all 126 cases were Swiss.

The different factors analysed and considered to be relevant for the disease under study are summarised in Tables 1–4. Due to the study design (consent had to be obtained both from the patient and his treating general practitioner), a significant delay between the start of the first symptoms and the completion of the patient questionnaire resulted. This period averaged 29 days (median, 24 days). For this reason, the contents of questionnaires were checked and verified by telephone in approximately one-third of all cases.

Table 1: Frequency of symptoms of NV illness, compiled from all 126 cases.

Symptoms	Number of cases (n = 126)	
Diarrhoea	124	(98.4%)
Vomiting	84	(66.7%)
Nausea	85	(67.5%)
Fever[(1)]	57	(45.2%)
Headache	45	(35.7%)
Abdominal cramps	87	(69.0%)
Other[(2)]	46	(36.5%)

[1] Mean temperature: 38.5°C (range, ±0.6°C)
[2] Other symptoms: dizziness, flatulence, loss of appetite, muscle pain, joint pain

The reported symptoms shown in Table 1 were compiled from all 126 cases and match the NV syndrome, which is based on Kaplan s criteria and is used by the SFOPH [5, 11, 12]. The mean duration of illness was 7.3 days (SD, 6.2 days; range, 0.25–28 days).

Forty-nine of the 126 (39%) NV cases reported having had contact with persons showing symptoms of gastroenteritis within 3 days before the onset of illness, and 42 (33%) cases reported such contact within 3 days after the onset of illness (Table 2). Additionally, 71 of the 126 cases reported absence from work or school due to their illness. A total of 63 of 71 patients provided information about the duration of absence, which was 4.3 days on average (SD, 3.1 days; range, 0.5–14 days). Only 25 of 126 (19.8%) patients reported a history of travel within 3 days before the onset of symptoms. Fifteen (60%) of them had travelled abroad, while the remaining 10 (40%) had travelled within Switzerland. Thirty-six (29%) patients reported having taken part at a mass event (such as parties or military service). In the 3 days before the onset of illness, 63 of the 126 (50%) patients had eaten meals in restaurants or cafeterias.

Table 2: Contact to ill persons before and after the own episode of NV gastroenteritis.

Contact to persons with symptoms of AGE(1)		**Number of cases (n = 126)**
Contact to ill persons before own illness:		49 (38.9%)
Contact in own family[2]	28 (57%)	
Contact at place of work[3]	16 (33%)	
Unknown	5 (10%)	
Total	*49 (100%)*	
Contact to ill persons after own illness:		42 (33.3%)
Contact in own family[2]	32 (76%)	
Contact at place of work[3]	4 (10%)	
Unknown	6 (14%)	
Total	*42 (100%)*	

[1] AGE: Acute Gastroenteritis [2] Within family and relationship
[3] At place of work, in school, in pre-school or kindergarten

Analytical Epidemiology

The results of the univariate analysis are presented in Tables 3 and 4. The findings of the conditional logistic regression (clogit) in Table 3 revealed that no group of the possible risk factors under study (consumption of foods and beverages, personal contacts, and ABO histoblood group type) was significantly associated with NV gastroenteritis. These results were confirmed by the random effects logistic regression (xtlogit), as shown in Table 4.

Table 3: Results of the conditional logistic regression (Stata 7.0, clogit) for the analysis of the matched case-control pairs.

Exposure	**Pairs**	**OR[(1)]**	**(95% CI)**	**p[(2)]**
Total	73	-	-	-
Consumed food and beverages:				
Salad	73	1.25	(0.34; 4.65)	0.74
Raw berries	72	0.75	(0.17; 3.35)	0.71
Tap water	73	1.33	(0.56; 3.16)	0.51
Mineral water	73	1.00	(0.46; 2.16)	1.00
Sweet beverages	71	1.06	(0.55; 2.05)	0.87
Personal contacts:				
Household with children ≤2 years	73	1.00	(0.29; 3.45)	1.00
Household with children ≤5 years	73	0.75	(0.26; 2.16)	0.59
Household with children ≤10 years	73	0.75	(0.26; 2.16)	0.59
Household with persons ≥65 years	73	0.75	(0.17; 3.35)	0.71
Household with >1 person	72	1.50	(0.53; 4.21)	0.44
Household with >2 persons	72	0.77	(0.34; 1.75)	0.53
Household with >3 persons	72	0.71	(0.32; 1.61)	0.53
Household with >4 persons	72	1.14	(0.41; 3.15)	0.53
ABO histo-blood group type:				
Type A	36	1.34	(0.55; 3.42)	0.49
Type B	36	0.33	(0.07; 1.65)	0.15
Type 0	36	1.00	(0.40; 2.52)	0.49
Type AB	36	1.50	(0.25; 8.98)	0.65
Type A/AB	36	1.44	(0.62; 3.38)	0.39
Type B/AB	36	0.63	(0.20; 1.91)	0.40

[1] OR: Maximum Likelihood Estimation Odds Ratio [2] p-value: Likelihood Ratio Test

Table 4: Results of the random effects logistic regression procedures (Stata 7.0, xtlogit). All data has been included, also of those cases with no controls.

Exposure	**Cases**	**Controls**	**OR[(1)]**	**(95% CI)**	**p[(2)]**
Total	126	76	-	-	-
Consumed food and beverages:					
Salad	126	76	1.41	(0.41; 4.78)	0.58
Raw berries	124	76	0.69	(0.17; 2.74)	0.59
Tap water	126	76	1.01	(0.53; 1.92)	0.98
Mineral water	124	76	1.15	(0.61; 2.15)	0.67
Sweet beverages	126	76	1.10	(0.60; 1.99)	0.76
Personal contacts:					
Household with children ≤2 years	126	76	0.89	(0.34; 2.10)	0.79
Household with children ≤5 years	126	76	0.83	(0.44; 1.55)	0.57
Household with children ≤10 years	126	76	0.77	(0.43; 1.37)	0.37
Household with persons ≥65 years	126	76	1.22	(0.40; 3.72)	0.72
Household with >1 person	125	76	2.18	(0.86; 5.54)	0.10
Household with >2 persons	125	76	0.87	(0.48; 1.59)	0.65
Household with >3 persons	125	76	0.91	(0.51; 1.62)	0.75
Household with >4 persons	125	76	1.25	(0.53; 2.95)	0.61
ABO histo-blood group type:					
Type A	62	45	1.20	(0.55; 2.61)	0.64
Type B	62	45	0.28	(0.07; 1.13)	0.07
Type 0	62	45	1.11	(0.51: 2.45)	0.79
Type AB	62	45	1.89	(0.35; 10.2)	0.46
Type A/AB	62	45	1.39	(0.64; 3.00)	0.40
Type B/AB	62	45	0.59	(0.21; 1.70)	0.32

[1] OR: Odds Ratio, corrected for random effect [2] p-value: Wald Test

4.5 Discussion

The sample size of 73 matched case-control pairs does not allow small risks to be detected with reasonably high confidence and power. The conducted power calculations showed that a minimal statistically significant odds ratio (OR) of 2.9 (value 0.05, power of 0.80) can be achieved with 70 matched case-control pairs (probability of event in exposed group: 0.5). A further limiting factor was the long interval between mailing and return of the questionnaires (median, 24 days) that may have introduced a considerable recall bias. The delay can be explained by the fact that the NV analysis of the patient stool samples was only performed after the return of a negative bacterial culture and after informed consent was obtained from both the patient and his general practitioner. This delay may have influenced the information on the food and beverages consumed. On the other hand, it probably had less influence on the results concerning the personal contacts, certainly within families.

The most important finding of the present study is that 39% of all cases reported contact with ill persons before the onset of illness, and 33% reported contact after their own illness (Table 2). Although no information regarding the contact with ill persons was obtained from the control subjects, it can be stated that the contacts previous to illness point to the relevance of ill persons as sources of infection, while the contacts following illness are an indication of the relevance of the ease of the person-to-person transmission route. Due to the high infectivity and simplicity of person-to-person transmission of NV, at least the contacts with ill family members are very probably indicative of real chains of infection. This kind of personal contact within and outside the family has been shown to be a relevant risk factor in earlier studies [13, 14]. A Dutch study has revealed a strong association between NV illness and household contacts with gastroenteritis [13]. The large number of contacts with ill persons within the family group suggests that a substantial proportion of the patients belonged to family mini-outbreaks. However, the comparison of the different household sizes revealed no statistically significant effect on NV gastroenteritis.

The results regarding the consumption of bottled mineral water (Tables 3 and 4) are interesting in relation to recent findings of NV sequences in different brands of mineral waters [15, 16]. The results from this study do not confirm this risk and are in concordance with the estimation of the SFOPH that NV-contaminated mineral water does not play a major role in the epidemiology of NV in Switzerland [5, 17].

The difference in risk associated with individual ABO histo-blood groups did not show statistical significance. Thus, the previous findings regarding a possible correlation between the histo-blood group type B (protective) and histo-blood group type O (risk factor) with respect to NV infections [18, 19] could not be confirmed in this study. The results must be seen in the context of other findings, which show that no general conclusion could be reached on the association between the ABO histo-blood group subtypes and the risk of illness, and additional histo-blood types, such as Lewis and ABH, seemed to play important roles in the probability of disease manifestation [18–22]. Furthermore, our results may be biased by the fact that the distribution of ABO histo-blood group types of the control group differs from that for Switzerland as a whole (p value, chi-squared xtlogit: 0.046; p value, chi-squared clogit: 0.005; detailed data not shown).

The reported symptoms of illness (Table 1) matched those of the known NV syndrome [5, 11, 12]. However, the mean duration of illness of 7.3 days was significantly higher than the average duration of NV illness of 12–72 h published in the literature [5, 11]. This may reflect the fact that our study only included patients who visited a physician, and hence their illness may have been generally more serious than that of NV patients in common-source outbreaks, which generally include all cases [23]. The findings on the absence from work indicate that sporadic community-acquired NV infections are of substantial economic importance.

In conclusion, the results of this study are consistent with person-to-person transmission being of importance for the spread of sporadic community-acquired NV infections, a finding that correlates well with the postulated main transmission pathway from person to person within confined NV outbreaks [5, 24, 25]. However, due to the limitations outlined, further work is necessary to identify all factors and critical points to be considered for prevention and control of NV outbreaks.

4.6 Acknowledgement

The authors would like to express their gratitude to the collaborating medical laboratory Viollier AG (especially Louis Herrmann and Olivier Dubuis) and to Urs Schibli from the Bakteriologisches Institut Olten AG. Furthermore, we wish to thank Jürg Grimbichler from the Cantonal Laboratory Basel-Landschaft for his support with the NV analysis. The study was fully financed by the SFOPH and the Cantonal Laboratory Basel-Landschaft

4.7 References

1. Tompkins DS, Hudson MJ, Smith HR, Eglin RP, Wheeler JG, Brett MM, Owen RJ, Brazier JS, Cumberland P, King V, Cook PE (1999) A study of infectious intestinal disease in England: microbiological findings in cases and controls. Commun Dis Public Health 2:108–113
2. de Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J, van Leusden F, Bartelds AI, van Duynhoven YT (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol 154:666–674
3. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, Griffin PM, Tauxe RV (1999) Food-related illness and death in the United States. Emerg Infect Dis 5:607–625
4. Centers for Disease Control and Prevention (2001) Norwalk-like viruses : public health consequences and outbreak management. MMWR Recomm Rep 50(RR-9):1–18
5. Fretz R, Svoboda P, Schmid H, Baumgartner A (2003) Durch Noroviren verursachte Gastroenteritis – eine Übersicht. Bull SFOPH 46:828–833
6. Lauritsen JM, Bruus M (2003) EpiData V3: a comprehensive tool for validated entry and documentation of data. EpiData Association, Odense, Denmark
7. StataCorp LP (2000) Intercooled Stata 7.0 for Windows 98/95/NT. College Station, TX, USA
8. Tappin D, Brooke H, Ecob R, Gibson A (2002) Used infant mattresses and sudden infant death syndrome in Scotland: case-control study. BMJ 325:1007
9. Hintze J (2001) NCSS 2000/PASS 2000. Number cruncher statistical systems, Kaysville, UT, USA
10. Beuret C (2003) A simple method for isolation of enteric viruses (noroviruses and enteroviruses) in water. J Virol Methods 107:1–8
11. Lüthi TM (1998) Ermittlung eines möglichen Ursprungs gastrointestinaler Gruppenerkrankungen durch die Kombination klinischer, bakteriologischer und epidemischer Kriterien. Mitt Lebensm Hyg 89:196–218
12. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW (1982) The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am J Public Health 72:1329–1332
13. de Wit MAS, Koopmans MPG, van Duynhoven YTHP (2003) Risk factors for Norovirus, Sapporo-like virus, and group A rotavirus gastroenteritis. Emerg Infect Dis 9:1563–1570
14. Letrilliart L, Desenclos JC, Flahault A (1997) Risk factors for winter outbreak of acute diarrhoea in France: case-control study. BMJ 315:1645–1649
15. Beuret C, Kohler D, Baumgartner A, Lüthi TM (2002) Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl Environ Microbiol 68:1925–1931
16. Beuret C, Kohler D, Lüthi T (2000) Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction in mineral waters imported into or bottled in Switzerland. J Food Prot 63:1576–1582

17. Baumgartner A (2001) Norwalk-like -Viren (NLV) und Lebensmittel – eine Situationsanalyse für die Schweiz. Bull SFOPH 46:909–916

18. Hutson AM, Atmar RL, Graham DY, Estes MK (2002) Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis 185:1335–1337 19.

19. Hennessy EP, Green AD, Connor MP, Darby R, MacDonald P (2003) Norwalk virus infection and disease is associated with ABO histo-blood group type. J Infect Dis 188:176–177

20. Meyer E, Ebner W, Scholz R, Dettenkofer M, Daschner FD (2004) Nosocomial outbreak of norovirus gastroenteritis and investigation of ABO histo-blood group type in infected staff and patients. J Hosp Infect 56:64–66

21. Huang P, Farkas T, Marionneau S, Zhong W, Ruvoen-Clouet N, Morrow AL, Altaye M, Pickering LK, Newburg DS, LePendu J, Jiang X (2003) Noroviruses bind to human ABO, Lewis, and secretor histo-blood group antigens: identification of 4 distinct strain-specific patterns. J Infect Dis 188:19–31

22. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R (2003) Human susceptibility and resistance to Norwalk virus infection. Nat Med 9:548–553

23. Tam CC, Rodrigues LC, O Brien SJ (2003) The study of infectious intestinal disease in England: what risk factors for presentation to general practice tell us about potential for selection bias in case-control studies of reported cases of diarrhoea. Int J Epidemiol 32:99–105

24. Lopman BA, Reacher MH, Van Duijnhoven Y, Hanon FX, Brown D, Koopmans M (2003) Viral gastroenteritis outbreaks in Europe, 1995–2000. Emerg Infect Dis 9:90–96

25. Lopman BA, Adak GK, Reacher MH, Brown DW (2003) Two epidemiologic patterns of Norovirus outbreaks: surveillance in England and Wales, 1992–2000. Emerg Infect Dis 9:71–77

5. Outbreaks of Gastroenteritis due to Infections with *Norovirus* in Switzerland, 2001 – 2003

R. Fretz[1,2], P. Svoboda[1], T.M. Lüthi[3], M. Tanner[2], A. Baumgartner[4*]

[1] *Cantonal Laboratory Basel-Landschaft, 4410 Liestal, Switzerland*

[2] *Swiss Tropical Institute, 4002 Basel, Switzerland*

[3] *University of Applied Sciences Wädenswil, 8820 Wädenswil, Switzerland*

[4] *Swiss Federal Office of Public Health, 3003 Bern, Switzerland*

* Corresponding author:
Dr. Andreas Baumgartner, Swiss Federal Office of Public Health, Division of Food Science, 3003 Bern, Switzerland.
Phone: ++41 31 322 95 82; E-mail: andreas.baumgartner@bag.admin.ch

Paper 3:

Outbreaks of gastroenteritis due to infections with *Norovirus* in Switzerland.

Published in: *Epidemiol Infect. In press (E-Pub 2005 Jan 25)*

5.1 Summary

Viral infections, especially those with noroviruses are the most common cause of acute gastroenteritis in Europe. To obtain information about the epidemic situation of noroviruses in Switzerland, an initial study was launched in the German-speaking part of the country to systematically compile *Norovirus* outbreak information between 2001 and 2003. In total, 73 outbreaks were registered. Most affected were closed settings, e.g. nursing homes (34%) and hospitals (25%). Transmission pathways were identified in 74% of *Norovirus* outbreaks. In 81% of these cases person-to-person transmission was the primary route of infection and on seven occasions (13%), a foodborne transmission was the possible cause. Furthermore, *Norovirus* outbreak characteristics of epidemiological importance are highlighted with a discussion of four selected events.

5.2 Introduction

Recent international studies have shown that viral infections, especially those with noroviruses (formerly known as Norwalk-like viruses), are the most frequent cause of gastroenteritis in the community with regard to the endemic and epidemic situation [1–6]. These viruses account for an estimated 6% and 11% of all infectious intestinal diseases in England and The Netherlands respectively [3, 5] and for an estimated 23 million cases in the United States annually [7]. Noroviruses are also the most common cause of outbreaks of infectious intestinal diseases in Western Europe and North America [3, 7]. The illness is characterized by acute onset of vomiting and diarrhoea, after an average incubation period of 12–48 h. The faecal–oral route is described as the most common route of transmission. Noroviruses are transmitted either by contaminated fomites (such as food and water) and the environment, or directly by person-to-person contact [8]. Noroviruses are often responsible for foodborne outbreaks due to contaminated water, ready-to-eat dishes, seafood, fruits and vegetables. Furthermore, various outbreaks have been associated with the ingestion of contaminated drinking or recreational surface water [9]. During the past 10 years, *Norovirus* outbreaks have been increasingly identified in Switzerland. However, solid epidemiological data were missing because noroviruses are not routinely searched for in diagnostic laboratories and there is no obligation to report known cases except for outbreaks registered by the cantonal (regional) health authorities. For this reason, the Swiss Federal Office of Public Health (SFOPH) launched a series of studies to learn more about the national

epidemiology of noroviruses [6]. In the context of this programme, systematic investigations of outbreaks between 2001 and 2003 were conducted. They are presented and discussed in summary with a closer look at four outbreaks of epidemiological importance.

5.3 Methods

Between 2001 and 2003 *Norovirus* outbreak information was systematically compiled. For the purpose of this study, a temporary network consisting of the cantonal (regional) food and health authorities (cantonal laboratories and cantonal surgeons) from the German-speaking part of Switzerland and the SFOPH was established. Outbreaks of gastrointestinal disease and clusters that were suspected of being caused by viral agents were reported by members of this network to the Cantonal Laboratory Basel-Landschaft. This institution was in charge of registering *Norovirus* outbreak information from the whole country and conducting separate investigations of outbreaks in close cooperation with the health authorities in the German-speaking part of the country. Switzerland is comprised of 7.4 million inhabitants and the German-speaking part accounts for approximately 64% of the country [10].

Classification of *Norovirus* outbreaks was performed by descriptive and analytical epidemiological investigations, by epidemiological profiling and by laboratory diagnosis. The profiling was based upon the *Norovirus* infection syndrome plus the following additional epidemiological characteristics [6, 11, 12]: (i) an incubation period varying between 1–2 days (range 12–48 h); (ii) major symptoms of vomiting (frequently explosive) and mainly diarrhoea (sometimes profuse), partially accompanied by nausea, abdominal pain and cramps, muscle pain, headache and sporadic low-grade fever; (iii) pathogenic bacterial and parasitic agents of gastroenteritis typically not detected in analysed patient stool samples; (iv) secondary cases typical within *Norovirus* outbreaks; (v) more than 50% of patients suffering from vomiting; (vi) more patients suffer from vomiting than fever, and adolescent patients suffer predominately from vomiting whereas adult patients suffer predominately from diarrhoea.

Consequently, a *Norovirus* outbreak was classified as confirmed by exhibiting the typical *Norovirus* profile and by laboratory diagnosis of the pathogen in at least one patient stool sample. In a probable *Norovirus* outbreak, the typical epidemiological profile was present but either no patient samples were obtained or the samples were not analysed for the presence of

noroviruses. In a possible *Norovirus* outbreak, either the clinical picture of the persons involved was incomplete, or the epidemiological links in terms of place, person and time were not established or could not be proven due to lack of information. The transmission routes (person-to-person, contaminated water, food or environment) were categorized in analogy to the outbreak classification scheme used.

The *Norovirus* reverse transcription–polymerase chain reaction (RT–PCR) diagnosis was performed at the Cantonal Laboratory Basel-Landschaft. The method used consisted of a genogroup-specific RT–PCR system for the detection of *Norovirus* GGII based on degenerate primers located in highly conserved regions of the RNA polymerase and of a second generic RT–PCR system also based on degenerate primers [13]. Furthermore, this combined system is part of the detection method for noroviruses in water samples recommended by the SFOPH in Switzerland [14].

5.4 Results

Overall Characteristics of 73 NV Outbreaks

Between January 2001 and December 2003, 73 *Norovirus* outbreaks were analysed. Ninety per cent (66/73) of the outbreaks were registered in the German-speaking part of Switzerland and a complete epidemiological outbreak investigation was carried out for 20 outbreaks (27%). Key information, e.g. primary transmission mode and number of cases, was collected from the remaining 53 outbreaks. Six out of 73 outbreaks (8%) were classified as *Norovirus* outbreaks by epidemiological profiling only, without laboratory confirmation. In the remaining incidents (92%), noroviruses were detected in patient specimens.

Twenty-five outbreaks (34%) occurred in nursing homes and accommodation for the disabled, 18 (25%) in hospitals and health resorts, nine (12%) in schools and boy-scout camps, six (8%) at social gatherings, five (7%) in hotels, four (5%) in the community, three (4%) in military settings, one (1%) at a pilgrimage and two (3%) in other settings.

Transmission pathways were identified in 54 of the 73 outbreaks (74%). In 44 of these 54 outbreaks (81%), person-to-person transmission was the primary route of infection. On seven occasions (13%), a foodborne transmission was a possible cause. One outbreak occurred due to an epidemiologically classified probable waterborne incident (discussed below) and

another due to a classified possible waterborne episode caused by contamination of the drinking water system by sewage leakage. Within the possible foodborne outbreaks, the attack rates were high (>70%) and a common meal took place during one incubation period (1–2 days) before the onset of illness. Epidemiological investigations identified either no contaminated foodstuff or insufficient information was available. One large outbreak affected different nursing homes and similar institutions during and after a pilgrimage [15]. Generally, it could be observed that outbreaks in hospitals, nursing homes and other similar settings frequently reflected the current *Norovirus* situation in the community. Consequently, community-acquired and imported *Norovirus* infections into various settings often acted as triggers of outbreaks. In almost all outbreaks with clear person-to-person transmission, the *Norovirus* agent was introduced into the setting by initially ill persons. The mean number of cases was found to be 60 (median 35, range 3–650) within all registered outbreaks and attack rates ranged between 30% and 90%.

Four selected *Norovirus* outbreaks will be presented in more detail below, because of their inherent characteristics such as transmission mode and setting.

Three Consecutive Outbreaks in Ski Camps

In January 2001, three consecutive *Norovirus* outbreaks occurred among ski camps, all located in the same accommodation (chalet) in the Swiss mountains. *Norovirus* infection was confirmed by epidemiological profiling and by RT–PCR diagnosis. Only one patient stool sample could be collected. The isolated *Norovirus* strain showed a sequence identity of 88% with the strain OS120458/01 (GenBank accession no. AB071035). Twenty-nine out of 34 persons (85%) were affected in the first ski camp, 21 out of 26 (81%) in the second and 13 out of 30 (43%) in the third. The epidemic curve in Figure 1 shows that in the first camp, a point-source infection occurred. A foodborne infection seems likely because of the high attack rate (85%) and also because the probable time of exposure could be fixed between late afternoon and midnight the day preceding onset of symptoms. One common meal (dinner) took place during this time period. Within 1.5 days (the average incubation period of noroviruses) after moving into the accommodation, the first symptoms occurred in persons from the second camp. In all probability the infections were caused by a heavy *Norovirus* contamination of the accommodation. The illness in the first camp started the night before and on the day of departure. Therefore, it appears that the toilets and residential rooms were not properly cleaned, as 85% of the persons became ill and suffered from heavy vomiting and diarrhoea.

The contamination was confirmed by persons of the second camp who reported the smell of vomitus and contaminated pillow covers. The computed time of exposure occurred on the first night after arriving at the accommodation. The *Norovirus* cases in the third camp showed a completely different pattern and a point-source of infection could be excluded. Nevertheless, the first patients of the third camp may have been infected by the environment of the accommodation during the day of arrival. The persons of the third camp were informed about the gastroenteritis illness in the two previous camps. Disinfectant was used and the toilets and kitchen were cleaned. This may explain the different dynamic of the outbreak that is typical for person-to-person transmission. After the guests' departure from the third camp the establishment was shut down and cleaned professionally before reopening. Since the re-opening no further cases have occurred.

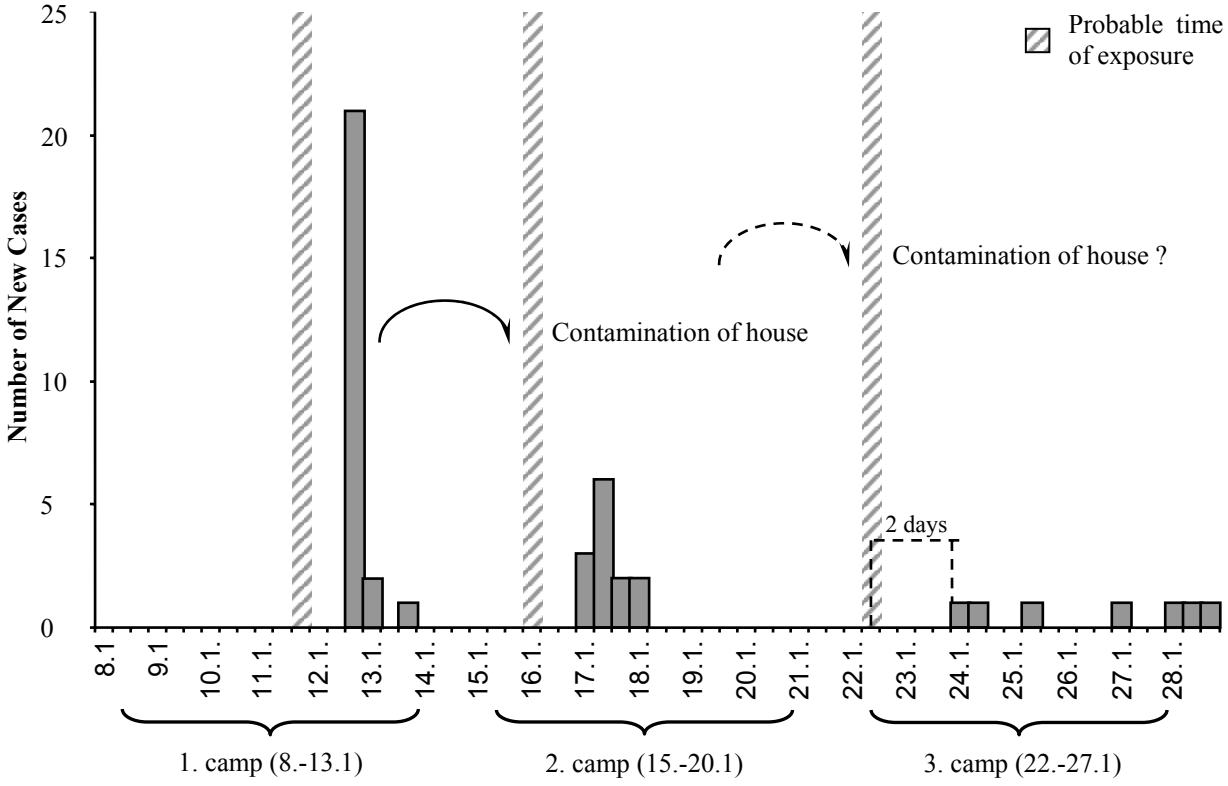


Figure 1: Epidemic curve of three consecutive *Norovirus* outbreaks in ski camps in January 2001, located at the same accommodation. Each division on the x-axis describes a time interval of 8 h. The position of the date illustrates the first time interval from 0 to 8 h. The probable time of exposure in the first and second camp was computed by subtracting the maximal incubation period of the noroviruses of 2 days from the last case and subtracting the minimal incubation period of 1 day from the first case. The overlapping time period corresponds to the probable time of exposure.

Outbreak within Two Communities

Within two weeks in January 2001, two communities were affected by a large outbreak with more than 650 cases. *Norovirus* illness was confirmed by epidemiological profiling and by *Norovirus* RT–PCR on patient stool samples. The two communities were mainly supplied with drinking water from a waterworks that distributes purified and processed water from a nearby lake. During 8 days within the 2-week outbreak, the water treatment plant in the waterworks exhibited major deficiencies regarding the application of chlorine and/or ozone. Two samples were drawn on the last day and following treatment failure. The samples neither exceeded the official bacteriological criteria for drinking water, nor were noroviruses detected by RT–PCR. However, there is evidence that Swiss surface waters are generally contaminated with noroviruses [6]. For an estimation of the number of cases, a selection of general practitioners, nursing homes and schools within the two communities were questioned and the extracted numbers of patients were then extrapolated to the whole population of the two communities. Parallel investigations of the local pharmacies supported the estimation. Eight patient stool samples were found to be *Norovirus*-positive by RT–PCR and seven RT–PCR products were sequenced. Three samples showed a sequence identity of 89.7% with the Norwalk virus (GenBank accession no. NC_001959.1), three samples showed a sequence identity of 88.0% with the Camberwell virus strain (NC_002614.1) and the last sample exhibited an identity of 98.0% to the Whiterose virus strain (HCA277610). This high variety within the discovered *Norovirus* isolates clearly supports the hypothesis of drinking water as the source of the outbreak.

Outbreak Following a Banquet

In a small outbreak, at a family gathering, 21 out of 25 persons (84%) were found to be suffering from gastroenteritis approximately 1.5 days (the average incubation period of noroviruses) following a banquet. *Norovirus* was confirmed by epidemiological profiling and by *Norovirus* RT–PCR on two patient stool samples. One *Norovirus* isolate was sequenced and showed a sequence identity of 94% with Chiba virus (GenBank accession no. NC_002613). A cohort study on the consumed food items could not define any foodstuff as a risk factor. Because of the low statistical power of the analysis due to the high attack rate and small size of the cohort, the foodborne route of infection cannot be excluded. However, the food was delivered to the banquet by a catering service. Investigations with the catering company showed that no further cases could be found within their clientele. Further investigations revealed that the *Norovirus* agent was most probably introduced into the setting

by a young girl from family A (probable time of exposure III). The further transmission pathway from the girl to the other guests at the banquet, e.g. by personal contact or by contamination of some food items, remained unclear. Looking back at the chain of infection, it could be seen that the brother of the young girl was suffering from *Norovirus* illness 3 days previously. Two days before, two ill children of family B had been looked after by the parents of the boy and girl (probable time of exposure II). The mother of family B had been incapacitated with gastroenteritis 24 h earlier. Finally, this mother was herself visiting a third family with an ill boy (probable time of exposure I). All cases were classified as probable *Norovirus* by epidemiological investigation (see Fig. 2).

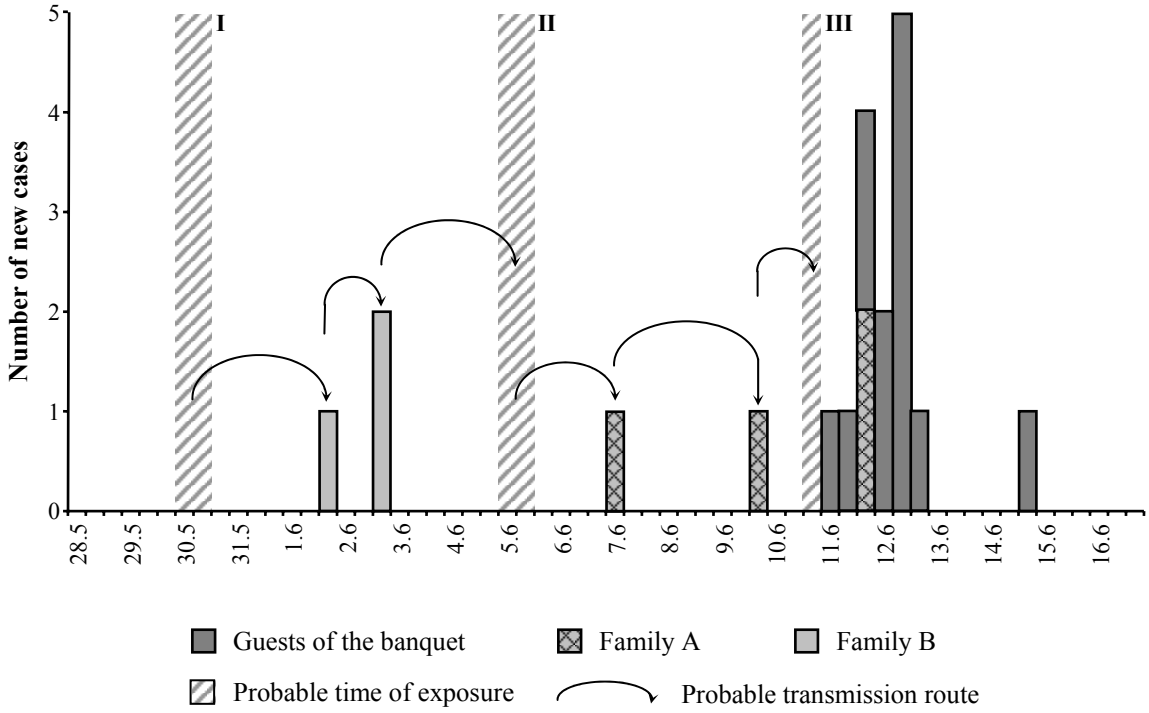


Figure 2: Epidemic curve of an outbreak following a banquet in June 2001. Each division on the x-axis describes a time interval of 8 h. The position of the date illustrates the first time interval from 0 to 8 h. The three different probable times of exposure (I–III) are indicated by hatched bars. See text for further explanation of the introduction and transmission of noroviruses.

Consecutive NV Outbreaks in a Hospital with an Affiliated Nursing Home

Between the end of January and the beginning of April 2003, a series of *Norovirus* cases occurred in a Swiss hospital and an affiliated nursing home. In total, 140 persons were affected by gastroenteritis : 34 patients from the hospital, 28 patients from the nursing home and 78 staff members. Twelve patient stool samples tested positive for *Norovirus* by RT–PCR. The epidemic curve with information from 132 patients is plotted in Figure 3.

Obviously, the curve is divided into three separate peaks. The *Norovirus* cases within the second and third peak were confirmed by laboratory results, whereas the cases from the first peak were classified as probable *Norovirus* cases by epidemiological profiling only. It is of interest that the incident consisted of three separate outbreaks rather than a single protracted one. From the 140 patients involved, 132 could be clearly allocated to the hospital, respectively to the nursing home. The 28 *Norovirus* cases (21% out of the 132 patients) allocated from the first peak originated exclusively from the hospital and the second peak consisted of 29 patients (22%) from the hospital and six patients from the nursing home (5%). Finally, the last peak was dominated by 48 patients (36%) from the nursing home and also included 21 patients (16%) from the hospital. The 12 *Norovirus*-positive stool samples all exhibited a sequence identity of 95% with the *Norovirus* strain Miami Beach (GenBank accession no. AF414424). Phylogenetic analyses, conducted with the software packages Clustal W, Phylip 3.6a3 and Emboss matcher 2.0u4, revealed that the *Norovirus* sequences discovered formed two separate clusters as shown in Figure 4. The division of the *Norovirus* sequences into the two clusters corresponded exactly to a local distribution of the patients. All *Norovirus* isolates of cluster I originated from patients from the second peak, whereas all isolates from cluster II came from patients from the third outbreak peak. The sequences within cluster I exhibited an average sequence identity of 100%, whereas the sequences within cluster II showed an average identity of 99.9%. The computed sequence identity between ID01 (cluster I) and ID10 (cluster II) was 97.9%. The predominant transmission mode in all three consecutive outbreaks was the person-to-person route. No information about the entry pathway of the noroviruses could be obtained. At the time of the outbreak, community-acquired *Norovirus* infections were reported and may have played an important role in transporting the agent into the hospital setting. Sequence information of the noroviruses from each cluster (ID01 and ID10) was submitted to GenBank (GenBank accession nos. AY551087 and AY551088).

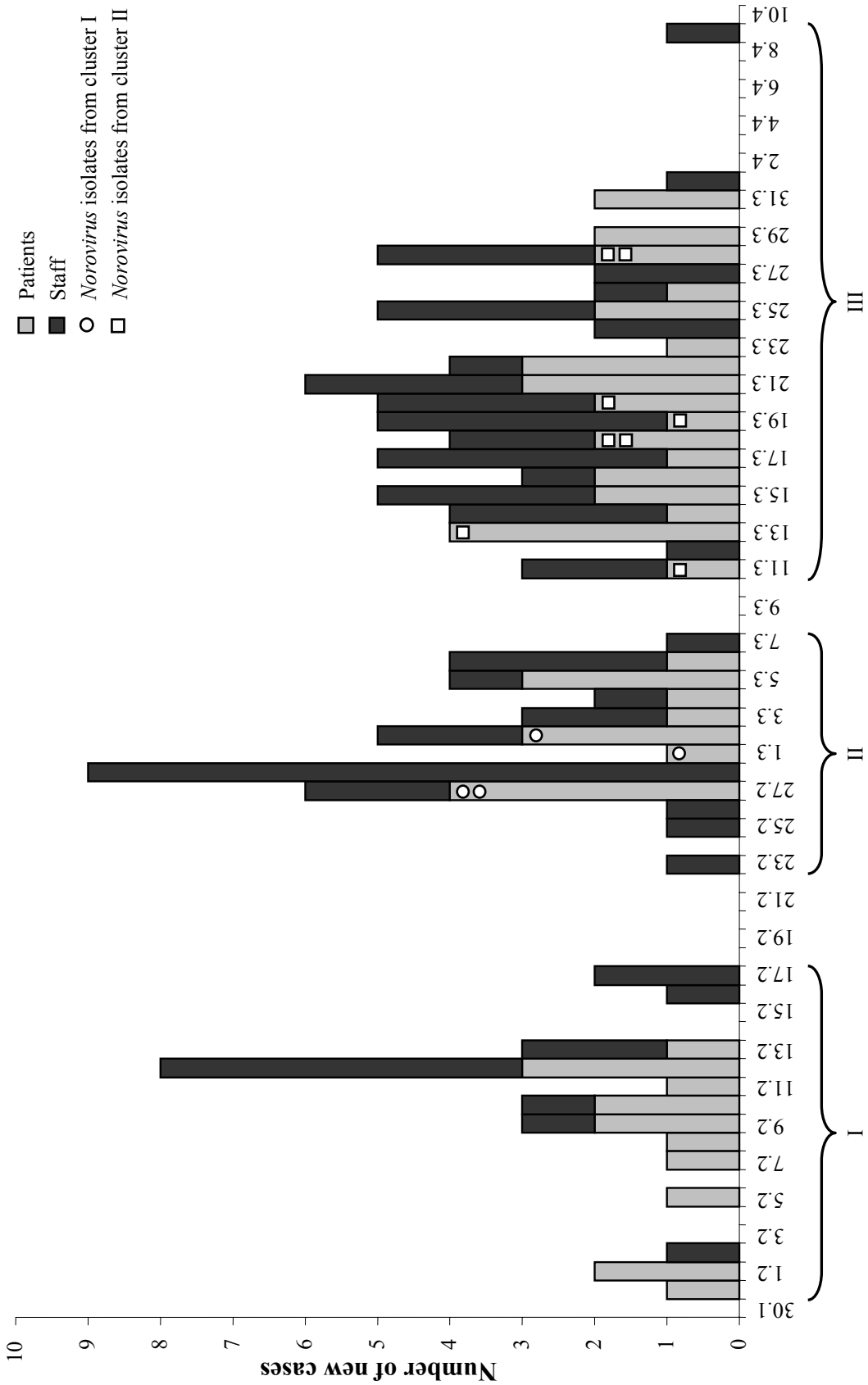


Figure 3: Epidemic curve of consecutive *Norovirus* outbreaks in a hospital with an affiliated nursing home between the end of January and beginning of April 2003. The three distinct peaks correspond to local distribution of cases and differences between *Norovirus* isolates.

Figure 4: UPGMA tree (Kimura matrix) showing the phylogenetic relationship between *Norovirus* sequences from patient stool samples (IDs) compared to the following reference strains: Camberwell virus (CAV; AF145896), Hawaii virus (HV; U07611), Lordsdale virus (LV; X86557) and the used positive control (Pos.). The *Norovirus* sequences of clusters I and II are allocated to the second peak (23 February to 7 March), and the third peak (11 March to 10 April) respectively of the epidemic curve as displayed in Figure 3.

5.5 Discussion

Between the years 2001 and 2003, 73 *Norovirus* outbreaks were registered by the study network consisting of cantonal food and health authorities and SFOPH. This figure will certainly not account for the real number of outbreaks during this time period in Switzerland, because first, 90% (66/73) of the outbreaks were registered in the German-speaking part of Switzerland due to the study design and second, because of the lack of an established nationwide reporting system for *Norovirus* infections. Germany, for example, has operated such a reporting system since 2001 [16]. Prior to 2001, only very rudimentary *Norovirus* outbreak data from Switzerland were available. Furthermore, within this period foodborne transmission was thought to be the dominant transmission pathway [8]. From the total 156 registered foodborne outbreaks in Switzerland in the six years from 1993 to 1998, only 25 (16%) were confirmed or possible *Norovirus* infections [17]. In England & Wales, Germany, and The Netherlands, a striking increase in *Norovirus* outbreaks occurred in 2002. This coincided with the detection and emergence of a new predominant *Norovirus* GGII variant [18]. This emergence of a new strain can most probably be used to explain the high number of registered outbreaks presented in this study.

With respect to settings, the registered outbreaks occurred predominantly in nursing homes (34%), hospitals (25%), camps (12%) and hotels (7%). The investigation of 1877 *Norovirus* outbreaks between 1992 and 2000 in England & Wales revealed a similar situation. In total, 40% of the outbreaks occurred in hospitals, 39% in residential-care facilities, 8% in hotels, 4%in schools, 6%were linked to food outlets and the remaining 4% occurred in other settings [3]. These proportions were confirmed in a further study for 2002 in England & Wales [19].

In our study, in only seven of the 54 outbreaks (13%) with a known infection route for noroviruses could a foodborne transmission have occurred. Outbreaks due to contaminated food and water vary from country to country. Finland reported 24%, The Netherlands 17%, Slovenia 14%, Spain, England & Wales 7% [4]. The predominance of the person-to-person transmission route (81% of all outbreaks with known transmission route) confirms the results of an English study, where a rate of 85% was found [3].

The three consecutive outbreaks in ski camps clearly demonstrated the epidemiological potential of *Norovirus*-contaminated environments. Earlier studies have clearly shown that *Norovirus* particles may keep their infectivity for lengthy periods [20–22]. For example, on carpets they stay infectious for up to 12 days [20]. Therefore, treatment of contaminated environments with an appropriate disinfectant (noroviruses are non-enveloped viruses) is of the utmost importance in halting the chain of infection [6]. Adequate treatment of contaminated clothes and linen, e.g. pillow covers, should also be performed [6]. Outbreaks in camps should generally be reported to the management of the establishments so that disinfection of the rooms can be organized. Waterborne outbreaks with noroviruses were shown to be associated with contaminated septic tanks, industrial water systems and swimming water as well as drinking water worldwide [23]. Two waterborne outbreaks occurred in Switzerland in 1998 and 1999 [6, 24, 25]. The first outbreak with 3500 cases was a result of a pump failure producing a spill of sewage into the groundwater [24], the second outbreak with 1400 cases occurred due to the use of contaminated and accidentally untreated surface water [6, 25]. In 2001, the probable waterborne outbreak described in the present study was registered. The most recent case occurred in 2002 in a ski region of the Swiss Alps. Here, 100–150 persons suffered from acute gastroenteritis during a period of 2 weeks. Noroviruses were detected in patient stool samples and investigations revealed that the drinking water system was contaminated by faeces from a sewage leakage [Schmid, H. (SFOPH), personal communication]. There is a strong tendency that such outbreaks in

Switzerland are most often the result of deficiencies in the infrastructure or in the water treatment process [6, 26].

In the previously reported banquet incident, various facts pointed to a foodborne scenario, however, a cohort study demonstrated that no association existed between consumed foodstuff and illness. The introduction of noroviruses into the setting by the young girl who was ill shortly before the banquet is a scenario often found in outbreaks, particularly in camps and nursing homes. The simplicity of transmission of noroviruses can be explained by the low infectious dose (10–100 particles) [6, 8], the effective transport of the agent by air after projectile vomiting of infected persons [6, 27] and by the prolonged shedding of viruses [6, 8]. Because of the simple and rapid transmission of noroviruses from person-to-person, every patient has a literally inherent potential to initiate outbreaks, at least within his own family. Public health institutions in particular, have to account for this possibility. Due to modern-day travel, noroviruses can easily cross national borders as demonstrated by two recent studies [15, 28]. Furthermore, a US study demonstrated the global circulation of a single *Norovirus* strain [29].

The previously presented example of consecutive outbreaks in a hospital with an affiliated nursing home illustrates how important it is not only to perform tests to detect noroviruses but also to conduct phylogenetic analysis of *Norovirus* RT–PCR products. Together with the results of the epidemiological investigation, it was feasible to determine that the incident was not one protracted outbreak, as initially thought, but consisted of different autonomous outbreaks. This was also meaningful in terms of the quality evaluation of the accomplished outbreak management. A very similar situation was found in another hospital, where from the beginning of Gastroenteritis outbreaks due to *Norovirus* 7 November to the end of December 2002, 130 persons (patients and staff) were affected by *Norovirus* infections. Nine *Norovirus* isolates from patient stool samples were phylogenetically analysed and again exhibited two different clusters. These clusters were allocated to patients that stayed locally and temporally on different floors and departments of the hospital (data not shown). It is also important to note that these hospital outbreaks all reflect the *Norovirus* situation in the community. In each hospital outbreak, a number of patients had acquired their infection outside the hospital, i.e. in the community. Thus far, there exists only one Swiss hospital outbreak which has been previously described; in March 2001 with 63 patients [30].

Epidemiological profiling, also recommended by authors from the United States, is a strong tool to conduct a fast and first assessment of a suspected *Norovirus* outbreak scenario [31]. This is important because of the current lack of routine analysis for noroviruses in Switzerland. Furthermore, the rapid implementation of outbreak control measures, even prior to the confirmation of *Norovirus* infection, is crucial.

5.6 Acknowledgement

The authors thank Jürg Grimbichler, Cantonal Laboratory Basel-Landschaft, for his support with the *Norovirus* analysis, Rosemarie Contre and Peter Wiedemeier, Hospital Limmattal, for their assistance and cooperation. The study was fully financed by the SFOPH and the Cantonal Laboratory Basel-Landschaft.

5.7 References

1. de Wit MAS, Koopmans MPG, van Duynhoven YTHP. Risk factors for Norovirus, Sapporo-like Virus, and Group A Rotavirus gastroenteritis. Emerg Infect Dis 2003; 9: 1563–1570.
2. de Wit MAS, Koopmans MPG, Kortbeek LM, van Leeuwen WJ, Bartelds AIM, van Duynhoven YTHP. Gastroenteritis in sentinel general practices in the Netherlands. Emerg Infect Dis 2001; 7: 82–91.
3. Lopman BA, Adak GK, Reacher MH, Brown DW. Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992–2000. Emerg Infect Dis 2003; 9: 71–77.
4. Lopman BA, Reacher MH, Van Duijnhoven Y, Hanon FX, Brown D, Koopmans M. Viral gastroenteritis outbreaks in Europe, 1995–2000. Emerg Infect Dis 2003; 9: 90–96.
5. de Wit MAS, Koopmans MPG, Kortbeek LM, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands, incidence and etiology. Am J Epidemiol 2001; 154: 666–674.
6. Fretz R, Svoboda P, Schmid H, Baumgartner A. Gastroenteritis due to norovirus – an overview [in German]. Bulletin SFOPH 2003; 46: 828–833.
7. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. Emerg Infect Dis 1999; 5: 607–625.
8. CDC. 'Norwalk-like viruses': public health consequences and outbreak management. MMWR Recomm Rep 2001; 50: 1–18.
9. Beuret C, Kohler D, Baumgartner A, Lüthi TM. Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl Environ Microbiol 2002; 68: 1925–1931.

10. Swiss Federal Statistic Office, 2004 (http://www.bfs.admin.ch).

11. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. Am J Public Health 1982; 72: 1329–32.

12. Lüthi TM. Identification of possible sources of gastrointestinal outbreaks by using a combination of clinical, bacteriological and epidemiological criteria [in German]. Mitt Lebensm Hyg 1998; 89: 196–218.

13. Beuret C. A simple method for isolation of enteric viruses (noroviruses and enteroviruses) in water. J Virol Methods 2003; 107: 1–8.

14. Beuret C, Baumgartner A. Empfohlenes Verfahren für den Nachweis von 'Norwalk-like' Viren (NLV) und Enteroviren in Wasser. Mitt Lebensm Hyg 2002; 93: 91–103.

15. Fretz R, Schmid H, Kayser U, Svoboda P, Tanner M, Baumgartner A. Rapid propagation of norovirus gastrointestinal illness through multiple nursing homes following a pilgrimage. Eur J Clin Microbiol Infect Dis 2003; 22: 625–627.

16. Höhne M, Schreier E. Detection and characterization of norovirus outbreaks in Germany: application of a one-tube RT-PCR using a fluorogenic real-time detection system. J Med Virol 2004; 72: 312–319.

17. Schmidt K, Tirado C (eds). WHO surveillance programme for control of foodborne infections and intoxications in Europe: Seventh Report 1993–1998. Berlin : BfR, 2001: 343–353.

18. Lopman B, Vennema H, Kohli E, et al. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet 2004; 363: 682–688.

19. PHLS. Norovirus outbreaks peak in 2002: England and Wales. J Public Health Med 2003; 25: 179–180.

20. Cheesbrough JS, Green J, Gallimore CI, Wright PA, Brown DW. Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. Epidemiol Infect 2000; 125: 93–98.

21. Evans MR, Meldrum R, Lane W, et al. An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiol Infect 2002; 129: 355–360.

22. Kuusi M, Nuorti JP, Maunula L, et al. A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. Epidemiol Infect 2002; 129: 133–138.

23. Nygard K, Torven M, Ancker C, et al. Emerging genotype (GGIIb) of norovirus in drinking water, Sweden. Emerg Infect Dis 2003; 9: 1548–1552.

24. Maurer AM, Stürchler D. A waterborne outbreak of small round structured virus, campylobacter and shigella co-infections in La Neuveville, Switzerland, 1998. Epidemiol Infect 2000; 125: 325–332.

25. Lüthi TM, Beuret C. What is the reason for virus detection in water? [in German]. Gas Wasser Abwasser 2001; 5: 283–290.

26. Baumgartner A. Norwalk-like viruses and foodstuffs – a situation analysis for Switzerland [in German]. Bulletin SFOPH 2001; 46: 909–916.

27. Lopman BA, Brown DW, Koopmans M. Human caliciviruses in Europe. J Clin Virol 2002; 24: 137–160.

28. Pedalino B, Feely E, McKeown P, Foley B, Smyth B, Moren A. An outbreak of Norwalk-like viral gastroenteritis in holidaymakers travelling to Andorra, January-February 2002. Euro Surveill 2003; 8: 1–8.

29. Noel JS, Fankhauser RL, Ando T, Monroe SS, Glass RI. Identification of a distinct common strain of 'Norwalk-like viruses' having a global distribution. J Infect Dis 1999; 179: 1334–1344.

30. Khanna N, Goldenberger D, Graber P, Battegay M, Widmer AF. Gastroenteritis outbreak with Norovirus in a Swiss university hospital with a newly identified virus strain. J Hosp Infect 2003; 55: 131–136.

31. Hall JA, Goulding JS, Bean NH, Tauxe RV, Hedberg CW. Epidemiologic profiling: evaluating foodborne outbreaks for which no pathogen was isolated by routine laboratory testing: United States, 1982–9. Epidemiol Infect 2001; 127: 381–387.

6. Rapid Propagation of *Norovirus* Gastrointestinal Illness through Multiple Nursing Homes Following a Pilgrimage

R. Fretz[1,2], H. Schmid[3], U. Kayser[4], P. Svoboda[1], M. Tanner[2], A. Baumgartner[3*]

[1] *Cantonal Laboratory Basel-Landschaft, 4410 Liestal, Switzerland*

[2] *Swiss Tropical Institute, 4002 Basel, Switzerland*

[3] *Swiss Federal Office of Public Health, 3003 Bern, Switzerland*

[4] *6403 Kuessnacht, Switzerland*

* Corresponding author:
Dr. Andreas Baumgartner, Swiss Federal Office of Public Health, Division of Food Science, 3003 Bern, Switzerland.
Phone: ++41 31 322 95 82; E-mail: andreas.baumgartner@bag.admin.ch

---

Paper 4:

Rapid propagation of *Norovirus* gastrointestinal illness through multiple nursing homes following a pilgrimage.

Published in: *Eur J Clin Microbiol Infect Dis (2003) 22:625-627*

6.1 Abstract

Reported here is an outbreak of gastroenteritis due to *Norovirus* infection that affected at least 450 persons from nursing homes and similar institutions in Switzerland during and after an organised pilgrimage to Lourdes in France. The outbreak was characteristic of direct person-to-person transmission, with the primary cases occurring in the hospital that harboured some of the pilgrims in Lourdes.

6.2 Introduction

In recent years, outbreaks of viral gastroenteritis due to human caliciviruses of the genus *Norovirus* (formerly called "Norwalk-like viruses") have been increasingly recognised in Switzerland. The growing awareness of these pathogens can be attributed to advances made in detection methods for the identification and typing of noroviruses (e.g., reverse transcriptase polymerase chain reaction technology). Illness is characterised by acute onset vomiting and diarrhoea following an average incubation period of 12–48 h. The faecal-oral route has been described as the most common route of transmission, but the very low infectious dose of these pathogens (probably $<10^2$ viral particles) allows easy and rapid spread from person-to-person via droplets, fomites, or faecal contamination [1]. Noroviruses are not routinely searched for in clinical microbiology laboratories in Switzerland, and there is no obligation to report infections caused by this viral agent to a governing health agency. For this reason, the Swiss Federal Office of Public Health launched a project to investigate outbreaks in which a viral aetiology was suspected [2]. The present report describes the investigation of one such outbreak that occurred in spring 2002 in association with an organised pilgrimage to Lourdes, France. Epidemiological investigations in Switzerland were conducted by the Cantonal Laboratory Basel-Landschaft and those in Lourdes by the district health office (Direction Départementale des Affaires Sanitaires et Sociales des Hautes Pyrénées).

6.3 Patients and Methods

On 2 May 2002 the health authorities of a Swiss canton informed the Swiss Federal Office of Public Health of an outbreak of gastroenteric illness occurring in a nursing home (institution A). The outbreak was affecting elderly residents as well as nursing staff. The observed symptoms and the spread of the illness were similar to those of earlier outbreaks in similar

settings caused by noroviruses. It very quickly became apparent that institution A was part of a much larger outbreak that included many similar institutions whose residents had participated in a pilgrimage to the city of Lourdes in France.

For the epidemiological investigation of the outbreak, the following two case definitions were used: (i) a person who participated in the pilgrimage to Lourdes between 19 and 25 April 2002 and who developed symptoms compatible with *Norovirus*-associated gastroenteritis either during the pilgrimage or within the 2-week period thereafter; (ii) a person who was a resident or employee of one of the institutions participating in the pilgrimage who developed symptoms compatible with *Norovirus*-associated gastroenteritis within 3 weeks following the return of the pilgrims. The main symptoms of *Norovirus*-associated illness were defined as projectile vomiting and/or diarrhoea, and constitutional symptoms including headache, nausea, myalgia, abdominal cramps and fever.

The pilgrimage to Lourdes was undertaken by individuals from 29 different institutions, and they were transported on four special trains. Those pilgrims who needed constant medical care (n=119) travelled on the so-called “blue train”, together with accompanying medical personnel, and they arrived at their destination in the morning of 20 April. The 119 pilgrims from this train stayed at a pilgrim’s hospital, and 107 of them were assigned to the third floor. The 89 accompanying personnel remained responsible for the care of the pilgrims during the whole stay, but they were accommodated in nearby hotels. On 21 April, the first cases of gastroenteric illness appeared among the pilgrims on the third floor of the hospital, and 1 day later the first cases of illness occurred among the accompanying personnel. By the end of the stay in Lourdes on 24 April, the number of new cases had reached 69. Thirty-three of these individuals were pilgrims, all of whom had resided on the third floor of the host hospital, and 36 were accompanying personnel, who had resided in hotels (Fig. 1). Despite frequent get-togethers during the stay in Lourdes, no cases occurred among pilgrims residing on other floors of the hospital or among other hotel guests.

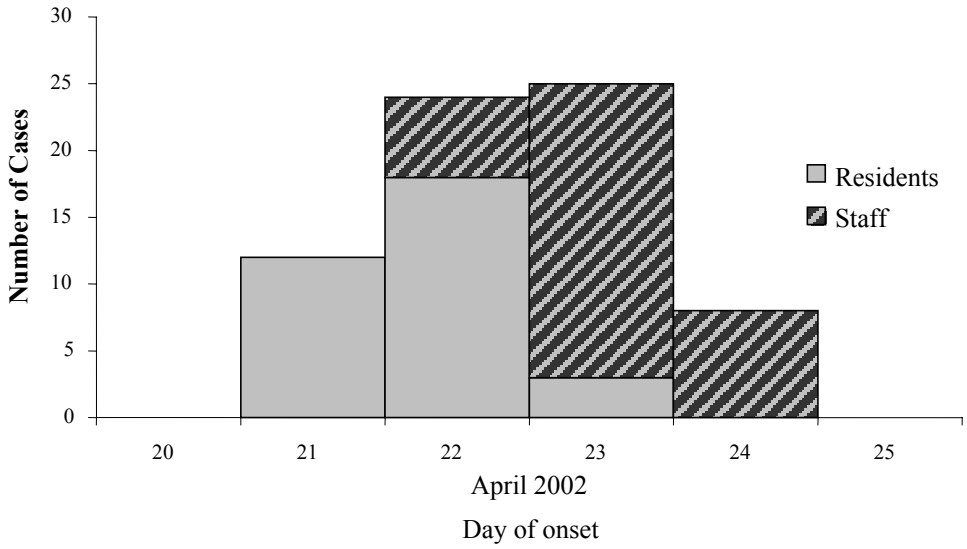


Figure 1. Epidemic curve of an outbreak of *Norovirus* gastroenteritis occurring in pilgrims and accompanying staff in Lourdes, France, in April 2002

During the return journey, which began on 24 April, no additional cases of illness occurred among the persons on the "blue train". On 25 April the pilgrims returned to their 29 different institutions of residence. During the following days, 11 (38%) of these institutions experienced new outbreaks of gastroenteric illness, amounting to more than 380 apparently secondary cases.

Institution A was the most severely affected, with 47 cases occurring among 64 residents (73%) and 32 cases among 63 staff members (51%). The accompanying physician from institution A was in contact with vomiting patients on the "blue train" and fell ill the day after return. Three residents of institution A had been ill in Lourdes and had travelled on the "blue train"; two staff members who had close contact with these three patients after their return fell ill on 27 April. Over the following days, the illness spread epidemically within the institution (Fig. 2). The outcome was fatal in one patient who had a severe underlying disease.

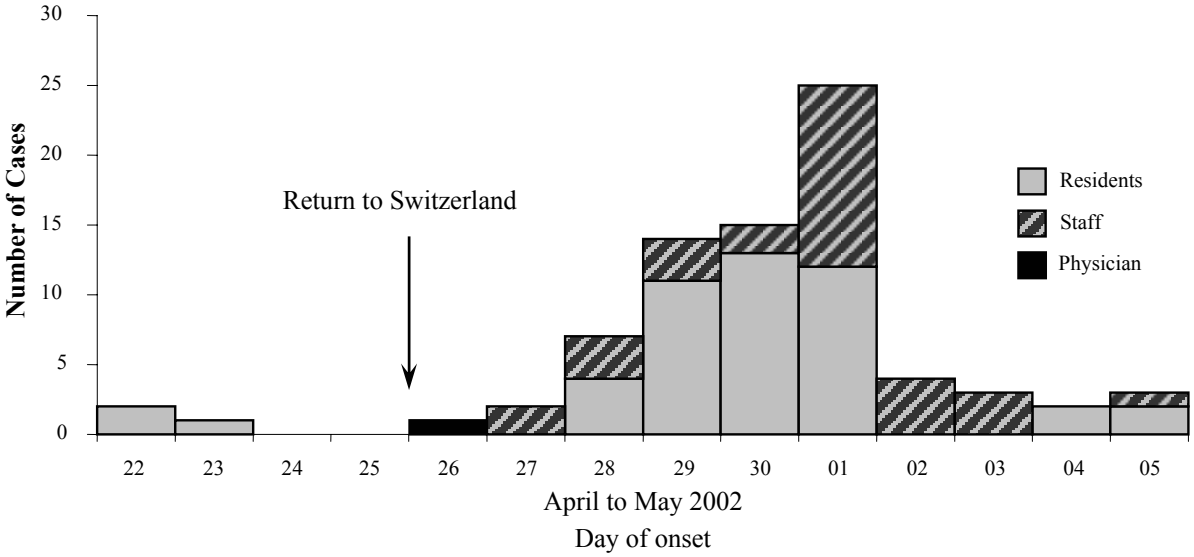


Figure 2: Epidemic curve of an outbreak of *Norovirus* gastroenteritis in a nursing home (institution A) after the return of three residents from a pilgrimage to Lourdes, France, in April 2002 where they had an episode of gastroenteritis. The physician accompanying the residents of institution A was in contact with vomiting patients during the return journey by train on 24 April and became ill himself on the day after return. Two staff members who had been in close contact with three pilgrims after their return became ill on 27 April.

In institution B a very similar outbreak pattern was recorded, with attack rates of 17% (38 illnesses) occurring among residents and 11% (28 illnesses) among staff. Furthermore, nine of the affected staff members in institution B reported similar illnesses among their family members outside of the institutional setting; however, these probable tertiary cases were not followed up. The epidemic curve for institution B was very similar to that of institution A and is therefore not shown. No staff member was shared between institutions A and B.

Six stool specimens from patients in institution A and six from patients in institution B, all of which had resulted negative for routine bacteriological tests, were further analysed using reverse transcriptase polymerase chain reaction at the Cantonal Laboratory Basel-Landschaft, which specialises in the diagnosis of noroviruses. The method used involves a genogroup-specific reverse transcriptase polymerase chain reaction system for the detection of *Norovirus* GG II based on degenerate primers and located in highly conserved regions of the RNA polymerase [3]. It is part of the method used to detect noroviruses in water samples, which has been approved by the Swiss Federal Office of Public Health [4]. All 12 specimens were positive for *Norovirus*, and 11 of the strains could be sequenced. A comparison of these 11 sequences with the DNA sequences publicly available in the GenBank database (National Center for Biotechnology Information, Bethesda, Md., USA) [5] showed 94% identity of all

sequences with "Norwalk-like virus NLV/Miami Beach/326/1995/US" (GenBank Accession No. AF414424). For the phylogenetic comparison of sequences, ClustalX 1.81, PAUP 4.0b10, EMBOSS matcher 2.0u4 and TreeView V1.6.6 were used. Among themselves, the 11 *Norovirus* sequences showed 99.4% identity. Cross-contamination with the positive control used for testing was excluded. Displayed in Fig. 3 is the NJ-phylogram of the RNA polymerase sequence information of the 11 Swiss *Norovirus* isolates from institutions A and B (deposited in GenBank under Accession No. AY339133–AY339143) plus the reference sequences. One *Norovirus* sequence isolated from a primary case patient in Lourdes (A4), who normally resided in institution A, showed 100% identity with the *Norovirus* sequence from a later case occurring in institution A (A2) and 99.4% identity with the *Norovirus* sequence of a case from institution B (B2).

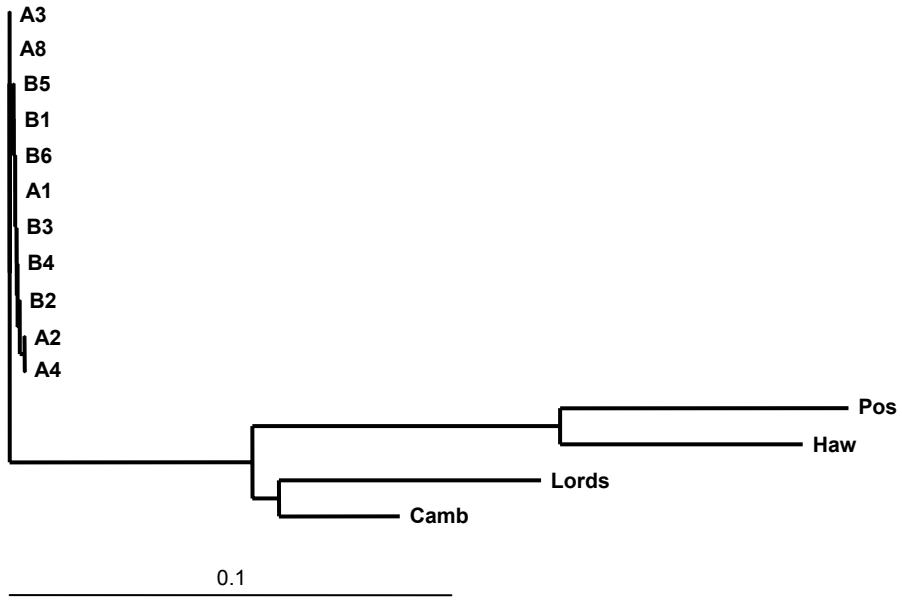


Figure 3: Phylogram created using the Neighbour Joining method of analysis with RNA polymerase sequence information of Swiss *Norovirus* isolates form institution A (A1, A2, A3, A4, A8), from institution B (B1, B2, B3, B4, B5, B6), the used internal positive control (Pos) and sequences from GenBank (Haw: Hawaii Calicivirus HCU07611; Lords: Lordsdale virus X86557; Camb: Camberwell virus AF145896). The internal positive control (Pos) has a 96% sequence identity with NV/Westover/302/1994/US{AF414418}])

Through further epidemiological investigations, it could be ascertained that in the week before the arrival of the Swiss pilgrims in Lourdes, and also in the week of their stay, gastroenteric illnesses also occurred in groups of French pilgrims. In the week following the departure of the Swiss pilgrims, 75 further cases of gastroenteric illness were recorded in the pilgrim's hospital, with most of them occurring again on the third floor.

6.4 Discussion

Many outbreaks due to *Norovirus* infection have been investigated in Switzerland in past years, and most of them showed an epidemic curve characteristic of a protracted outbreak with primary, secondary, and sometimes tertiary cases. In most of these events, the sequence of illness onset observed in the affected persons provided evidence for the direct transmission of a *Norovirus* from person to person [2], with foodstuffs or water being implicated in only a few instances. The outbreaks occurred in institutional settings (e.g. nursing homes, nursery schools, youth centres, hostels) or places where many people gather (e.g. sports events, youth camps, parties). Similar settings for *Norovirus* outbreaks have been described in other countries [6, 7, 8]. There is accumulating evidence that person-to-person transmission is the most common route of *Norovirus* infection [9, 10].

In summer 2000, a *Norovirus* outbreak affected about 120 attendees of a boy scout camp in southern Switzerland. The index case had vomited prior to his arrival in the camp, while travelling by train. When the children returned to their homes in the northern part of the country, they caused new outbreaks among their family members [11]. A very similar pattern was observed in the pilgrimage outbreak described here, demonstrating again the highly infectious nature of noroviruses.

The very close similarity (99.4%) determined in the viral sequences of strains from two different institutions pointed to a common origin of infection. The primary infections were probably acquired in the pilgrim's hospital in Lourdes. However, it could not be conclusively determined whether the viral agent had been carried to the centre by visitors preceding the Swiss group or if a Swiss passenger on the "blue train" had been ill before the journey and was still shedding the virus while travelling. Due to the 100% identity shared by one *Norovirus* sequence isolated from a primary case in Lourdes, who normally resided in institution A (A4) and a *Norovirus* sequence from a later case occurring in institution A (A2) and the 99.4% identity shared by sequences from secondary cases in institution A (A2) and institution B (B2), it is very likely that returning virus shedders had caused secondary cases in their institutional settings. Patients in nursing homes and similar institutions can be regarded as particularly susceptible collectives.

This report clearly emphasises the importance of sequencing *Norovirus* isolates in outbreak investigations, since, in most instances, an epidemiological link between outbreaks is not as obvious as in the present case. Furthermore, it is hoped that this report may stimulate discussions in Switzerland regarding the need for an obligatory reporting system for *Norovirus* infections to ensure the future detection of linked outbreaks.

6.5 Acknowledgement

The authors thank P. Capdepon, Direction Départementale des Affaires Sanitaires et Sociales des Hautes Pyrénées for his investigations in Lourdes, H. de Valk, Institut de Veille Sanitaire, Paris, for her cooperation; C. Sacher, Cantonal Physician of Schwyz, for his precious collaboration on site; P. Helbling, Swiss Federal Office of Public Health, for critical review of the manuscript.

6.6 References

1. Centers for Disease Control and Prevention (2001) „Norwalk-like Viruses:“ public health consequences and outbreak management. MMWR 50 (No. RR-9): 1-17
2. Fretz R, Svoboda P, Lüthi TM, Baumgartner A (2002) Gastroenteritis outbreaks due to infections with «Norwalk-like viruses» (NLV) in Switzerland. In: Program and abstracts, 12th European Congress of Clinical Microbiology and Infectious Diseases, Abstract no. P648
3. Beuret C (2003) A simple method for isolation of enteric viruses (noroviruses and enteroviruses) in water. J Virol Methods 107 (1): 1-8
4. Beuret C, Baumgartner A (2002) Empfohlenes Verfahren für den Nachweis von “Norwalk-like” Viren (NLV) und Enteroviren in Wasser. Mitt Lebensm Hyg 93: 91-103
5. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL (2002) GenBank. Nucleic Acids Res 30 (1): 17-20
6. Becker KM, Moe CL, Southwick KL, McCormack JN (2000) Transmission of Norwalk virus during a football game. N Engl J Med 343: 1223-1227
7. Götz H, Ekdahl K, Lindbäck J, de Jong B, Hedlund KO, Giesecke J (2001) Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: Findings from large community outbreak in Sweden. CID 33: 622-628
8. Public Health Laboratory Service (2002) Outbreaks of Norwalk-like virus infections. Commun Dis Rep CDR Weekly [serial online] 12(51): 2-3
9. Koopmans M, von Bonsdorff CH, Vinje J, de Medici D, Monroe S (2002) Foodborne viruses. FEMS Microbiol Rev 26(2): 187-205
10. Green KY (1997) The role of human caliciviruses in epidemic gastroenteritis. Arch Virol Suppl 13: 153-165

11. Swiss Federal Office of Public Health (2002) Virale Gastroenteritis durch "Norwalk-like"-Viren: Epidemiologie, Diagnose und praktische Empfehlungen. Bulletin SFOPH 14: 274-275

7. Phylogenetic Analyses of *Norovirus* Isolates from Human Stool Samples, Mineral Waters and Oysters in Switzerland

R. Fretz[1,2], C. Beuret[3], P. Svoboda[1], M. Tanner[2], A. Baumgartner[4*]

[1] *Cantonal Laboratory Basel-Landschaft, 4410 Liestal, Switzerland*

[2] *Swiss Tropical Institute, 4002 Basel, Switzerland*

[3] *Spiez Laboratory, 3700 Spiez, Switzerland*

[4] *Swiss Federal Office of Public Health, 3003 Bern, Switzerland*

* Corresponding author:
Dr. Andreas Baumgartner, Swiss Federal Office of Public Health, Division of Food Science, 3003 Bern, Switzerland.
Phone: ++41 31 322 95 82; E-mail: andreas.baumgartner@bag.admin.ch

Paper 5:

Phylogenetic analyses of *Norovirus* isolates from human stool samples, mineral waters and oysters in Switzerland.

Submitted to: *Mitt Lebensm Hyg*, April 2005

7.1 Summary

Reported here are firstly the results of the phylogenetic analyses of human *Norovirus* (NV) isolates obtained from patients with sporadic infections and from outbreak cases in Switzerland between January 2001 and July 2003. Secondly, the results of the phylogenetic comparison between NV sequences from human stool specimens from Switzerland with NV sequences obtained from a recent study with European mineral waters and from an oyster screening in Switzerland (oysters originating from France) are presented. 63 of the 74 (85%) analysed human NV sequences belonged to NV Genogroup II and a temporal clustering was observed. The phylogenetic comparison of NV sequences from mineral waters and oysters with such of human origin revealed that the mineral water sequences were highly related and clustered predominantly separate to the human NV sequences. However, human NV sequences were also found within the mineral water cluster. Additionally, a temporal correlation between the dates of the stool specimen with the period of bottling of the mineral waters was observed. The oyster sequences displayed a far greater variability and no specific clustering with either mineral water or human NV sequences was found.

7.2 Introduction

Recent international studies have shown that viral infections, especially noroviruses (NV) (former name *"Norwalk-like viruses"*) are the most frequent cause of gastroenteritis in the community regarding the endemic and the epidemic situation (1-6). These viruses account for an estimated 6% and 11% of all infectious intestinal diseases in England and the Netherlands (3,5), respectively and for an estimated 23 million cases in the United States each year (7). NV are also the most common cause of outbreaks of infectious intestinal diseases in Western Europe and North America (3,7). The illness is characterised by acute onset of vomiting and diarrhoea, after an average incubation period of 12-48 hours. The faecal-oral route is described to be the most common route of transmission. NV are transmitted either by contaminated fomites (such as food and water) and environment, or directly from person-to-person (8). Furthermore, outbreaks have been associated with the ingestion of contaminated drinking or recreational surface water (9). Two recent Swiss studies found RNA sequences of NV in bottled mineral waters of various brands (9-10). In one of these investigations, samples of three European brands of mineral water without gas were monitored by RT-PCR during a period of a year. NV sequences were detected in 33% (53 of 159) of the analysed samples (9).

A further monitoring study found that 8 of 87 samples of oysters (originating from France) (9%) imported into Switzerland were positive for NV (11). Reported here are the results of the phylogenetic analyses of Swiss human NV isolates obtained from January 2001 to July 2003 and the results of the phylogenetic comparison between these human isolates with NV sequences from the mineral waters and oysters.

7.3 Methods

NV samples

NV-positive stool samples were obtained from sporadic cases and from NV outbreak patients between January 2001 and July 2003. Stool samples were randomly chosen from all available patient samples. Sequence information from a monitoring of oysters (November 2001 to February 2002; used NV sequences isolated from French oysters) (11) and from a monitoring of three European brands of mineral water (April 2000 to April 2001) (9) were included in the phylogenetic comparison.

RT-PCR methodology, sequence analyses and phylogeny

All samples were analysed with the same NV RT-PCR methodology. The method used consisted of a genogroup-specific RT-PCR system for the detection of NV GGII based on degenerate primers, located in highly conserved regions of the RNA polymerase (system B) and of a second generic RT-PCR system also based on degenerate primers (system A) (12). This combined system is a part of the detection method for NV in water samples recommended by the SFOPH in Switzerland (13). The NV RT-PCR of the human stool samples was performed at the Cantonal Laboratory Basel-Landschaft and the sequencing of the RT-PCR products was carried out by Microsynth GmbH (9436 Balgach, Switzerland). Phylogenetic analyses were performed on sequences from both NV detection systems. Alignment of the sequences was conducted by the software ClustalX 1.81. The distance-based (Kimura matrix) neighbor-joining (NJ) method in the software package PAUP 4.0b10 was the algorithm used for analysis and the phylogenetic trees were constructed with the software Treeview 1.6.6. The robustness of the NJ-trees generated were assessed by bootstrapping (1000 replications) in PAUP 4.0b10. Pairwise sequence comparisons were carried out with the software EMBOSS matcher 2.0u4s. The strains found in this study were compared with the following published reference sequences (GenBank accession numbers in parentheses): Desert Shield Virus (DSV; U04469), Bristol Virus (BV; X76716), Camberwell Virus (CAV;

AF145896), Hawaii Virus (HV; U07611), Lordsdale Virus (LV; X86557), Melksham Virus (MKV; X81879), Mexico Virus (MXV; U22498).

7.4 Results

Phylogenetic comparison of Swiss human NV isolates

In total, 74 NV sequences isolated from human stool samples were phylogenetically analysed. 32 isolates were detected by the generic primer system (system A) and the other 42 isolates by the GGII – specific system (system B). The appropriate NJ-trees are plotted in the figures 1 and 2.

A region of the RNA polymerase of approximately 120bp was amplified by RT-PCR and used for the phylogenetic analysis in the NV generic system A. Three distinct sequence clusters were detected. Cluster I revealed an overall sequence identity of 76.0% in respect to the sequence "A08" and of 77.9% in respect to the GGI reference strain Desert Shield Virus (DSV). Cluster II showed an overall sequence identity of 83.9% compared to the sequence "A09". The isolates within Cluster II grouped together with the following GGII reference strains: Hawaii Virus (HV), Camberwell Virus (CAV), Bristol Virus (BV), Lordsdale Virus (LV), Mexico Virus (MXV) and Melksham Virus (MKV). The isolates from cluster II showed an overall sequence identity of 85.0% with the Camberwell Virus. Finally, cluster III exposed a high sequence identity of 97.4% to the sequence "A30", as seen in figure 1.

For the NV GGII – specific system B, another region of the RNA polymerase of about 120bp was amplified by RT-PCR and used for the phylogenetic analysis. Two distinct clusters within NV GGII were found, as seen in figure 2. Cluster I showed an overall sequence identity of 96.1% compared to the sequence "B44" and cluster II demonstrated an overall sequence identity of 97.4% with the sequence "B43". Cluster II included the reference strain Bristol Virus (BV) and Lordsdale Virus (LV). The sequence identity between "B43" and "B44" was 80.0%. Compared to the reference sequence of the Camberwell Virus (CAV), cluster I showed a sequence identity of 87.4% and cluster II an identity of 91.1%.

In total, 11 of the 74 sequenced NV isolates (15%) belonged to the *Norovirus* GGI and 63 samples (85%) to GGII. No local clustering of the patients where the NV sequences were derived from could be observed (data not shown). Interestingly, a temporal clustering of the

NV sequences was observed: 8 of 10 NV GGII samples from the year 2001 and detected by the system B could be assigned to the found cluster I, as seen in figure 2. The cluster II on the other hand is mainly formed by sequences obtained in the year 2002 (17 of 26) and in the early months of 2003 (8 of 26). Of further interest is the fact that all samples positive for GGI were only found in the year 2001 and in the early months of 2002 by the system A, as seen in figure 1. This corresponds to the observation that GGI isolates are hardly found in Switzerland since the middle of 2002 (data not shown). The NV sequences “A08”, “A09”, “A30”, “B43” and “B44” were submitted to GenBank (GenBank accession numbers: AY545060-AY545064).

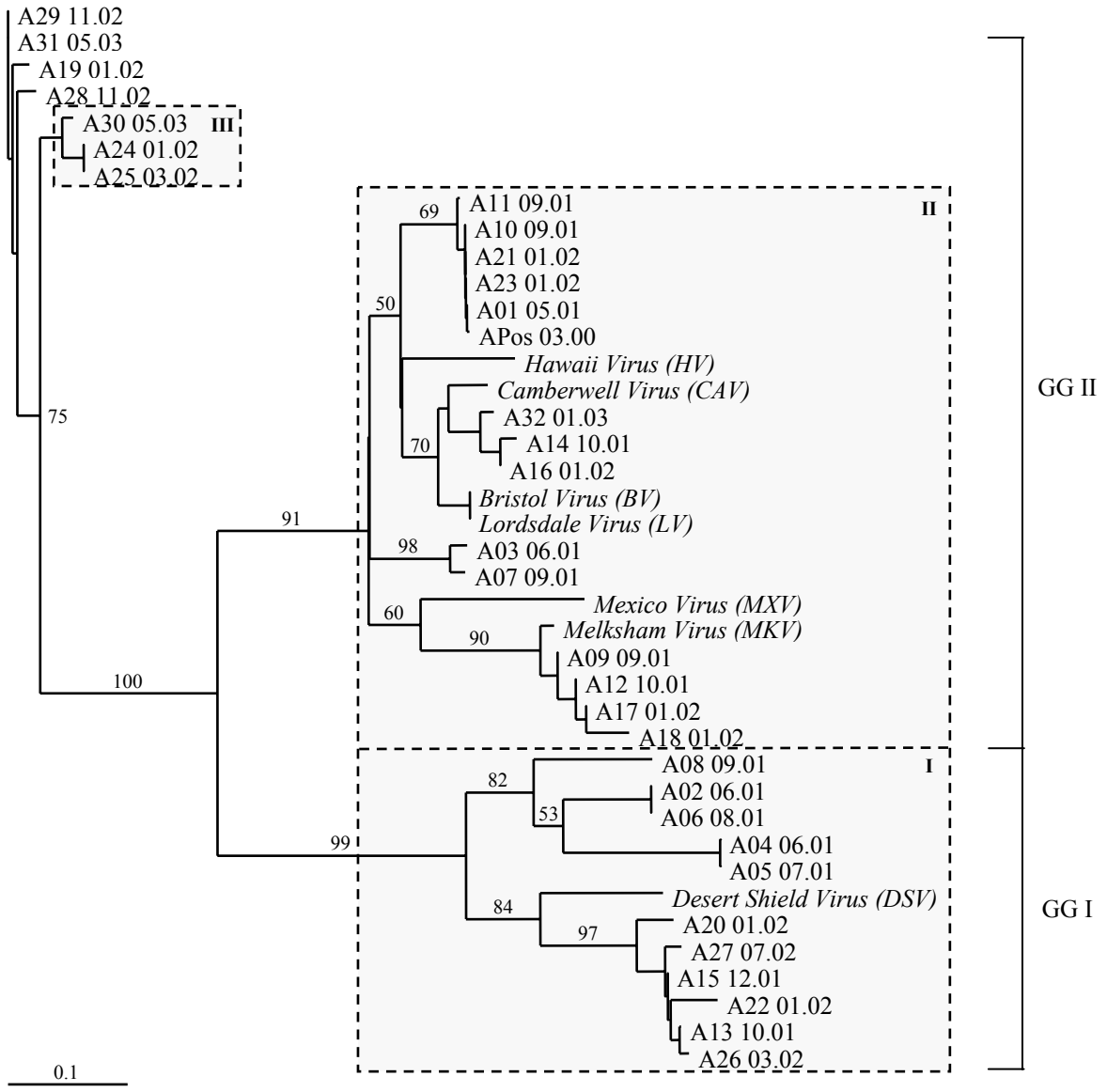


Figure 1: NJ-tree showing the phylogenetic relationship between human NV sequences from Switzerland (generated with the generic RT-PCR system A) in comparison with the following reference strains: Desert Shield Virus (DSV; U04469), Bristol Virus (BV; X76716), Camberwell Virus (CAV; AF145896), Hawaii Virus (HV; U07611), Lordsdale Virus (LV; X86557), Melksham Virus (MKV; X81879), Mexico Virus (MXV; U22498). Bootstrapping values are indicated above the major branches. The sequence IDs are describing the used primer system [A], ID-number and date of illness [mmyy]. A contamination with the used positive control ("APos") can be excluded.

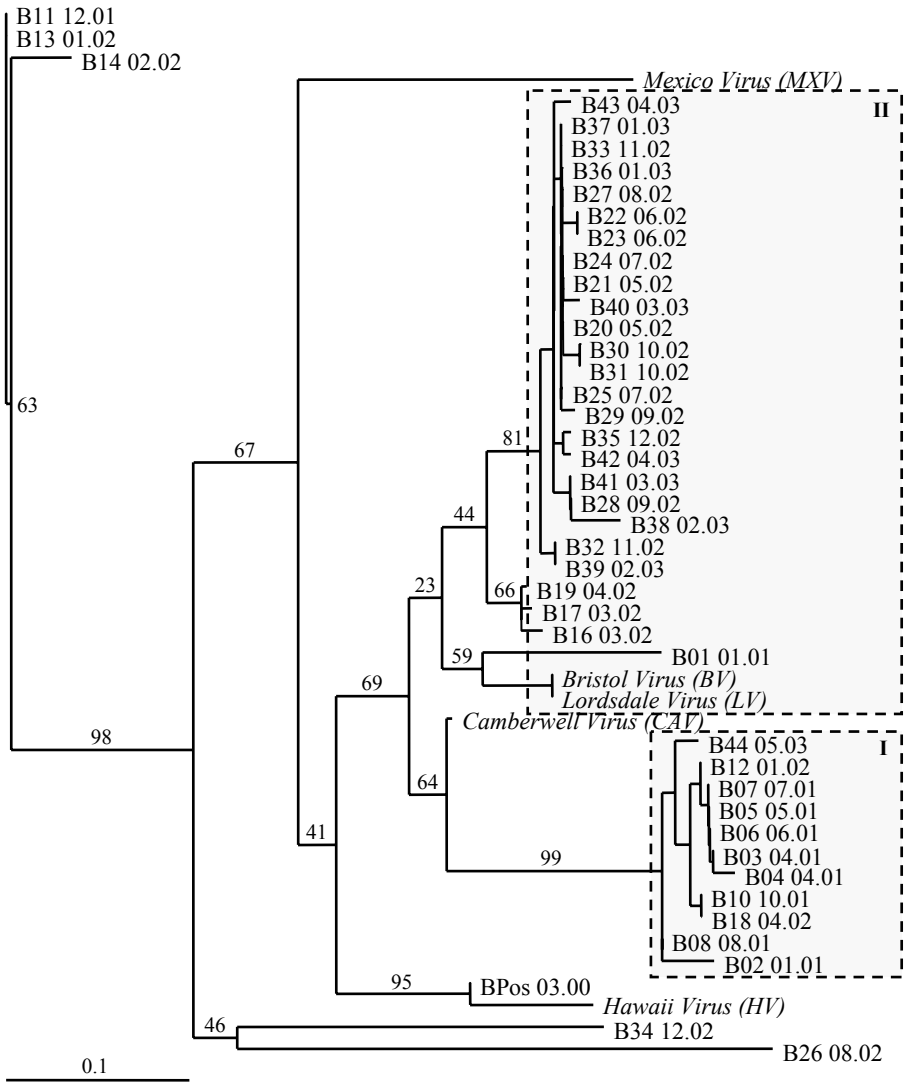


Figure 2: NJ-tree showing the phylogenetic relationship between human NV sequences from Switzerland (generated with the NV GGII – specific RT-PCR system B) in comparison with the following reference strains: Bristol Virus (BV; X76716), Camberwell Virus (CAV; AF145896), Hawaii Virus (HV; U07611), Lordsdale Virus (LV; X86557), Mexico Virus (MXV; U22498). Bootstrapping values are indicated above the major branches. The sequence IDs are describing the primer system used [B], ID-number and date of illness [mmyy]. A contamination with the used positive control ("BPos") can be excluded.

Phylogenetic Comparison between NV isolates from human stool samples from Switzerland, European mineral waters and French oysters

Sequence data from 18 additional NV patients from Switzerland, collected between 1999 and spring 2001, were added to the 74 human NV isolates of the period between January 2001 and July 2003. With this completion, the underrepresentation in the number of NV sequences from spring 2001 and before was corrected. This was meaningful in respect to the date of the NV analyses with mineral water samples which started in April 2000 and lasted to April 2001. The NV analyses with the oyster samples were originally performed between November 2001 and February 2002. Although the same NV RT-PCR method was applied on all samples, only a fragment of approximately 90bp for system A, respectively a fragment of approximately 70bp for system B, could be used for phylogeny. Even if the complete sequence information (120bp for the human isolates, 150bp for the mineral water isolates and 140bp for the oyster isolates) were used for an improper phylogenetic comparison due to massive sequence overlaps, the main conclusions drawn on the following phylogenies remain the same (data not shown).

Figure 3 shows the results of the phylogenetic comparison based on the system A. All NV sequences obtained from European mineral waters (IDs "ABAG#") clustered entirely together in GGII and were distinct from all others except for one Swiss human NV sequence, "AAdd1", which clustered within the mineral water sequences. This additional sequence derived from a NV positive tested water sample belonging to an outbreak in July 2000. The French oyster isolates (IDs "A60623" and "A54818") clearly clustered separately form the human isolates and showed the highest relation to the reference sequences of Bristol Virus (BV) and Lordsdale Virus (LV), respectively to Melksham Virus (MKV). In figure 4, the results of the phylogenetic comparison within system B are presented. The majority of all sequences was very closely related. Remarkably, the European mineral water sequences were once more clustering together and closely related to Swiss human NV isolates (especially to "BAdd9", "BPos" and "BAdd10"), whereas the sequences of the French oyster isolates were spread over the whole phylogram and showed no distinct clustering. The following NV sequences were submitted to GenBank: "AAdd1", "BAdd9", "BPos" and "BAdd10" (GenBank accession numbers: AY545065-AY545068).

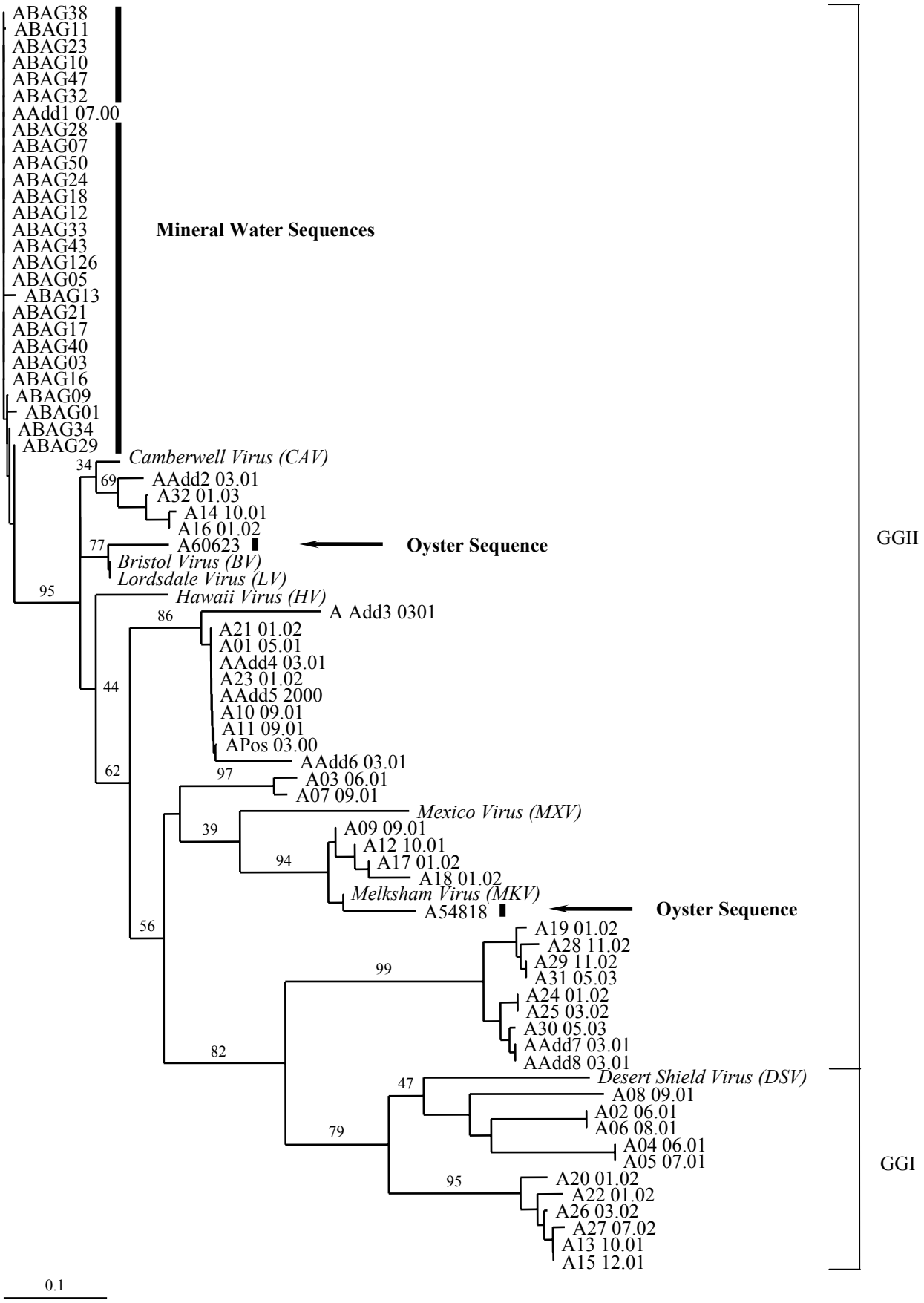


Figure 3: Legend on page 73.

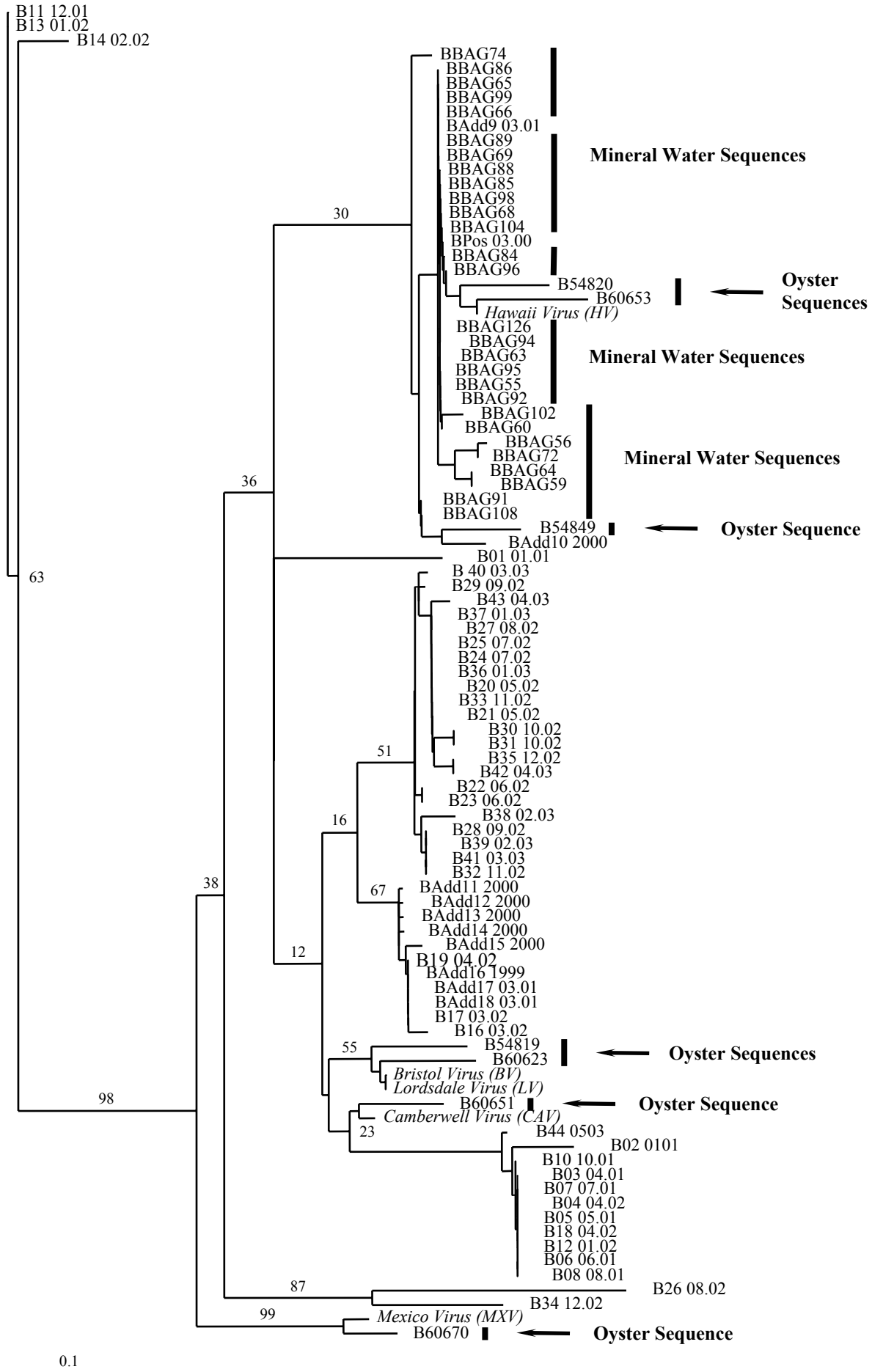


Figure 4: Legend on page 73.

Legends of figures 3 and 4

Figure 3: NJ-tree showing the phylogenetic relationship between human NV sequences from Switzerland (generated with the generic RT-PCR system A) in comparison with the sequences obtained from European mineral waters ("ABAG#"; indicated by black vertical bars) analysed between April 2000 and April 2001 and from French oyster samples (A60623 and A54818; indicated by black vertical bars) analysed between November 2001 to February 2002 and with the following reference strains: Desert Shield Virus (DSV; U04469), Bristol Virus (BV; X76716), Camberwell Virus (CAV; AF145896), Hawaii Virus (HV; U07611), Lordsdale Virus (LV; X86557), Melksham Virus (MKV; X81879) and Mexico Virus (MXV; U22498). Bootstrapping values are indicated above the major branches. The sequence IDs of the human samples are describing the primer system used [A], ID-number and date of analysis [mmyy].

Figure 4: NJ-tree showing the phylogenetic relationship between human NV sequences from Switzerland (generated with the NV GGII – specific RT-PCR system B) in comparison with the sequences obtained from European mineral waters ("BBAG#"; indicated by black vertical bars) analysed between April 2000 and April 2001 and from French oyster samples ("B60623" and "B54818"; indicated by black vertical bars) analysed between November 2001 to February 2002 and with the following reference strains: Bristol Virus (BV; X76716), Camberwell Virus (CAV; AF145896), Hawaii Virus (HV; U07611), Lordsdale Virus (LV; X86557) and Mexico Virus (MXV; U22498). Bootstrapping values are indicated above the major branches. The sequence IDs of the human samples are describing the primer system used [B], ID-number and date of analysis [mmyy].

7.5 Discussion

Phylogenetic comparison of Swiss human NV isolates

15% (11 of 74) of the NV isolates from human stool samples obtained between January 2001 and July 2003 were allocated to the NV GGI, the remaining 85% (63 of 74) NV isolates to the NV GGII. Furthermore, temporal clustering within the sequences could be observed. All GGI isolates were found in the year 2001 and early months of the year 2002 (figure 1). Analyses of the NV isolates detected by the GGII – specific system B showed that 8 of 10 NV isolates from the year 2001 grouped together and that 23 of 26 isolates from the year 2002 and early months of 2003 clustered jointly (figure 2). The temporal dominance of different NV strains or genotypes is described in various studies. A review of the internationally available NV sequences between the years 1992 and 2002 revealed a clear dominance of GGII strains and genotypes from both sporadic cases and outbreaks and the cyclic emergence and disappearance of distinct NV strains (14-21). A well cited study showed that a subset of 60 strains, the so-called "95/96-US" strain (GGII), was predominantly associated with NV outbreaks during the 1995-1996 season in the US. Furthermore, this strain showed a global distribution (15,18). In Europe, it could be observed that NV sequences found in the years 2000 and 2001 clustered around a newly emerging variant of a GGII strain (so-called

"GGIIb" strain) (19-20). Likewise, a cluster of NV isolates around a new GGII/4 strain (so-called "Farmington Hills" strain) could be detected during the year 2002 in the US (21). A striking increase in NV outbreaks occurred in 2002 in England and Wales, Germany, and in The Netherlands. This coincided with the emergence of a new predominant NV GGII/4 variant (22). In the GGII – specific system B 23 of 26 NV isolates from the year 2002 and early months of 2003 were clustering together. Within this specific cluster, Bristol virus, a GGII/4 reference strain (23) can be found (figure 2, cluster II). Most probably, these NV isolates are therefore corresponding to the new variant found in Europe. This assumption seems to be reasonable, because of the first occurrence of these new variant in Germany (Switzerland lies next to Germany) in January 2002 (22). Furthermore, a Swiss NV screening study from July 2001 to July 2003 revealed that the detection rates of NV positives peaked between January and July 2002 (manuscript in preparation). This circumstance may explain the grouping of certain NV GGII sequences found in this study.

Phylogenetic comparison between NV isolates from human stool samples from Switzerland, European mineral waters and French oysters

Phylogenetic analyses with both systems (NV generic system A and NV GGII – specific system B) revealed similar results with respect to the NV sequences from the European mineral water samples (figures 3 and 4). All mineral water sequences were highly related and clustered predominantly separate to the human NV sequences. Nevertheless, single human NV isolates from Switzerland were also found within these mineral water clusters. Furthermore, it was shown that the NV sequences found during the mineral water screening from April 2000 to April 2001 (9) were closely related to human NV isolates. The analysed European mineral waters were all bottled between October 1999 and August 2000. Interestingly, Swiss human NV isolates clustering within the European mineral water sequences ("AAdd1", "BAdd9", "BPos" and "BAdd10") came from patients of the same time period. From an epidemiological point of view, the NV positive results obtained by RT-PCR of mineral waters have to be treated with caution because the used detection method is not able to distinguish between infective and inactivated virus (9,10,24). But it was demonstrated that the NV sequences from the analysed mineral waters were stable after 6 and 12 months of storage (9). Furthermore, a recent study could display that enteric viruses, inactivated at moderate temperatures, still have a capsid that protects the RNA from RNase (25). The origin of NV sequence contamination in bottled mineral waters also remains unclear. Water sources, packing materials or the bottling process were considered as possible origin of contamination

(9). Contamination of the water source, e.g. by surface waters, may play a role. If so, could NV derive from an animal reservoir ? This way can be excluded because to date, no zoonotic transmission of NV was demonstrated (26). Additionally, it is established that the NV strains in animals (calf and pig) were genetically distinct to any NV found in humans (26-28). Regarding the close relationship between NV sequences found in mineral waters and human stool samples, it can be stated that the detected NV in the mineral waters are of human origin. The sensitivity of the method used was estimated to be around 10 viral particles. This suggested a low concentration of NV sequences per litre of the tested mineral water brands (9). Additionally, a case-control study to investigate risks for NV infections did not identify mineral waters to be a measurable risk factor (manuscript in preparation). Therefore, it can be supposed that mineral waters do not play a relevant role in the epidemiology of NV in Switzerland. This estimate confirms a former assessment of the Swiss Federal Office of Public Health (SFOPH) (6).

The sequence analyses NV from the French oysters showed a far greater variability compared to the NV sequences generated from the mineral water samples. Oyster sequences were not found within specific clusters, but grouped together with NV sequences from mineral waters and human samples. Together with the low NV positivity rate of 9% (8 of 87 samples) of the oyster monitoring study (11) and since the overall consumption of oysters in Switzerland is rather low (11), the direct impact on food safety and the epidemiology of the NV can be stated as rather low.

7.6 Acknowledgement

The authors would like to thank Jürg Grimbichler, Cantonal Laboratory Basel-Landschaft, for his support with the NV-analysis and Marius Grand, Swiss Federal Office of Public Health, for the French summary. The study was fully financed by the SFOPH and the Cantonal Laboratory Basel-Landschaft.

7.7 References

1. de Wit MAS, Koopmans MPG, van Duynhoven YTHP. Risk factors for Norovirus, Sapporo-like Virus, and Group A Rotavirus gastroenteritis. Emerg. Infect. Dis. 9, 1563-1570 (2003)
2. de Wit MAS, Koopmans MPG, Kortbeek LM, van Leeuwen WJ, Bartelds AIM, van Duynhoven YTHP. Gastroenteritis in sentinel general practices in the Netherlands. Emerg Infect Dis 7, 82–91 (2001)
3. Lopman BA, Adak GK, Reacher MH, Brown DW. Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. Emerg. Infect. Dis. 9, 71-77 (2003)
4. Lopman BA, Reacher MH, van Duijnhoven Y, Hanon FX, Brown D, Koopmans M. Viral gastroenteritis outbreaks in Europe, 1995-2000. Emerg. Infect. Dis. 9, 90-96 (2003)
5. de Wit MAS, Koopmans MPG, Kortbeek LM et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands, incidence and etiology. Am. J. Epidemiol. 154, 666–674 (2001)
6. Fretz R, Svoboda P, Schmid H, Baumgartner A. Durch Noroviren verursachte Gastroenteritis – eine Übersicht. Bull. SFOPH 46, 828-833 (2003)
7. Mead PS, Slutsker L, Dietz V et al. Food-related illness and death in the United States. Emerg. Infect. Dis. 5, 607-625 (1999)
8. CDC. "Norwalk-Like Viruses": public health consequences and outbreak management. MMWR Recomm. Rep. 50, 1-18 (2001)
9. Beuret C, Kohler D, Baumgartner A, Lüthi TM. Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl. Environ. Microbiol. 68, 1925-1931 (2002)
10. Beuret C, Kohler D, Lüthi T. Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction in mineral waters imported into or bottled in Switzerland. J. Food. Prot. 63, 1576-1582 (2000)
11. Beuret C, Baumgartner A, Schluep J. Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland. Appl. Environ. Microbiol. 69, 2292-2297 (2003)
12. Beuret C. A simple method for isolation of enteric viruses (noroviruses and enteroviruses) in water. J. Virol. Methods 107: 1-8 (2003)
13. Beuret C, Baumgartner A. Empfohlenes Verfahren für den Nachweis von "Norwalk-like" Viren (NLV) und Enteroviren in Wasser. Mitt. Lebensm. Hyg. 93: 91-103 (2002)
14. Hale A, Mattick K, Lewis D et al. Distinct epidemiological patterns of Norwalk-like virus infection. J. Med. Virol. 62, 99-103 (2000)
15. Noel JS, Fankhauser RL, Ando T, Monroe SS, Glass RI. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. J. Infect. Dis. 179, 1334-1344 (1999)

16. Iritani N, Seto Y, Haruki K, Kimura M, Ayata M, Ogura H. Major change in the predominant type of "Norwalk-like viruses" in outbreaks of acute nonbacterial gastroenteritis in Osaka City, Japan, between April 1996 and March 1999. J. Clin. Microbiol. 38, 2649-2654 (2000)

17. Gonin P, Couillard M, d'Halewyn MA. Genetic diversity and molecular epidemiology of Norwalk-like viruses. J. Infect. Dis. 182, 691-697 (2000)

18. White PA, Hansman GS, Li A et al. Norwalk-like virus 95/96-US strain is a major cause of gastroenteritis outbreaks in Australia. J. Med. Virol. 68, 113-118 (2002)

19. Buesa J, Collado B, Lopez-Andujar P et al. Molecular epidemiology of caliciviruses causing outbreaks and sporadic cases of acute gastroenteritis in Spain. J. Clin. Microbiol. 40, 2854-2859 (2002)

20. Nygard K, Torven M, Ancker C et al. Emerging genotype (GGIIb) of norovirus in drinking water, Sweden. Emerg. Infect. Dis. 9, 1548-1552 (2003)

21. CDC. Norovirus Activity – United States, 2002. MMWR 52, 41-45 (2003)

22. Lopman B, Vennema H, Kohli E et al. Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet 363, 682-688 (2004)

23. Vinje J, Vennema H, Maunula L et al. International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. J. Clin. Microbiol. 41, 1423-1433 (2003)

24. Gassilloud B, Schwartzbrod L, Gantzer C. Presence of viral genomes in mineral waters: a sufficient condition to assume infectious risk ? Appl. Environ. Microbiol. 69, 3965-3969 (2003)

25. Nuanualsuwan S, Cliver DO. Capsid functions of inactivated human picornaviruses and feline calicivirus. Appl. Environ. Microbiol. 69, 350-357 (2003)

26. Lopman BA, Brown DW, Koopmans M. Human caliciviruses in Europe. J. Clin. Virol. 24, 137-160 (2002)

27. Sugieda M, Nagaoka H, Kakishima Y, Ohshita T, Nakamura S, Nakajima S. Detection of Norwalk-like virus genes in the caecum contents of pigs. Arch. Virol. 143, 1215-1221 (1998)

28. van der Poel WH, van der Heide R, Verschoor F, Gelderblom H, Vinjé J, Koopmans MP. Epidemiology of Norwalk-like virus infections in cattle in The Netherlands. Vet. Microbiol. 92, 297-309 (2003)

8. Discussion and Conclusions

8.1 General Considerations

The present thesis was launched in the second half of the year 2000. Since then, the awareness of the relevance of NV infections has risen worldwide, especially in high-income countries. Prior to these studies, little epidemiological data was available for an assessment of the situation of the NV in Switzerland. Single incidents of foodborne NV outbreaks (25 confirmed or possible NV infections) were reported to the Swiss Federal Office of Public Health (SFOPH) between 1993 and 1998 (1). Between 1999 and 2000 "several outbreaks due to Norwalk-like viruses" were recorded (2). In March 2000, a pilot study was carried out by the Cantonal Laboratories Solothurn and Basel-Landschaft. This study revealed that 44 of 100 stool samples previously analysed negative for enteric bacterial and parasitic pathogens were NV-positive by RT-PCR (3). This result – together with the realisation of a gap in epidemiological data concerning NV – lead to the carrying out of the present studies. In the next sections, the methodology, the different study designs and the key results from these studies will be reviewed and a final conclusion concerning the present NV situation in Switzerland will be drawn.

8.2 RT-PCR Methodology and Phylogenetic Analysis

Using sequence information derived from an increasing number of NV strains, several international research groups successfully developed RT-PCR assays – similar to the one adopted in the present study – based on primers targeting a conserved region of the open reading frame 1 (ORF1) coding for the viral RNA polymerase. These assays have been used successfully in epidemiological studies for the diagnosis of NV in faecal specimens from both outbreaks and sporadic cases (4). A crude estimation of sensitivity of the used RT-PCR system was carried out and found to be approximately 10 NV particles in water samples (5). However, there is no data about sensitivity and specificity concerning the detection of NV in stool samples, a matrix far more complex than drinking water. Based on the high number of virus particles found in stool specimens (approximately 10^7 particles per ml) (6), the sensitivity of this assay may be nevertheless compromised by the possible occurrence of inhibitors effecting the PCR. Concerning the ability to detect current NV strains circulating in the population, it can be stated that the assay covered at least a great number of strains

circulating between 2000 and 2003 in Switzerland. This can be explained firstly by the creation of the assay in 1999 and its first use in 2000 (7) and secondly by the ability to detect sequences that belong to the newly emerged variant of NV as described in chapter 7.

8.3 Study Designs

NV Screening

From July 2001 to July 2003, a screening for the presence of NV in previously bacteriological-negative tested stool samples (negative at least for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp.) has been undertaken. The NV analysis was conducted on stool samples from patients that had to fulfil specific criteria, like age constraint (patients only between 6 months and 75 year of age) and the exclusion of known outbreak and hospital cases. The frequency of NV-positive stool samples was influenced by several selection biases introduced e.g. by the stringent inclusion criteria and by the local dominance of patients from cantons within the northern and the central part of the study area. A recent study revealed valuable information about possible selection biases concerning the presentation of cases of diarrhoea to general practice (GP) (8). The most relevant results from the present study were that severe illness, recent foreign travel, leaving full-time education at an early age and housing conditions (representing lower socio-economic status) all were associated with GP presentation (8). A further selection bias was brought into the study by the circumstance that parallel to the screening a case-control study was performed on the patients. From these patients, an informed consent prior to the NV analyses had to be obtained. Therefore, it is obvious that the NV positivity-rate may not be extrapolated from the study population to the general population. Nevertheless, this screening was very valuable to generate a baseline of the occurrence and distribution of NV infections in patients suffering from gastrointestinal illness or disorder.

NV Case-Control Study

A general practitioner-based matched case-control study was performed between July 2001 and July 2003. NV patients were identified by collaboration with two medical diagnostic laboratories and cases had to fulfil the following case definition: 1.) Episode of diarrhoea and/or vomiting in patients consulting a medical practitioner in the study area. 2.) Stool sample tested negative at least for *Campylobacter* spp., *Shigella* spp. and *Salmonella* spp. 3.) Stool sample tested positive for NV by RT-PCR. 4.) Cases residing within the defined study

area. 5.) Only one case per family was allowed to take part in the study. Furthermore, the following exclusion criteria applied: Babies under the age of 6 months, elderly patients above 75 years, patients with a history of possible nosocomial disease and known cases from an NV outbreak. One "friend-control" per case was selected. The control had to be of the same sex, to belong to the same age group and was not allowed to be related with the corresponding case. In addition, the control had to live at a close range to the case. Unfortunately, the use of a "friend-control" introduces automatically an unplanned matching, because it is likely that the case and his friend (control) share many lifestyle characteristics (9).

In total, 126 patients and 76 control persons were approved for the study. In comparison with other European case-control studies on gastrointestinal illness this sample size is relatively low. For example, the case-control component within the British "Study of Infectious Intestinal Disease in England" between 1992 and 1996 (10) was performed on over 1300 matched case-control pairs, a French study carried out in 1997 (11) recruited over 560 matched case-control pairs and finally a Dutch study was carried out with 878 cases and 581 controls (12). There exists one recent case-control study with a comparable number of participants (152 matched pairs), where all the persons were recruited from an antecedent large cohort study (13). The initially goal of 190 case-control pairs in the present study (as considered sufficient for detection of an OR 2.0 by sample size calculations prior to the study) could not be achieved. This can be explained by the circumstance that the overall positivity-rate within the screening (where cases were recruited from) was lower than the initially though 40%, obtained during the pilot study in March 2000 (3). Consequently, the through-put of NV-analyses of stool samples would have been increased which was impossible due to limitations of financial and human resources.

Cases and controls were matched for sex, age and domicile. The characteristics most appropriately specified for matching are those that are potential confounders of the exposure-disease association. Reflecting the present study, only the matching on age was really necessary because of the uneven distribution of risk for NV illness regarding age groups (14). Therefore, besides the appropriate matched analysis by logistic regression, the matched pairs were broken and analysed by using a random effects logistic regression. The latter procedure allowed the inclusion of all the data, including those of cases with no corresponding control.

The self-administered questionnaires obtained demographic information and contained questions on the date of onset and the nature of symptoms (cases only), date of inquiry and further factors considered relevant for the disease under study (e.g. consumed food items and beverages, travel history, contact to ill persons etc.). Although the questionnaire was designed primarily on issues of food consumption because at that time the foodborne spread was thought to be the predominant transmission mode. This was then supported by the estimation of the US Centers for Disease and Prevention (CDC) that 39% of all NV outbreaks registered in the US between 1996 and 2000 happened due to foodborne transmission (6). This assessment heavily influenced the complete design and direction of the used questionnaire. Nevertheless (and luckily), the questionnaire allowed to gather at least some important information about the relevance of the person-to-person spread. Copies of questionnaires are available upon request.

NV Outbreak Investigations

Between 2001 and 2003, an inter-cantonal network in the German speaking part of Switzerland was established. Integrated were initially the cantonal laboratories and cantonal surgeons and all investigations were done in accordance with the SFOPH. Very soon this network became more a web of flow of information concerning various questions about NV-associated illness. Within this great cooperation, several institutions and persons other than cantonal health authorities were involved (like medical laboratories, physicians, infection nurses, directors from nursing homes etc.). With this background, NV outbreak information were compiled on the one hand systematically by the cooperation with the health authorities and on the other hand information was collected very stochastically by attaining information from the different participants of the network. One of the advantages of this web of information was that many different outbreaks regarding the number of ill persons and settings were recorded, starting by family outbreaks of three persons, going further to hospital outbreaks and ending by a pilgrimage where NV incidents must be seen in an international context. But, the most inherent characteristic of this network was its temporality. Mainly because of the possibility to offer NV analyses on patient and environmental samples and the carrying out of outbreak investigations at no charge. With the ending of this three year's cooperation, it must be assumed that the number of registered NV outbreaks will decrease. Mainly because of the ending of this 'epidemiological service' and because of the lack of a mandatory reporting of known NV cases (apart from the obligatory reporting of outbreaks registered by the cantonal health authorities, Epidemiengesetz SR 818.101).

In total, 73 NV outbreaks were registered within three years. But it has to be pointed out that 90% of the registered incidents happened in the German speaking part of Switzerland. Most NV outbreaks within the French and the Italian speaking part of the country have not been recorded at all.

8.4 Results of the Studies

This section covers only the main outcomes and reference should be made to the single discussion chapters within the papers.

8.4.1 Results of the NV Screening and of the Phylogenetic Analysis of NV Isolates obtained from Human Stool Samples

A striking increase in NV outbreaks in Europe and in the US occurred in 2002. This coincided with the emergence of a new predominant NV GGII/4 variant (15-16). Furthermore, US data from 1995–1997 suggested also the emergence of a globally common strain that accounted for 55% of all *Norovirus* outbreaks investigated by the CDC during that period. Like the newly emerging strain in 2002, the "common strain" of 1995–1997 was also classified as a GGII/4 strain (15). In the present NV screening (see chapter 3) the highest rate of NV-positives was observed in the first quarter of the year 2002 and corresponds exactly to the first appearance of the new NV variant in Germany in January 2002 (16). This estimation is supported by the results of the phylogenetic comparison of NV isolates from Swiss human stool samples (see chapter 7). It was shown that 23 of 26 NV isolates from the year 2002 and early months of 2003 clustered together and around Bristol virus, a GGII/4 variant. The dramatic decrease of 50% in the NV positivity-rates from the first year (July 2001 to July 2002) to the second year (July 2002 to July 2003) under study may be reflected by changes in the circulation of NV strains.

But it must be pointed out that the overall rise in the number of recognised NV cases can certainly be co-explained by the recent rise in awareness of the public health institutions worldwide and by the broader implementation of molecular detection methods (17). The finding of the similarity within the age structure between NV-positive and NV-negative tested persons is remarkable. This can be explained that certain parts of the population (toddlers and

the elderly) were not included in the screening due to the case definition. Therefore, the expected accumulation of NV infections in these groups (18) could not be shown.

A second screening revealed information about the possible relevance of NV mixed-infections. Totally, 132 stool samples positively tested at least for one bacterial pathogen were analysed for the presence of NV and only in one specimen NV were detected. The only found mixed-infection was caused by NV and *Salmonella enteritidis* and the patient had a travel history to Greece. There are only few European studies on gastroenteritis where a broad panel of intestinal pathogens were tested by using recently developed techniques. During the conduction of a population-based prospective study on gastroenteritis in The Netherlands, more than one pathogen was found in 8% of all stool samples studied (19). Mixed-infections in a Dutch general-practice based case-study were detected in 2.3% of all patients (20). Compared to the present results, the Dutch studies showed a higher number of NV mixed-infections. The discrepancy may be explained by the limitation that only bacteriologically-positive stool samples were analysed for NV and by the exclusion of children under 2 years of age, who are often infected by *Cryptosporidium* sp. and rotaviruses. Nevertheless, the results from the present study suggest that NV mixed-infections are playing a marginal role in Switzerland.

8.4.2 Assessment of Risk Information for NV Infection

NV Transmission Routes

Person-to-Person Transmission

Two studies with the objective to collect information about risk factors for NV infections in Switzerland were conducted. A case-control study (see chapter 4) covered sporadic NV infections whereas the systematic outbreak investigations (chapter 5) revealed information from the epidemic situation. Both studies clearly showed the dominance of the person-to-person transmission pathway. This finding is consistent with current knowledge (14,21). Further, results from the case–control study showed that a surprisingly large number of the originally thought ‘sporadic NV cases’ were truly belonging to determinable chains of infections. 39% of all cases reported had contact to ill persons before, respectively 33% after their own illness. At least the antecedent and the following case of acute gastroenteritis within a family or relationship strongly suggest a chain of infection. This kind of personal contacts within and outside the family has been shown as a relevant risk factor in earlier studies

(11,13). Probably, the effect of family-external contacts to ill persons were narrowed, because persons known to belong to a NV outbreak were excluded from the case-control study. Concerning the outbreak situation, the finding is similar: in 54 of the 73 registered NV outbreaks (74%) the transmission pathways were known and in 44 incidents (81%) the person-to-person transmission was found to be the primary infection route.

Foodborne Transmission

The case-control study covered questions about the consumption of a variety of foodstuff and beverages in relation to NV illness. But the consumption of salads, raw berries, tap water, mineral water and sweet beverages could not be associated with the NV gastroenteritis. However, due to the low sample size, risk associations smaller than OR 3.0 may not have been detected. But, it can be stated that the foodstuffs and the beverages in question may probably not exhibit a high risk for NV infection. This assessment is supported by the results of the outbreak investigations. In only 7 of the 54 outbreaks (13%) with known transmission routes, a foodborne spread was a possible reason for the outbreak. Generally, the registration of outbreaks due to contaminated foods and waters varies from country to country. Finland reported 24%, The Netherlands 17%, Slovenia 14%, Spain, England and Wales 7% (22). The predominance of the person-to-person transmission route (81% of all outbreaks with known transmission route) confirms the results of an English study, where a rate of 85% was found (21).

Two recent Swiss studies found RNA sequences of NV in bottled mineral waters of various brands (5,7). In one of those screenings, samples of three European brands of mineral waters without gas were monitored by RT-PCR during a period of a year. NV sequences were detected in 53 of 159 samples (33%) analysed (5). A further monitoring study found that 8 of 87 samples of oysters (9%) imported into Switzerland were positive for NV (23). The phylogenetic comparison between NV isolates from human stool samples, mineral waters and oysters (see chapter 7) revealed the following results: All mineral water sequences were highly related and clustered predominantly separate to the human NV sequences. Nevertheless, single human NV isolates were also found within these mineral water clusters and it can be stated that the NV sequences from the mineral waters were all closely related to human NV isolates. Interestingly, the human NV isolates clustering within the mineral water sequences were derived from patients of the same time period like the bottling of the mineral waters.

However, the NV positive results obtained by RT-PCR on mineral waters have to be treated with caution because the detection method used is not able to distinguish between infective and inactivated viruses. Additionally, the stability of NV sequences in mineral water was estimated. NV sequences were found after 6 and 12 months of storage of the mineral waters and this suggests a high stability of NV sequences (NV particles) in mineral water (5). The origin of NV sequence contamination in bottled mineral waters remains unclear. Three main contamination routes are considered: contamination of the water sources, of the packing materials or during the bottling procedure (5). Contamination of the water source, e.g. by NV contaminated surface water, may play a role. Regarding the close relationship between NV sequences found in mineral waters and human stool samples, it can be stated that the detected NV in the mineral waters are of human origin. The sensitivity of the method used was estimated to be approximately 10 viral particles. This suggests a low concentration of NV sequences per litre of the tested mineral water brands (5). Together with the mentioned results of the case-control study, it can be concluded that mineral waters are not playing a relevant role in the overall epidemiology of NV in Switzerland. This estimate confirms a former assessment of the SFOPH (17).

The sequence analyses of oyster isolates, showed a far greater variability compared to the NV isolates derived from mineral water samples. Oyster sequences were not found within specific clusters, but grouped together with NV sequences from mineral waters and human samples. Together with the low NV positivity rate of 9% (8 of 87 samples) found in the originating oyster monitoring study and since the overall consumption of oysters in Switzerland is rather low (23), a direct impact on food safety and the epidemiology of NV might be rather low.

Other Transmission Modes

Two waterborne outbreaks occurred in 1998 and in 1999 in Switzerland (17,24-25). In 2001, a probable waterborne outbreak with more than 650 patients was registered and in 2002 the last possible waterborne outbreak with approximately 100-150 persons was recorded (see chapter 5). Generally, there is a strong tendency that such outbreaks in Switzerland are most often the result of deficiencies in the infrastructure or in the water treatment process, e.g. the leakage of the sewage water system with consequent contamination of the drinking water system or deficiencies in the application of chlorine and/or ozone to the drinking water (3,17). Environmental contamination is a logical consequence due to heavy diarrhoea and projectile vomiting and to the high environmental stability of the viruses (17) and plays an important

role especially in prolonged and protracted NV outbreaks (26-28). The description of the three consecutive outbreaks in ski camps in 2001 in Switzerland (chapter 5) demonstrates clearly the importance of environmental contamination and the necessity of an appropriate cleaning and disinfection during and after an outbreak.

In summary, the person-to-person transmission plays by far the most important role within outbreak settings and in sporadic NV infections. Waterborne transmissions occur and are mostly the result of 'engineering problems' and may result in a high number of cases. The foodborne transmission plays an important but not the major role in comparison to the overall epidemiology. But the key to all the different transmission pathways are always ill persons. To exemplify, at the beginning of every foodborne chain of infection stands a foodhandler infected with NV. Also oysters are contaminated by filtrating of sea water polluted by human faeces and consequently virus particles are accumulated in the oyster tissue (23,29). However, environmentally contaminated shellfish may introduce new strains to an area or cause infection with multiple strains thus providing the right circumstances for genetic recombination to occur (14). To date, no zoonotic transmission of NV was demonstrated and it could be shown that the NV strains in animals (calf and pig) were genetically distinct to any NV found in humans (30).

Settings of NV Outbreaks

A clear dominance of closed and semi-closed settings was observed within the 73 registered NV outbreaks. 25 (34%) outbreaks occurred in nursing homes and asylums for disabled, 18 (25%) in hospitals and health resorts, 9 (12%) in school and boy scout camps, 6 (8%) at festivities, 5 (7%) in hotels, 4 (5%) in the community, 3 (4%) in military settings, 1 (1%) at a pilgrimage and 2 (3%) in other settings. These findings confirm the results of earlier studies, where a very similar distribution of outbreak settings was found (21,31). For a number of reasons, NV pose a particular health risk in hospitals and nursing homes. NV can be introduced into institutions by an ill patient or visitor from the community (often recognised in Switzerland), by food or water. Since living quarters in both nursing homes and hospitals are tight and personal hygiene may be reduced as a result of poor health or incontinence, conditions are prime for person-to-person transmission (14). The high attack rates (30-50%) found in NV outbreaks are not only limited to residents and patients. It is obvious that such high infection rates pose a real risk for maintaining the regular operation, e.g. of a nursing

home, during an outbreak. Of highest importance is a fast and accurate reaction in terms of outbreak control. A first assessment of a NV suspicious incident within a closed setting has to be done immediately, even prior to laboratory proof of NV infection. Only with a rapid response, outbreaks can be contained and the number of affected people controlled (17).

High Infectivity of NV

The outstanding high infectivity of the NV can be explained by a number of factors: By the low infectious dose of about 10-100 virus particles, by the high number of excreted viruses during the course of illness (approximately 10^7 particles per ml stool in the first days of illness), by the aerosolisation of the NV during the physical act of vomiting and by the high environmental stability (also against commonly used disinfectants) (17). The high infectivity is very descriptive explained in the case report of the propagation of NV through multiple nursing homes following a pilgrimage (see chapter 6). Further, this incident demonstrated very clearly how easy NV may be transmitted over longer distances and even across national borders. These characteristics emphasise the building of interregional or international networks such as the leading European Food-borne Viruses Network (16)

8.5 Conclusion and Outlook

It could be clearly shown that NV infections in Switzerland are an important issue in terms of public health. The NV screening revealed that a high number of infected persons per year must be anticipated. A first and very crude assessment of the SFOPH resulted in an estimate of approximately 400'000 NV cases per year in Switzerland (17). A recent study in The Netherlands found that the incidence of infectious gastroenteritis was determined to be 283 cases per 1000 person-years. In the case-control component of the study, viral agents accounted for 34% of all cases, with NV the most common viral pathogen, accounting for 11% of cases. (14). Similar, results from the England's IID study revealed an overall rate of 194 cases per 1000 person-years and the rate of NV infection was 13 cases per 1000 person-years (6% of all cases) (19). In Germany, an incidence for NV infection based on the reporting system was found for the year 2002 to be 57 per 100'000 persons (32). Germany has introduced its NV-specific reporting system in 2001 (33).

The high number of outbreaks registered (mainly from the German speaking part of Switzerland) with numbers of affected patients ranging from 3 to 650 per outbreak was really

outstanding and exceeded the expectations. It is obvious that within a closed setting, a fast and adequate outbreak controlling is absolutely indicated. Information about the correct outbreak measures have to be disseminated from the cantonal and federal health systems to the concerning closed and semi-closed institutions.

The person-to-person transmission in comparison to the food- and waterborne spread was coming to the fore. This is truly an important conclusion and must be beard in mind especially during NV outbreaks. But the foodborne background must not be forgotten. As an example, the cantonal food inspectorates and its inspectors have to be informed about the NV and their epidemiological potential. On the other hand, the water industry must remain cautious about their quality of the treatment and processing of drinking water. The multiple waterborne outbreaks in the past have demonstrated the severe consequences of failures in the (drinking) water treatment.

The recent reports on NV sequences in mineral waters have risen a lot of dust internationally. But bacterial and fungal contamination of bottled mineral waters have been reported repeatedly (5,34) which suggests that viral contamination is also possible. In the present phylogeny, it could be stated that the NV sequences are of human origin. However, the most crucial questions about the infectivity and amount of NV particles found in mineral waters must remain unanswered to date. However, with the state of today's knowledge, the conclusion can be drawn that mineral waters contaminated by NV play not a relevant role in the overall epidemiology of NV in Switzerland. This estimate confirms the current assessment of the SFOPH.

The future trend of the NV situation in Switzerland is unpredictable. Therefore, strategies have to be implemented by the health authorities to remain on track. Meanwhile, several medical laboratories have already implemented the NV detection methodology. With the broader application of NV analysis, it should be considered, if NV should be included in the laboratory-based mandatory reporting system in Switzerland. Germany may serve herewith as an example. Also, the future development and enhancement of alternative cost-efficient essays to the RT-PCR detection methodology (e.g. ELISA) should be followed. The recent (real-time) PCR protocols are fast and accurate but still very cost intensive. Further, the future trend concerning the outbreak situation is unknown and is most probably linked to the circulation of certain NV strains in the community and to the general awareness of the NV.

However, the next goal that has to be approached is the broad dissemination of information concerning the NV in Switzerland. A first step was taken by a recent publication in the Bulletin of the SFOPH (17). Following to this article, a national reference brochure on NV will be generated in 2004. This brochure will cover the biology, the epidemiology and the prevention of NV illness in Switzerland and should be propagated widely. This brochure should also serve as a guideline for an accurate reaction to NV illness and prevention of – or at least minimising – the spread of infection.

Finally, it can be concluded that although the results of the recent methodological and epidemiological studies have allowed to catch up with the current situation of the NV in Switzerland, the future development in this field has to be closely observed.

8.6 References

1. Schmidt K, Tirado C, eds. WHO surveillance programme for control of foodborne infections and intoxications in Europe: Seventh Report 1993-1998. Berlin: BfR, 2001: 343-53.
2. Schmidt K, Gervelmeyer A, eds. WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe: Eight Report 1999-2000. Berlin: BfR, 2003: http://www.bfr.bund.de (date of download: 12.03.04).
3. Baumgartner A. (2001) «Norwalk-like»-Viren (NLV) und Lebensmittel – eine Situationsanalyse für die Schweiz. Bulletin SFOPH 46:909-916
4. Vinje J, Vennema H, Maunula L, von Bonsdorff CH, Hoehne M, Schreier E, Richards A, Green J, Brown D, Beard SS, Monroe SS, de Bruin E, Svensson L, Koopmans MP (2003) International collaborative study to compare reverse transcriptase PCR assays for detection and genotyping of noroviruses. J Clin Microbiol 41:1423-1433
5. Beuret C, Kohler D, Baumgartner A, Lüthi TM (2002) Norwalk-like virus sequences in mineral waters: one-year monitoring of three brands. Appl Environ Microbiol 68:1925-1931
6. CDC (2001) "Norwalk-Like Viruses": public health consequences and outbreak management. MMWR Recomm Rep 50(RR-9):1-18
7. Beuret C, Kohler D, Lüthi T (2000) Norwalk-like virus sequences detected by reverse transcription-polymerase chain reaction in mineral waters imported into or bottled in Switzerland. J Food Prot 63:1576-1582
8. Tam CC, Rodrigues LC, O'Brien SJ (2003) The study of infectious intestinal disease in England: what risk factors for presentation to general practice tell us about potential for selection bias in case-control studies of reported cases of diarrhoea. Int J Epidemiol 32:99-105
9. Gordis L. Epidemiology. W.B. Saunders Company, 2000: 149.

10. IID Study Executive (2000). A report of the study of infectious intestinal disease in England. The Stationery Office Books, UK

11. Letrilliart L, Desenclos JC, Flahault A (1997) Risk factors for winter outbreak of acute diarrhoea in France: case-control study. BMJ 315:1645-1649

12. de Wit MA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Bartelds AI, van Duynhoven YT (2001) Gastroenteritis in sentinel general practices, The Netherlands. Emerg Infect Dis 7:82-91

13. de Wit MA, Koopmans MP, van Duynhoven YT (2003) Risk factors for norovirus, Sapporo-like virus, and group A rotavirus gastroenteritis. Emerg Infect Dis 9:1563-1570

14. Lopman BA, Brown DW, Koopmans M (2002) Human caliciviruses in Europe. J Clin Virol 24:137-160

15. CDC (2003) Norovirus Activity – United States, 2002. MMWR 52:41-45

16. Lopman B, Vennema H, Kohli E, Pothier P, Sanchez A, Negredo A, Buesa J, Schreier E, Reacher M, Brown D, Gray J, Iturriza M et al. (2004) Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant. Lancet 363:682-688

17. Fretz R, Svoboda P, Schmid H. Baumgartner A (2003) Durch Noroviren verursachte Gastroenteritis – eine Übersicht. Bulletin SFOPH 46:828-833

18. Mounts AW, Ando T, Koopmans M, Bresee JS, Noel J, Glass RI (2000) Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. J Infect Dis 181(Suppl 2):S284-S287

19. de Wit MAS, Koopmans MPG, Kortbeek LM, Wannet WJB, Vinjé J, van Leusden F, Bartelds AI, van Duynhoven YT (2001) Sensor, a population-based cohort study on gastroenteritis in the Netherlands, incidence and etiology. Am J Epidemiol 154:666–674

20. de Wit MA, Koopmans MP, Kortbeek LM, van Leeuwen NJ, Vinje J, van Duynhoven YT (2001) Etiology of gastroenteritis in sentinel general practices in the Netherlands. Clin Infect Dis 33:280-288

21. Lopman BA, Adak GK, Reacher MH, Brown DW (2003) Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. Emerg Infect Dis 9:71-77

22. Lopman BA, Reacher MH, Van Duijnhoven Y, Hanon FX, Brown D, Koopmans M (2003) Viral gastroenteritis outbreaks in europe, 1995-2000. Emerg Infect Dis 9:90-96

23. Beuret C, Baumgartner A, Schluep J (2003) Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland. Appl Environ Microbiol 69:2292-2297

24. Maurer AM, Stürchler D (2000). A waterborne outbreak of small round structured virus, campylobacter and shigella co-infections in La Neuveville, Switzerland, 1998. Epidemiol Infect 125: 325-332

25. Lüthi TM. Beuret C (2001) Weshalb ein Virennachweis in Wasser ? GWA 5: 283-290

26. Cheesbrough JS, Green J, Gallimore CI, Wright PA, Brown DW (2000) Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. Epidemiol Infect 125: 93-98

27. Evans MR, Meldrum R, Lane W, Gardner D, Ribeiro CD, Gallimore CI, Westmoreland D (2002) An outbreak of viral gastroenteritis following environmental contamination at a concert hall. Epidemiol Infect 129: 355-360

28. Kuusi M, Nuorti JP, Maunula L, Minh NN, Ratia M, Karlsson J, von Bonsdorff CH (2002) A prolonged outbreak of Norwalk-like calicivirus (NLV) gastroenteritis in a rehabilitation centre due to environmental contamination. Epidemiol Infect 129: 133-138

29. Shieh Y, Monroe SS, Fankhauser RL, Langlois GW, Burkhardt W 3rd, Baric RS (2000) Detection of norwalk-like virus in shellfish implicated in illness. J Infect Dis 181(Suppl 2): S360-S366.

30. Koopmans M, Duizer E (2004) Foodborne viruses: an emerging problem. Int J Food Microbiol 90: 23-41

31. PHLS (2003) Norovirus outbreaks peak in 2002: England and Wales. J Public Health Med 25: 179-180

32. RKI (2003) Erkrankungen durch Norwalk-ähnliche Viren (Noroviren). Epidemiologisches Bulletin 6:39-43

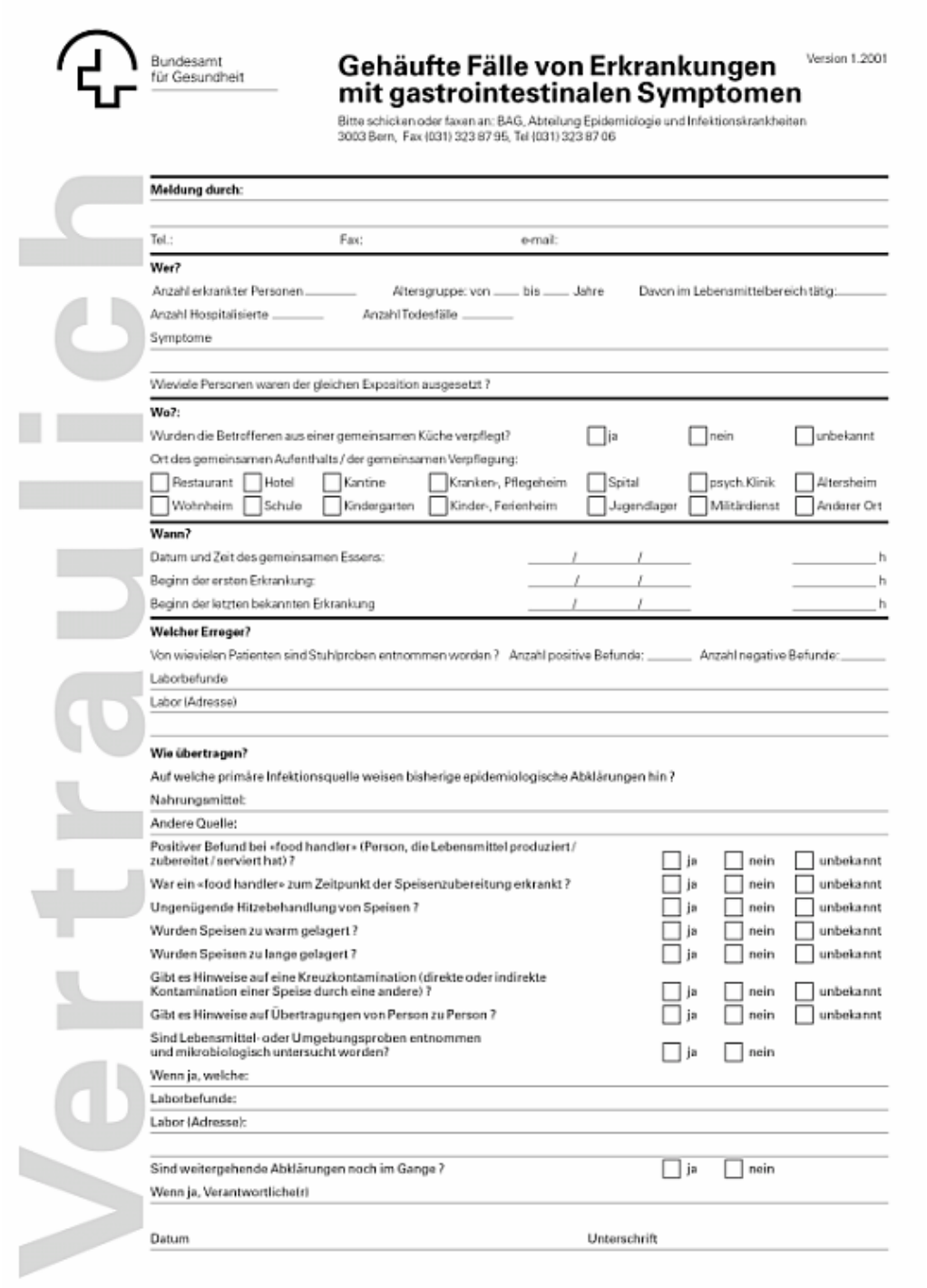
33. Höhne M, Schreier E (2004) Detection and characterization of norovirus outbreaks in Germany: application of a one-tube RT-PCR using a fluorogenic real-time detection system. J Med Virol 72:312-319

34. Cabral D, Fernandez P (2002) Fungal spoilage of bottled mineral water. Int J Food Microbiol 72: 73-76.

Annex SFOPH Reporting Form:

"Gehäufte Fälle von Erkrankungen mit gastrointestinalen Symptomen"

It would be very appreciated if any accumulated cases of gastrointestinal illness or disorder would be optionally reported to the Swiss Federal Office of Public Health (SFOPH). The reporting form may be downloaded in German or in French from the following website: http://www.bag.admin.ch/infreporting.



Bundesamt für Gesundheit

Gehäufte Fälle von Erkrankungen mit gastrointestinalen Symptomen

Version 1.2001

Bitte schicken oder faxen an: BAG, Abteilung Epidemiologie und Infektionskrankheiten 3003 Bern, Fax (031) 323 87 95, Tel (031) 323 87 06

Vertraulich

Meldung durch:

Tel.: Fax: e-mail:

Wer?

Anzahl erkrankter Personen ____ Altersgruppe: von ____ bis ____ Jahre Davon im Lebensmittelbereich tätig: ____

Anzahl Hospitalisierte ____ Anzahl Todesfälle ____

Symptome

Wieviele Personen waren der gleichen Exposition ausgesetzt ?

Wo?:

Wurden die Betroffenen aus einer gemeinsamen Küche verpflegt? ☐ ja ☐ nein ☐ unbekannt

Ort des gemeinsamen Aufenthalts / der gemeinsamen Verpflegung:

☐ Restaurant ☐ Hotel ☐ Kantine ☐ Kranken-, Pflegeheim ☐ Spital ☐ psych. Klinik ☐ Altersheim
☐ Wohnheim ☐ Schule ☐ Kindergarten ☐ Kinder-, Ferienheim ☐ Jugendlager ☐ Militärdienst ☐ Anderer Ort

Wann?

Datum und Zeit des gemeinsamen Essens: ___/___/___ ____ h

Beginn der ersten Erkrankung: ___/___/___ ____ h

Beginn der letzten bekannten Erkrankung ___/___/___ ____ h

Welcher Erreger?

Von wievielen Patienten sind Stuhlproben entnommen worden ? Anzahl positive Befunde: ____ Anzahl negative Befunde: ____

Laborbefunde

Labor (Adresse)

Wie übertragen?

Auf welche primäre Infektionsquelle weisen bisherige epidemiologische Abklärungen hin ?

Nahrungsmittel:

Andere Quelle:

Positiver Befund bei «food handler» (Person, die Lebensmittel produziert / zubereitet / serviert hat) ?	☐ ja	☐ nein	☐ unbekannt
War ein «food handler» zum Zeitpunkt der Speisenzubereitung erkrankt ?	☐ ja	☐ nein	☐ unbekannt
Ungenügende Hitzebehandlung von Speisen ?	☐ ja	☐ nein	☐ unbekannt
Wurden Speisen zu warm gelagert ?	☐ ja	☐ nein	☐ unbekannt
Wurden Speisen zu lange gelagert ?	☐ ja	☐ nein	☐ unbekannt
Gibt es Hinweise auf eine Kreuzkontamination (direkte oder indirekte Kontamination einer Speise durch eine andere) ?	☐ ja	☐ nein	☐ unbekannt
Gibt es Hinweise auf Übertragungen von Person zu Person ?	☐ ja	☐ nein	☐ unbekannt
Sind Lebensmittel- oder Umgebungsproben entnommen und mikrobiologisch untersucht worden?	☐ ja	☐ nein	

Wenn ja, welche:

Laborbefunde:

Labor (Adresse):

Sind weitergehende Abklärungen noch im Gange ? ☐ ja ☐ nein

Wenn ja, Verantwortliche(r)

Datum Unterschrift

Curriculum Vitae of Rainer Fretz-Männel

Date of birth	April 1st, 1972
Nationality	Swiss
Marital status	Married
Address	Sierenzerstrasse 3, CH-4055 Basel
Profession	Biologist (MSc) PhD candidate in epidemiology (date of completion: April 2004)

1. Education and Qualifications

2002	October 2002: four weeks' training in epidemiology at the Centers for Disease Control and Prevention (CDC) and at Rollins School of Public Health (RSPH) in Atlanta, USA
1994 – 2000	Studies in Integrative Biology at the University of Basle, Switzerland; MSc thesis at the Swiss Tropical Institute in Basle
1992 – 1996	Training as an expert in radiation protection for the handling of open and sealed sources of radiation and for the transportation of dangerous goods (CH SDR class 7) at the School of Radiation Protection at the Paul Scherrer-Institute (PSI) in Villigen, Switzerland

2. Professional Experience

2002 – 2003	Tutorship for a MSc thesis at the Swiss Tropical Institute and at the Cantonal Laboratory Basel-Landschaft
from 2002	Instructor in the annual course "Introduction to the Epidemiological Investigation of Foodborne Outbreaks", organised by the Institute for Quality Management & Food Safety in Wädenswil, Switzerland
July 2000	Start of doctoral dissertation in epidemiology (PhD) at the Cantonal Laboratory Basel-Landschaft and at the Swiss Tropical Institute in Basle (date of completion: April 2004)
Mai – June 2000	Practical training at the Cantonal Laboratory Basel-Landschaft in Liestal, Switzerland
1993 – 1999	Simultaneous with university studies: part-time job (25%-75%) as radiation protection officer with responsibility for the sealed sources of radiation at the Centre for Radiation Protection of Novartis Services AG
1992 – 1993	Full-time job as radiation protection officer at the Zentralstelle für Strahlenschutz (Centre for Radiation Protection) at Novartis Services AG

3. Achievements

3.1 Professional Experiences

Outbreak Management	Systematic registration of outbreaks of gastroenteritis due to infections with *Norovirus* (NV) in the community and partially in hospitals in Switzerland. Active role in the controlling and management of outbreaks in terms of counselling and diagnostics
Conducting of scientific studies	Performing of a matched case-control study with the objective to gain information about relevant risk factors of *Norovirus* (NV) infection in Switzerland. Contribution in other research topics in the same scientific field (see list of scientific publications)
Build-up of centre of competence	Cooperated in the formation of the first epidemiological centre of competence for *Norovirus* (NV) infections in Switzerland in accordance with the Swiss Federal Office of Public Health (SFOPH)

3.2 Scientific Publication List

2003

Christen A, Fretz R, Tanner M, Svoboda P. Evaluation of a commercial ELISA kit for the detection of *Norovirus* antigens in human stool specimens. Mitt Gebiete Lebensm Hyg 2003; 6: 594-602

Fretz R, Svoboda P, Schmid H. Baumgartner A. Durch Noroviren verursachte akute Gastroenteritis – eine Übersicht. Bull BAG 2003; 46: 828-833

Fretz R, Schmid H, Kayser U, Svoboda P, Tanner M, Baumgartner A. Outbreak of gastroenteritis due to *Norovirus* infection associated with pilgrimage. Eur J Clin Microbiol Infect Dis; 2003 22: 625-627

Fretz R, Svoboda P, Ryan UM, Thompson RC, Tanner M, Baumgartner A. Genotyping of *Cryptosporidium* spp. isolated from human stool samples in Switzerland. Epidemiol Infect 2003; 131: 663-667

Fretz R, Svoboda P, Schmid H. Baumgartner A. Epi-Notiz: Häufung von Noroviren-Ausbrüchen im Winter 2002/2003. Bull BAG 2003; 21: 348-349

Fretz R, Herrmann L, Dubuis O, Svoboda P, Baumgartner A, Viollier EH. One-year screening of bacteriological-negative stool samples for "Norwalk-like viruses" (NLV) in Switzerland. 1st Joint Annual Meeting of the Swiss Society for Microbiology, the Swiss Society for Infectious Diseases, and the Swiss Society of Tropical Medicine and Parasitology. Basel, 6th-7th March 2003

2002

Fretz R, Svoboda P, Lüthi TM, Baumgartner A. Gastroenteritis outbreaks due to infections with «Norwalk-like viruses» (NLV) in Switzerland. 12th European Congress of clinical microbiology and infectious diseases. Milano, 24th–27th April 2002

4. Various

from 2001	Expert for biochemistry and medicine at "Schweizer Jugend forscht" (Swiss Youth Research Competition)
1999	Nine weeks' stay at Murdoch University in Perth, Division of Health Sciences, Australia (as part of the MSc thesis at the Swiss Tropical Institute)
Military Service	From 2003 assigned to the Swiss Army's Biological Service (BDA-18), section epidemiology

5. Attended Courses During the Studies at the University of Basel

Courses at the university of Basel were attended given by the following lecturers:

C. Baroni Urbani, H.P. Beck, Th. Boller, R. Brun, P. Duelli, I. Felger, W. Gehring, U. Gisi, M. Hall, H. Im Hof, S. Jacomet, L. Jenni, C. Körner, C. Lengeler, E. Lüdin, P. Oelhafen, G. Pluschke, H. Reichert, H. Riezman, H. Rowel, W. Rudin, M. Rüegg, V. Schmid, H. Schneider, D. Senn, U. Sequin, H. Sigel, T. Smith, M. Spiess, S. Stearns, J. Stöcklin, M. Tanner, P. Vounatsou, N. Weiss, A. Wiemken