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Generic protocols for

(i) hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children and (ii) a community-based survey on utilization of health care services for gastroenteritis in children

Field test version

Vaccines and Biologicals

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Contents

Preface

The WHO Department of Vaccines and Biologicals (V&B) has been involved in research on rotavirus diarrhoea for many years. A number of candidate rotavirus vaccines are being field-tested, partly in studies sponsored by WHO. It is expected that one or more of these vaccines will become available within the next few years. This document has been developed so that countries can obtain data on the local disease burden attributable to rotavirus in young children. Such information will be needed in the future when countries consider the introduction of rotavirus vaccine.

This document was cosponsored by the V&B Steering Committee on Diarrhoeal Diseases and the V&B Steering Committee on Epidemiology and Field Research. Part I of the document contains a generic protocol for hospital-based surveillance of paediatric rotavirus gastroenteritis. This was developed by scientists from various countries, under the leadership of the WHO Collaborating Centre for Rotavirus and the Agents of Viral Gastroenteritis located at the United States Centers for Disease Control and Prevention. Part II of the document contains a generic protocol for a survey on utilization of health care services for paediatric gastroenteritis. This survey will allow investigators to ascertain whether particular hospitals are suitable for the hospital-based surveillance study and will also provide data to adjust hospital-based findings to better reflect the disease burden of paediatric rotavirus gastroenteritis in the entire population.

Each of the two generic protocols provides guidance on the main procedures as well as suggested forms for data collection. However, each protocol has to be adapted to the local situation. Thus, details of fieldwork and operational procedures should be added by local investigators with experience in conducting studies of diarrhoeal diseases.

WHO provides these generic protocols free of charge. In return, V&B would appreciate being informed about studies in which they are used. In addition, this document should be referenced in any publication resulting from its use.

Comments or suggestions for improving these generic protocols are welcome and should be sent to the Department of Vaccines and Biologicals, World Health Organization, CH-1211 Geneva 27, Switzerland.

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Part I:

Generic protocol for hospital-based surveillance to estimate the burden of rotavirus gastroenteritis in children under 5 years of age

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1. Introduction

Rotaviruses are the most common cause of severe gastroenteritis and dehydration in young children in both industrialized and developing countries. A WHO-sponsored review of rotavirus studies found that 20–70% of all hospitalizations and 20% of deaths from diarrhoea were attributable to rotavirus (*de Zoysa and Feachem 1985*). Recent studies have estimated that 500 000 to 600 000 children die each year because of rotavirus gastroenteritis (*Miller and McCann 2000, Molbak et al. 2001*). In response to this disease burden, several vaccines against rotavirus have been or are being developed (*Bresee et al. 1999*). In many countries, however, the disease burden and epidemiology of rotavirus are unknown because of a lack of adequate data or because no studies have been conducted recently. The lack of data is particularly notable in developing countries. The anticipated availability of an effective vaccine highlights the need for new data on the rotavirus disease burden in developing countries, where rotavirus-associated morbidity and mortality are high.

The purpose of this generic protocol is to present in detail a method for hospital-based surveillance of rotavirus gastroenteritis in children under 5 years of age. The data collected in such studies should allow investigators to summarize local epidemiological and virological features of rotavirus and to develop estimates of disease burden in the populations under surveillance. The protocol is designed as a guide for ministries of health, local health officials and researchers to facilitate such studies. The data generated by them should be useful for policy-makers seeking to determine the need for rotavirus vaccination in their countries.

1.1 Clinical characteristics of rotavirus diarrhoea

Rotavirus infections cause acute gastroenteritis, characterized by the acute onset of watery diarrhoea, fever and vomiting *(Linhares et al. 1983, Mata et al. 1983a, Rodriguez et al. 1977, Zaki et al. 1986)*. Diarrhoea usually persists for 3–8 days and is self-limited *(Bass and Greenberg 1995, Black et al. 1989, Mata et al. 1983b, Wyatt et al. 1979)*. However, diarrhoea has been documented to last as long as 22 days *(Wyatt et al. 1979)*, and in infants its duration may be longer than in older children *(Black et al. 1982a)*. Fever and vomiting are most prominent during the first few days of the illness. Rotavirus infections are more often severe than other common causes of diarrhoea, and are more likely to be associated with dehydration *(Black et al. 1982b, Rodriguez et al. 1977 and 1985, Zaki et al. 1986)* and hospitalization *(Brandt et al. 1983)*. Consequently, the proportion of children hospitalized for diarrhoea caused by rotavirus is generally greater than the proportion seen in the community or in outpatient clinics *(Bhan et al. 1988, Black et al. 1982a, Cunliffe et al. 2001)*.

Many rotavirus infections are asymptomatic, particularly among infants aged under 3 months, older children, and adults *(Abiodun et al. 1985, Araya et al. 1986, Black et al. 1989, Champsaur et al. 1984, Cravioto et al. 1990, Georges-Courbot et al. 1988, Linhares et al. 1989, Losonsky and Reymann 1990, Mata et al. 1983b, Schorling et al. 1990, Simhon et al. 1985)*. The high proportion of asymptomatic infections in neonates and young infants has not been clearly explained. The presence of maternal antibody and the physiological immaturity of the neonatal gut may each play a role *(Hoshino et al. 1985)*. Immunity from repeated exposures to rotavirus probably accounts for the high infection-to-illness ratio among older children and adults. Rotavirus infections can lead to severe disease *(Kapikian and Chanock1990, Wood et al. 1988, Yolken et al. 1982)* and prolonged shedding of virus *(Eiden et al. 1985, Hundley et al. 1987, Oishi et al. 1991, Pedley et al. 1984)* in immunocompromised persons. However, a recent study has indicated that children in Malawi with HIV infection do not develop more serious disease or shed virus for longer than children who are HIV-negative *(Cunliffe et al. 2001)*.

1.2 Burden of rotavirus disease

In a 1985 review, rotaviruses were estimated to account for 20–70% of hospitalized cases of diarrhoea and 20% of all diarrhoeal deaths in children under 5 years of age worldwide *(de Zoysa and Feachem 1985)*. In 1986 it was estimated that 130 million children developed diarrhoea caused by rotavirus each year, 18 million of whom experienced moderate or severe dehydration, resulting in 873 000 deaths *(United States Institute of Medicine 1986)*. Recent conservative estimates have indicated that between 500 000 and 600 000 children die each year from rotavirus disease *(Miller and McCann 2000, Molbak et al. 2001)* and that up to 85% of these deaths occur in low-income countries as defined by the World Bank *(Miller and McCann 2000)*. A review of epidemiological studies conducted in developing countries, found that rotavirus accounted for some 6% of all diarrhoeal episodes, a median of 28% of outpatient or clinic visits for diarrhoea and 34% of hospitalizations for diarrhoea in young children *(de Zoysa and Feachem 1985)*. More recent reviews and studies from developing countries have similar findings *(Cook et al. 1990, Cunliffe et al. 1998 and 2001, Van Man et al. 2001)*.

Hospitalization rates are quite variable, both between developed and developing countries and between countries with similar income levels (Table 1). Limited data from developing countries indicate that rates of severe disease, i.e. hospitalizations, are likely to be higher than in developed countries.

Table 1. Annual incidence of hospitalizations attributable to rotavirus gastroenteritis in children under 5 years of age in 12 countries

Country	Years	Reference	Annual incidence per 100 000 children under 5 years of age	Risk of hospitalization per child by age 5 years
Spain	1989–1995	Visser et al., 1999	250	1:80
Netherlands	1998	de Wit et al. 2000	270	1:74
USA	1993–1995	Parashar et al. 1997	274*	-
Poland	1996	Mrukowicz et al. 1999	310	1:65
Sweden	1993–1996	Johansen et al. 1999	370**	-
United Kingdom	1993–1994	Ryan et al. 1996	520	1:39
Finland	1985–1995	Vesikari et al. 1999	610	1:33
Argentina	1991	Gomez et al. 1998	645	1:31
Australia	1993–1996	Carlin et al. 1998	750	1:27
Hungary	1993–1996	Szucs et al. 1999	840**	-
Australia	1991–1993	Ferson 1996	870	1:23
Ireland	1997–1998	Lynch et al. 2001	1080	1:19
Venezuela	1990	Perez-Schael et al. 1997	3000***	-

* Incidence derived from data for diarrhoea of all causes.

** Incidence for children under 4 years of age.

*** Incidence for children under 2 years of age.

1.3 Rotavirus epidemiology

Almost all children are infected with rotavirus in early childhood. In prospective community cohort studies conducted in developing countries the incidence of rotavirus diarrhoea among young children has varied from 0.07 to 0.8 episodes per child per year and almost all children experienced at least one episode of rotavirus diarrhoea by the age of 24 months *(Black et al. 1982a, Grinstein et al. 1989, Kantharidis et al. 1987, Linhares et al. 1989, Oishi 1985, Reves et al. 1989, Simhon et al. 1985, Zaki et al. 1986)*. In developing countries, 65–80% of children have antibodies to rotavirus by 12 months and 95% have been infected by 24 months. In general, children infected with rotaviruses during the first 3 months of life are asymptomatic, while those infected for the first time after the age of 3 months are usually symptomatic. Because natural infection confers some immunity to disease on subsequent exposures and because this protection increases with each subsequent exposure, the highest rates of rotavirus disease occur between 3 months and 2 years of age *(Velazquez et al. 1996)*. The incidence of symptomatic disease decreases rapidly after 24 months of age in most settings.

In developed countries, 80% of children develop rotavirus diarrhoea in their first 3 years of life *(Gurwith et al. 1981, Rodriguez et al. 1987)* and the highest rates of illness occur during the second year. In many developing countries, however, the highest rates of illness occur among children aged 6–11 months. In some developing countries it is common for neonates to acquire nosocomial rotavirus infections, which usually occur without symptoms *(Kilgore et al. 1996, Omoignerale and Abiodun 1995, Perez-Schael et al. 1984, Sukumaran et al. 1992)*. In India, rotavirus infections were documented among 40–50% of neonates hospitalized for three days or more *(Cicirello et al. 1994)*. Since children with neonatal infections may be protected from subsequent severe illness, high rates of neonatal infection may affect the outcomes of vaccine efficacy studies in some developing countries.

In temperate climates, rotavirus diarrhoea is predominantly a winter disease and few or no cases occur other than at the seasonal peak *(Ho et al. 1988, LeBaron et al. 1990, Ryan et al. 1996)*. In some tropical countries there are seasonal peaks *(Molbak et al. 2001, Sitbon et al. 1985)* but many tropical countries experience rotavirus diarrhoea throughout the year and have no seasonal peaks or only small ones *(Cook et al. 1990, Cunliffe et al. 1998)*.

Table 2. Key features of rotavirus epidemiology

- Almost all children are infected with rotavirus in early childhood.
- First infections occurring after 3 months of age are commonly associated with diarrhoea.
- Repeat infections are asymptomatic or associated with mild diarrhoea, indicating that immunity is acquired and protective against repeat severe disease.
- The incidence of rotavirus diarrhoea is similar among children in developed and developing countries. Attempts to control infection by improving water or food are therefore unlikely to alter the incidence of infection (Bresee et al. 1999).
- Humans appear to be the main reservoir of rotavirus infections. The exact modes of transmission are unknown but are presumed to involve droplet or direct contact spread via the faecal-oral route.

1.4 Virological characteristics

Rotaviruses are 100-nm viruses with a characteristic wheel-shaped structure (rota) and belong to the family Reoviridae. The virus has three shells: an outer capsid, an inner capsid and a core. They surround 11 segments of double-stranded RNA, which encode for six structural proteins (VP1–VP4, VP6, VP7) and five non-structural proteins (NSP1–NSP5). Two structural proteins, VP7 (the glycoprotein or G protein) and VP4 (the protease-cleaved protein or P protein), make up the outer shell and are considered important for vaccine development since they define the serotype of the virus and are the major antigens involved in virus neutralization *(Prasad et al. 1990, Yeager et al. 1990)*. The protein making up the inner capsid, VP6, is the most abundant protein in the virus and the target of most simple antigen detection assays. Of the non-structural proteins, NSP4 is a putative virulence factor, although other proteins are also involved *(Ball et al. 1996)*.

1.5 Rotavirus strain prevalence

The classification of rotaviruses is based on differences in the VP7 (G) and VP4 (P) capsid proteins. G serotypes 1–4, and P genotypes P[8] and P[4] predominate worldwide *(Gentsch et al. 1996, Gunasena et al. 1993, Mphalele and Steele 1995, Steele et al. 1993, Wu et al. 1994)*. A review of G and P types from over 2700 specimens found that the P[8] genotype was almost always associated with either G1, G3 or G4, and that P[4] was almost always associated with G2 *(Gentsch et al. 1996)*. In this study, 96% of strains that could be typed and were from faecal specimens with a single strain contained one of these four common combinations. Among them, P[8]G1 was predominant, accounting for 53% of all strains, followed by P[8]G3 (14%), P[4]G2 (11%) and P[8]G4 (5%). Various less common types were detected. Although they were individually uncommon, each accounting for under 1% of strains, they constituted 3% overall. Additionally, two uncommon P types, P[6] and P[9], which were found with common G types, and a natural reassortant, P[4]G1, were each reported from six countries, indicating a wide geographical distribution. Subsequently, however, additional seroytypes have been reported, including serotypes G5, G8, G9 and G10. Each has been reported in a number of countries, often bearing a variety of P proteins and sometimes as the dominant strain within a country. This suggests that these rare serotypes are potentially much more common than previously believed *(Bon et al. 2000, Cunliffe et al. 1999, Gouvea et al. 1994 and 1999, Griffin et al. 2000, Holmes et al. 1999, Leite et al. 1996, Palombo et al. 2000, Santos et al. 1998, Unicomb et al. 1999)*. Especially intriguing have been recent reports of serotype G9 strains in Bangladesh, the United Kingdom, the USA, and several other countries. These strains have been found at high prevalences (0.4–13%) since 1995, are widespread in individual countries and exist in at least five genotype combinations. It is thus possible that G9 strains represent a fifth of the globally important serotypes that either have recently emerged or were underdiagnosed in the past because of inadequate surveillance.

Different patterns of strain distribution occur in some developing countries. In Brazil, one-third of single infections involved serotypes that were uncommon elsewhere, including P[6]G1, P[6]G3, P[6]G4 and P[3]G1 *(Timenetsky et al. 1994)*. In addition, P[8]G5 accounted for 13% of single infections in some regions of Brazil, making it the second commonest strain detected. In Bangladesh, 10% of strains were either natural reassortants (P[4]G1 or P[4]G4) or uncommon strains (P[6]G1) *(Bern et al. 1992)*. In India, P[6] strains with common G types accounted for 43% of typeable strains detected from stools of children with diarrhoea, while the four common types represented only 33% of the total *(Ramachandran et al. 1996)*; strains that had previously been recognized mostly or only in asymptomatic neonates (P[6]G9, P[11]G9 and P[11]G10) were recovered from infants with diarrhoea. P[6] strains were identified in 8% of specimens from hospitalized children in South Africa *(Mphalele and Steele 1995)* and in 38% of children surveyed in Guinea-Bissau *(Fischer et al. 2000)*.

Because of the regional variability in strain prevalence and the finding that some serotypes thought to be rare may be far more important globally than previously believed, all countries interested in conducting surveillance for rotavirus should include a component of strain surveillance.

1.6 Rotavirus vaccines

No rotavirus vaccines are currently included in national immunization systems. Two vaccines, however, are currently licensed. In the USA, a tetravalent rhesus-human reassortant vaccine (RRV-TV)(Rotashield®, Wyeth Laboratories, Inc., USA) was licensed in August 1998 and recommended for routine immunization of infants in the USA at 2, 4, and 6 months of age (*Centers for Disease Control and Prevention 1999b*). In 1999, however, Rotashield® was found to be associated with intussusception among vaccinees, and as a result has been withdrawn from the USA market and vaccine schedule (*Centers for Disease Control and Prevention 1999a and 1999b*) (see section 1.6.1 below). Prior to licensure, seven large efficacy trials were conducted with RRV-TV vaccine (*Bernstein et al. 1995, Joensuu et al. 1997, Lanata et al. 1996, Linhares et al. 1996, Perez-Schael et al. 1997, Rennels et al. 1996, Santosham et al. 1997*). The resulting efficacy estimates were approximately 50–60% against all cases of rotavirus diarrhoea and 70–90% against severe rotavirus disease, e.g. involving dehydrating diarrhoea and hospitalizations. In addition, a lamb strain vaccine, LLR, (Lanzhou Institute for Biological Products, China) is currently licensed for use in China but has not been included in the routine system of childhood immunizations (*Vaccines and Biologicals 2000*).

Other candidate rotavirus vaccines are in late stages of development and may be commercially available within a few years. A polyvalent human-bovine rotavirus reassortant vaccine is being developed. In the USA, an earlier, quadrivalent version of this vaccine conferred approximately 70% protection against rotavirus gastroenteritis in a large field trial (*Clark et al. 1995*), and a candidate monovalent live human G1 strain vaccine, 89–12, had an efficacy of 89% against severe rotavirus gastroenteritis in a single trial (*Bernstein et al. 1999*). Additional field trials are planned with both of these vaccines. Various other vaccines and approaches to creating rotavirus vaccines are being tested, including the use of other live human strains and neonatal strains as vaccine candidates, animal strains, inactivated parenteral vaccines, and subunit vaccines and others (*Bresee et al. 1999*).

All the leading candidate vaccines are live, orally administered vaccines designed to be given in a multidose schedule early in infancy along with other routinely administered immunizations. Like natural rotavirus infection, vaccines are expected to confer partial immunity after a single dose and greater protection with subsequent doses. Because immunization against rotavirus is expected to be most effective against severe disease, it may be desirable for surveillance to focus on severe outcomes such as hospitalizations.

1.6.1 RRV-TV vaccine and intussusception

The receipt of the licensed formulation of RRV-TV vaccine (Rotashield®, Wyeth Laboratories, USA) has been associated with an increased risk of developing intussusception (*Centers for Disease Control and Prevention 1999a, Kramarz et al. 2001, Murphy et al. 2001*). The risk appears to be limited to the two weeks following the first two doses of vaccine, and is highest three to seven days after the first dose (*Kramarz et al. 2001, Murphy et al. 2001*). The overall risk appears to be small. Estimates based on studies led by the United States Centers for Disease Control and Prevention indicate an attributable risk of approximately one case for every 4600 to 11 000 vaccinees (*Kramarz et al. 2001, Murphy et al. 2001*).

However, subsequent ecological studies produced considerably lower risk estimates (one case for every 66 000 to 302 000 vaccinees) (*Chang et al. 2001, Simonsen et al. 2001*). At a Workshop on Intussusception, Rotavirus, and Oral Vaccines held 5-7 September 2001 in Arlington, Virginia, USA, a group of rotavirus experts reached a consensus figure for the attributable risk of intussusception as 1 case per 10 000 rotavirus vaccine recipients (*G. Peter, personal communication, 2002*). The biological mechanism of the association remains unclear. Nevertheless, these risks led to the withdrawal of the vaccine from the schedule of immunizations in the USA. It is not known whether intussusception would be associated with other rotavirus vaccines or with this vaccine used in other settings, e.g. developing countries, or in neonates. Even if the attributable risk of intussusception following vaccination is as high in developing countries as in the USA the benefits of vaccination against rotavirus may far exceed the risks. However, all future vaccine trials should include surveillance for this possible adverse reaction, as recommended by WHO (*Vaccines and Biologicals 2000*). Because of the importance of this finding, rotavirus surveillance systems designed to assess rates of hospitalization may wish to incorporate surveillance for intussusception. This would provide baseline data on rates of intussusception that will be useful for assessing the safety of a vaccine following its introduction.

2. Objectives

The main reason for developing a system of rotavirus surveillance is to collect data that will facilitate and support the introduction of rotavirus vaccination, once a vaccine becomes available. The general objectives of surveillance described in this generic protocol are therefore to determine the disease burden and the epidemiology of rotavirus in a country or other defined geographical area. In the long term, after rotavirus vaccines are available, the surveillance system can be used to monitor their impact.

The specific objectives are as follows:

1. To estimate the incidence of hospitalizations associated with rotavirus in a defined population of children under 5 years of age.
2. To determine the age and seasonal distribution of hospitalizations associated with rotavirus in the population of children under 5 years of age under surveillance.
3. To estimate the proportion of diarrhoea hospitalizations in children under 5 years of age which are attributable to rotavirus.
4. To identify the prevalent strains of rotavirus in the population under surveillance.
5. To monitor temporal trends in the incidence of hospitalization, age distribution, seasonality and strains.

3. Selection of surveillance population

3.1 Rationale for focusing on hospitalizations

This generic protocol describes hospital-based surveillance for rotavirus-associated gastroenteritis among children under 5 years of age. This type of surveillance requires an accurate estimation of the demographic characteristics of the population under surveillance, the ability to conduct surveillance for acute gastroenteritis in hospitals that serve the population, and the capacity to obtain stool specimens for confirmation of the presence of rotavirus and to document serotype prevalences. The rationale for focusing on hospitalizations is as follows:

- *Hospitalizations allow for surveillance of severe rotavirus disease that would be targeted for prevention by vaccines.* Hospital-based data will therefore be important in evaluating the need for a vaccine. Once a rotavirus vaccine becomes available, such surveillance would allow reliable and rapid assessment of the success or failure of a vaccine programme.
- *Hospitalizations are easy to detect.* Where most children with severe rotavirus diarrhoea are likely to present to a hospital for treatment, case-finding will be easier than in other settings, e.g. in the community, and require fewer resources. To learn where children receive care for paediatric gastroenteritis, a survey needs to be conducted as described in part II of this document.
- *Hospitalizations represent a significant cost in health resources.* The demonstration of the burden of rotavirus diarrhoea on health resource utilization may be helpful in assessing whether rotavirus vaccine should be included in the national immunization schedule.
- *Hospitals are likely to have a laboratory capability,* making it comparatively easy to collect specimens from children and test them for rotavirus.
- *The incorporation of surveillance for intussusception is feasible in a hospital-based study.* It is important to determine the baseline rate of intussusception in populations where rotavirus vaccine will be introduced. A system for monitoring hospitalizations attributable to acute gastroenteritis can easily be modified to cover intussusception as well, since the disease occurs in the same age group and is likely to result in hospitalization. Guidelines on conducting surveillance for intussusception are being developed by WHO.

3.2 Selection of surveillance population and participating hospitals

Several factors may affect the choice of a population for surveillance. It should be demographically and geographically well defined, and data should be available on, e.g. age distribution and numbers of births and deaths. The numbers of children in specific age groups (e.g. 0–2, 3–5, 6–8, 9–11, 12–17, 18–23, 24–35, 36–47 and 48–59 months) should be known so that age-specific incidence rates of hospitalization can be calculated. Surveillance for hospitalizations attributable to rotavirus requires the inclusion of children under 5 years old, as the disease burden in both developed and developing countries affects this age group almost exclusively.

Surveillance is best initiated in a population that has been stable and is expected to be stable during the period of surveillance. The characteristics of such a population might include little expected change in age-specific population size, access to care, and patterns of immigration and emigration. Populations in which many children die from diarrhoea before presenting to hospital are likely to yield underestimates of morbidity attributable to rotavirus diarrhoea and, therefore, are unsuitable for the purposes of surveillance. The investigators should obtain or prepare a map of the study area showing the exact geographical boundaries.

A population that uses a single hospital or a small number of hospitals and has good access to it or them would be ideal. A study is easiest to conduct if a single large hospital serves the population and if the services it provides are free. Importantly, all government and private hospitals serving the defined population should be included and they should admit children under 5 years of age. Although the protocol does not call for surveillance in outpatient clinics or other health care settings apart from hospitals, it is advisable to be aware of the sources of health care in the population under surveillance and the numbers of patients they care for. Providers of care to outpatients may include government services, diarrhoea treatment centres, private physicians, pharmacists and traditional healers. Access to a hospital may be determined by measuring the distance to the hospital from different areas under surveillance, taking into account any areas with poor roads or none, a lack of public transport, and natural barriers such as rivers.

Each hospital in which surveillance for rotavirus cases is conducted must have either the laboratory capacity to perform rotavirus detection by the use of rapid antigen detection methods, or a reliable system for transporting specimens to a reference laboratory. This capability should include the resources necessary for collecting stools appropriately and in a timely fashion, the storage of specimens in a refrigerator or freezer until testing is performed, personnel trained in testing methods, and adequate record-keeping practices in order to allow the coordination of laboratory and clinical data.

3.3 Size of surveillance population

The population served by the participating hospitals should be reasonably large so that a sufficient number of hospitalizations associated with rotavirus can be expected during the period of surveillance. Because the hospitalization rate for rotavirus diarrhoea among children in developing countries is expected to be several times greater than that among those in developed countries, the population required for

the observation of a sufficient number of rotavirus-associated hospitalizations during a two-year surveillance period would be greater in developed countries than in developing countries.

Each site could select hospitals expected to treat at least 250–500 children for gastroenteritis each year. On the basis of a conservative estimate of 30% of severe gastroenteritis cases being attributable to rotavirus, this should provide 75–150 cases of rotavirus annually. Assuming a conservative estimate of an annual incidence of rotavirus-associated hospitalization in developing countries of 12 per 1000 children under 5 years of age (on the basis of the Venezuelan annual rate of 30/1000 children aged under 2 years shown in Table 1, and assuming that no additional cases occur among children aged 24–59 months), a population of 6250 to 12 500 children under 5 years of age would probably be sufficient to allow precise estimates of disease burden to be obtained. However, the actual size of a surveillance population that would yield accurate estimates is difficult to determine, given the paucity of data on hospitalizations in developing countries and the unknown sensitivity of each system in detecting and testing cases of gastroenteritis. The investigators may therefore wish to conduct surveillance in a larger population in order to allow for some error and random variation in expected rates of hospitalization attributable to rotavirus.

3.4 Health care services utilization

Since this generic protocol is based on monitoring outcomes in hospitals, members of the population should have good access to medical care within the surveillance site. Moreover, residents in the surveillance area should not seek care to a great extent in hospitals outside this area. An effort should be made to include all appropriate hospitals that are in the surveillance area.

To ensure that the hospitals selected for the surveillance study capture enough patients with rotavirus diarrhoea, the investigators may wish to conduct a census of all hospitals, clinics and other places where children with acute diarrhoea can be treated. The locations of the institutions can be mapped and the number of children aged under 5 years who were treated for diarrhoea during the previous year can be determined. This information will provide preliminary information on whether the proposed hospitals appear to be covering the population. Community-based information will be needed, however, on health care utilization patterns of the proposed surveillance population for paediatric gastroenteritis.

Each site wishing to conduct rotavirus surveillance in accordance with the methods outlined in this protocol should conduct a survey of health care services utilization for paediatric gastroenteritis at the beginning of the study. Part II of this document provides the methodology for a 30-cluster survey involving 600 children, and this survey will provide data on where parents seek care when their children have severe diarrhoeal disease. Knowledge of the settings in which care for gastroenteritis is received, and of the attitudes and practices of the population concerning gastroenteritis and its treatment, are vital for designing the best possible surveillance to understand disease burden and for the comparison of data between studies in different settings. Ideally 100% of patients with severe diarrhoea would receive care at a hospital participating in the study. If a survey indicates that the figure is below 70%,

consideration should be given to the enrolment of additional hospitals in the area or to the use of another population. If, however, there is an indication that the site is acceptable for surveillance, the survey may provide information permitting the investigators to adjust their final data to account for the proportion of children with gastroenteritis who are not likely to be hospitalized. The methods for conducting such a survey are explained in Part II of the present document.

3.5 Review of data from previous studies

Several considerations are important for determining the best methods of surveillance for a country or region, such as the financial and personnel resources that are available, the structure and referral patterns of the local health care system, the local scientific expertise and interest, the presence of surveillance systems for other vaccine-preventable diseases, and a history of previous epidemiological and laboratory research on rotaviruses. A reasonable first step is to review and analyse local rotavirus data collected in previous studies. General estimates of the rotavirus disease burden can be developed from retrospective data such as national death surveys and hospital and local laboratory records. Data may be obtained from regional medical literature, local medical society publications and hospital newsletters, as well as from the peer-reviewed literature. Some valuable information may not have been published, including data on diarrhoeal disease obtainable from health ministries or local hospitals and rotavirus testing results obtainable from local hospital laboratories. A review of all available sources of data helps to define the local epidemiology of rotavirus, including the likely age of disease and the primary setting of the disease burden, e.g. hospitals and rehydration clinics.

When selecting studies for review it is important to consider their duration and the methods of testing for rotavirus. Because rotavirus diarrhoea is a seasonal disease, studies lasting at least a year are desirable so that the detection rates are not affected by seasonal fluctuations in incidence. Annual differences in rotavirus activity are possible and can be assessed by examining trends in study outcomes (e.g. hospitalizations) for rotavirus over several years.

A line-list should be created with information on the location and time of studies, the age range and number of the subjects studied, the tests used to detect rotavirus and the number and percentage of children positive for rotavirus in each study (see Annex 1 for examples of tables for the analysis). A summary estimate of the median and range of the rotavirus detection rate should be provided. Age-specific detection rates of rotavirus and the cumulative frequency of rotavirus-associated outcomes (e.g. hospitalizations) by age should be examined in order to determine the age groups of children with the greatest burden of disease. Seasonality should be assessed by examining the monthly detection rates of rotavirus.

4. Surveillance for rotavirus-associated hospitalizations in children under 5 years of age

4.1 Overview

This generic protocol describes a two-year prospective surveillance for hospitalizations associated with rotavirus among children under 5 years of age. A stool sample from every eligible child with acute gastroenteritis who is admitted to a participating hospital is tested for the presence of rotavirus. A minimal set of data is collected during the hospital stay, including details of the patient's place of residence. The study is conducted in a well-defined population to allow calculation of the incidence of hospitalization, the principal measure of disease burden. Each site should have the capacity to determine the serotypes present among a subset of the rotavirus-positive stool samples or should arrange collaboration with a reference laboratory.

4.2 Case definitions

The establishment and use of uniform case definitions is essential for conducting accurate surveillance in multiple settings and over time in the same setting.

For the purposes of surveillance, a **suspected case of rotavirus diarrhoea** should be defined as a child under 5 years of age who is admitted for treatment of diarrhoea to a hospital participating in the study. A **confirmed case of rotavirus diarrhoea** should be defined as a suspected case in whose stool the presence of rotavirus is demonstrated by means of an enzyme immunoassay.

4.3 Surveillance system

4.3.1 Diarrhoeal hospitalization logbook

The diarrhoeal hospitalization logbook is used to count and track all children aged under 5 years who are admitted to hospital because of diarrhoea, i.e. suspected cases. Every possible case in a child under 5 years of age who is admitted should be entered in this logbook. The completion of logbook entries may require daily surveys of inpatient wards, admission logs, or other methods depending on the setting. Information kept in the logbook should include the date of admission, the patient's name or unique identification number, the admission diagnosis and the location of the patient in the hospital. Each site, depending on its specific needs, may wish to enter additional information.

4.3.2 Rotavirus diarrhoea case report form

A case report form covering demographic, clinical and laboratory information should be completed for each case of rotavirus diarrhoea identified (see Annex 2 for an example of a case report form). Before surveillance begins the case report form should be pretested and care should be taken to ensure that information is recorded in a standardized manner. Data can be entered into an EpiInfo.QES file.

4.4 Laboratory procedures

4.4.1 Stool specimen logbook

Each laboratory should keep a stool specimen logbook for recording information on all children who are admitted because of diarrhoea (see Annex 3 for an example of a stool specimen logbook). As laboratory data, e.g. results of tests for rotavirus, become available they should be added to the specimen logbook. The information kept in the stool specimen logbook should match the information in the diarrhoeal hospitalization logbook. Frequent comparison of the entries in the two logbooks will help to ensure that patients are not being missed by the surveillance and that each patient is properly identified.

4.4.2 Collection and handling of specimens

A sufficient amount of bulk stool, approximately 5 ml, should be obtained from each suspected case during the acute illness, preferably on the day of presentation to hospital. Rectal swabs, or swabs placed in bacterial culture media, are not optimal for rotavirus detection or characterization; thus such swabs should be avoided. Attempts should be made to obtain a stool specimen from all possible cases within 48 hours of hospital admission so as to avoid the detection of nosocomial infection. The stool specimen should be placed in a sterile screw-top container, e.g. a urine collection cup, and properly labelled with information that includes a unique identification number and the date of collection. It is necessary to ensure that the information on the specimen label matches that in the stool specimen logbook. All stool specimens should be stored in a freezer at -20°C until testing is performed, and care should be taken to avoid freeze-thaw cycles where possible. For prolonged storage a temperature of -70°C is preferable because there is evidence suggesting that the ability to characterize rotaviruses declines during storage for years at –20°C *(O'Ryan et al. 1990, Unicomb et al. 1989).*

Special consideration should be given to the long term storage conditions of stool specimens collected from countries that are polio endemic. Stool specimens collected for rotavirus investigation may also unknowingly harbor wild polioviruses if collected in endemic countries. Such specimens may represent the only remaining source of wild poliovirus once the goal of the Polio Eradication Initiative has been achieved. In order to prevent reintroduction of wild polioviruses, the Polio Eradication Initiative has produced a Global Action Plan for Laboratory Containment of Wild Polioviruses. The Action Plan describes the safety considerations laboratories should take into account when storing specimens that knowingly or potentially contain wild polioviruses *(WHO Department of Vaccines and Biologicals, 1999).* All laboratories with such specimens are requested to register with their country's National Inventory of Laboratories with Wild Poliovirus materials and to store these materials under the

conditions outlined in the document. For further information, please consult the referenced document or contact the Polio Eradication Initiative, WHO Department of Vaccines and Biologicals, CH-1211 Geneva 27, Switzerland (+41 22 791 4524 / 4372; labnet@who.int).

If specimens need to be stored temporarily before being placed in a freezer they should be refrigerated at 4 to 8°C. Rotavirus infectivity in both faeces and culture medium is relatively stable at these temperatures *(Meng et al. 1987, Ramos et al. 2000)*. If specimens need to be transported before freezing, ice packs should be used to keep them at 4 to 8°C. Nonetheless, rotaviruses are relatively stable in a variety of conditions; this is true of some strains stored at room temperature in culture medium *(Meng et al. 1987)*. Consequently, even if there is a breakdown in handling, e.g. in the event of the storage of specimens at room temperature for more than four hours, they may still be tested, although the potential loss of sensitivity should be considered in later analyses.

If stool samples are also tested for bacterial or parasitic pathogens the specimens should be transported to the laboratory within two hours of collection and placed on the appropriate media. Specific guidelines on the collection, handling and testing of specimens for other enteropathogens should be obtained from other sources.

4.4.3 Methods for detection of rotavirus

All stools collected should be tested for rotavirus antigen by means of a commercial enzyme immunoassay kit. Other methods of detection – electron microcopy, RNA electrophoresis, and the use of the polymerase chain reaction – are generally more laborious and resource-intensive and demand more training. They are not, therefore, recommended for routine use in surveillance. Several enzyme immunoassay kits for the rapid detection of rotavirus are available. Except for the ID EIA test, the kits indicated in Table 3 have been widely used and evaluated *(Dennehy et al. 1990 and 1999, Lipson et al. 2001, Lipson and Zelinsky-Papez 1989, Sanchez et al. 1993)*. The ID EIA test is included because it has a convenient format and has been used in one previous study *(Wilhelmi et al. 1999)*.

Table 3. Rotavirus enzyme immunoassay kits* for use in surveillance studies

Product	Manufacturer	Format	Number of tests in each kit
Premier Rotaclone® qualitative or quantitative antigen enzyme immunoassay	Meridian Diagnostics, Inc., USA	12 well strips	48
Abbott Testpack® qualitative antigen enzyme immunoassay	Abbott Laboratories, USA	Individual reaction discs	20
Pathfinder® rotavirus qualitative antigen enzyme immunoassay	Sanofi Diagnostics Pasteur, France	Individual tubes	50
ID EIA Rotavirus Test	DAKO Diagnostics Ltd, United Kingdom	12 well strips	96

* Note: The above list of products and manufacturers of rotavirus enzyme immunoassay kits does not indicate special endorsement of these products and/or manufacturers. Please follow carefully the manufacturer's instructions when using these products.

Table 3 is not a complete list of available tests but, for the most part, represents kits with comparable sensitivity and specificity that are widely available. Each site should consult its nearest laboratory for more information about the kits and their local availability. It is best to use a single manufacturer's kit during the entire study in order to achieve comparability and uniformity of data. Once antigen detection is completed, any remaining stool and stool suspension should be stored at approximately -20°C, unless extracts are to be tested immediately by serotype analysis. In the latter event, suspensions can be kept at 4 to 8°C for a few days.

4.5 Data analysis

The first step in data analysis should be to characterize the reported cases of rotavirus diarrhoea by person, place or time for children living in the referral population defined at the beginning of surveillance. Specific analysis should cover the following matters.

1. The number of hospitalizations for diarrhoea in children under 5 years of age.
2. The percentage of hospitalizations caused by diarrhoea in children under 5 years of age.
3. The distribution of diarrhoeal hospitalizations by etiology, including rotavirus diarrhoea.
4. The percentage of diarrhoea-associated hospitalizations caused by rotavirus.
5. The rates of hospitalizations associated with diarrhoea and rotavirus per 1000 children per year in the surveillance population overall and by age groups 0–2, 3–5, 6–8, 9–11, 12–17, 18–23 and 24–59 months.
6. The percentages of diarrhoea hospitalizations associated with rotavirus by age groups 0–2, 3–5, 6–8, 9–11, 12–17, 18–23 and 24–59 months.
7. The numbers and percentages of hospitalizations for diarrhoea and for rotavirus diarrhoea by month of year.
8. The median and range of duration of hospital stay for all diarrhoea hospitalizations and for rotavirus diarrhoea hospitalizations.
9. The number and rate of deaths associated with rotavirus diarrhoea.
10. Calculation of the above data by year of surveillance.

4.6 Monitoring data quality

Periodic review of the data collected can help to identify problems in data collection, patient enrolment, or specimen collection and handling. Some signs that problems may exist in study procedures are indicated below.

Fewer subjects tested for rotavirus than expected

The data collected before the study on the number of hospitalizations for acute gastroenteritis in children under 5 years of age by month should indicate for each site approximately how many children can be expected to be hospitalized during the study period. If the number enrolled in the study, i.e. children with acute diarrhoea (suspected cases) is below 75% of the expected number for two consecutive months, this may indicate that the procedures for case-finding are inadequate. The expected

number can be calculated as the average number of admissions for gastroenteritis by month during the preceding three years, on the assumption that there are no major changes in referral patterns or population size. If, however, such changes occur, the methods for detecting new admissions should be checked.

Fewer than 15% of children positive for rotavirus

Since most hospital-based studies have detected rotavirus infection in 20–50% of children admitted for acute diarrhoea, a rate of detection below 15% may indicate a problem. A rate of detection below 15% should lead the investigators to ask the following questions:

1. Is the surveillance system missing the youngest age groups?
2. Is the quantity of stool collected insufficient?
3. Are stool specimens handling procedures not optimal?
4. Are there problems in using the enzyme immunoassay test kits?
5. Have the reagents in the enzyme immunoassay test kits expired?
6. Are stools being tested in a timely manner?
7. Is storage of samples adequate?
8. Have the personnel been properly trained?

Since many settings can be expected to have seasonal peaks and troughs in rotavirus circulation the rate of detection at which problems should be investigated may be reduced in some months. Outbreaks of other enteric agents in a surveillance area may lead to a decrease in the proportion of rotavirus-positive samples but the absolute number of positives should not change dramatically.

4.7 Duration of surveillance

Ideally, surveillance should be conducted for at least two complete years in order to assess annual and seasonal variations in the disease burden. Once rotavirus vaccine becomes available, such a system should be introduced at least a full year before vaccine introduction so as to provide a reliable baseline estimate from which to calculate the effectiveness of rotavirus immunization.

5. Strain surveillance and characterization

Information on the prevalence of circulating rotavirus strains is important in the assessment of the likely impact of vaccine and in understanding reasons for any programme failure that may occur. In each setting where rotavirus surveillance is conducted, information should be obtained on local rotavirus strains. At a given site it may be desired to conduct strain serotyping with the help of local expertise and laboratories, to participate in a regional strain surveillance network (see Annex 4), and/or to collaborate with an international reference laboratory.

5.1 Background on methods of strain characterization

Clinical and epidemiological studies of rotavirus have depended on the availability of sensitive methods to detect rotaviruses in faecal specimens obtained from infected children. The early work was conducted by means of electron microscopy. However, the development of immunoassays, latex tests and electrophoresis have made diagnosis feasible throughout the world. These assays are rapid, sensitive, specific, inexpensive and easy to perform, and can be used in the simplest laboratory settings. The sensitivity of routine diagnostics is high since the number of viruses excreted by a child with rotavirus diarrhoea, approximately 10^{10}–10^{12} viruses per gram of stool, is much higher than the detection limit of approximately 10^{7} viruses per gram of stool using the enzyme immunoassay. Virus can be cultivated from stool specimens, and molecular detection methods, e.g. dot blot hybridization and reverse transcription-polymerase chain reaction (RT-PCR) are available for special studies but are not needed for routine diagnosis.

Because the genes encoding the two proteins associated with neutralization segregate independently, a dual serotyping system has been adopted to account for serotype specificities of VP7 (G) and VP4 (P) *(Gentsch et al. 1996)*. G serotypes are determined by using an enzyme immunoassay with four monoclonal antibodies directed to serotype-specific epitopes on the VP7 protein of the four common G types. G serotypes can be predicted by molecular methods, such as RT-PCR, hybridization with oligonucleotide probes, and nucleotide sequencing. P serotypes are difficult to determine using traditional methods of virus neutralization and, although immunoassays incorporating monoclonal antibodies have been described *(Coulson 1993, Masendycz et al. 1997, Padilla-Noriega et al. 1993 and 1998)*, they have not been extensively applied to P-serotyping. Consequently, P genotypes are defined on the basis of predicted amino acid differences and compared to strains of known P serotype. This method correlates with virus neutralization, and viruses with over 89% similarity in VP4 amino acid sequence are usually serologically indistinguishable *(Gorziglia et al. 1990)*. Exceptions to these correlations have been

noted *(Hoshino et al. 1998, Li et al. 1996)*. On the basis of correlation between serotype and amino acid sequence, genotype-specific primers and probes have been developed to determine P genotypes by RT-PCR and hybridization methods *(Gentsch et al. 1992)*.

5.2 Selection of specimens for further characterization

A subset of rotavirus-positive stools obtained from routine surveillance should be chosen for further characterization. The type and size of sample depends on the number of positive tests, the resources of the institution in question, the number and heterogeneity of participating hospitals, the variability of strains in past studies, and other factors. All hospitals in the surveillance system should contribute rotavirus-positive stool samples for further characterization so as to enhance the representativeness of the system. Samples for examination should be collected throughout the year. Only specimens of adequate volume for several tests (more than 3 ml) should be chosen in order to avoid running out of material before testing is complete. Finally, the selection should be as random and standardized as possible so as to minimize selection bias. The decision as to which samples to test could be made at the end of each year of surveillance on the basis of the monthly and age-specific distribution of strains and the above considerations.

5.3 Strain characterization methods

Rotaviruses are most often characterized with respect to their VP7 (G) and VP4 (P) proteins. These are the viral antigens to which neutralizing antibodies are directed and are the basis of rotavirus nomenclature. The rotavirus vaccines under development are designed to induce protection from viruses with common G and P types, so that knowledge of prevalent types may prove important for decisions on vaccine use. Two main methods have been extensively employed for typing: serotyping with monoclonal antibodies to determine the G protein, and RT-PCR to identify both G and P types.

In one typing strategy, strains are first G-serotyped using enzyme immunoassay with type-specific monoclonal antibodies for predominant serotypes (usually G1–G4), and possibly for other strains, depending on previous local data. For instance, G5, G8, G9 and G10 viruses have been found to be quite common in some countries, and G6 and G12 have also been detected at various sites. Monoclonal antibodies have been produced for both common and unusual types G5, G6, G8 and G10, together with methods for their use *(Coulson et al. 1987, Taniguchi et al. 1987)*. Investigators can expect to G-type 50–80% of strains with monoclonal antibodies alone. Samples stored for the minimum amount of time before analysis have yielded the highest percentage of typeable strains, presumably because the outer capsid degrades slowly during storage so that serotype-specific monoclonal antibodies eventually do not bind *(Matson et al. 1990)*. The freezing and thawing of stools may also reduce the ability to serotype rotaviruses with monoclonal antibodies. The strains that are not typeable by means of monoclonal antibodies should be genotyped using RT-PCR and hybridization techniques *(Das et al. 1994, Gentsch et al. 1992, Gouvea et al. 1990, Gunasena et al. 1993)*. The analysis of untypeable strains by these methods has been important in identifying unusual strains that could have a bearing on vaccine strategies *(Griffin et al. 2000, Unicomb et al. 1999)*.

The second strategy, and the one we recommend, is to type specimens exclusively by RT-PCR. In the first strategy, i.e. monoclonal antibody serotyping, 20–50% of specimens analysed remain untypeable and subsequently have to be reanalysed by genotyping methods, regardless of how carefully the specimens are collected and stored. It is therefore probably easier for most laboratories to set up a single method for typing, namely RT-PCR. Furthermore, RT-PCR genotyping has proved highly efficient in some settings, allowing 90–95% of strains to be typed *(Bon et al. 2000, Griffin et al. 2000, Iturriza-Gomara et al. 1999, Ramachandran et al. 1998)*, and this approach is required for the P-genotyping of a representative subset of strains which we recommend. For the subset of strains P-genotyped, uncommon G serotypes should also be represented. Strains that have unusual G and/or P types can be further analysed by polyacrylamide gel electrophoresis, which helps to understand the amount of diversity among strains by the determination of RNA migration patterns (long versus short). A complete set of strain characterization protocols with details of sources of reagents, including some limited reagents such as RT-PCR primers, can be obtained by contacting Dr Jon Gentsch, WHO Collaborating Centre for Rotavirus and Agents of Viral Gastroenteritis, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., MailstopG-04, Atlanta, GA 30333, USA (Email: jrg4@cdc.gov; Fax: + 1 404 639 3645).

6. Other issues

6.1 Role of surveillance coordinator

In each study a coordinator should be designated with responsibility for implementing and monitoring the system and for report-writing. At the sites conducting surveillance (e.g. laboratory, hospital, health ministry) the coordinator should appoint contact persons to collect data. The coordinator should periodically contact the sites, say at weekly intervals, to ensure that reliable data collection is continuing, and should make site visits, say at monthly intervals, to assess whether complete records are being kept. The coordinator should also ensure that missing information is obtained and that erroneous information is corrected, and, if possible, should supervise data entry into a computer database for easy analysis.

6.2 Surveillance for other outcomes

Hospital-based surveillance for rotavirus gastroenteritis is likely to be the most practical type of surveillance in many settings. In some situations, however, surveillance for other outcomes or the use of alternative methods may be desirable. For instance, household-based surveillance or surveillance of paediatric gastroenteritis cases that present to community clinics may be considered in some settings. The increasing use of prolonged stays in rehydration areas of hospitals, instead of admitting children with gastroenteritis, may require the inclusion of these clinics in surveillance for severe disease. The use of ICD-coded databases has been described for the estimation of rotavirus-associated hospitalizations and mortality, and this is appropriate for settings where such data are available *(Parashar et al. 1998 and 1999)*. Where resources are insufficient to conduct hospital-based surveillance as described in this protocol, participation in a regional rotavirus strain surveillance network may be appropriate (see Annex 4). Since the most appropriate outcome to consider may vary between sites, local treatment patterns should be reviewed at each site before surveillance begins.

The estimation of rotavirus-associated mortality may be particularly important for establishing public health priorities. It can be estimated in settings where information on monthly age-specific mortality data is available along with some syndrome-specific data (obtained, for example, through verbal autopsies) combined with the data from hospital-based surveillance for the rotavirus fraction. The use of this method has recently been reported from Guinea-Bissau *(Molbak et al. 2001)*.

6.3 Inclusion of data on cost and resource use

Since an implicit goal of surveillance is to direct public policy and health resources appropriately, it may be desirable for a surveillance system to collect data on the costs of rotavirus, either in resources or of money spent on the treatment and care of ill children. This is particularly useful in countries where rotavirus mortality is low but rates of hospitalization are high. The costs to be surveyed depend on the surveillance setting, the concerns of the agency collecting or monitoring the data, and where the data are collected. For instance, if a health ministry is responsible for funding hospitals that care for children with rotavirus disease, it may be useful to collect data related to the monetary costs associated with staffing and materials for the care of these children in hospitals. Information on the costs associated with rotavirus can be used to make decisions about the cost-benefit of vaccine introduction. Guidelines for costing studies on rotavirus gastroenteritis are under development.

6.4 Opportunity for regional networks and collaboration

Once a hospital-based surveillance study is established, several countries may wish to coordinate activities and form a regional surveillance group (see Annex 4). The advantages of such collaboration include the possibility of sharing resources (e.g. by organizing a regional strain surveillance laboratory), learning more about the epidemiology of rotavirus disease, and comparing data with a view to determining the best possible preventive strategies. In addition, research opportunities may arise. Finally, agencies such as WHO and the United States Centers for Disease Control and Prevention may wish to collaborate or provide technical consultation for some activities, such as laboratory testing and the design and implementation of surveillance (see contact addresses in box below).

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Annex 1:

Examples of data forms for analysis of previous studies of rotavirus diarrhoea

Table A. Line-listing of previous local studies of rotavirus diarrhoea

Location and setting of study	Duration of study	Age range of subjects (months)	Test used to detect rotavirus	Test results	
				Number of specimens tested	Number (%) rotavirus-positive

Table B. Age-specific gastroenteritis hospitalizations associated with rotavirus among children aged 0 to 59 months

Age group (months)	Number of gastroenteritis hospitalizations	Number (%) of gastroenteritis hospitalizations associated with rotavirus	Cumulative number (%) of hospitalizations for rotavirus gastroenteritis
0–2			
3–5			
6–8			
9–11			
12–17			
18–23			
24–35			
36–47			
48–59			
Total			

Annex 2:
Diarrhoea case report form

Reporting hospital: _____ **Medical record No.:** _____

Date of admission: _____/_____/_____ **(day/month/year)**

Patient information

Family name: _____ First name: _____

Address: _____ City: _____ District: _____

Age (months): _____ Date of birth _____/_____/_____ (day/month/year) Sex: M F (circle one)

Clinical information

Temperature: _____ °C

Vomiting: _____ (Yes/No) No. of episodes/24 hr: _____ Duration (days): _____

Diarrhoea: _____ (Yes/No) No. of episodes/24 hr: _____ Duration (days): _____

Outcome

Date of discharge or death: _____/_____/_____ (day/month/year)

Laboratory information

Date stool specimen collected: _____/_____/_____ (day/month/year)

Bacteria identified in stool? _____ (Yes/No) If "Yes", which bacteria? _____

Parasites identified in stool? _____ (Yes/No) If "Yes", which bacteria? _____

***For rotavirus, please complete the following*:**

Rotavirus identified in stool? _____ (Yes/No) If "Yes", by what method? _____

What was the G-serotype (if known)? _____

Person completing form:

Name: _____ Signature: _____

Date of report: _____/_____/_____ (day/month/year)

Annex 3:
Sample stool specimen logbook

ID No.	Date received (day/month/year)	Patient's name	Age (months)	Estimated stool amount (grams)	Stool screening results			Rotavirus-positive stool characterization			Location of stool extract storage	Location of specimen storage
					Bacterial testing	Parasitic testing	Rotavirus enzyme immunoassay	E-type	G serotype	G genotype		
	____/____/____											
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Annex 4:
Sentinel regional laboratory surveillance for rotavirus

A laboratory component is essential for any rotavirus surveillance activity. It may involve support only for a hospital-based system or may include a network of laboratories that report rotavirus detections and a small amount of demographic data or strain characterization. The following discussion refers to a network of laboratories that primarily report detection data. However, it would be helpful in connection with the development of a laboratory component of any system, e.g. sentinel hospital-based surveillance.

Selection of laboratories

A network of laboratories that report data on the number of weekly rotavirus detections could be used to assess temporal and geographical trends in rotavirus activity in a given region. Efforts should be made to ensure that the participating laboratories are geographically representative, that they process a sufficient number of stool specimens each month and that they use methods with similar sensitivities and specificities for the detection of rotavirus so that the data they provide are robust and comparable. If possible, the participating laboratories should be associated with a paediatric or general hospital so that the laboratory data can be linked with clinical information on the children whose stool specimens yield rotavirus.

Surveillance system

The participating laboratories should use a case report form to make a weekly record of the total number of faecal specimens processed, the type of test performed (e.g. rapid antigen detection test, electron microscopy), and the number of specimens testing positive for rotavirus (see example of a laboratory case report form). While demographic information on a subset of patients, including their age, gender, disease severity and location (i.e. outpatient or inpatient) is desirable, it may be difficult to obtain because of the extra effort required by laboratory personnel and because some referral laboratories may not have access to the data in question. Even without demographic information, data on the number of monthly rotavirus detections should be sufficient to assess geographical and temporal trends in rotavirus activity. In order to enhance communication the laboratories could provide weekly information through an automated telephone polling system or through a computerized system in countries where these facilities are available

Analysis of data

For each laboratory an examination should be made of indicators of rotavirus activity, including the number of faecal specimens processed, the number of rotavirus detections and the percentage of specimens testing positive for rotavirus. In order to examine seasonal trends the monthly or weekly detection rates of rotavirus should be examined. In regions where rotavirus exhibits a distinct seasonality the peak of rotavirus activity should be defined as the two consecutive weeks with the greatest number of rotavirus detections. The onset of the rotavirus season should be defined as the week in which the number of rotavirus detections first exceeds the mean number of rotavirus detections per week for the entire year. The duration of the rotavirus season should be defined as the number of weeks during which the detections exceed the weekly mean. Spatial differences in the timing of seasonal rotavirus activity should be examined by assessing the timing of peak rotavirus activity at the individual sites. They could be plotted as contour maps by means of sophisticated computer software.

Strain characterization

The characterization of locally prevalent rotavirus strains before a vaccine programme is undertaken, usually by serotyping, is of value when assessing the likely impact of a vaccine and the effectiveness of a programme, and in gaining an understanding of the potential reasons for programme failure. Strain characterization requires skilled laboratory personnel and special equipment and is relatively resource-intensive. Consequently, there is no expectation that all surveillance sites could incorporate it into their systems locally or that all rotavirus-positive samples could be further characterized. The possibility exists, however, of establishing regional networks or collaborations allowing strain characterization to be performed by other interested laboratories.

Selection of specimens for further characterization

A sample of rotavirus-positive stools obtained from either hospital-based or laboratory-based surveillance should be chosen for further characterization. The type and size of the sample depend on the number of positive tests, the resources of the institution in question, the number and heterogeneity of surveillance sites, the variability of strains in past studies, and other factors. All the sites in a surveillance system should be covered in order to make the results as representative as possible. Samples collected throughout the year should be examined. Only specimens that are large enough in volume for several tests should be chosen so as to avoid running out of material before adequate testing has been completed. The selection should be as random and standardized as possible in order to decrease selection bias, and should be simple so as to ensure that there is no loss of data and that compliance occurs. For instance, a site might include the first three rotavirus-positive stools each month at each participating laboratory or hospital.

Review of methods

Rotaviruses are most often characterized with respect to their VP7 (G) and VP4 (P) proteins. These are the viral antigens to which neutralizing antibodies are directed and are the basis of rotavirus nomenclature. Moreover, the vaccines under development are designed to induce protection from viruses with specific G and P types, so that a knowledge of prevalent types is potentially important in relation to decision-making on the optimal vaccine for local use.

Local strain characterization should include G-serotyping by means of enzyme immunoassay with type-specific monoclonal antibodies for predominant serotypes (usually G1–G4), and possibly for other strains, depending on previous local data. Since P-typing requires the use of the polymerase chain reaction and probe hybridization, it may be too difficult to complete for all rotavirus-positive samples tested. However, it should be considered for a subset. Another common method for characterizing rotaviruses is polyacrylamide gel electrophoresis, which may be included in a testing algorithm, although it is probably less helpful than G-serotyping.

Example of data form for use in laboratory-based surveillance

Rotavirus laboratory reporting system – weekly report

Laboratory ID number: _____

Surveillance week		Test used (Type of test and manufacturer)	Number of tests performed	Number rotavirus-positive
Date begun	Date ended			
____/____/____	____/____/____			
____/____/____	____/____/____			
____/____/____	____/____/____			
____/____/____	____/____/____			
____/____/____	____/____/____			
____/____/____	____/____/____			
____/____/____	____/____/____			

Part II:

Generic protocol for a community-based survey on utilization of health care services for gastroenteritis in children under 5 years of age

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1. Introduction

Rotavirus is a major cause of acute diarrhoea in children throughout the world and a leading cause of diarrhoeal deaths in early childhood (*de Zoysa and Feachem 1985, Miller and McCann 2000*). Improvements in sanitation and in food and water quality have failed to alter the incidence of rotaviral infection in many countries. The use of vaccines is considered to be the intervention most likely to prevent the disease, and a number of candidate rotavirus vaccines are undergoing clinical testing (*Vaccines and Biologicals 2000*).

With a view to preparing countries for decisions on the introduction of new rotavirus vaccines a method for hospital-based surveillance of rotaviral gastroenteritis in children under 5 years of age is described in Part I of this document. As an adjunct to hospital surveillance it is important to determine the hospital utilization pattern among carers of children with severe diarrhoea in catchment areas. It has been reported that the care-seeking behaviours and treatments for children with diarrhoeal illnesses vary considerably between countries. Recent surveys suggest an increasing tendency to seek care for diarrhoea outside the home (*Mururi et al. 1996*). Because of this, a knowledge of the settings in which care for gastroenteritis is received and of the attitudes and practices of the population towards paediatric gastroenteritis and its treatment is important in connection with designing the best possible study of rotavirus disease burden and comparing data between studies in different settings.

2. Objectives

The primary purpose of this community-based survey is to determine the proportion of children with severe diarrhoea who receive treatment at hospitals that have been proposed for participation in a rotaviral gastroenteritis surveillance study. This information provides a means of determining whether particular hospitals would be appropriate sites for such surveillance. Since the goal of hospital-based surveillance is to capture severe cases of rotavirus gastroenteritis which occur among children under 5 years of age, care-seeking patterns in the population concerned are an important indicator of the appropriateness of settings for surveillance activities. The survey is designed to reveal: (i) what action was taken by mothers whose children had severe diarrhoea during a specified period of one month preceding the survey; and (ii) what mothers would do if their children experienced severe diarrhoea.

The results of this survey will give an estimate of the proportion of gastroenteritis in children under 5 years of age whose disease will be missed by a hospital-based surveillance system. This information can be used to adjust hospital-based data to better reflect the true disease burden in the community.

3. Methods

3.1 Study design

The survey uses a cluster sampling procedure to select households within the referral area of the proposed study hospital(s). The mother (or other primary caretaker) of an eligible child is identified for interview with a standard questionnaire (Annex 1). The cluster sampling technique allows a small number of members of the target population to be sampled while providing data that are statistically valid. It is necessary to know the catchment area of the proposed study hospital(s), the number of sampling units within the catchment area and the size of the population in each sampling unit.

The hospital referral or catchment area will serve as the sampling frame. For many hospitals, the geographical catchment area may be defined administratively with a map available to show the administrative areas where the catchment population resides. Where the hospital catchment area is not clearly defined, the investigator will need to consider factors such as the distance from the hospital(s) or the addresses on the admission records of children admitted to the proposed study hospital(s) over a specified period. Once the sampling frame has been defined, the investigator will need to determine all the identifiable sampling units within that geographic area. Sampling units can be defined in a variety of ways: the definition of a sampling unit ultimately depends on the type of information available locally. Examples of sampling units from past surveys include city blocks in urban areas, villages in rural areas, census tracts, neighbourhoods and other administrative districts. Within the sampling frame, each area needs to be included in one of the sampling units. Mapping the sampling frame and the sampling units will help to ensure that no geographic areas are missed.

For this generic protocol we have selected an example adapted (with some modifications) from a submetropolitan area of Accra, Ghana, served by a large public hospital. This geographic area is the survey sampling frame. Within the sampling frame there are 16 administrative blocks, which are the sampling units for the study. Figure 1 shows a map with the survey sampling frame, the location of the hospital (marked "H"), and the 16 sampling units.

It is necessary to know the most current estimated population for each sampling unit. If there has been a recent census, this should be easy. In practice, this may be difficult if it has been a long time since the last census was carried out. The main concern is to ensure that the relative sizes of the sampling units are approximately correct.

Figure 1. Map of showing the survey sampling frame and the 16 sampling units, H indicates the hospital

3.2 Sample size

The well-established EPI 30-cluster coverage survey uses a sample size of 210 children, i.e. 30 clusters of seven children each *(Expanded Programme on Immunization 1991, Henderson and Sundaresan 1982, Lemeshow and Robinson 1985)*. This is based on an anticipated level of immunization coverage of 50%, a precision of 10% and a 95% confidence level. Several modifications of the procedure have been applied to different situations *(Malilay et al. 1996)*.

In the present survey it is assumed that the prevalence of severe diarrhoea in a population of children aged under 5 years is 30% and that 90% of mothers would take cases of severe diarrhoea to hospital. With a precision of 5% and a 95% confidence level, a sample size of 600 is needed. Further details of the sample size calculation are given in Annex 2.

The sample size of 600 will be covered by interviews of 20 mothers in each of 30 clusters.

3.3 Selecting 30 clusters

A table should be set up with four columns with the name of each sampling unit in the first column and the population size or the estimated population size in the second column (see Table 1). The third column is for the cumulative population, which will be calculated by the investigator. The fourth column indicates the particular clusters selected for the survey on the basis of a sampling interval (S) and a selection of a random number (N) between 1 and the sampling interval.

Table 1. List of the sampling units within the sampling frame, with their population and cumulative population

Sampling units	Population	Cumulative population	Clusters selected for survey
Chorkor	34 846	34 846	
Lower Zongo	17 904	54 750	
Mamprobi	37 300	91 050	
Laterbiokorshie	15 925	106 975	
Zamrama Line	12 145	119 120	
Banana-inn	34 840	153 960	
Upper Zongo	15 440	169 400	
Town Council Line	12 082	181 482	
Sukura/Rassia	12 875	194 357	
Mabruk	18 717	213 074	
Agege	10 582	223 656	
Abose-Okai	10 807	234 463	
Old Dansoman	19 000	253 463	
Mataheko	73 000	326 463	
Korle Gonno	90 100	416 563	
Korle Bu	4 200	420 763	
Gbebu	29 837	450 600	
Total	**450 600**		

In the example given in Table 1 the total population of the sampling frame is 450 600. The sampling interval S is obtained by dividing the total population by 30.

$$S = 450\ 600/30 = 15\ 020.$$

Next, the investigator will need to select a random number from a table of random numbers (Annex 3). A column and a row are selected randomly according to the instructions. The five-digit random number at their intersection is the starting point. The first number which falls between 1 and the sampling interval is selected as N. In this example, we randomly selected column 3 and row 21, giving a starting point of 26 382. Staying within row 21, the first random number to the right of the starting point which is equal to or less than the sampling interval (S) is 01100, located in column 8.

$$\text{Thus, } N = 1100.$$

The calculations for selecting the 30 clusters are outlined in Table 2. The first of the 30 clusters to be selected is located in the first sampling unit with a cumulative population equal to or more than N. The second cluster is located in the sampling unit with a cumulative population containing the random number plus one sampling interval (N + S). The third cluster is located in the sampling unit whose cumulative population equals or exceeds (N + S + S). The remaining clusters are selected in the same manner by adding the sampling interval to the previous figure and identifying the corresponding sampling unit with a cumulative population equal to or more than the calculated sum.

Table 2. Calculations for selecting the 30 clusters

Cluster number	Formula	Calculation	Cumulative population	Location of cluster
1	N	1100	1 100	Chorkor
2	N + S	1100 + 15 020	16 120	Chorkor
3	N + S + S	1100 + (2 * 15 020)	31 140	Chorkor
4	N + S + S + S	1100 + (3 * 15 020)	46 160	Lower Zongo
5	N + S + S + S + S	1100 + (4 * 15 020)	61 180	Mamprobi
.				
.				
29	N + (28 * S)	1100 + (28 * 15 020)	421 660	Gbebu
30	N + (29 * S)	1100 + (29 * 15 020)	436 680	Gbebu

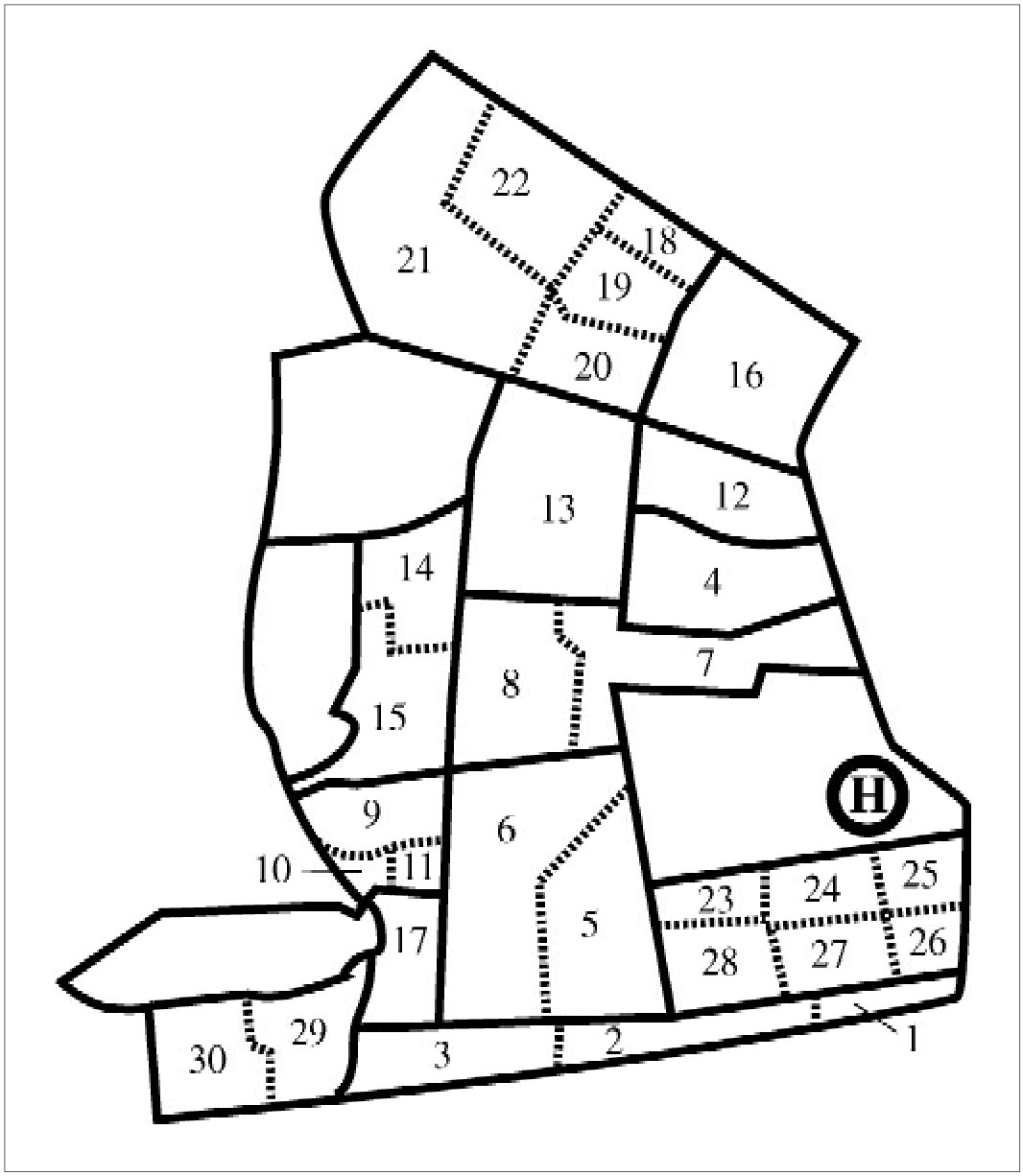
Table 3 shows the final results of the cluster selection procedure. Where two or more clusters are located in a single sampling unit, as in Chorkor, Mamprobi, Laterbiokorshie, etc. in the example, the sampling unit will need to be subdivided into the number of expected clusters, which should not overlap.

Table 3. The 30 clusters selected for the survey

Sampling units	Population	Cumulative population	Clusters selected for survey
Chorkor	34 846	34 846	1,2,3
Lower Zongo	17 904	54 750	4
Mamprobi	37 300	91 050	5,6
Laterbiokorshie	15 925	106 975	7,8
Zamrama Line	12 145	119 120	
Banana-inn	34 840	153 960	9,10,11
Upper Zongo	15 440	169 400	12
Town Council Line	12 082	181 482	13
Sukura/Rassia	12 875	194 357	
Mabruk	18 717	213 074	14,15
Agege	10 582	223 656	
Abose-Okai	10 807	234 463	16
Old Dansoman	19 000	253 463	17
Mataheko	73 000	326 463	18,19,20,21,22
Korle Gonno	90 100	416 563	23,24,25,26,27,28
Korle Bu	4 200	420 673	
Gbebu	29 837	450 600	29,30
Total	**450 600**		

In our example there are several clusters located within a single sampling unit. In this situation, the sampling unit will need to be subdivided, and the map revised. Figure 2 shows a map which indicates the location of all 30 clusters; where required, sampling units have been subdivided.

Figure 2. Map showing the locations of the 30 clusters within the sampling frame, H indicates the hospital



3.5 Selecting the first household

In each sampling unit (or subdivision of the sampling unit) where a cluster has been selected, a house is selected at random as the starting household of the survey. A household is defined as a group of persons who live and eat together. A house may contain one or more households.

To select the starting household use the following three steps. Step 1: a unique location is identified in the selected cluster; this could be a cinema, a market, a restaurant or a popular spot. Step 2: one of the roads or lanes joining the location is chosen at random. Step 3: the first house for the survey is selected at random along the selected road or lane on either the left or right side.

3.6 Selecting at random

Balloting can be used to select either the road or the house at random. If there are four roads or streets leading to the location, strips of paper are used, each with the name of one of the roads written on it. The paper strips are placed in an envelope so that the names are not visible and someone is asked to select a strip. The same method can be used for the houses on the selected road (e.g. if there are nine houses along a reasonable length of the selected road, one of them can be selected as the starting house by balloting). Interviews are conducted in all the eligible households of the selected house by means of the questionnaire (Annex 1).

3.7 Population

The study population comprises children under 5 years of age (i.e. under 60 months) who are resident within the study sampling frame, which is the catchment area of the hospital(s) being considered for the rotavirus surveillance study.

3.8 Time frame

The questions in the survey are of two types. First, mothers are asked about illnesses in any of their children who are under 5 years of age which have occurred during the preceding month. Second, mothers are asked about what they would do if their child experienced severe diarrhoea with dehydration. Rotavirus gastroenteritis in children tends to be seasonal in temperate regions, with peak incidence in winter. In tropical areas, the seasonality of rotavirus is less distinct, although there is often a slight peak in the dry season *(Biritwum et al. 1984, Molbak et al. 2001)*. It would therefore be wise for investigators to consult paediatricians in the proposed study hospitals in order to discover when the local rotavirus season occurs. Carrying out the survey during the peak season is likely to yield more cases and secure better recall of events from mothers.

3.9 Administering the survey questionnaire

The survey questionnaire is reproduced in Annex 1. The data collected include information about the household and family structure, recent diarrhoeal illnesses among children under 5 years of age who live in the household, care practices for diarrhoeal illnesses, and health care utilization for hypothetical severe diarrhoea among children. The questions probably require to be modified so that they are appropriate for each site. For the purpose of comparison between sites, however, investigators should try to adhere to the main elements of the questionnaire. In some countries, for example, the birth dates of children may not be known and it may be necessary to use an event calendar in order to estimate ages.

The first question on the survey asks if any child under 5 years of age lives in the household in question. If the answer is in the negative, this should be indicated on the questionnaire and the interviewer should proceed to the next household. A new questionnaire is used for each household, so that every household, whether it contains children of the appropriate age or not, has a questionnaire in the database. However, only questionnaires concerning children under 5 years of age count as part of the total of 600 which is required. The questionnaire should always be administered to a mother or primary carer, since these are most likely to provide the best data for the survey. If the mother or primary carer is unavailable, this is indicated on the questionnaire and the interviewer proceeds to the next household. A message should be left at each household where no response is obtained, indicating when the interviewer is likely to return. If there is still no response when two further visits are made the household is replaced by continuing after the last household where interviewing was completed in the cluster concerned.

After completion of the questionnaire in the first household, subsequent households are selected on the basis of closest proximity to the household where interviewing has been completed *(Henderson and Sundaresan 1982, Lemeshow and Robinson 1985)*. Interviews are conducted in all the eligible households in a house until the expected sample size of 20 per cluster is reached.

4. Data outcomes and analysis

The question of primary interest is as follows: "What is the proportion of children under 5 years of age with severe diarrhoea who ultimately receive care at one of the hospitals participating in the rotavirus surveillance study?" This information allows an assessment to be made of the appropriateness of the hospital(s) for the rotavirus surveillance study. The survey results may also make it possible to estimate the true incidence of severe rotaviral diarrhoea in the community at the end of the period of hospital-based surveillance.

4.1 Definitions

The following can be used as definitions of **severe diarrhoea**.

Table 4. Definitions of severe diarrhoea

1	Acute watery diarrhoea (more than three loose or watery stools in a 24-hour period and no blood in them) and at least two of the following: • lethargy/unconsciousness; • sunken eyes; • inability to drink, or drinking poorly.
2	Local name for severe diarrhoea (if applicable).
3	Child has received intravenous rehydration.

If ***any*** of the above definitions apply the child can be considered to have had severe diarrhoea.

4.2 Data analysis

The data set consists of the 600 completed surveys, and an initial frequency analysis should be completed for all variables to scan for missing or incomplete data.

A. The proportion of severe diarrhoea cases that received treatment at hospital(s) participating in the surveillance

Numerator = number using the participating hospital(s) (**Q13**).

Denominator = number who have had severe diarrhoea during the specified period (a variable to be created from **Q9**, **Q10** and **Q11**).

Conditions

1. "Yes" for **Q9** and **Q10a** with any of **Q10b** to **Q10h**.
2. "No" for **Q11**.

B. Estimation of proportion of mothers who would use the participating hospital(s) if their children had severe diarrhoea

Numerator = number of mothers who would choose a participating hospital.

Denominator = number of mothers interviewed.

It should be noted that mothers who would choose a non-participating hospital or another source of health care, such as a clinic or a pharmacy, could eventually be referred to a participating hospital. The above estimates should therefore be regarded as minimum values.

All these estimates can be arrived at by means of existing statistical packages such as EPI INFO and SPSS, or they can be calculated manually. For each estimate a 95% confidence interval should be calculated.

4.3 Use of estimate

The estimate provides a measure of the relative magnitude of the cases being managed at the participating hospital(s). However, irrespective of the level of cases being seen at the hospital(s), surveillance activities, which are designed to monitor trends, remain valid if the pattern or use of facilities is unchanged. The incidence rate can be estimated if a count is made of all the children under 5 years of age with severe diarrhoea in the one-month period and of all children belonging to this age group in the population. However, in many situations the sample size from this survey is likely to be too small for any meaningful estimate of incidence to be made.

4.4 Other information from the questionnaire

The rest of the data from the questionnaire should be analyzed to gain further insight into the factors related to the causes and quality of management of severe diarrhoea. For example, the socioeconomic status of the family can affect the level of care given (**Q6** to **Q8**, **Q19**, **Q20**). The quality of medical care provided during episodes of severe diarrhoea can be deduced from **Q14** to **Q18**.

5. Points to consider when planning the survey

Several points should be considered at the start of the planning phase.

1. The survey should be reviewed and approved by the appropriate local ethical committee.
2. The team leader should have experience in study design and in conducting and analysing surveys.
3. A map or sketch of the hospital catchment area (the sampling frame for the survey) should be available, with a clear indication of sampling units. In addition, the investigator will need to know the estimated population sizes for each sampling unit in the sampling frame.
4. Survey staff should be carefully trained using pilot survey areas or role plays.
5. There should be clear instructions for the survey staff on household selection.
6. When survey staff identify a household where the mother (or primary caretaker) of a child under 5 years of age is not present, persons in the household should be given clear information about the time of the interviewer's return visit.
7. The team leader should ensure the availability of persons with skills in data entry and analysis.

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Annex 1:

Questionnaire on health care services utilization for gastroenteritis in a child under 5 years of age

Name of sampling unit (village/community/neighbourhood) _____

Cluster no. _____

Range of birth dates allowed: from _____/_____/_____ (day/month/year) to _____/_____/_____ (day/month/year)

Date of interview: _____/_____/_____ (day/month/year)

Name of interviewer: _____

Address/location of household: _____

Part A

1. Are there any children under 5 years old living in this household? Yes No
   If "No", say 'Thank you' and proceed to the next household.
   If "Yes", go to question 2.

   If the household contains more than one child under 5 years of age, use a separate form for each child.

2.
   a. Can you tell me how old this child is? _____months

   b. Can you tell me his/her birth date? _____/_____/_____ (day/month/year)

3. What is the sex of the child? M / F

4. Are you the child's mother or primary caretaker? Yes No
   If "No", ask if the mother or primary caretaker is available; if not, make an appointment for a return visit and proceed to the next household.
   If "Yes", proceed to the next question.

5. What is your relationship to *[child's name]*? _____Mother
   _____Father
   _____Grandmother
   _____Grandfather
   _____Aunt
   _____Sister
   _____Other: _____

6. How many years of schooling did you complete? _____

7. What is the occupation of the father? _____

8. Do you own any of the following?
(This list may need to be modified in accordance with the local situation.)

Radio	Yes	No
Television	Yes	No
Motorcycle	Yes	No
Refrigerator	Yes	No
Car or truck	Yes	No

Now I am going to ask you about recent illnesses, if any, that *[child's name]* has had.

9. Has *[child's name]* had an illness with diarrhoea in the last month? Yes No

If "No", go to Part 2, Q22.

10. If "Yes", during this illness did he/she have any of the following?

a. More than 3 loose or watery stools in a day	Yes	No
b. Increased thirst	Yes	No
c. Irritability or restlessness	Yes	No
d. Decreased liquid intake or inability to drink fluids	Yes	No
e. Decreased activity or lethargy	Yes	No
f. Loss of consciousness	Yes	No
g. Decreased urination	Yes	No
h. Dehydration (sunken eyes)	Yes	No
i. Blood in his/her stools	Yes	No
j. Skin rash	Yes	No

11. Did the illness last longer than 14 days? Yes No

12. Did you seek medical care for *[child's name]* outside your home? Yes No

If you did not seek care outside your home, what were the reasons?

a. Clinic too far from house
b. Unable to find transport
c. Cost for travel too high
d. Cost for treatment too high
e. Other children at home who could not be left alone
f. Other

13. If "Yes", where did you seek care for *[child's name]*?

[The specific hospitals included in the rotavirus surveillance system should be mentioned by name.]

____Private clinic in your community

____Government/public clinic in your community

____Pharmacy

____Hospital outpatient department

____Hospital emergency centre

____Friend or relative

____Traditional healer

____Hospital 1 .....

____Hospital 2 .....

____Hospital 3 .....

____Other (specify) .....

14. During the illness, was your child admitted to a hospital for treatment of diarrhoea and dehydration? Yes No

15. If "Yes", to which hospital was he/she admitted?
[The specific hospitals included in the rotavirus surveillance system should be mentioned by name.]

____Hospital 1 .....

____Hospital 2 .....

____Hospital 3 .....

16. Did *[child's name]* receive oral rehydration solutions (ORS) during his/her treatment? Yes No

17. If "Yes", was ORS first given at home or at the clinic/hospital?
_____Home _____Clinic/hospital

18. Did *[child's name]* receive intravenous fluids during his/her treatment? Yes No

19. If *[child's name]* did not receive care at a hospital, were you advised to take him/her to a hospital? Yes No

20. If "Yes", why was *[child's name]* not taken to a hospital?
    a. The hospital was too far from home
    b. Unable to find transportation
    c. Cost of travel too high
    d. Cost of treatment too high
    e. Other children at home who could not be left alone
    f. Other

21. Have any of your children been admitted (kept overnight) to a hospital in this area for treatment of diarrhoea and dehydration during the past year? Yes No

Part B

Now I am going to ask you what you might do if *[child's name]* had severe diarrhoea.

22. If *[child's name]* had severe diarrhoea (more than 3 watery stools without blood in a 24-hour period) that lasted less than 14 days, where would you take him/her for care?

____Private clinic in your community

____Government/public clinic in your community

____Pharmacy

____Hospital outpatient department

____Hospital emergency centre

____Friend or relative

____Traditional healer

____Surveillance hospital (name .....

____Other (specify .....

23. If, during this illness, he/she developed signs and symptoms of dehydration, such as irritability, restlessness, decreased urine output, increased thirst, dry mouth or sunken eyes, where would you take him/her for care?

____Private clinic in your community

____Government/public clinic in your community

____Pharmacy

____Hospital outpatient department

____Hospital emergency centre

____Friend or relative

____Traditional healer

____Surveillance hospital (name) .....

24. If, during this illness, he/she also experienced loss of consciousness or lethargy and inability to drink, where would you take him/her for care?

____Private clinic in your community

____Government/public clinic in your community

____Pharmacy

____Hospital outpatient department

____Hospital emergency centre

____Friend or relative

____Traditional healer

____Surveillance hospital (name) .....

25. If a doctor or nurse recommended that you take *[child's name]* to a hospital for care, would you be able to do this? Yes No

26. If "No", why not?

_____

27. If "Yes", to which hospital would you take him/her?
[The specific hospitals included in the rotavirus surveillance system should be mentioned by name.]

____Hospital 1 .....

____Hospital 2 .....

____Hospital 3 .....

28. If a doctor or nurse recommended that *[child's name]* be kept overnight at the hospital for care, would you agree to this? Yes No

29. If "No", why not?

_____

30. What is your opinion of the care your child might receive? At:
[The specific hospitals included in the rotavirus surveillance system should be mentioned by name.]

a. Hospital 1 ..... Excellent Good Fair Bad Don't know

b. Hospital 2 ..... Excellent Good Fair Bad Don't know

c. Hospital 3 ..... Excellent Good Fair Bad Don't know

Thank you very much for participating in our survey.

Annex 2:
Sample size calculations

The sample size calculations are based on a standard formula (Henderson and Sundaresan, 1982):

$$N_o = \frac{z^2 p q}{d^2}$$

where N_o is the number of children required in the survey;

z is the normal deviate (1.96 for an alpha of 0.05);

p is the proportion of children with severe diarrhoea expected to be treated at the hospital;

q = (1-p);

d is the precision (acceptable error) of the estimate;

Since the value of p is precisely what the survey is designed to collect, it is relatively arbitrary. For the purposes of these calculations p has been set at 0.27 (that is, 27%). The value of d can be set at 0.05, to indicate that we will accept a precision of our estimate within 5%; that is, the true proportion of children with severe diarrhoea treated at a participating hospital is between 22% and 32%. Incorporating these estimates into the equation yields a sample size of 302. That is, the sample of households should be large enough to include 302 children who may have severe diarrhoea that might result in hospitalization.

Since the study design includes cluster sampling, the effect of the cluster design must be factored in to the sample size. The *design effect* accounts for possible clustering of cases that one would expect. Few data exist which would help to help quantify this effect for rotavirus hospitalizations, although health utilization may certainly be similar within closely situated households. A design effect of 2, often used when no direct data are available, has been adopted in the present calculations. Instead of 302 children, therefore, the survey requires 604 children. In order to have equal numbers of children from each cluster this number is rounded down to 600 to give 20 per cluster.

Annex 3:
Table of random numbers

Method for choosing a random number from the table

1. Using a currency note, select a single digit random number between 0 and 9 to identify a column. Select a two-digit random number to identify a row (note that the numbers 01–09 count as two-digit numbers). The five digit number that is at the intersection of the column and row is the starting point.

2. Read to the right across that row from the starting point until you come to a five digit random number that is equal to or smaller than your sampling interval.

	Column									
	0	1	2	3	4	5	6	7	8	9
Row										
01	19289	93144	95340	30285	11083	88238	70387	97121	68924	78828
02	52013	34168	50026	63695	97161	76180	30119	21059	24233	19072
03	51923	17987	40495	36750	90764	84754	25504	69325	14494	28615
04	85188	35557	59556	17835	49317	30957	68224	83494	68361	63836
05	33865	60916	85080	61805	41162	64238	26199	77822	75933	61311
06	55811	82788	43985	16848	50340	87732	54580	58581	15339	98337
07	16650	86180	10382	65131	48434	38562	02875	99975	07213	27155
08	12800	48589	80380	74042	25345	70783	58110	47579	38287	63004
09	36653	03457	17568	23186	79328	35509	63547	46628	64067	76684
10	27238	05858	46983	49613	96575	57242	32000	82228	20696	67929
11	03769	63510	21463	16059	89100	64232	70747	97079	18500	47826
12	36094	09879	80907	78051	30458	01722	91738	55742	61922	86259
13	47274	65956	41027	89573	59260	41175	45136	94124	47732	01105
14	06728	25795	54919	80457	25401	63698	93399	59861	35281	58867
15	18067	74766	10773	13910	32803	57406	70427	78357	45716	24485
16	28519	10640	92569	17874	30560	14098	67406	93728	53203	78583
17	84332	32264	01524	93786	00692	79817	80680	74503	95874	94716
18	55310	08997	52383	39182	16561	68922	29334	70000	45258	58807
19	42341	75993	97579	07503	65899	15260	25739	56852	52541	89205
20	96644	54398	49707	40193	52321	95949	51268	54025	38083	03049
21	20644	04581	37056	26382	35641	29688	44475	42118	01100	81046
22	46597	56716	35108	37190	78569	86025	92893	88353	22636	88374
23	50543	29533	46259	37429	71060	36121	04913	37496	97095	71557
24	38658	41814	32171	76513	89135	39217	07240	82646	11085	26858
25	20054	42726	57198	90448	20618	28893	19375	17367	56035	07123

The Department of Vaccines and Biologicals was established by the World Health Organization in 1998 to operate within the Cluster of Health Technologies and Pharmaceuticals. The Department's major goal is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases.

Five groups implement its strategy, which starts with the establishment and maintenance of norms and standards, focusing on major vaccine and technology issues, and ends with implementation and guidance for immunization services. The work of the groups is outlined below.

The *Quality Assurance and Safety of Biologicals team* team ensures the quality and safety of vaccines and other biological medicines through the development and establishment of global norms and standards.

The *Initiative for Vaccine Research* and its three teams involved in viral, bacterial and parasitic diseases coordinate and facilitate research and development of new vaccines and immunization-related technologies.

The *Vaccine Assessment and Monitoring team* assesses strategies and activities for reducing morbidity and mortality caused by vaccine-preventable diseases.

The *Access to Technologies team* endeavours to reduce financial and technical barriers to the introduction of new and established vaccines and immunization-related technologies.

The *Expanded Programme on Immunization* develops policies and strategies for maximizing the use of vaccines of public health importance and their delivery. It supports the WHO regions and countries in acquiring the skills, competence and infrastructure needed for implementing these policies and strategies and for achieving disease control and/or elimination and eradication objectives.

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