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Rotavirus disease in Germany - A prospective survey of very severe cases

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This dissertation is dedicated to

my parents,

my sons Noah & Henry

and my loving muse Heike

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Zusammenfassung

Rotaviren sind weltweit die häufigste Ursache von Gastroenteritis bei Kindern. Bis zum Alter von fünf Jahren hat nahezu jedes Kind in der Welt mindestens eine Gastroenterits durch Rotaviren gehabt. In der Europäischen Union sterben jährlich ca. 230 Kinder an den Folgen einer Rotavirus-Erkrankung und etwa 87,000 Kinder pro Jahr benötigen eine stationäre Behandlung.

Es gibt zwei sichere und effektive Impfstoffe gegen Rotaviren. Vor dem Jahr 2013 war die Impfung gegen Rotaviren nicht Bestandetil der allgemeinen Impfemfehlung in Deutschland.

Besonders schwere Verläufe bei Rotavirus-Erkrankungen sind beschrieben worden. Die Datenlage hierzu basiert vorwiegend auf Fallbeschreibungen oder retrospektive Zusammenfassungen. Prospektiv erhobene Daten über besonders schwere Verläufe bei Rotavirus-Erkrankungen fehlen. Solche Daten könnten wichtig bei der Entscheidung für oder gegen eine deutschlandweite Impfempfehlung bezüglich Rotaviren sein.

Ziel der Studie war die prospektive Erfassung von schweren Rotavirus-Erkrankungen bei Kindern und Jugendlichen in Deutschland mit Ermittlung demographischer Daten und Daten über den einzelnen Krankheitsverlauf.

Alle Fälle von besonders schweren Verläufen von Rotavirus-Erkrankungen wurden durch die Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland (ESPED) ermittelt. Die Datenerhebung erfolgte mittels anonymisierter Fragebögen und Arztberichten und begann im April 2009 für eine Dauer von 2 Jahren. Die Einschlusskriterien waren: Rotavirusnachweis im Stuhl, Patient 0 - 16 Jahre sowie eins oder mehrere der folgenden Kriterien: Behandlung auf einer Intensivstation, Hypo- oder Hypernatriämie (>125 mmol/l bzw. 155 mmol/l), klinische Zeichen einer Enzephalopathie (Somnolenz, zerebrale Krämpfe, Apnoen), Tod durch Folgen der RV-Erkrankung.

Während der Studienperiode wurden 130 Fälle gemeldet. Nach Abzug von 10 fehlenden Rückmeldungen, einer Doppel-Meldung und 18 Meldungen bei denen die Einschlusskriterien nicht erfüllt waren, konnten 101 Fälle ausgewertet werden. Siebzehn Fälle waren nosokomial erworben, 14 davon waren Neugeborene in intensivmedizinischer Behandlung, von denen 12 den Verdacht auf oder eine gesicherte nekrotisierende Enterokolitis hatten. Vierundachtzig waren ambulant erworben. In dieser Gruppe war das mediane Alter 10,5 Monate, 48 Patienten benötigten eine intensivmedizinische Behandlung. Die mediane stationäre Behandlungsdauer war 6 Tage. Bei Kindern unter 5 Jahren war die jährliche Inzidenz einer ambulant-erworbenen besonders schweren Rotavirus-Erkrankung 1,2/100.000 . Es wurden drei Todesfälle gemeldet.

In Deutschland, ein Land mit hohem Standard der medizinischen Versorgung können besonders schwere Verläufe bei Rotavirus-Erkrankungen auftreten. Im Juli 2013 wurde die Impfung gegen Rotaviren in die nationale Impfempfehlung der Ständigen Impfkommision implementiert.

Summary

Rotavirus is worldwide the most common cause of gastroenteritis in children. By the age of five years, nearly every child has had at least one case of rotavirus gastroenteritis. In the European Union annually about 230 deaths are attributable to rotavirus in young children and about 87,000 children need hospital treatment.

There are two safe and effective vaccines against rotavirus available. Before 2013, the vaccination against rotavirus was not part of the German national vaccine recommendation.

Very severe courses of rotavirus infections have been described. Information on very severe rotavirus disease is primarily based on case reports and retrospective analyses, whereas prospectively collected data are missing. Such data could inform the decision for or against a national vaccine recommendation against rotavirus.

Aim of study was to prospectively query all cases of very severe rotavirus disease in children in Germany to gather demographic data and information about each individual case.

All cases of very severe rotavirus disease were collected by the German Paediatric Surveillance Unit for rare diseases (ESPED) using anonymous questionnaires based on hospitalized patients between April 2009 and March 2011. Inclusion criteria were detection of rotavirus antigen in feces, patient age 0 - 16 years and one or more of the following criteria: intensive care treatment, hypernatremia (>155 mmol/l) or hyponatremia (<125 mmol/l), clinical signs of encephalopathy (somnolence, cerebral seizures, apnea), rotavirus associated death.

During the study period 130 cases of very severe rotavirus disease were reported. After exclusion of 10 cases with no feedback, one double report and 18 reports not fulfilling the inclusion criteria, 101 reports were included to the study. Seventeen cases were nosocomially acquired of which 14 patients were neonates in intensive care treatment of whom 12 were diagnosed with or had a suspected necrotizing enterocolitis. Eighty-four community acquired cases were reported. In this group, the median age was 10.5 months, 48 patients needed intensive care treatment. Mean duration of hospital stay was 6 days. In children less than 5 years of age, the annual incidence of community acquired very severe rotavirus disease was 1.2/100,000. Three deaths were reported.

In Germany, a country with a high standard of medical care, rotavirus infections can have a life-threatening course. In July 2013 the vaccine against rotavirus was implemented in the national vaccine recommendation in Germany.

Abbreviations

CDC	Centers for Disease Control and Prevention
dsRNA	Double-stranded ribonucleic acid
ER	Endoplasmic reticulum
ESPED	Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland (German Paediatric Surveillance Unit for rare diseases)
GE	Gastroenteritis
ICU	Intensive care unit
mRNA	Messenger ribonucleic acid
NEC	Necrotizing enterocolitis
NSP	Non structural protein
NTPase	Nucleoside triphophatase
PCR	Polymerase chain reaction
RER	Rough endoplasmic reticulum
RNA	Ribonucleic acid
RV	Rotavirus
SCID	Severe combined immunodeficiency
ss	Single-stranded
STIKO	Ständige Impfkommision (German Standing Committee on Vaccination)
VP	Virus protein
vsRVd	Very severe rotavirus disease
WHO	World Health Organization

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1. Introduction

1.1. Epidemiology

Regardless of cause, diarrheal diseases are among the most common illnesses in infants and young children throughout the world (Bryce et al., 2005). Of the 10.6 million yearly deaths in children younger than five years of age world wide, diarrhea is attributable to 18% (Bryce et al., 2005). Rotavirus (RV) is the most common cause of gastroenteritis (GE) in children in Germany (Koch and Wiese-Posselt, 2011) and worldwide (Parashar et al., 1998). By the age of five years, nearly every child in the world has been infected by RV at least once (Bernstein, 2009). In early childhood, the single most important cause of severe dehydrating GE is RV infection. Not only is GE a major cause of pediatric mortality but also a cause to significant morbidity. Repeated diarrheal infections may be a precipitating factor in the development of malnutrition by damaging the intestinal mucosa (Mata et al., 1983). However, malnutrition is also considered to play an important role in increasing the severity of clinical manifestation of RV infections (Brown et al., 1981). Rotavirus disease causes a tremendous health burden. In a global setting, it is estimated that RV disease annually causes about 530,000 deaths in children less than 5 years of age. Most of these fatal disease courses occur in developing countries (Parashar et al., 2009). In developed countries RV GE is usually a mild and self-limiting disease. Nevertheless, it is estimated that in the European Union more than 87,000 hospitalizations and about 230 deaths per year in children less than five years of age are attributable to RV (Soriano-Gabarró et al., 2006).

1.1.1. Impact on health system

With an estimated annual 3.6 million cases of RV GE in young children in the European Union, RV causes a very heavy burden on society and the healthcare system (Soriano-Gabarró et al., 2006). It is estimated that RV infections result in more than $400 million per year in direct medical costs in the United States, primarily for hospitalizations and more than $1 billion in total societal costs, primarily from loss of work time of parents or caregivers (Fields and Knipe, 2006).

Rotaviruses have been detected throughout the world wherever they have been sought and they constitute the major etiologic agents of severe infantile diarrhea in every country where this disease has been studied (Fields and Knipe, 2006). Rotaviruses display a seasonal pattern of infection in developed countries, such as in Germany, with epidemic peaks occurring in the cooler months of each year (Koch and Wiese-Posselt, 2011).

In 2001, laboratory confirmed acute RV disease with diarrhea and/or vomiting became a notifiable disease in Germany under the new German Protection against Infection Act (Koch and Wiese-Posselt, 2011). Each RV case is to be reported to the Robert Koch Institute, the German federal institution responsible for disease control and prevention. A minimal data set includes information on age, sex, onset of symptoms, hospitalization, fatal outcome and laboratory diagnostic method. However, detailed information about the course and complications of the case being reported is lacking.

As RV GE usually is mild and self limiting, the RV infection is often managed at home and the patient is not presented to a physician. In population-based studies of infectious gastroenteritis in Europe, only 5% to 20% of those who develop symptoms of an acute GE consult a primary care physician or other health care professionals (Piednoir et al., 2003). The patients who are presented to a physician usually remain in ambulatory care. Furthermore, there is little incentive for physicians to collect stool samples from patients with acute GE, because the detection of RV and most other pathogens in stool does not influence the medical treatment. Therefore, a high number of mild or moderate RV diseases will not be detected by a passive surveillance system. As most mild or moderate RV infections do not require hospitalization, it can be assumed that an underestimation is a particular problem for the assessment of the outpatient disease burden. The diagnosis of an acute gastroenteritis is usually based on a clinical and epidemiological basis. Feces are usually not tested for RV, especially in an ambulatory medical setting. For this reason there is a significant underreporting of RV GE. The true number of cases is probably 5 – 10 times higher than the reported cases (Karsten et al., 2009).

Interestingly, Koch et al. observed an increase in RV notifications to the Robert Koch Institute since 2004 (Koch and Wiese-Posselt, 2011). The authors conclude that this increase might be related to changes in the German reimbursement system for hospitalization charges. In 2004, the diagnosis-related group system, a system to classify hospitalized patients, was implemented in German hospitals and acts as a basis for payment of services provided by the hospitals. As hospitals receive higher payments for each acute GE case with a confirmed causative agent than for microbiologically unconfirmed GE, a higher number of stool samples might have been tested for RV in German hospitals and might have resulted in a higher number of reported RV cases since 2004.

1.2. Rotavirus – A short history

In 1943, Light and Hodes described the isolation of an agent in the feces of children with infectious GE which causes diarrhea in calves (Light and Hodes, 1943). In 1974, preserved samples of the agent were shown to be rotavirus. The first reports of RV were about murine rotaviruses by Adams and Kraft in 1963 (Adams and Kraft, 1963). In 1973 RV was discovered in duodenal biopsies of children with acute GE. This discovery was achieved without the benefit of tissue culture technology. Their identification relied on direct visualization by electron microscopy (Bishop et al., 1973).

Electron microscopy images of the RV reveal the particle to resemble a wheel with short spokes and a well-defined outer rim. Wheel in Latin is *rota* and thus it was given the name rotavirus (Matthews, 1979). It soon became apparent that RV was an important etiologic agent of diarrhea in infants and young children, causing about 35% to 50% of the hospitalizations due to GE in young children (Bishop, 1996).

Rotavirus serotypes were first described in 1980. In 1981 RV from humans were first grown in cell cultures derived from monkey kidneys, by adding trypsin

to the culture medium. The ability to grow rotavirus in culture accelerated the pace of research, and by the mid-1980s the first candidate vaccines were being evaluated (Ward and Bernstein, 2009).

1.3. Rotavirus – Structure

Rotaviruses belong to the family *Reovirida.* The virions are about 100 nm in diameter and possess a triple-layered icosahedral protein capsid as illustrated in Figure 1 (Angel et al., 2007). The core layer consists of virus protein 1 (VP1), VP3 and VP2. The intermediate layer is made up of VP6 where as VP7 makes up the outer layer with VP4 protruding as spikes. The RV has 60 protein spikes that extend from the smooth surface of the outer shell. The RNA-dependent RNA polymerase and other enzymes such as virus VP1 and VP3 contained in the virions are capable of producing RNA transcripts. The viral double-stranded RNA (dsRNA) genome consists of 11 segments containing a total of 18,555 nucleotides. Each segment, a double helix molecule, is a gene numbered 1 to 11 by decreasing size. Deproteinized RV dsRNS are not infectious, reflecting that the virus particles contain their own RNA-dependent RNA polymerase to transcribe the individual RNA segments into active mRNA (Fields and Knipe, 2006). In addition to the viral proteins, there are six nonstructural proteins that are only produced in cells infected by the RV (Kirkwood, 2010)

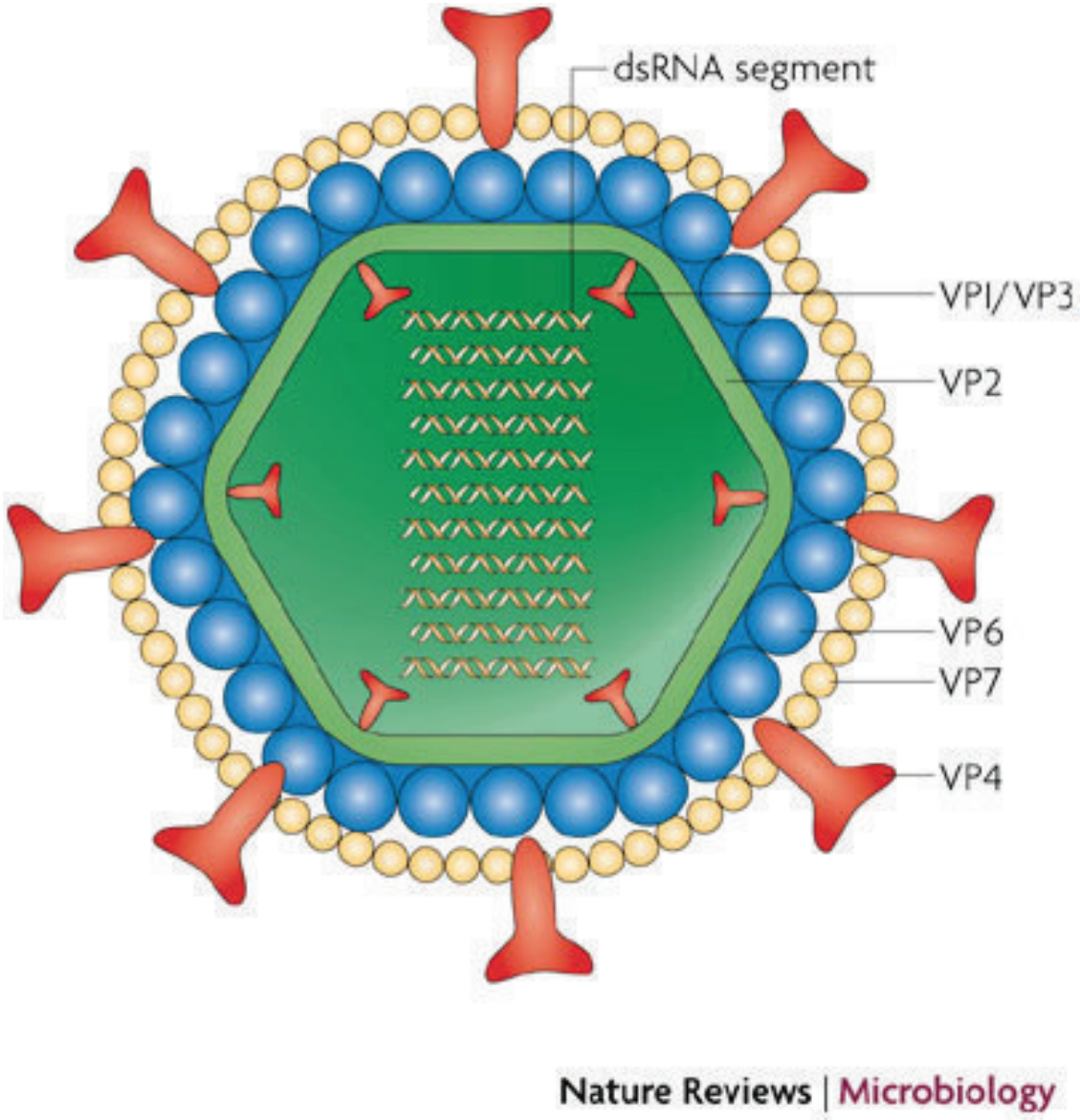

Figure 1: Schematic illustration of a rotavirus virion. VP, virus protein.
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1.3.1. Classification of rotaviruses

Rotaviruses are classified serologically into serogroups. A RV serogroup includes viruses that share cross-reacting antigens detectable by a number of serologic methods (Fields and Knipe, 2006). There are seven distinct RV groups (A ,B, C, D, E, F and G). Groups A, B and C rotaviruses are found both in humans and animals, whereas RV Group D, E, F and G have only been found in animals. Group A RV cause significant diarrheal disease in infants and the young of various mammalian species. Group B RV has been associated with epidemics of severe diarrheal disease primarily in adults in China. Group C RV has been sporadically reported in children with GE (Fields and Knipe, 2006).

Within each RV group, the rotaviruses are further classified into serotypes. The serotype classification is a dual classification system based on serological properties of the outer layer proteins (VP7 and VP4) of the RV. The glycoprotein VP7 defines the G serotype (G1, G2, G3, and so forth). The protease-sensitive protein VP4 defines the P serotype. Currently there are 16 G and 20 P types known (Greenberg and Estes, 2009).

An overview of the RV proteins and their known functions is listed in Table 1 (Angel et al., 2007).

Protein	Function
VP1	RNA-dependent RNA polymerase; ssRNA binding; located at the five–fold axis inside the inner capsid; forms a transcription complex with VP3.
VP2	Inner capsid structural (core) protein; non-sequence-specific RNA-binding activity; required for replicase activity of VP1.
VP3	Guanylyltransferase and methyltransferase; part of the virion transcription complex with VP1.
VP4	Trimers of VP4 form the outer capsid spike; P-type-specific neutralization antigen; virulence determinant; haemagglutinin; cell-attachment protein; cleavage by trypsin into VP5* and VP8* enhances infectivity.
VP6	Major virion protein; middle capsid structural protein; homotrimeric structure; subgroup antigen; required for transcription.
VP7	Outer capsid structural glycoprotein; G-type neutralization antigen; RER transmembrane calcium-binding protein.
NSP1	Associates with the cytoskeleton; extensive sequence diversity between strains; has a role in suppressing the host IFN-α response; non-essential in some strains.
NSP2	NTPase and helicase; non-specific ssRNA binding; involved in viroplasm formation; binds NSP5 and VP1; essential for dsRNA synthesis.
NSP3	Homodimer; specifically binds 3'-end of rotavirus mRNA; binds elongation factor eIF4G1; involved in translational regulation.
NSP4	Viral enterotoxin; receptor for budding of double-layered particles through the ER membrane; glycoprotein; modulates intracellular calcium levels and RNA replication; secreted cleavage product.
NSP5	Interacts with NSP2 and NSP6; forms homomultimers; *O*-linked glycosylation; hyperphosphorylated; binds ssRNA; component of viroplasm; essential for viral replication.
NSP6	Product of the second out-of-frame open-reading frame of gene segment II; interacts with NSP5; localizes to the viroplasm.
VP, virus protein; NSP, non-structural proteins, double-stranded; ER, endoplasmic reticulum; IFN, interferon; RER, rough endoplasmic reticulum; ss, single-stranded; NTPase, nucleoside triphosphatase.	

Table 1: Characteristics and known functions of rotavirus proteins
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1.4. Rotavirus – Pathophysiology

The route of transmission is feco-oral and the incubation period is 24 – 48 hours (Davidson et al., 1975). In a study from 1983, in which 18 volunteers were challenged orally with human RV, four volunteers developed a diarrheal illness two to four days after inoculation. Viral shedding was detected in five of the 18 volunteers, whereas 12 developed serologic evidence of infection (Kapikian et al. 1983).

The triple layered protein coats make the RV resistant to the acidic pH of the stomach and the digestive enzymes in the gut. The natural cell tropism for RV is the differentiated enterocyte in the small intestine, suggesting these cells express specific receptors for RV (Fields and Knipe, 2006). Recent studies have shown that extraintestinal spread of rotaviruses occurs (Crawford et al., 2006),(Fenaux et al., 2006),(Fischer et al., 2005). The understanding of the molecular details of RV adsorption, entry and uncoating is incomplete. Rotaviruses attach to the cell through sialic acid containing receptors. VP4 spikes are implicated in facilitating optimal interface between viral and host components (Baker and Prasad, 2010). The RV enters the cell through endocytosis, in which an RV containing vesicle is formed and transported from the extracellular milieu to the interior of the cell (Gutiérrez et al., 2010). The role of VP7 is less clear, although it has been shown that it interacts with the cell surface molecules at a post-attachment step (Graham et al., 2003). A change in calcium concentration in the intracellular microenvironment may be responsible for uncoating and in turn activation of the RNA polymerase of the RV (Cohen et al., 1979). Despite the fact, that in vivo, RV primarily infects the mature enterocyte of the small intestine, studies on the infection of this type of cells have been limited due to the lack of established intestinal cell lines of small intestinal origin. Given the absence of a better model, most of the studies on the entry and replication cycle of RV have been conducted either in the epithelial monkey kidney cell line or in the human colon carcinoma cell line, which are highly permissible to these viruses (Gutiérrez et al., 2010).

RV particles contain the enzymatic activities needed for messenger RNA (mRNA) synthesis. Viral mRNAs are capped and viral proteins are translated by the cellular translation machinery. Most of the RV structural proteins and the nonstructural proteins are synthesized on the free ribosomes (Fields and Knipe, 2006). The synthesis of RNA in infected cells commences about 3 hours after infection (Stacy-Phipps and Patton, 1987). The selective packaging mechanism that leads to the presence of genome segments within rotaviruses remains a challenging puzzle (Patton and Spencer, 2000).

A distinctive feature of RV morphogenesis is that subviral particles, which assemble in the cytoplasmic viroplasms, bud through the membrane of the endoplasmic reticulum (Ruiz et al., 2009). Rotaviruses are normally cytocidal and rapidly kill the permissive cells they infect (Fields and Knipe, 2006). Cell death is preceded by the shut-off of host RNA, DNA and protein synthesis (Carpio et al., 1981). NSP4 is one protein that mediates cell death by causing intracellular calcium levels to increase as well by affecting the plasma membrane permeability and tight junctions of cells (Michelangeli et al., 1991),(Newton et al., 1997),(Tian et al., 1994).

Despite the term gastroenteritis, the gastric mucosa is not affected in RV infections. After transmission, infection is initiated in the upper intestine and typically leads to a series of histological and physiological changes involving destruction of villus tip cells in the small intestine, in particular in the duodenum and jejenum. There is also an increased infiltration of the lamina propria with mononuclear cells (Nelson et al., 2004). Rotaviruses replicate in the non-dividing, mature enterocytes near the tips of the villi, suggesting that differentiated enterocytes express factors required for efficient infection and replication (Fields and Knipe, 2006). RV replication in continuous cell cultures derived from monkey kidneys is fairly rapid with a maximal virus count found after 10 to 12 hours at 37° Celsius. Recent studies examining RV replication in differentiated human intestinal cell lines show that the virus replication cycle is slower, with a maximum viral particle count reached after 20 to 24 hours after infection (Fields and Knipe, 2006). Electron microscopy has revealed numerous RV particles in the epithelial cells. Biopsies obtained four to eight weeks after

onset of illness demonstrate apparently normal histological findings (Barnes and Townley, 1973).

The upper villus enterocytes have both digestive functions such as hydrolysis of disaccharides and absorptive functions such as transport of water and electrolytes via glucose co-transporters (Nelson et al., 2004). Acute RV illness is associated with decreased levels of intestinal brush border enzymes such as lactase (Bishop et al., 1973). Viral infection of intestinal villus cells can thus lead to an imbalance between intestinal fluid absorption and secretion and to malabsorption of complex carbohydrates such as lactose (Nelson et al., 2004).
A third component to the pathogenesis of RV disease is the activation of the enteric nervous system resulting in a net intestinal fluid and electrolyte secretion (Lundgren et al., 2000). Luminal enterochromaffin cells, a type of enteroendocrine cells, can release mediators to activate the enteric nervous system. Following activation, enterochromaffin cells mobilize intracellular calcium ions which causes a release of serotonin. Serotonin is involved in regulation of gut motility, intestinal secretion and blood flow (Kordasti et al., 2004). It has been shown, that RV stimulates release of serotonin from human enterochromaffin cells causing diarrhea. A recent study has shown that RV induced serotonin release also activates brain structures involved in nausea and vomiting (Hagbom et al., 2011). Mechanisms of RV pathogenesis are summarized in Figure 2 (Angel et al., 2007).

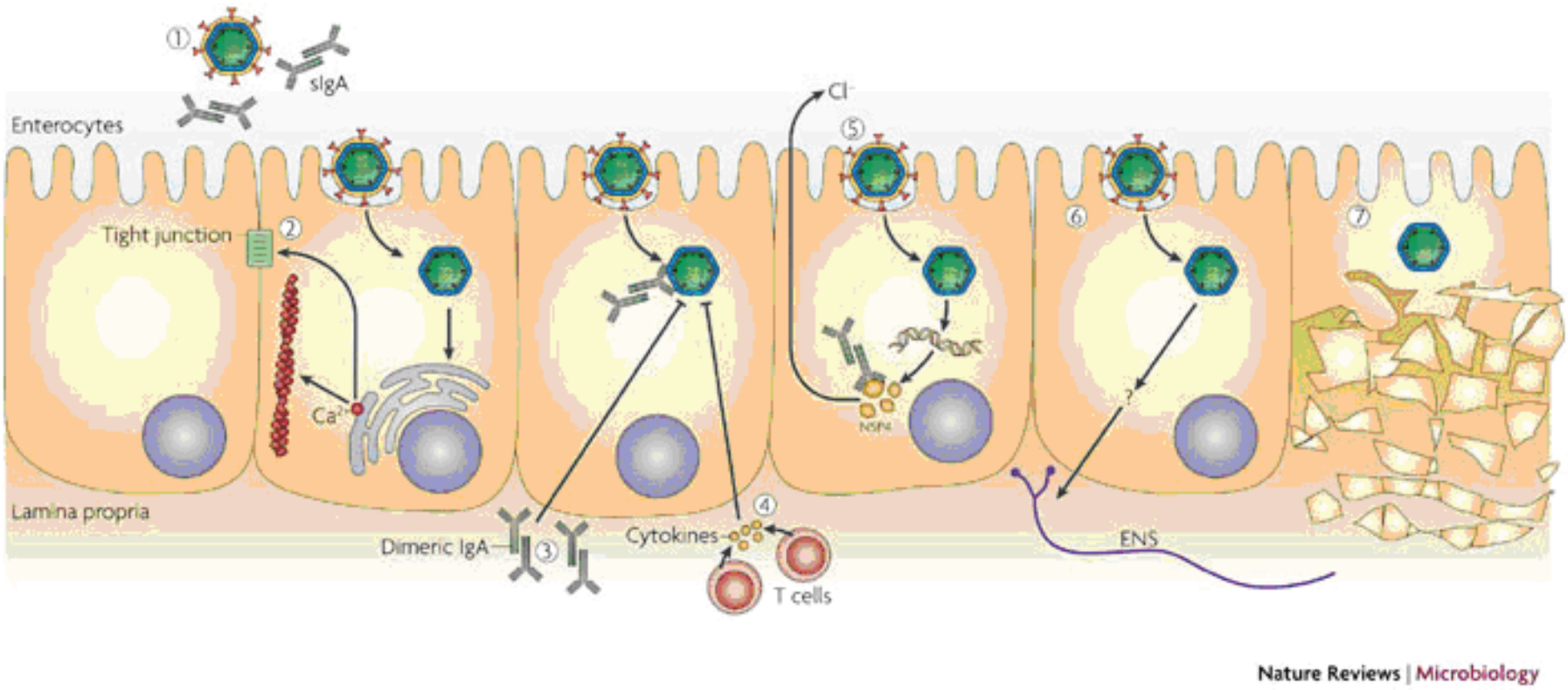

Figure 2: Potential mechanisms of rotavirus pathogenesis and immunity.
Step 1: Neutralizing antibodies directed against VP4 and/or VP7 can prevent vital binding to the host cell.
Step 2: If step 1 fails, rotavirus replication inside enterocytes causes altered metabolism of enterocyte membrane proteins inducing malabsorptive or osmotic diarrhoea. Rotavirus also increases the concentration of intracellular calcium, which disrupts the cytoskeleton and the tight junctions, raising the paracellular permeability.
Step 3: Intracellular viral replication can be inhibited by secretory anti-VP6 immunoglobulin A (IgA) during transcytosis across enterocytes.
Step 4: Cytokine-secreting RV-specific T cells can also inhibit viral replication.
Step 5: If viral replication is not stopped, replicating RV produces non-structural protein 4, a toxin which induces a secretory non-cystic fibrosis transmembrane conductance regulator mediated diarrhea.
Step 6: It is proposed, that RV can also stimulate the enteric nervous system, inducing secretory diarrhea and increasing intestinal motility.
Step 7: Late in the infectious process, RV kills the host cell, further contributing to malabsorptive or osmotic diarrhea.
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1.5. Rotavirus – Clinical manifestation

Infection in children may range from asymptomatic RV shedding to severe dehydration and death (Champsaur et al., 1984). RV infection typically begins with mild to moderate vomiting followed by the onset of frequent, watery diarrhea and fever. Fever typically abates during the second day but diarrhea often continues for 5 – 7 days. Dehydration may develop and progress rapidly, particularly in infants (Nelson et al., 2004). Respiratory symptoms such as rhinitis and cough, may be present in many of the children with RV GE (Rodriguez et al., 1987), however ill children are frequently simultaneously infected with both respiratory and gastrointestinal viruses (Brandt et al., 1986).

Rodrguez et al compared the clinical manifestations of 78 patients hospitalized with RV diarrhea and 72 patients hospitalized with a diarrheal illness that could not be associated with RV. The mean duration of vomiting was longer in the RV positive group than in those without RV (2.6 days vs. 0.9 days). RV diarrhea also lasted longer (5 days versus 2,6 days) (Rodriguez et al., 1977). A summary of this study is presented in Table 2 (adopted from (Fields and Knipe, 2006) and (Rodriguez et al., 1977).

Clinical Finding	Percent having each clinical finding	
	RV positive	RV negative
Vomiting	96[1]	58[1]
Fever 37.9° - 39° C	46	29
Fever >39° C	31	33
Dehydratation	83[1]	40[1]
- Hypertonic	5	16
- Isotonic	95	77
- Hypotonic	0	6
Irritability	47	40
Lethargy	26	27
Pharyngeal erythema	49	32
Tonsillar exudate	3	3
Rhinitis	26	22
Red tympanic membrane	19	9
Rhonchi or wheezing	8	8
Palpable cervical lymph nodes	18	9

Table 2: Clinical characteristics of 150 children with acute GE divided into RV positive and RV negative patient group.
[1]:Significant difference between the groups ($p < 0.01$) (adopted from (Fields and Knipe, 2006) and (Rodriguez et al., 1977))

1.6. Rotavirus - Diagnosis

1.6.1. Laboratory findings

Dehydration with metabolic acidosis is a common laboratory finding in children with severe viral enteritis (Palumbo et al., 2010). Peripheral blood leukocyte counts are usually normal in uncomplicated cases. Mild elevations in the serum apspartase aminotransferase have been reported during acute illness without other evidence of hepatic injury (Akelma et al., 2013).

The clinical manifestations of RV illnesses are not sufficiently distinctive to permit a definitive diagnosis on this basis alone. Epidemiologic pattern of RV disease and clinical features may indeed suggest the diagnosis, but laboratory confirmation with detection of the virus or a viral antigen is required for the definite diagnosis.

1.6.2. Confirmation of RV disease

Many assays have been developed for the detection of RV in stools (Mathewson et al., 1989). Enzyme immunoassays for stool samples, which offer approximately 90% specificity and sensitivity, are available for detection of group A RV. Specimens from the first to the fourth day of illness are optimal for virus detection using conventional assays. However, RV shedding can continue for up to 3 weeks after infection. (Fields and Knipe, 2006)

Electron microscopy or detection by polymerase chain reaction (PCR) comprise further methods for RV detection, in particular for more obscure cases in which standard procedures as described above fail to confirm the diagnosis. PCR has also been applied to the detection of group B and C RV (Eiden et al., 1991). A variety of techniques are available to measure a serologic response to RV infection (Ishida et al., 1997). However, in most cases a reliable diagnosis of RV disease can be made on basis of a stool assay.

1.7. Rotavirus - Treatment

The primary aim of treatment of RV GE is to replace fluids and electrolytes lost by vomiting and diarrhea. Because facilities for parenteral administration of fluids and electrolytes are not readily available in many parts of the world, intensive efforts have been made to evaluate the efficacy of oral fluid replacement therapy (Khin-Maung-U et al., 1991). Various formulations of oral rehydration salts have been shown to be effective in the treatment of dehydration caused by RV GE. In particular, an oral rehydration therapy with electrolyte solutions containing glucose has been shown to be a highly effective treatment for RV GE (Sack et al., 1978). The World Health Organization has proposed a standard glucose electrolyte formula. The current recommended WHO oral glucose electrolyte formula is composed of 75 mmol/L sodium chloride, 75 mmol/L glucose, 20 mmol/L potassium chloride and 10 mmol/L trisodium citrate (Hahn et al., 2002). After rehydration has been achieved, resumption of a normal diet appropriate to the child's age has been shown to result in a more rapid recovery. Prolonged administration of exclusive clear liquids for more than 12 hours or dilute infant formula is without clinical benefit and may even prolong the duration of diarrhea. If oral rehydration does not correct the fluid and electrolyte loss, or if the patient is severely dehydrated or in shock, intravenous fluids must be administered immediately (Fields and Knipe, 2006).

The use of loperamide, anticholinergic agents or adsorbents, opiates and atropine combination drugs is not recommended (AAP, 1996).

1.8. Rotavirus - Immunity

The mechanisms responsible for immunity to RV infections are not completely understood. Animal models have been used to derive a better understanding of immunity against RV, which consists of both local and systemic immunity.

1.8.1. Local immunity

Antibodies in the lumen of the small intestines seem to play a major role, as has been shown in newborn mice (Offit and Clark, 1985). Systemic RV antibodies are present in the gastrointestinal tract of neonatal calves if the level of circulating antibodies is sufficiently high (Besser et al., 1988). For instance, serum immunoglobulin G mediates mucosal immunity against RV infection (Westerman et al., 2005). A preexisting level of serum neutralizing antibodies to certain RV types correlates with resistance to diarrheal disease when being re-exposed to the RV type (Kapikian et al., 1983). Salivary immunoglobulin A antibodies are proposed to surrogate for the level of intestinal RV antibodies (Ward et al., 1992). RV serum immunoglobulin A levels have also been shown to correlate with resistance to severe RV illness (Hjelt et al., 1987). RV specific fecal immunoglobulin A titers correlate with protection against RV infection as do preexisting serum levels of immunoglobulin A and G (Matson et al., 1993). These antibodies are primarily against VP4 and VP7 of the RV (Hoshino et al., 1988). Furthermore, neutralization epitopes on VP4 or VP7 are shared among viruses within the same serotypes, so that reinfection with a virus of the same serotype amplifies the cross-reactive antibody response (Conner et al., 1988). Thus an infection caused by a certain RV serogroup can induce protection against numerous RV serogroups within the same serotype (Brüssow et al., 1988), (Brüssow et al., 1991).

1.9. Rotavirus – Prevention

1.9.1. Contagiosity

RV is a highly contagious pathogen that remains infectious on hands and on environmental surfaces for up to weeks (Wilde et al., 1992),(Ansari et al., 1988). In addition, RV is very resistant to disinfectants and hand-washing agents (Ansari et al., 1991). Even chlorine solutions for disinfection seem inadequate to sufficiently disable RV (Xue et al., 2013). Further adding to the contagiosity of RV is the small minimum infectious dose and high virus concentration in stool during acute illness (Ward et al., 1986),(Ward et al., 1984).

1.9.2. Hygiene

Good hygiene reduces the transmission of viral GE. However, as the incidence of viral GE is about the same in industrialized countries compared to developing countries, sanitary measures do not seem to be adequately effective in preventing RV disease (Nelson et al., 2004).

1.9.3. Vaccination against rotavirus

Natural RV infection protects against severe disease associated with re-infection (Velázquez et al., 1996). However, natural infection does not provide complete protection against a subsequent reinfection (Matson, 1996),(Velázquez et al., 1996). The RV surface proteins VP4 and VP7 are targets of neutralizing antibodies which can mediate protection (Franco et al., 2006). On this basis a human-simian reassortant RV vaccine containing distinct VP7 components was developed in the 1990s and licensed in the United States in 1998 under the name RotaShield®. Clinical trials in the United States, Finland and Venezuela had found it to be 80 – 100% effective at preventing severe diarrhea caused by RV A. RotaShield® was recommended by the Advisory Committee on Immunization Practices for all full-term infants in 1999 (ACIP, 1999), The American Academy of Pediatrics also recommended the vaccine (AAP, 1998).

RotaShield® was administered to almost 1 million children before a temporal association between vaccine administration and intestinal intussusception was detected. 15 cases of intussusception were reported to the Vaccine Adverse Events Reporting System (Centers for Disease Control and Prevention (CDC), 1999a). Intussusception occurred with significantly increased frequency in the first 1 - 2 weeks after vaccination, in particular after the first dose (Centers for Disease Control and Prevention (CDC), 1999b). RotaShield® was withdrawn from the market in 1999 (Angel et al., 2007). After the withdrawal of RotaShield® there have been numerous retrospective analyses on the risks and

benefits of RotaShield® with a general consensus that the risk of intussusceptions associated with RotaShield® was lower than initially estimated (Murphy et al., 2003). There was no increase in hospitalizations due to intussusceptions among children less than one year of age during the period in which RotaShield® was administered (Simonsen et al., 2001). It was also observed, that age at vaccination was a critical factor in the occurrence of intussusception. Children who received RotaShield® at 90 or more days of age had a higher risk of intussusception (Simonsen et al., 2005). In addition, no cases of intussusception were registered during the first two weeks after vaccination with RotaShield® in children less than 60 days of age (Simonsen et al., 2005).

Despite the controversies with the withdrawal of RotaShield®, development of other RV vaccines continued. Currently there are two new oral vaccines against RV available. Rotarix® from GlaxoSmithKline is a live attenuated monovalent vaccine. RotaTeq® from Merck is a live, bovine human reassortant pentavalent vaccine. As mentioned above, the risk of intussusception associated with RotaShield® appeared to be strongly related to the age at vaccination, so that the recommended age at first vaccination for the new vaccines is earlier than that for RotaShield®.

The vaccination with Rotarix® consists of two doses whereas Rotateq® vaccination consists of three doses. The first dose of both vaccines should be administered at an age of 6 weeks. The following dose or doses can be administered after a minimum period of four weeks respectively. Prior to the licensing of the vaccines, large-scale safety and efficacy studies had been conducted. Both vaccines were safe and effective and were not associated with an increased risk of intussusception (Soares-Weiser et al., 2012),(Ruiz-Palacios et al., 2006),(Vesikari et al., 2006).

In 2009, the World Health Organization recommended global use of RV vaccines (WHO, 2009). By the year 2011, 45 countries including the United States, Austria, Finland and Australia have introduced RV vaccines in their

national immunization programs. RV immunization was not part of the national vaccination program in Germany by 2011. In absence of a national recommendation for RV vaccination, the German federal states Saxony, Thuringia, Mecklenburg-Western Pomerania, Brandenburg and Schleswig-Holstein implemented the RV vaccine in their local vaccination recommendations before 2009.

1.10. Rotavirus – Severe disease course

The severity of RV illness varies, although most cases are self limiting. To better evaluate and objectify the severity of gastrointestinal infections, two severity scoring scales have been introduced. The Vesikari Scale is a 20 point scale (Ruuska and Vesikari, 1990) whereas the Clark Scale is 24 point scale (Clark et al., 1988). In both scales, increasing scores are associated with more severe disease. The features of the scales are shown in table 3 (Givon-Lavi et al., 2008).

Scores higher than 10 points using the Vesikari-Scale or Clark scores higher than 16 points are considered as severe GE and usually require hospital treatment. Considering that most cases of RV GE are not life threatening, such scores may be an adequate measure to objectify the severity of RV GE. However, very severe courses of RV infections associated with clinical features such as encephalopathy, severe electrolyte disturbance, need for intensive care treatment and death have been described (Kobayashi et al., 2010),(Kaiser et al., 2012). Neither of the two described severity scores yields information about these features of very severe RV disease, although seizure is one criterion for a maximal score using the Clark scale. Thus even a maximum score using either scale, does not necessarily associate with a life-threatening GE.

	Point value		
	1	2	3
Clark Scale			
Diarrhea			
- Number of stools/day	2–4	5–7	≥8
- Duration in days	1–4	5–7	≥8
Vomiting			
- Number of emeses/day	1–3	4–6	≥7
- duration in days	2	3–5	≥6
Rectal temperature			
- Temperature (°C)	38.1–38.2	38.3–38.7	≥38.8
- Duration in days	1–2	3–4	≥5
Behavioral symptoms	Irritable	Lethargic/	Seizure
- Description	1–2	3–4	≥5
- Duration in days			
Vesikari Scale			
Duration of diarrhea (days)	1–4	5	≥6
Maximum number of diarrhea stools/24 h	1–3	4–5	≥6
Duration of vomiting (days)	1	2	≥3
Maximum number of vomiting episodes/24 h	1	2–4	≥5
Temperature (°C)	37.1–38.4	38.5–38.9	≥39.0
Dehydration	–	Mild	Moderate to severe
Treatment	Rehydration	Hospitalization	–

Table 3: Description of the Clark- and Vesikari Scale. (Adopted from (Givon-Lavi et al., 2008))

Information on very severe RV disease (vsRVd) is primarily based on case reports and retrospective analyses, whereas prospectively collected data are lacking. Especially, prospective studies to specifically query vsRVd and collect epidemiological data are not available. A PubMed search using the query string “rotavir*[ti] AND (encephal*[ti] OR neurol*[ti] OR severe*[ti] OR complication*[ti] or nervous*[ti] OR seizure*[ti])” performed in January 2009 (prior to the presented study on vsRVd) yielded 80 publications published between 1977 and 2009. These contain 21 publications reporting a total of 40 patients (19 male, 21 female). Thirty of these patients were reported to have had seizures whereas 3 patients had neurological symptoms such as tremor, ataxia or paralysis. Five patients had signs of encephalopathy and 3 patients were reported to suffer from circulatory failure. 59 publications were retrospective epidemiological studies and studies on political economy or studies on pathophysiology.

Prospective data on life threatening RV disease in children could document the virulent properties of RV. Furthermore, a better understanding of the severe morbidity of RV could contribute to inform decision on a national RV vaccine recommendation in Germany and in other developed countries as well.

The study protocol was approved by the ethics committee of the University of Essen (Germany) and registered with the file reference 09-4203.

2. Aims of study

This study aimed to prospectively detect all cases of very severe rotavirus disease in children younger than 17 years in Germany in a 2-year study period between April 2009 and March 2011. The goal was to gather demographic and incidence data as well as details about each individual case such as ambulatory or nosocomial origin of infection, course and duration of disease and clinical and laboratory findings as well as to determine whether a very severe RV disease might cause persisting complications.

Data acquisition was coordinated by the German Pediatric Surveillance Unit for rare diseases (ESPED) which collaborates with all pediatric departments in Germany.

3. **Rotavirus Disease in Germany - A prospective Survey of Very Severe Cases,** Shai, S., Perez-Becker, R., Wirsing von König, C-H., von Kries, R., Heininger, U., Forster, J., Huppertz, H-I., Roos, R., Göbel, U., Niehues, T., The Pediatric Infectious Disease Journal, Volume 32, e62 - e67, (2013)

Rotavirus Disease in Germany—A Prospective Survey of Very Severe Cases

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Objective: Rotavirus (RV) gastroenteritis is a notifiable disease in Germany. The reports to the authorities contain few data concerning the severity of disease. The aims of this study were to determine incidence and outcome of very severe cases of RV disease.

Methods: Cases of very severe RV disease were collected by the German Paediatric Surveillance Unit for rare diseases (Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland) using anonymous questionnaires based on hospitalized patients between April 2009 and March 2011. Inclusion criteria were detection of RV antigen in feces, patient aged 0–16 years and 1 or more of the following criteria: intensive care treatment, hypernatremia or hyponatremia (>155 mmol/L or <125 mmol/L), clinical signs of encephalopathy (somnolence, seizures, apnea) and RV-associated death.

Results: During 2 years, 130 cases of very severe RV disease were reported, 101 of 130 were verified. Seventeen patients had nosocomial infection, of whom 14 were neonates in intensive care. Among those, 12 infants had verified or suspected necrotizing enterocolitis. Eighty-four community-acquired cases were reported, median age was 10.5 months (0–108 months). The median hospital stay was 6 days, and 48 patients needed intensive care treatment. Among children less than 5 years of age, the yearly incidence of community-acquired very severe RV disease was 1.2 of 100,000 (95% confidence interval: 0.9–1.4/100,000). A total of 26 of 84 and 10 of 84 patients had severe hypernatremia or hyponatremia, respectively, and 58 of 84 patients had signs of encephalopathy. Three deaths were reported (1 nosocomial and 2 community acquired).

Conclusions: RV infection in Germany can have a life-threatening course. A substantial number are nosocomial infections.

Key Words: rotavirus, severe prospective epidemiology, Germany

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The fees (5000 Euro) for the case query through ESPED and collection of the monthly reports during the 2-year study period were covered by GlaxoSmith-Kline (GSK; München,Germany). None of the authors received personal grants or funding for this study. JF has been a member of the advisory board of GSK Germany on rotavirus vaccine and had travel expenses paid by GSK and Pasteur to give a talk on rotavirus epidemiology. UH is a member of and has been a consultant to the independent Data Monitoring Board for GSK, Rixensart, Belgium, and has received payment for lectures by most major vaccine manufacturer. H-IH receives payments by GSK and Sanofi-MSD for lectures. RR was a member of the advisory board on rotavirus vaccine by GSK. C-HWvK has received payments by GSK for lectures. RvK's institution receives grants. The authors have no other funding or conflicts of interest to disclose.

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Rotavirus (RV) is the most common cause of gastroenteritis (GE) in children in Germany[1] and worldwide.[2] It is estimated that in a global setting, RV disease annually causes about 530,000 deaths in children less than 5 years of age.[3] However, in industrialized countries, RV GE is usually a mild and self-limiting disease. Nevertheless, it is estimated that in the European Union more than 87,000 hospitalizations and 231 deaths per year are attributed to RV in children less than 5 years of age.[4] With an estimated annual 3.6 million cases of RV GE in children less than 5 years of age in the European Union, RV causes a heavy burden on society and the healthcare system.[4] Since 2001, acute RV disease with either diarrhea and/or vomiting in Germany is to be reported to the Robert Koch Institute (Berlin, Germany), the German federal institution responsible for disease control and prevention. However, due to underreporting, the true number of cases is probably 5–10 times higher than the reported cases.[5]

The 2 available vaccines against RV are safe and effective.[6–8] In 2009, the World Health Organization recommended global use of RV vaccines.[9] RV immunization is not part of the national vaccination program in Germany but is under consideration by the German Standing Committee on Vaccination.[1] In the absence of a national recommendation for RV vaccination, some individual federal states have still recommended RV vaccination. The RV vaccine is thus used on a limited level in Germany.

Very severe courses of RV infections associated with clinical features such as encephalopathy, severe electrolyte disturbance, intensive care treatment and death have been described.[10,11] Information on very severe RV disease (vsRVd) is primarily based on case reports or retrospective analyses, whereas prospectively collected data are lacking. Especially, prospective studies to query the vsRVd are not available.

This study aimed to prospectively detect all vsRVd in children younger than 17 years in Germany in a 2-year period, to gather demographic and incidence data as well as details about each individual case. The data could help to better understand the virulent properties of RV and could inform decision on a national RV vaccine recommendation.

METHODS

Prospective data collection was coordinated by the well-established German Paediatric Surveillance Unit for rare diseases (ESPED; Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland). Data on the reliability of the ESPED tool have been published previously.[12–14] Briefly, ESPED sends a report card to each pediatric department in Germany asking pediatricians for incident diagnoses with specific inclusion criteria of up to 12 conditions on a monthly basis. Each condition is investigated by a principal investigator. Report cards are to be returned to ESPED even if no incident diagnosis is reported. Reports of incident diagnoses are forwarded to the respective principal investigator. If an incident diagnosis is reported, the reporting department is asked to complete a questionnaire that is returned to the principal investigator to collect the relevant data. The current return rate of the report cards to ESPED is more than 95%.[15] Between April 2009 and March 2011,

vsRVds were collected on the monthly report cards. The case definition was patient 0–16 years of age, detection of RV in feces and a very severe course of RV disease defined by 1 or more of the following criteria: intensive care treatment, hypernatremia >155 mmol/L, hyponatremia <125 mmol/L, clinical signs of encephalopathy (somnolence, seizures and apneas) and death due to complications related to RV disease.

Patients with a nosocomial RV disease already in intensive care treatment were also included because the underlying diseases for admission to the intensive care unit (ICU) might increase the severity of the RV disease. Once a case was reported, an anonymous questionnaire was sent to the reporting clinic and an anonymized medical discharge report was asked for. Through the questionnaires, basic demographic data were acquired in addition to data related to RV disease such as preadmission duration of symptoms, RV vaccine status, clinical and laboratory findings, therapy, duration of hospital stay and health status on discharge. RV disease was classified as nosocomial infection if onset of RV symptoms occurred more than 48 hours after hospital admission.[1]

The population number of children and adolescents between 0 and 16 years of age in Germany was 12,636,179 and 12,515,138 in 2009 and 2010, respectively, adding up to a total of 25,151,317 of whom 6,818,728 were less than 5 years of age and 2,701,794 were less than 2 years of age (German Federal Statistical Office—https://www-genesis.destatis.de; accessed April 27 2012). Population data for 2011 were not available at the time of data retrieval.

Collected data were continuously entered into LibreOffice Calc (version 3.4.4). Data were imported to and analyzed using R (version 2.14.1), a language and environment for statistical computing.[16] Medians with interquartile ranges (IQRs: 25–75 percentile), complete ranges and means where appropriate were used for descriptive analysis. For comparison of categorical variables Fisher exact test was used. For assessing continuous variables, the Mann–Whitney–Wilcoxon test was applied because parametric nature of the variables could not be assumed. Comparison of categorical variables with an assumed or estimated probability distribution was performed with Pearson χ^2 test (Goodness of Fit). The Poisson distribution was used to calculate 95% confidence intervals (CIs) of count data. Level of significance was set to $P < 0.05$. The study protocol was approved by the ethics committee of the University of Essen, Essen, Germany.

RESULTS

Between April 2009 and March 2011, 130 cases of vsRVd were reported. A completed questionnaire or written or oral feedback could not be retrieved in 10 cases despite multiple enquiries, yielding a questionnaire return rate of 92%. After deducting reports of patients not fulfilling the inclusion criteria (18 reports) and 1 double report, 101 cases were evaluable from 58 different pediatric departments in Germany. The majority of reports (96/101; 96%) concerned children less than 5 years of age. There were 17 nosocomial and 84 community-acquired vsRVd, none of which had occurred in a child who had received an RV vaccine.

Incidence

To estimate the yearly incidence of vsRVd with a 95% CI, only community-acquired cases were taken into account as the reports of nosocomial disease cannot be regarded as independent occurrences (eg, multiple cases within 1 clinic). There were 79 reports of community-acquired vsRVd in children under the age of 5 years yielding an incidence of 1.2/100,000/year in children less than 5 years of age; 95% CI: 0.9–1.4/100,000. Considering patients less than 2 years of age with a community-acquired disease (65 patients), the yearly incidence was 2.4/100,000/year; 95% CI: 1.9–3.1/100,000.

Seasonal Variation

The monthly incidence of vsRVd varied in parallel to the total number of reported RV infections in children less than 14 years of age reported to the Robert Koch Institute as seen in Figure 1. Yearly peak incidences were observed in the months March–May.

Nosocomial vsRVd

Seventeen of the 101 cases (17%) were nosocomially acquired, of which 16 patients needed or were already in intensive care treatment during onset of RV symptoms. One child, a 4-month-old boy, was reported due to hypernatremia >155 mmol/L.

Fourteen patients were neonates in intensive care treatment. Three neonates were reported to have apneas as a sign of encephalopathy. A total of 8 neonates were diagnosed with necrotizing enterocolitis (NEC); in addition, NEC was suspected in 4 neonates who were treated with antibiotics and withdrawal of enteral nutrition. There was a significant male preponderance (13/17; $P = 0.03$).

Seven of the 16 neonates were reported by a single hospital between July and October 2009; 6 were preterm neonates (29–34 weeks of gestation) diagnosed with NEC and detection of both RV and adenovirus in feces. Additionally, this hospital reported a 2-month-old infant with tetralogy of Fallot and pyloric stenosis already in the ICU at the onset of RV disease.

Among the 17 reported nosocomial cases 1 death occurred—a preterm neonate with 25 weeks of gestation. The child suffered

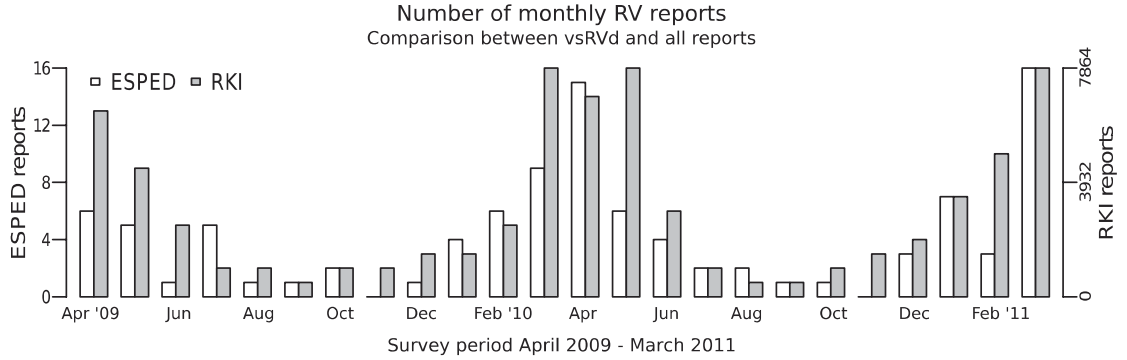

FIGURE 1. Monthly reports of RV disease. Left scale: ESPED reports (age group, 0–16 years). Right scale: All RV reports to the Robert Koch Institute (age group, 0–14 years). (Source of RKI data: with permission from Robert Koch-Institut: SurvStat, http://www3.rki.de/SurvStat).

TABLE 1. Descriptive Characteristics of the Patients With Community-acquired vsRVd Grouped by Inclusion Criteria (Multiple Entries per Patient Possible)

	Total cohort	ICU	Hypernatremia (>155 mmol/L)	Hyponatremia (<125 mmol/L)	Clinical signs of encephalopathy
n	84	48	26	10	58
n, male (%)	45	29 (60)	13 (50)	5 (50)	31 (53)
Median age (mo)	10.5	7	7	17.5	10.5
Median preadmission duration of symptoms (h)	48	24	48	30	36
Median duration of hospitalization (days)	6	8.5	6	6.5	6

There was no significant statistical difference between median age and hypernatremia ($P = 0.09$) or hyponatremia ($P = 0.12$) compared with the median age of the patients without severe electrolyte disturbances.

a bilateral third-degree intraventricular hemorrhage with secondary hydrocephalus, which was treated with a Rickham reservoir. Additionally, the boy needed a surgical intervention due to a patent ductus arteriosus. After these procedures, he was in a stable condition. However, at the age of 2 months, he showed a rapid clinical deterioration with a suspected NEC and clinical and laboratory signs of septic shock (C-reactive protein, 107 mg/L; interleukin-6 >1000 pg/mL), although no bacteria could be isolated in the cerebrospinal fluid (CSF) or blood cultures. The CSF contained 98 leukocytes/mL.

Community-acquired vsRVd

A total of 84 community-acquired cases were reported (45 male, 39 female). The median age was 10.5 months (IQR: 4.8–21.3 months; range: 0–109 months). The median preadmission duration of symptoms was 48 hours (IQR: 23.5–72 hours; range: 0– 168 hours) and the median duration of hospital stay was 6 days (IQR: 5–11 days; range: 1–85 days). There were no relevant gender differences regarding patient age, preadmission duration of symptoms or duration of hospital stay. Seventy-nine of the 84 patients (94%) were less than 5 years of age. The incidence in children less than 5 years of age in the various federal states ranged from 0 to 2.3/100,000/year. The characteristics of the patients grouped by inclusion criteria are shown in Table 1. The 2 oldest patients were a 8.6-year-old boy with a short bowel syndrome and need for total parenteral nutrition being admitted to the ICU due to gastrointestinal hemorrhage and a 9-year-old girl with an RV encephalitis without any known prior conditions.

Intensive Care Treatment

Forty-eight of the 84 patients (57%) needed treatment in an ICU, and their median age (7 months) was significantly lower than those who were not admitted to the ICU (median age, 12.5 months; $P = 0.01$). The hospital stay was significantly longer if the patient needed treatment in an ICU (median, 8.5 days versus 5 days in patients not treated in an ICU; $P < 0.001$).

Electrolyte Disturbances

In 26 of 84 (31%) patients hypernatremia (>155 mmol/L) was observed, and 10 (12%) patients had severe hyponatremia (<125 mmol/L). Patients with severe hypernatremia tended to be younger (median age, 8 months) compared with all other patients (median age, 12 months; $P = 0.08$). Further details are listed in Table 1.

Encephalopathies

A total of 58 of 84 (69%) patients had clinical signs of encephalopathy as defined by the inclusion criteria. Somnolence was reported in 44 patients. Five patients had apneas. Twenty-three patients suffered convulsions (multiple criteria for encephalopathy per patient possible). Most convulsions were associated with fever. However, 5 of the seizures were afebrile and not associated with severe electrolyte disturbance or severe dehydration. Six of the 23 patients with convulsions also had very severe hypernatremia or hyponatremia (n = 2 and n = 4, respectively).

Fourteen of the 58 patients with encephalopathy (24%) had pathological electroencephalography. In 22 patients (38% of the patients with encephalopathy, 61% of patients with convulsions), the CSF was examined revealing pathological findings in 6 patients: 5 patients had pleocytosis and were diagnosed with encephalitis. In a 9-year-old girl who had no convulsions but severe hyponatremia, somnolence and pathological electroencephalography (general deceleration), RV was identified in her CSF containing 2000 copies/mL of RV. There was no pleocytosis or abnormal levels of glucose, protein or lactate. Magnetic resonance imaging on the day of admission first showed an increased signal intensity in the splenium of the corpus callosum whereas the control magnetic resonance imaging results showed signal changes in the cerebellum compatible with cerebellitis 5 days later. She was discharged with persisting neurologic symptoms. The CSF was tested for RV by polymerase chain reaction only in this one case. Of the remaining children with encephalopathy, 1 child was discharged with hemiparesis after an intraventricular hemorrhage, 1 child had residual convulsions and 1 child was discharged with developmental delay, although the delay was present before the RV disease. One patient was lost to follow-up after he was transferred to another hospital. The remaining patients had fully recovered at the time of hospital discharge.

Clinical and Laboratory Findings

Severe dehydration (>10% of the body weight) was observed in 39 patients; the median age of these patients (8 months) was significantly lower than those with a moderate or no dehydration (median age, 15.5 months; $P = 0.04$). A synopsis of the laboratory findings is shown in Table 2. Fourteen of the 39 patients (36%) with severe dehydration also presented with severe hypernatremia (>155 mmol/L); 6 of 39 patients (15%) had severe hyponatremia (<125 mmol/L).

Demographic Data

Ethnicity-based data of the patients were obtained in 68 of the 84 reports of ambulatory-acquired RV disease. Fifty-four of the 68 patients (79%) were Caucasians from Central Europe.

Natural History and Deaths

Seventy-one of the 84 patients (94%) with community-acquired RV disease were classified as healthy at the time of discharge, 3 patients had residual gastrointestinal symptoms, 4 patients had persisting neurologic symptoms and 1 patient was discharged with a joint effusion, which was associated with a coinfection caused by *Staphylococcus aureus*. Discharge data were missing from 2 patients.

TABLE 2. Descriptive Characteristics of the Laboratory Findings

	WBC (/nL)	CRP (mg/L)	pH	BE (mmol/L)	pCO_2 (mm Hg)	Bicarb.(mmol/L)	ALT (U/L)
Range	0.4 to 39.5	0 to 443	6.77 to 7.53	−28.3 to 5.2	2.48 to 59.0	6.0 to 30.5	4 to 1364
IQR	6.7 to 16.2	3 to 23.5	7.18 to 7.33	−16.1 to −7.9	24.8 to 35.4	12.0 to 17.3	30 to 81
Median	10.3	8.9	7.27	−12.0	28.4	14.9	43
Mean	12.3	27.9	7.24	−12.0	28.8	14.8	123

WBC indicates white blood cell count (normal values (×1000): less than 2 years of age: 6.5–15.0, 2–6 years 5.0–12.0, 6–16 years 4.5–10.5); CRP, C-reactive protein (normal values <5 mg/L); BE, base excess (normal values −2 to +3 mmol/L), pCO_2, partial pressure of carbon dioxide (normal values 35–45 mm Hg), Bicarb., bicarbonate (normal values 21–26 mmol/L); ALT, alanine transaminase (normal values age 0–30 days <90 U/L, 1–12 months < 80 U/L, 1–12 years <50 U/L, 12–16 years <30 U/L). (Normal values from Thomas, L.; Labor und Diagnose: Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik, 7th ed., Frankfurt: TH Books-Verlags-Geschellschaft, 2008.)

Two deaths among the community-acquired vsRVd were reported. A 6-month-old infant with trisomy 21 with diarrhea beginning on the day of admission with fever and hyponatremia (124 mmol/L). RV and clostridium difficile toxin were identified in the stools. Hirschsprung disease was diagnosed during the acute exacerbation. The child died due to cardiac and respiratory failure and presumed septic shock, although the blood and CSF cultures were sterile.

A 12-month-old boy with symptoms of GE beginning 48 hours before hospital admission with severe dehydration (>10% of the body weight) was admitted to the ICU during cardiopulmonary resuscitation. Maximum therapy with parenteral rehydration, mechanical ventilation and catecholamines was initiated but the boy died on the day of admission due to hypovolemic shock. The autopsy revealed no further or concomitant causes.

DISCUSSION

We present a 2-year prospective survey of vsRVd in children in Germany. Our estimation of the incidence of vsRVd is 1.2/100,000 in children less than 5 years of age. This estimate is a minimum. The true incidence might be twice as high because ESPED does not capture all cases as shown by capture–recapture analyses.[17,18] Comparing this incidence with the overall reported incidence of RV disease in the same age group in Germany (115/10,000),[1] a vsRVd occurs in about 1% of all reported RV cases in children under 5 years of age.[19] The median duration of hospitalization (6 days) is considerably longer than the median duration of all reported hospitalized RV cases (2–3 days).[1]

A recent retrospective analysis of the epidemiology of RV infections in children less than 5 years of age in Germany describing the burden of disease was recently published by Koch and Wiese-Posselt.[1] This publication shows a detailed, descriptive analysis of national RV surveillance data collected in Germany between 2001 and 2008. Although epidemiologic data of hospitalizations and numbers of deaths are described in detail, the cases of vsRVd are not described or listed. The RV mortality rate we observed fits the data of this study that reported 8 deaths in children less than 5 years of age between 2004 and 2008. Another study estimated a death rate due to RV infections of 13 per year in children under the age of 5 years in Germany.[4]

Nosocomial RV infections cause a high disease burden.[20–23] In particular, neonates are prone to severe illness. We observed 14 neonates treated in an ICU. There were significantly more male neonates with nosocomial vsRVd. Male preterm neonates had more complications and a poorer outcome than their female counterparts.[24] This association may explain why male patients dominated this group.

RV in neonatal ICUs is a well-known problem, especially so, as the clinical signs in preterm neonates differ greatly from those in older children.[25] In particular, diarrhea may not be the leading symptom.[26,27] Most of the 14 neonates with nosocomially acquired RV disease (86%) were diagnosed with or had a suspected NEC. It has been reported that about 30% of NEC cases in preterm neonates are associated with RV.[28] The pathogenesis of this association remains unclear. It has been hypothesized that a viral nonstructural protein evokes abnormal intracellular calcium mobilization in enterocytes.[29] There was a cluster of 6 neonates with NEC excreting RV and adenovirus reported by a single hospital over a period of 4 months. In that hospital, of course, nosocomial RV infection added seriously to the common neonatal pathology.[25] Three neonates were reported to have apneas. Although this might be a clinical sign of encephalopathy, there were no data to distinguish the RV-associated apneas from apneas due to prematurity.

Ninety-four percent of patients with community-acquired RV disease were less than 5 years old. This is in accordance to epidemiologic findings in Germany and Europe.[1,30]

The overall median duration of admission was 6 days, which is considerably longer than the overall hospitalization duration of all acquired RV infections in children less than 5 years of age in Germany (2–3 days).[1]

The majority of patients with community-acquired vsRVd (69%) had clinical signs of encephalopathy. The direct role of RV in these cases is not clear because neurologic symptoms may be unspecific and related to fever, dehydration or electrolyte disturbances. However, in 6 of these patients there was no severe dehydration, electrolyte disturbance or fever reported. Five children had afebrile seizures. One of them was coexcreting adenovirus, having severe hyponatremia at the time of admission as well. Afebrile convulsion associated with RV GE has been described numerous times.[31–34]

Although patients with RV GE and convulsions were included in this study, convulsions in patients with GE generally have a good prognosis.[35] Thus, in most cases excessive diagnostic measures and therapeutic attempts can be avoided.[36,37] Moreover, convulsions in case of GE are not a feature unique to RV. There are reports of other pathogens such as norovirus that are the primary cause of this clinical feature.[38]

The CSF was tested for RV only in 1 patient, containing 2000 copies/mL. The cranial magnetic resonance imaging of this patient showed signal intensity alterations in the cerebellum which appears to be a recurrent finding in patients with RV-associated cerebellitis.[39,40]

Although the detection of RV in the CSF of patients with RV-associated encephalopathy may have no direct therapeutic or prognostic value, it should be considered if a lumbar puncture is performed due to other reasons because this might improve our understanding of the RV.

Severe hypernatremia >155 mmol/L was observed in 26 patients. A recent retrospective study from Germany showed a significantly higher proportion of children with RV GE with hypernatremia >150 mmol/L compared with RV negative GE cases, proposing hypernatremia to be a particular feature of RV.[11] Another analysis from Sweden showed a hypernatremia in more than 9% of RV GE

cases over a period of 11 years.[41] The alanine transaminase was normal or slightly elevated in most cases. There have been observations of RV causing a moderate elevation of liver transaminase.[42,43]

RV vaccination is currently not generally recommended in Germany. However, health authorities of each federal state can establish local vaccination recommendations. In 5 federal states in Germany, the RV vaccine has been implemented in their local vaccination program (Saxony, Thuringia, Mecklenburg-Western Pomerania, Brandenburg and Schleswig-Holstein). In these states, the coverage is almost 60% as opposed to 20% in the 11 remaining federal states.[44] There has been a significant drop in RV notifications in 2010 compared with 2006 in states with a high RV vaccination coverage.[44] Estimates show that a 90% RV vaccination coverage would significantly decrease disease burden in European regions.[45,46] It has also been suggested that an RV vaccination coverage rate of about 90% might induce herd immunity, which may play a vital role in reducing the number of nosocomially acquired RV diseases..[47,48]

A limitation of this study is that the survey began after the new RV vaccine was approved. As some federal states have already implemented the RV vaccine in their local vaccine recommendations, this may have caused a reduction in the number of vsRVd as the RV vaccine reduces the rate of RV hospitalizations.[49]

As detailed population data for 2011 were not available at the time of data retrieval from the German Federal Statistical Office, the estimation of incidence may be slightly inaccurate. No stool samples were collected to determine the RV serotype, as this might have increased the understanding of the RV virulence. It has been suggested that disease severity might vary with RV serotype.[50] RV antigenemia might be observed during an acute RV GE as well as in asymptomatic children.[51–53] Analysis of the patient serum might have contributed to determine the role of RV antigenemia in courses of vsRVd. Furthermore, a long-term follow-up of the patients might have given insight to the long-term effects of vsRVd.

Even in a country with guaranteed medical care for all and a high standard of pediatric care, vsRVd does occur. Although rare, vsRVd constitutes a considerable burden to the healthcare system and the families of the affected children.

Conclusion

Over a period of 2 years, 101 cases of vsRVd in Germany were reported. Although the incidence is relatively low compared with all RV cases, significant RV morbidity could be identified in a country with free access to health care for all and a highly developed healthcare system.

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4. Discussion

A recent retrospective analysis of the epidemiology of RV infections in children less than 5 years of age in Germany describing the burden of disease was published by Koch and Wiese-Posselt in 2011 (Koch and Wiese-Posselt, 2011). This publication shows a detailed, descriptive analysis of national RV surveillance data collected in Germany between 2001 and 2008. Although epidemiologic data of hospitalizations and numbers of deaths are described in detail, the cases of very severe disease course are not described or listed.

4.1. Summary of results

A total of 130 cases of vsRVd were reported during the data acquisition period of two years. A feedback from the reporting clinics could be achieved in 120 cases, of which a double report was identified. In 18 of the reports, the inclusion criteria were not fulfilled. Of the 101 verified cases of vsRVd, 84 patients had a community acquired vsRVd, whereas 17 were nosocomially acquired. A summary is shown in Figure 3.

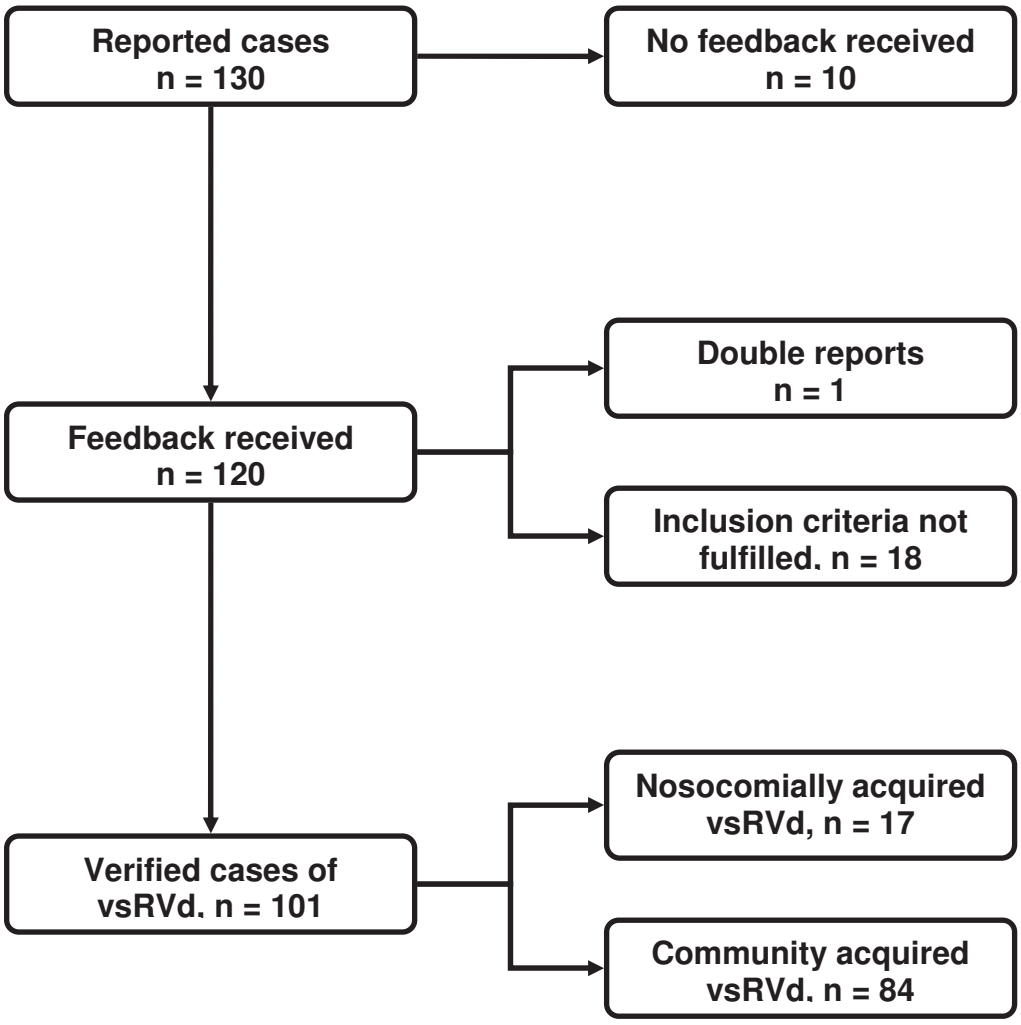

Figure 3: Chart showing the distribution of the 130 reported cases.

A total of 84 community-acquired cases of vsRVd were reported. The median age at diagnosis in this patient group was 10,5 months with an age range from 0 – 109 months. Seventy-nine of these patients (94%) were under 5 years of age. Further epidemiological data of the community-acquired cases of vsRVd are shown in Figure 4. None of the reported patients had received a vaccine against RV.

4.1.1. Incidence

The yearly incidence of community acquired very severe RV disease in children under the age of 5 years in German is 1.2/100,000. In comparison, the yearly incidence of all RV reported diseases in children less than 5 years of age in Germany is 1150/100,000 (Koch and Wiese-Posselt, 2011). One vsRVd occurs in about 1000 cases of RV infection (1 ‰).

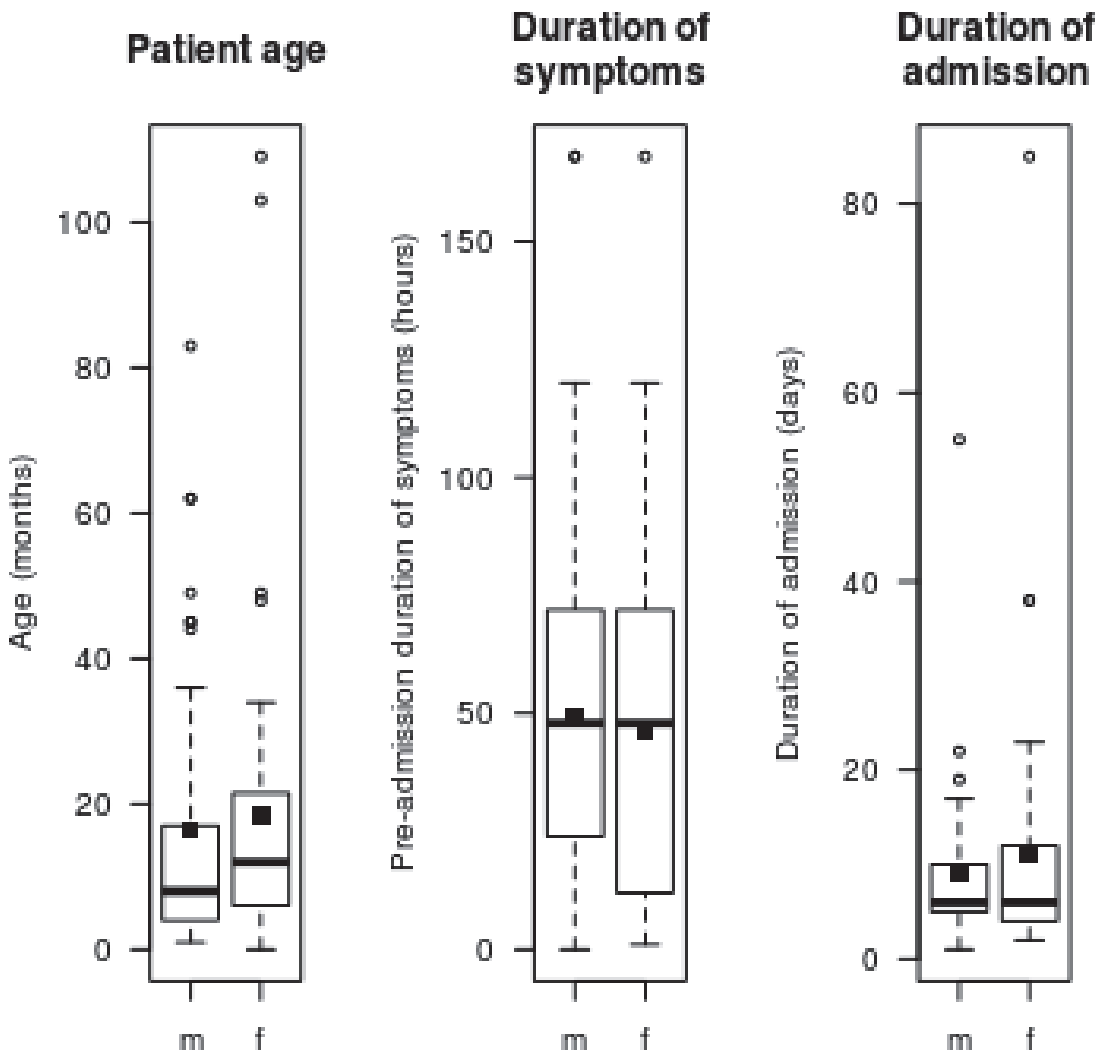

Figure 4: Box-whiskers plots showing gender (m = male, f = female) specific data for age (months), preadmission duration of symptoms (hours) and duration of hospital admission (days) for the patients with community acquired vsRVd. The boxes represent the 25 - 75 percent interquartile range with the median as a bold horizontal line, the whiskers depict 1.5 interquartile range from the 25 and 75 percentile respectively, outliers are represented as ○ and means are shown as ▪.

4.1.2. Mortality

There were 3 RV associated deaths reported. Two of these patients had severe concomitant conditions; a preterm neonate with extremely low birth weigh, necrotizing enterocolitis (NEC) and septic shock. This child had a nosocomially acquired vsRVd. A 6 month old infant with trisomy 21 and Hirschsprung's disease and a 12 month old boy who had symptoms of GE beginning 48 hours prior to hospital admission and was brought to the intensive care unit during cardiopulmonary resuscitation. The two latter cases of vsRVd were community acquired. The observed RV mortality rate of about 1.5 per year corresponds to the study by Koch and Wiese-Posselt in which 8 reported deaths were reported between 2004 and 2008 (Koch and Wiese-Posselt, 2011).

4.1.3. Neonates - a patient group at risk

Our study on vsRVd shows that neonates in particular are prone to have a very severe course of nosocomial RV disease. Of the 17 nosocomial vsRVd cases, 14 were neonates in intensive care treatment. The male proportion of this group was significantly larger than the female. Male neonates born very prematurely in neonatal intensive care treatment generally have more complications related to death, oxygen dependency, pulmonary hemorrhage and major cranial ultrasound abnormalities. The outcome of male neonates born very prematurely is poorer compared to their female counterparts (Peacock et al., 2012). Information on whether male neonates are also more prone to infections is lacking. The findings of this study might imply such an association regarding RV infections but information on how many neonates were admitted to the intensive care unit at the time of the report of vsRVd and their gender distribution required for such estimations is lacking.

RV in neonatal intensive care units (ICUs) is a recognized problem and the clinical signs in preterm neonates may differ greatly compared to older children. In particular diarrhea may not be the dominating clinical feature (Sharma et al., 2002),(Verboon-Maciolek et al., 2005). The majority of the neonates with

nosocomially acquired vsRVd (86%) was diagnosed with or had a suspected NEC. It has been reported that about 30% of NEC cases in preterm neonates are associated with RV (Sharma et al., 2004).

There was a cluster of 6 neonates with NEC and detection of RV and adenovirus over a period of 4 months reported by a single pediatric department. This observation sustains the indication, that nosocomial RV infection can have a very serious impact on a neonatal ICU and seriously adds to the common neonatal pathology (Tai et al., 2012).

4.1.4. Signs of encephalopathy

Clinical signs of encephalopathy was the most common criterion for inclusion which was reported in 58 of 84 patients (69%) of the patients with community acquired vsRVd. However, neurologic symptoms may also be unspecific and related to fever, dehydration or electrolyte disturbances. Five children had afebrile seizures without severe dehydration or electrolyte abnormalities. In particular afebrile convulsions have been described in Asian children numerous times. Whether convulsions associated with RV disease are limited to certain RV types is unknown (Komori et al., 1995),(Hung et al., 2003). None of the children reported to this study were of Asian origin.

Four of the 5 children with vsRVd and afebrile seizure were classified as healthy at the time of discharge from the hospital. One patient had residual ataxia at the time of discharge. Although convulsions in patients with RV GE were classified as very severe RV disease in this study, reported cases in the literature, often described as convulsions with gastroenteritis, mostly present with a mild GE and the prognosis is good (Verrotti et al., 2011). Better recognition and understanding of this fact may help avoid unnecessary diagnostic measures and therapy in affected children (Fasheh Youssef et al., 2011),(Durá-Travé et al., 2011). Furthermore, convulsions related to GE are not a feature unique to RV. There are reports of other pathogens such as norovirus as a primary cause of convulsion with GE (Chen et al., 2009).

4.1.5. Nosocomial infections

Our study shows that nosocomial infections play a vital role in the occurrence of vsRVd. A study reported that 17% of children hospitalized for nondiarrheal disease during a period of RV endemic developed a diarrheal illness associated with a RV infection (Ryder et al., 1977).

4.2. Method

To acquire prospective data on vsRVd on a nation wide scale, a well established surveillance system is required. The German pediatric surveillance unit (ESPED; Erhebungseinheit für seltene pediatrische Erkrankungen in Deutschland) was founded in 1992 and is based on the principles of the British surveillance unit for rare paediatric diseases which was founded in the early 1980s (Göbel et al., 2010).

Every month the ESPED office sends a mailing card to the heads of all pediatric departments requesting for the incident diagnosis of up to 12 conditions. The report card is to be returned even if there are no occurrences of either condition. If the report card is not returned, a reminder is sent to the department. Each report of an incident diagnosis to the ESPED office is anonymous and is promptly forwarded to the corresponding principal investigator. The reporting department receives a questionnaire related to the incident diagnosis. In addition, sometimes patient consent is asked for and relevant specimens such as blood samples or infectious isolates are asked for, depending on the condition under surveillance. The feedback of the report cards to ESPED is about 95 % (Göbel et al., 2010).

The two year study period was between April 2009 and March 2011. Case definition was patient 0 – 16 years of age, detection of RV in feces and a very severe course of RV disease defined by 1 or more of the following criteria: intensive care treatment, hypernatremia > 155 mmol/l, hyponatremia < 125

mmol/l, clinical signs of encephalopathy (somnolence, seizures or apnea) and death due to complications related to RV disease.

The query of cases of vsRVd was coordinated by ESPED. During the study period ESPED sent report cards to each pediatric department in Germany asking clinicians for incident diagnoses with specific inclusion criteria, of which one was vsRVd.

The data were collected using anonymous questionnaires and anonymous discharge reports. Through the report cards to ESPED 130 cases of vsRVd were reported. A feedback from the reporting department was retrieved in 120 of 130 reported cases yielding a questionnaire return rate of 92 % which approximately corresponds to the return rate of the ESPED report cards.

4.3. Limitations

4.3.1. Data acquisition

Even though ESPED is very well established and the return rate of the report cards is very high, one can not assume that all cases of the incident diagnosis are queried. This is a limitation of the presented study on vsRVd as well. Theoretically, using another independent surveillance system parallel to ESPED could have increased the statistical sensitivity to the incidence data considerably using a capture-recapture analysis (von Kries et al., 2001). However, there are no other established surveillance systems in Germany able to capture vsRVd prospectively.

4.3.2. Lack of blood and stool samples

There were no stool or blood samples collected from the patients reported. Detection of RV is usually done by enzyme immunoassay kits which can detect serotypes of RV Group A. Information about the serotype, however, is lacking. Thus it was not possible to gather specific information about the RV serotype

causing the vsRVd. It has been suggested that disease severity might vary with RV serotype (Mota-Hernandez et al., 2003).

Presence of RV antigen in serum, RV antigenemia, has often been described (Ramani et al., 2010). Recent studies also show that complete RV RNA segments can also be present in serum (Ahmed et al., 2013). Information about the RV serotype and whether RV antigen or RNA was present in the patient serum might have contributed to a better understanding of the RV virulence properties.

4.3.3. Inclusion criteria

Nosocomial infections in ICUs are a common problem and cause a heavy burden. Knowing that infectious GE such as RV GE is highly contagious, this might influence the threshold to admit a patient with GE to the ICU out of fear of causing a nosocomial outbreak of infectious GE. This in turn might lead to fewer patients with severe GE being admitted to the ICU. Thus it is possible that fewer patients with a clinical very severe RV disease were admitted to the ICU, causing an under-estimation of the incidence rate of vsRVd. On the other hand, it is probable, that the younger a patient with a severe GE is, the lower the threshold will be to admit the patient to the ICU. Forty-eight patients (57 %) with community acquired vsRVd in this study were admitted to the ICU. Their median age of 7 months was significantly lower than those not admitted to the ICU. However, the reason for their admittance to the ICU might also in part have been due to safety measures, such as the ability to better monitor these patients. This aspect might have caused an over-estimation of the incidence of vsRVd.

4.3.4. Lack of patient follow-up

Immunodeficiencies (e.g. severe combined immunodeficiency (SCID)) may predispose patients to vsRVd. With the study design only anonymized data were collected, a further follow-up of the patients was not possible. None of the

patients included in the study of vsRVd were reported to suffer from an immunodeficiency, however a detailed analysis of the immune system of the patients might have contributed to a better understanding of the pathophysiology of vsRVd. Reports to the Vaccine Adverse Events Reporting System in the United States concerning RV vaccines have shown that patients with SCID might present with diarrhea among other symptoms following vaccination against RV (Bakare et al., 2010). These findings caused the manufacturers of the RV vaccines to include SCID as a contraindication for the RV vaccine in 2010 (Centers for Disease Control and Prevention (CDC), 2010). Studies have shown that rotaviruses have the ability to establish persistent infections in mice with SCID (Riepenhoff-Talty et al., 1987). Patel et al published a case report in 2012 describing an infant with SCID with a chronic RV infection who was successfully treated by hematopoietic stem cell transplantation (Patel et al., 2012), sustaining the notion, that SCID might play a vital role in some patients with vsRVd. Although none of the patients reported were reported to suffer from SCID or other diseases of the immune system, it is possible, that a vsRVd might be the first manifestation of immunodeficiency.

4.3.5. Limitation of the questionnaire

The questionnaire on vsRVd which was sent to the reporting departments did not query for serum calcium levels of the patients as was done for sodium levels. Recent studies show that RV and in particular NSP4 seems to disrupt calcium homeostasis. A theoretical link between calcium dysregulation and seizures in patients with RV GE has been suggested (Yeom et al., 2012), so that more information about serum calcium levels in patients with vsRVd could have shed more light on the pathophysiology of vsRVd, in particular in patients with neurological complications.

4.3.6. Availability of RV vaccine during data acquisition period

The two available RV vaccines were approved in 2006, three years prior to data query for this study. The five German federal states Saxony, Thuringia,

Mecklenburg-Western Pomerania, Brandenburg and Schleswig-Holstein implemented the RV vaccine in their local vaccination recommendation prior to the data acquisition for this study. In these states, the RV vaccination coverage was almost 60 % compared to about 20 % RV vaccination coverage in the 11 remaining federal states during the study period. The incidence information acquired in this study is therefore based on a study population partially vaccinated against RV. Especially when discussing whether a general vaccination against RV should be implemented or not, exact incidence data based on a population prior to the introduction of the RV vaccine would have been more helpful. It is probable that the incidence of vsRVd would have been higher, had this study been conducted prior to the introduction of the RV vaccines.

4.4. RV prevention – currently licensed vaccines

Observations made during epidemiologic and hospital-based studies worldwide indicate the need for prevention of RV disease (Fields and Knipe, 2006). The results of this study sustain the notion, that RV disease should be prevented during the first two years of life, the period when RV disease is most serious and takes its greatest toll. Natural infection does not provide complete protection against a subsequent re-infection or mild disease (Velázquez et al., 1996).

There has been a significant drop in RV notifications in 2010 compared to 2006 in the five states with higher RV vaccination coverage (Uhlig et al., 2011). Eighteen percent of the community acquired vsRVd were reported from the five states with higher RV vaccination coverage. Fourteen percent of the children less than 5 years of age in Germany live in these states. This indicates that the frequency of reports of vsRVd from these states did not differ much comparing to the 11 federal states with less RV vaccination coverage. A lower number of reports from the five states with higher vaccination coverage would have been expected. A reason might be that the vaccination coverage is not yet high

enough to show effects of herd immunity on the incidence of vsRVd. Estimates show that a 90% RV vaccination coverage would significantly decrease disease burden in Europe (Diez-Domingo et al., 2010) and might induce herd immunity (Paulke-Korinek et al., 2011),(Buttery et al., 2011). Herd immunity is likely to play a vital role in reducing the number of nosocomially acquired RV diseases. Another reason for the number of cases of vsRVd in the federal states with higher RV immunization coverage might also be, that vsRVd might be caused by RV strains not covered by the RV vaccine.

4.5. Introduction of RV vaccine to the national vaccination recommendations in Germany

A goal of the study on vsRVd was to possibly inform decision on a national RV vaccine recommendation. In July 2013, the German Standing Committee on Vaccination (STIKO) recommended the routine use of an oral RV vaccine for infants (Oberpichler-Schwenk, 2013). The first dose should be administered between 6 and 12 weeks of age. STIKO strongly recommends beginning the vaccination series as early as possible due to the possible associated risk of intussusception which rises with increasing age at administration of the first dose. Koch et al published an extensive scientific background paper for the current STIKO recommendation (Koch et al., 2013). In this paper, current epidemiological data on RV disease and the available evidence regarding safety and efficacy of the two currently licensed RV vaccines are presented as well as the publication of the study presented (Shai et al., 2013) were summarized in the background paper.

4.5.1. Expected impact of a national vaccine recommendation against RV in Germany

Several impact studies have shown that RV vaccines reduce RV infections and hospitalizations (Zeller et al., 2010),(Field et al., 2010),(Hanquet et al., 2011), Koch et al conclude, that reduction of RV disease incidence in age groups not

eligible for vaccination is likely to occur (Koch et al., 2013). The authors conclude, that regarding the German healthcare system, RV vaccination could prevent a substantial number of RV cases and hospitalizations, however, the healthcare cost-savings would only partially compensate the costs for vaccination. They estimate, that there would be an incremental cost of up to about 48 million Euro for the vaccinations. If the vaccine prices would be reduced by 62 - 68%, vaccination could become a cost saving measure. Furthermore, the authors state, that children not eligible for RV immunization may benefit from indirect vaccination effects through herd protection.

RV has been associated with the occurrence of NEC in neonates (Sizmaz et al., 2012), so that there might even be a reduction in the incidence of NEC following a nation wide vaccination against RV. Vaccination coverage of other vaccines recommended by STIKO in Germany in 2009 varied between 87% and 95% in the 16 federal states in 2009 (Reiter and Poethko-Müller, 2009), so that it can be assumed that herd immunity against RV is an achievable goal after introduction of the RV vaccine by STIKO.

A recently published article by Vesikari et al describes the impact and effectiveness of the pentavalent RV vaccine following the introduction of RV immunization program in Finland. In Finland, RotaTeq® is used exclusively (Vesikari et al., 2013). Vaccination is scheduled at age 2, 3 and 5 moths. The authors conclude, that RV GE requiring hospitalization was virtually eliminated in vaccine-eligible children in the three years following implementation of universal RotaTeq® vaccination in Finland. However, a direct extrapolation to a German setting is not possible, as both RotaTeq® and Rotarix® are available in Germany.

4.6. RV vaccines – future considerations

4.6.1. Possible new vaccines

An Australian study described neonates in an Australian nursery who experienced a subclinical RV infection during the first 14 days of life. The follow

up of these children showed that they were protected against clinically significant RV diarrhea for up to 3 years (Bishop et al., 1983). This inspired another approach to RV immunoprophylaxis involving the use of a live, orally administered neonatal human RV strain that appears to be naturally attenuated. Clinical studies with a neonatal strain from the Australian nursery have been initiated (Barnes et al., 1997),(Barnes et al., 2002).

4.6.2. Outlook - developing countries

Clinical studies with two neonatal strains isolated in India have been initiated. The two strains are naturally occurring bovine-human reassortants. Infected neonates had no symptoms, shed virus for up to two weeks after infection, mounted a robust immune response and demonstrated protection against severe RV diarrhea after re-infection (Glass et al., 2005). With a proposed cost of only Rs. 54 or 0,65 Euro per dose, this vaccine, Rotavac®, could improve the vaccination rate drastically, especially in the developing countries where vaccination costs play a vital role.

4.6.3. Cost-effectiveness

In developed countries lacking a general RV vaccination recommendation could be influenced by the licensing of Rotavac® when considering the cost-effectiveness of a general RV vaccination program. Because most cases of RV infection do not require emergency department visits or hospital admission, a recent Canadian study concluded that a universal vaccination program against RV will be both cost saving and more effective than no vaccination when considered from a societal perspective. However, from a health care perspective, the authors conclude that a RV vaccination program would not be considered cost effective (Coyle et al., 2012). European studies on the cost effectiveness of RV vaccination have shown similar results (Tu et al., 2013). A vital parameter in the analysis was vaccine cost. Thus, if a vaccine, such as Rotavac® is licensed, the health care cost effectiveness analyses could be

influenced. Furthermore, it is to be expected, that if a new and much cheaper vaccine is licensed, the cost of RotaTeq® and Rotarix® will also drop markedly.

4.7. Emergence of other GE pathogens following RV vaccination

After introduction of the RV vaccine to the national immunization program in Finland, there has been a 57% decrease in all hospital admissions and 62% in all outpatient clinic visits for GE of any cause (Hemming et al., 2013).

Similarly to the German situation with a partial implementation of the RV vaccine is the situation in Taiwan where RV vaccines are available in the private sector. A recent study published in July 2013 showed a slow but modest impact on severe GE caused by RV. Particularly in infants there was a substantial decrease in severe RV GE (P = 0,056). A significant increase of norovirus infection was observed in the post-vaccine period (Chen et al., 2013).

The emergence and increase of other pathogens causing acute GE as a result of mass vaccination against RV is being discussed. A recent study by Payne et al shows that after the introduction of the RV vaccine, norovirus has become the leading cause of medically attended acute GE in the United States. Norovirus was detected in 21% of the patients enrolled in this study compared to a detection of RV in 12% of the patients (Payne et al., 2013). Another study over a period of 8.5 year in which the prevalence of RV decreased by 64% after the introduction of the RV vaccine, causing norovirus to become the most prevalent enteric pathogen in children (Koo et al., 2013). Similar findings have been observed in Finland where norovirus has become the leading cause of acute GE (Hemming et al., 2013).

4.8. Relevant publications after the publication of the study on vsRVd

Performing the same PubMed search in August 2013 using the search query “rotavir*[ti] AND (encephal*[ti] OR neurol*[ti] OR severe*[ti] OR complication*[ti] or nervous*[ti] OR seizure*[ti])” and limiting the results to publications newer than January 2009, revealed 54 hits. The term severe RV disease was used in 19 publications to describe patients requiring medical attention or hospital admission. However these cases were not being described as life threatening. 14 studies were case reports describing a total of 26 patients with encephalopathy.

Kang et al. have recently presented a retrospective summary of 755 patients admitted due to RV GE. 59 of these patients had seizures of which 42 were afebrile. Despite the seizures, most of these cases were classified as mild and had a favorable prognosis (Kang et al., 2013). Another retrospective study described the characteristics of 59 patients with seizures associated with acute RV GE (Lloyd et al., 2010).

There was a case report of a young child with a severe RV GE and a duodenal ulcer (Nejihashi et al., 2011). There were two prospective studies: one was a study on the association between RV GE and intussusceptions in Ghana (Enweronu-Laryea et al., 2012), in which no clear association could be determined. The other publication described a prospective query of bacteremia of normal intestinal flora in patients with RV GE (Ciftçi et al., 2009). The authors described 4 patients who had positive blood cultures (Klebsiella pneumoniae in 1 patient, Escherichia coli in 1 patient, Pseudomonas aeruginosa and Candida albicans in 1 patient, and Candida albicans in 1 patient) . The remaining studies were related to veterinary reports or molecular aspects of the RV.

5. Conclusion

Over a period of 2 years between April 2009 and March 2011, 101 verified cases of very severe rotavirus disease in Germany were reported. The majority of reports (96%) concerned children less than 5 years of age. Seventeen percent of the reported cases were nosocomially acquired. None of the reported children had received an RV vaccine prior to the acute RV illness.

The incidence of community acquired vsRVd in children less than 5 years of age in Germany is 1.2/100,000 per year. This is likely an underestimate as it is not to be expected, that all occurrences of vsRVd were reported to the German Paediatric Surveillance Unit for rare diseases. Comparing the incidence of vsRVd to all reported cases of RV disease, about 1 of 1000 children with RV disease has a very severe course of disease.

Fourteen reports of vsRVd were of neonates in intensive care treatment, showing that nosocomially acquired vsRVd causes a heavy burden on neonatal intensive care units. The clinical signs of RV disease in neonates differ greatly compared to older children. In particular, diarrhea may not be the leading symptom. Most of the reported neonates with vsRVd were diagnosed with or had a suspected necrotizing enterocolitis, sustaining the notion that there might be an association between RV disease and necrotizing enterocolitis in neonates.

The majority of patients with community-acquired vsRVd (69%) had clinical signs of encephalopathy. In particular seizures seem to be a clinical feature associated with RV disease as has been shown in numerous publications. However, the direct role of RV still remains unclear. The outcome of encephalopathy associated with RV disease is generally good.

In Germany, a country with guaranteed medical care for all and a high standard of pediatric care, yearly about 50 cases of vsRVd were observed between 2009

and 2011. Although rare, vsRVd constitutes a considerable burden to the families of the affected children and to the healthcare system.

In July 2013, the German Standing Committee on Vaccination introduced the RV vaccine to the national immunization program in Germany. It is estimated that there will be a significant reduction of the incidence of RV GE. Children with Rotavirus GE needing hospital admission and vsRVd might be virtually eliminated. Through herd immunity, it is estimated that if a vaccination coverage of about 90% is achieved, there will also be a remarkable reduction in nosocomially acquired RV GE, a great benefit in particular for neonates in intensive care treatment who are prone to nosocomially acquired vsRVd as was shown in our study. Furthermore, by protecting preterm neonates from RV infections, there might be a reduction in the number of neonates suffering from necrotizing enterocolitis.

By a mass vaccination against RV, other pathogens, notably norovirus might emerge to become the leading causative agent for GE in Germany.

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7. Annex

7.1. Questionnaire on vsRVd

Following is the questionnaire which was sent to the reporting clinics to acquire information about each reported case of vsRVd.

Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland

Forschungsstelle für Pädiatrische Epidemiologie bei der Deutschen Gesellschaft für Kinder- und Jugendmedizin e.V.

KKS düsseldorf

Besonders schwere Verläufe bei Rotavirus-Erkrankungen

HELIOS Klinikum Krefeld
Zentrum für Kinder- und Jugendmedizin
ESPED, z. Hd. Hr. Shai
Lutherplatz 40
47805 Krefeld

⇦ *Rücksendung an nebenstehende Adresse erbeten!*

IDNO: «Idno»
LNR: «Lnr»
Meldemonat: «Lnr»
ESPED-Eingang:

Ansprechpartner für Rückfragen:

Name: .....

Tel.: .....

Fax: .....

E-Mail.....

Berichtende Kinderklinik

«Klinik»
«Abt»
«Str»
«Plz» «Ort»

1. Patientendaten

Geburtsdatum *(mm.jj)*: I____I____I

Geschlecht: O weiblich
O männlich

Ehemaliges Frühgeborenes *(<37. SSW)*: O ja, ... SSW
O nein
O nicht bekannt

Ethnische Herkunft: .....

Eltern konsanguin: O ja
O nein
O nicht bekannt

*Falls **ja***, Art der Konsanguinität: .....

2. Anamnese

Stationäre Aufnahme *(mm.jj)*: I____I____I

Dauer des stationären Aufenthalts: ..... Tage

Erkrankungsdauer vor stationärer Aufnahme: ..... Stunden
(Erkrankungsbeginn: Temperatur > 38,5°C und/oder erster auffälliger Stuhl und/oder erstmaliges Erbrechen)

RV-Impfung: O ja
O nein
O nicht bekannt

*Falls **ja***, Welcher Impfstoff: O Rotarix™
O RotaTeq®

3. Rotavirus-Nachweis

RV-Nachweis im Stuhl positiv *(mm.jj)*? I____I____I

4. Befunde / Laborparameter (bitte auf Einheiten achten bzw. angeben)

Exsikkose: Gewichtsabnahme: O <10 %
O >10 %
O nicht bekannt

Maximales CRP: ..... mg/l

Welcher Behandlungstag (*Aufnahmetag=1*): .....

Leukozyten (*maximal oder minimal*): .....

Welcher Behandlungstag (*Aufnahmetag=1*): .....

Bitte wenden!

Na^+ > 155 mmol/l: O ja O nein *Falls **ja***, welcher Behandlungstag (*Aufnahmetag=1*): .....
Wert bei erneuter Bestimmung bestätigt: O ja O nein

Na^+ < 125 mmol/l: O ja O nein *Falls **ja***, welcher Behandlungstag (*Aufnahmetag=1*): .....
Wert bei erneuter Bestimmung bestätigt: O ja O nein

Blutgasanalyse (*bitte jeweils die auffälligsten Werte angeben*): pH ..... BE ..... pCO2 .....
Stand. Bikarbonat .....

Maximale Werte: GPT (*ALAT*): ..... GOT (*ASAT*): .....

5. Verlauf

Behandlung auf einer Intensivstation: O ja *Falls **ja***, Grund zur Aufnahme: .....
O nein .....
.....

Therapie (*Art der Therapie, Beatmung, Zusammensetzung i.v. Lösungen, etc*): .....
.....
.....

Symptome und Zeichen einer Encephalopathie:

Somnolenz:	O ja	O nein	Apathie:	O ja	O nein
Krämpfe:	O ja	O nein	mit Fieber assoziiert?:	O ja	O nein
Apnoen:	O ja	O nein			
Auffälliger EEG-Befund:	O ja	O nein	mit Encephalopathie/Encephalitis vereinbar?	O ja	O nein
Lumbalpunktion:	O ja	O nein			
RV Nachweis im Liquor:	O ja	O nein	O nicht untersucht		

Liquorstatus: .....
.....

6. Status bei Entlassung

Patient: O geheilt
O Residualsymptome Bitte spezifizieren: .....
O verstorben .....
.....

Verlauf / Kommentare: .....
.....
.....
.....
.....

Bei Rückfragen zum Fragebogen stehen wir Ihnen als Studienleiter selbstverständlich jederzeit zur Verfügung. Telefonnummer Hr. Prof. Niehues/Hr. Shai: 02151-32-2301

Bitte schicken Sie zusätzlich zu diesem Fragebogen einen anonymisierten Arztbrief des Kindes mit. (Aus datenschutzrechtlichen Gründen bitte **unbedingt** Name, Adresse und weitere **patientenbezogene Daten unkenntlich machen**.)

VIELEN DANK FÜR IHRE MITARBEIT!

Eidesstattliche Versicherung

Ich versichere an Eides statt, dass die Dissertation selbstständig und ohne unzulässige fremde Hilfe erstellt worden ist und die hier vorgelegte Dissertation nicht von einer anderen Medizinischen Fakultät abgelehnt worden ist.

19.03.2014, Sonu Shai