3rd African Rotavirus Symposium

15 - 17 September 2002, Accra, Ghana

The following abstracts are from talks presented at the 3rd African Rotavirus Symposium held in Accra, Ghana, on 15 - 17 September 2002. This Symposium was the culmination of 5 years of research, training and collaboration between African researchers. In 1998, the World Health Organisation (WHO) advised that the surveillance and diversity of rotavirus strains in Africa needed urgent attention. With a small research grant made available by the WHO the concept of the African Rotavirus Network was born.

In this time, during the course of four Rotavirus Workshops, 34 African scientists, technicians and students from 18 different countries have been trained in basic techniques for the identification and characterisation of rotaviruses from across the continent. Over 15 000 stools have been processed in the MRC Diarrhoeal Pathogens Research Unit at MEDUNSA. Almost one-third of these were positive for rotaviruses and most have been characterised at the genomic and antigenic levels.

In addition, the African Rotavirus Network has helped in the training of four doctoral students and another four are currently in training. Master's dissertations have also been successfully completed and several are currently ongoing.

This research is extremely relevant and will make a significant contribution to the possibility of attaining rotavirus immunisation for African children. It has recently been estimated that approximately 110 000 - 150 000 African children under the age of 5 years die annually due to rotavirus infection. This is one region of the globe where a rotavirus vaccine is urgently required.

The ongoing strain surveillance and characterisation of rotaviruses in Africa, as discussed at this Symposium, forms a basis to extend rotavirus research in this region. Two new rotavirus vaccine candidates are approaching licensure — one is currently being tested in South Africa. For the implementation and integration of these vaccines into national EPI programmes, we need to generate the requisite information on rotavirus-associated burden of disease and advocacy.

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Epidemiology of rotavirus infection in South Africa

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Rotavirus infection is associated with acute infantile gastro-enteritis in infants and young children globally. In the developing world, rotavirus is associated with high levels of morbidity and mortality and is estimated to account for 650 000 deaths in young children annually. Approximately a quarter of these deaths occur in African children, yet the epidemiology of rotavirus infection and the characterisation of rotavirus strains in Africa is poorly understood.

In South Africa, rotavirus infection has been shown to be associated with approximately 25% of all diarrhoeal admissions to hospital. Rotavirus infection predominantly occurs in infants less than 12 months of age (83%) and has a peak in shedding during the cooler, drier months of the year. A secondary peak during the spring has been observed. Clinically rotavirus infection has been statistically associated with increased fever and vomiting and dehydration in these children.

The circulating VP7 serotypes and VP4 genotypes have been determined in various regions of South Africa and show a geographic specific distribution. Although in the past, P[8]G1or G4 strains predominated, P[4]G2 strains occurred in peaks every 3-4 years, and sometimes contributed to unusually significant rotavirus seasons. More recently, rotavirus strains with P[6] genotype have become common and novel VP7 serotypes are occurring across the country. G9 strains have been reported from Cape Town to Vendaland. The circulating rotavirus types observed in this study add to the knowledge of the natural history of rotavirus infection and should be considered when considering future vaccine strategies.

Epidemiology of rotavirus infection in Saharan Africa

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Rotavirus is the single most important etiological agent implicated in severe dehydrating diarrhoea especially in developing countries. In Saharan Africa, some studies have documented the high prevalence of rotavirus but we have a very few information about appearance of infection in this region. In this paper we would like to present the results of some published studies in Tunisia, Morocco, Algeria and Egypt about prevalence of rotavirus infection, age distribution, seasonal trend, clinical data and the circulating rotavirus strains in Sahara Africa.

The prevalence of rotavirus infection varies between 17 - 40% with a median at 27%. The age distribution showed a predominance of rotavirus infection in children less than 2 years of age. Rotavirus

infection was found to occur predominantly in the cool season in Saharan Africa. Genomic analysis of the rotavirus strains detected showed some differences among some countries in Sahara Africa. In some regions (such as Casablanca in Morocco), a high diversity of the rotavirus RNA electrophoretypes was noted. This contrasts with the situation in Tunisia where limited strains where seen to be circulating in the city.

In limited studies to examine the distribution of the VP7 and VP4 genotypes in Egypt and Tunisia, a predominance of G1P[8] and G4P[6] strains were observed. The common rotavirus strains were observed and unusual strains were not identified.

The analysis of clinical data in rotavirus diarrhoea in Tunisia showed a range of symptoms from asymptomatic infection to high severity with severe dehydration with even death. The most common clinical signs and symptoms were diarrhoea, vomiting and fever with some respiratory signs witch caused hospitalisation in 68% of cases.

Rotavirus infection in a rural community in Ghana

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Background: Diarrhoea continues to be a great cause of infantile mortality and morbidity worldwide. Rotaviruses have been identified as the single most important cause of infantile diarrhoea and responsible for more that 25% of all diarrhoea related deaths. Rotavirus vaccines are being presently developed and tested to help alleviate the burden of rotavirus-associated disease. Basic information on the epidemiology of rotavirus infection in a community will be crucial for the evaluation of these vaccines in selected communities. This paper reports on the epidemiology of rotavirus infection in the Kassena Nankana district of Ghana.

Methods: Diarrhoeic stools samples, basic demographic data and clinical information were obtained from children under 2 years of age on presentation at selected health facilities in the Upper East Regions of Ghana during the rotavirus surveillance study. The Upper east region is very rural with the main subsistence\being agriculture on small family holdings. The stools samples were analysed for rotavirus by ELISA and all rotavirus strains genotyped by RT-PCR.

Results: A total of 2085 episodes of diarrhoea were recorded out of which 834 (40%) were found to be shedding rotaviruses. Diarrhoea episodes increased with age and was much more common in males. Rotavirus infection was seasonal with peak infection during the cool dry months of November to February. Infection was highest in children aged between 6 – 18 months. Severity of diarrhoea was associated\with rotavirus infection. Thirteen different G/P combinations were observed with G2P[6], G3P[4] and G9P[8] constituting more than 54% of strains typed. Rotavirus with the G9P[8] genotype was the predominate genotype detected in 1999 and 2000.

Conclusion: Rotavirus infection was common and increased with age in the Kasena Nankana District and episodes with severe Versikari scores were more likely to be linked to rotaviruses. The study demonstrates a great diversity of rotavirus strains circulating in Ghana. This has implication for vaccine design.

Community based surveillance of rotavirus infections in Guinea-Bissau

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The strategy for rotavirus vaccine development is to mimic the immunity provided by natural rotavirus infection. However, existing protection estimates range widely. Rotavirus vaccines are designed to provide specific immunity against the most prevalent viral surface proteins. As there is a substantial geographical and temporal variation in the distribution of the different rotavirus serotypes, extensive rotavirus strain surveillance is required to guide the incorporation of relevant antigens in future vaccines. To address these issues, data on rotavirus pathogenicity, morbidity, mortality, strain diversity and on naturally acquired immunity were generated during extensive epidemiological studies in Guinea-Bissau, one of the poorest countries in the world. Although rotavirus infections were confined to four epidemic winter months, at least 74% of all children would experience such infections before their second birthday. To give an indication of the potential impact of a future rotavirus vaccine, national program, we assessed the mortality from rotavirus infections in Guinea-Bissau and extrapolated the figure to Sub-Saharan Africa. By multiplying the proportion of cases with severe rotavirus diarrhea (hospital data) with the diarrheal mortality, the yearly rotavirus mortality was estimated to be 3.4 per 1000 in infants and 0.8 per 1000 in children aged 1-4 years. These estimates were applied to the approximated Sub-Saharan child population, and a total of 145,000 deaths due to rotavirus were estimated to occur yearly in this region. Characterization of the infecting rotavirus strains revealed a wide range of different G and P genotype combinations. Moreover, there was a substantial year-to-year shift in predominating genotypes. The globally most common G and P combinations were underrepresented, whereas the uncommon P[6], G2 type constituted a considerable fraction. The estimated 52% and 70% protection conferred by natural rotavirus infection against reinfection and rotavirus diarrhea, respectively, supports current vaccine development strategies. Although statistically somewhat imprecise, the apparent reduction in protection from one season to the next underscores the need for follow-up for a minimum of two seasons subsequent to the evaluation of a vaccine candidate, in order to estimate any such loss of protection. The present review of studies corroborates the idea that rotavirus is an important cause of diarrheal disease in children less than two years of age, even in areas where the incidence of infections with other enteropathogens is high. Immunization with an effective vaccine, which mimics the protection conferred by natural infection, is probably the best approach to reduce rotavirus morbidity and mortality and is likely to have a substantial public health impact in developing countries.

Rotavirus infection in domesticated animals: An important reservoir of infection in Africa?

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Introduction: Rotaviruses affect the young of many domesticated and companion animals. As the virus is able to survive for extended periods in the environment, animal rotaviruses may cross the species barrier and infect children. In African studies, approximately 25-30% of human rotavirus strains cannot be typed for the VP7 and VP4 by the routine RT-PCR typing methods. Up to 4% of these untypeable strains have a subgroup I specificity with a long polyacrylamide gel electrophoresis (PAGE) pattern, which together are indicative of animal rotaviruses. Human G6 rotaviruses have been detected in South Africa and recently a G5 rotavirus was identified in Cameroon. Studies of animal rotaviruses are therefore important in Africa, where a close proximity of man and his domesticated animals exists. Rotaviruses were first identified pigs with diarrhoea in South Africa in 1977. Additional studies on porcine rotaviruses have only been undertaken in Zimbabwe and Nigeria where they were associated with diarrhoea in 33% and 45% of cases. The VP6 subgroup (SG) specificity was ascertained in the Nigerian strains with 27% SGI and 40% SGII.

Materials and methods: Faecal specimens were collected from 20 farms throughout South Africa with an overall incidence of 33,7% rotaviruses in the litters. At one farm, which was visited from 1993 to 1996, the VP7 characteristics determined were G5 (45,2%), G3 (9,5%), and mixed genotypes (8,3%); 31% remained untyped. All the strains at this farm were of SGI specificity and 67% of the VP4 genotypes were P[6] with the remainder being P[7]. At the other 19 farms throughout South Africa, G5 strains also predominated (43,5%) followed by G3 (16,7%). In contrast, a P[7] VP4 was detected more commonly and 36,6% of the strains had an SGII VP6. Problems to amplify the VP7 gene were overcome with the development of a degenerate primer for the 3′ terminus of the gene.

Results: Bovine rotaviruses were detected in 30% of calves in Morocco although exposure to the virus was identified serologically in 66% of herds throughout the country. Two other studies indicate that 17% of dairy calves were infected with the virus in Ethiopia, whilst a high percentage of cattle in Western Zaire had been exposed to rotavirus. No further characterisation of the virus was done. In the South Arica specimens were collected from two beef and two diary herds. Rotaviruses were identified in 74% of the specimens, 50% of which were typed as G6 after hybridisation to G6, G8 and G10 probes. Caprine rotaviruses were isolated at MEDUNSA and the strains were characterised as G6P[14]. Sequence analysis of the VP4 and VP7 genes indicated that they shared a high level of homology with two human rotavirus strains namely PA169 and HAL1166, thus raising the possibility of interspecies transmission of the virus.

Discussion: There is a dearth of literature on the African continent about the incidence and characterisation of rotaviruses in domesticated animals. In view of the close proximity of people with their animals and the detection of rotaviruses with putative "animal" characteristics causing severe gastroenteritis in African children, it is important to expand such surveillance.

Genetic Diversity of Human Rotaviruses in West Africa

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Introduction: Human rotaviruses have been identified as the most common cause of diarrhoea in children worldwide and responsible for 6% of deaths of children less than 5 years in developing countries. Rotavirus vaccines are being developed and soon will be evaluated in trials in developed countries including African countries. Knowledge of the epidemiology of rotavirus infection and circulating strains is hence essential for the evaluation of these vaccines The paper reports on the molecular epidemiology of rotavirus infection in West Africa over 2 rotavirus seasons spanning 1998 to 2000.

Materials and methods: Diarrhoea stool samples were collected from children less than 5 years who presented with diarrhoea in selected hospitals in the West African cities Ouagadougou (Burkina Faso), Navrongo (Ghana), Abidjan (Ivory Coast), Ibadan, Lagos and Kano (Nigeria), West and South West Province (Cameroon) from January 1998 to December 2000, as part of the African Rotavirus Network rotavirus surveillance programme. Rotavirus infections were determined by ELISA\and the genotypes by RT-PCR.

Results: The putative neonatal VP4 P[6] type was very common inn the region and varied from a high of 82% in detected rotavirus strains in Ghana to 26% in Guinea Bissau. The global strains G1,G2 and G3 and the new strains G8 and G9 were very common. Rotavirus strain G4 was notably absent and strains with the with unusual G/P combinations G3P[6] and G8P[6] with short electropherotypes and G2P[6] were present. P[4] genotypes were uncommon except in Ghana and Cameroon

Conclusion: A high diversity in rotavirus genotypes and the presence of strains with unusual G/P combinations were detected in this region of Africa. The emergence of strains with G9 VP7 specificity, the high proportion of untypable strains the observance of strains with unusual G/P combinations may be an indicator of an on-going genetic reassortment in the region.

Molecular epidemiology and characterisation of rotavirus in Cameroon

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Introduction: Rotavirus is the most common cause of severe infantile diarrhoea worldwide accounting for a quarter of all the deaths due to diarrhoea among children less than 5 years old in developing countries. The objective of this study was to investigate the epidemiology of rotavirus infections in Cameroon and to determine the characteristics of the strains of rotavirus circulating in Cameroon.

Methods: Faecal specimens were collected from 890 infants and children under 5 years of age who presented with diarrhoea to clinics and hospitals in the South West and Western Provinces of Cameroon between June 1999 and May 2000. Group A rotavirus antigen was detected by enzyme immuno-assay (Rotavirus IDEIA™, DAKO, UK). Viral RNA was extracted by phenol-chloroform treatment from the rotavirus positive stools and electrophoresed through 10% polyacrylamide gels overnight. The viral RNA bands were subsequently visualized by silver staining. In addition the viral RNA was reverse transcribed and strains genotyped with specific primers. Selected untypeable strains were cloned and sequenced for G-type determination.

Results: Rotaviruses were detected in 195/890 (21.9%) of the diarrhoeal specimens. Rotavirus infection occurred throughout the year with no seasonal peak observed. The detection rate of rotavirus was much higher in the dry highlands of the Western Province (26.7%) than in the Coastal South West Province (16.9%). Thirteen RNA electrophoretic patterns were observed, of which 76.6% were long and 19.4% were short. Subgroup II strains occurred more commonly than Subgroup I strains (61.8% vs. 14.5%). Ten rotavirus strains (6.6%) reacted to both Subgroup I and II group-specific monoclonal, whilst 25 rotavirus strains (16.5%) could not be assigned any specific subgroup. The four globally common serotypes P[8]G1, P[4]G2, P[8]G3 and P[8]G4 accounted for only 36 (42.9%) of the strains typed, while unusual and partially characterized strains accounted for 24.2% and 29% respectively. The predominant G and P-types were P[8] (73.3%) and G1 (45.5%) and the most commonly isolated genotype was G1P[8] (32.6%). Of the 10 untypeable G-types that were cloned and sequenced, four were determined by sequence analysis to be G1, four as G9 and one each of G4 and G5 genotypes.

Conclusion: Some strains could not be genotyped indicating the need for extended surveillance of rotavirus genotypes in Cameroon and other African countries. This study is the first in Cameroon and defines the epidemiology of rotavirus infection. Also, rotavirus G5 was identified in humans and is being reported in Cameroon and outside South America for the first time.

Molecular techniques for characterisation of rotavirus strains in Africa

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Background: In Africa, rotavirus gastroenteritis remains a major cause of high mortality among infants and young children. Therefore, the application of a safe and effective rotavirus vaccine will expectedly reduce the level of mortality and morbidity attributable to the disease. To be effective, such vaccines will need to take into cognizance the diversity of circulating strains of rotavirus types in the target population in various countries of the continent. However the application of new molecular diagnostic techniques has shown varying diversity of rotavirus strains in African settings posing serious challenges to future vaccine application.

Materials and methods: A literature search on characterization of rotavirus strains in Africa was conducted. Published studies were reviewed to obtain data on techniques utilized for electropherotype, serotype and genotype distribution of rotaviruses in Africa.

Results: In most African countries where rotavirus has been reported, characterization methods have utilized serological techniques of antigen detection and serotyping ELISA as well as electropherotyping by PAGE. Whereas the serological and molecular methods have only partially characterized the virus leaving out a significant number of strains untypeable, recent reports from Egypt, Ghana, Malawi, Nigeria and South Africa utilizing PCR and sequencing analysis have revealed unusual diversity of rotaviruses in the continent. PCR techniques is one of the most sensitive approaches to characterizing rotavirus strains, albeit infrequently leading to errors in typing of rotaviruses due to small numbers of mutations in the primer binding region. However, sequencing analysis have permitted the design of new primers that have led to the identification of previously uncommon strain types in unusually high proportion than previously thought, thus altering the epidemiology of the viruses in Africa.

Conclusion: The broad applications of advanced molecular techniques of PCR and sequencing analysis to further characterize rotavirus strains in several African countries across species boundary will no doubt advance our knowledge and appreciation of the burden of rotavirus strain diversity and provide fundamental data necessary for vaccine design and implementation in Africa.

Emergence and characterisation of serotype G9 rotavirus strains from across the African continent

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In sub-Saharan Africa, one out of every 30 children born will die from diarrhoea before the age of five. Rotaviruses contribute up to 24% of all childhood diarrhoeal episodes in Africa. Efforts to

improve sanitation and provide clean water have not decreased the high mortality due to rotavirus infection in developing countries, focusing the need for an effective rotavirus vaccine. A properly administered vaccine could potentially prevent one in 20 children dying.

Rotavirus particles are composed of three protein layers surrounding 11 distinct segments of dsRNA and are classified according to three antigenic markers. The VP6 inner capsid protein specifies group and subgroup epitopes, while the VP4 outer spike protein and the VP7 outer capsid protein, carry epitopes specifying neutralizing antibody responses. VP7-specific serotypes are termed G types and VP4-specifc serotypes are termed P types. At least 10 G types and 11 P types are know to infect humans, although serotypes G1P[8], G2P[4], G3P[8] and G4P[8] were thought to be of major epidemiological importance due to their worldwide distribution

First identified in the United States in 1983, serotype G9 human rotavirus strains are currently being detected globally. Characterization of global G9 strains have revealed that most display the P[6] genotype, subgroup I specificity and the 'short' RNA profile. Serotype G9 strains have been isolated in various African countries, including South Africa, Botswana, Malawi, Kenya, Cameroon, Nigeria, Ghana, Ivory Coast, Guinea-Bissau and Libya. African G9 strains from South Africa, Botswana, Ghana and Cameroon were analyzed to investigate genogroup characteristics and the genetic composition of the VP7 and VP4 outer capsid genes. While serotype G9 strains cause localized outbreaks in certain areas, large-scale epidemics similar to those seen in Bangladesh have not been reported. Continuous surveillance of circulating rotavirus strains is vital to aid in vaccine development and to prepare for future vaccine implementation.

Evaluation of non-human primates as models for testing rotavirus candidate vaccines

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Background: Previous studies have indicated that antibodies to a rotavirus (SA11) were prevalent among a wide spectrum of animals including New and Old World primates. A number of rotavirus vaccines (including rhesus rotavirus, RRV) are at different stages of development and these vaccines need to be tested in animal models. In order to critically evaluate the safety and efficacy of these vaccines, an appropriate non-human primate model is desirable.

Materials and methods: Studies in our laboratory have focused on development of the olive baboon (*Papio anubis*) and the vervet monkey (*Cercopithecus aethiops*) models for human rotavirus infection. Initial studies involved screening for natural rotavirus infection in 21 baboons and vervet monkeys maintained at the Institute of Primate Research (IPR), Nairobi, Kenya. Subsequently, we tested the safety and efficacy of the RRV in baboons. Also, inoculating seven primates conducted rotavirus challenge experiments with human rotavirus recovered from a child suffering from rotavirus diarrhoea

Results: The presence of naturally occurring antibodies was demonstrated in monkeys maintained at IPR. Parenteral vaccination of pregnant baboons resulted in production of specific rotavirus antibodies (IgA, IgG and virus neutralising antibodies) in baboon serum and breast milk. These experiments showed the excretion of rotavirus in the stool of 5 out of 5 vervet monkeys and in 1 out of 2 baboons. Further characterization by SDS-PAGE, EM and RT-PCR of the excreted rotavirus showed similarities with the inoculum. The inoculated animals also showed presence of antibody responses and virus neutralizing antibodies

Conclusion: Our results showed that African primates are potential models for evaluating rotavirus candidate vaccines and understanding potential clinical manifestation of vaccination and rotavirus diarrhoea, including intussusception.

Lack of association of rotavirus infection and HIV status in young children in Nairobi, Kenya

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Background: Although diarrhoeal disease is a presenting symptom for HIV infection, the aetiology of diarrhoea in HIV-infected children is not well understood. In Africa, rotavirus is one of the most common aetiological agents associated with diarrhoeal disease in young children.

Materials and methods: In this study, we examined stool samples from 207 children for enteric viral pathogens. One hundred and thirteen children were infected with HIV while 94 children were visiting clinics in Nairobi for treatment for diarrhoea. The samples from HIV-infected children included 43 from those with diarrhoea and 70 from those without diarrhoea. Diagnosis was performed using commercially available enzyme immunoassays for rotavirus, astrovirus and adenovirus. The rotavirus strains were examined further by polyacrylamide gel electrophoresis and PCR of the RNA genome to monitor the strains in HIV-infected children versus those HIV-negative children with rotavirus-associated diarrhoea.

Results: There was no significant difference in prevalence of the

three viruses in diarrhoea samples from the two groups of HIV infected or uninfected children. Among the HIV infected children, rotavirus was more prevalent in children with diarrhoea than in those without (23.3% versus 2.9%; $p = 0.0004$). The results also show the prevalent rotavirus strain was G3P[6] (30.4% of all type rotaviruses), and was common in both HIV infected and HIV uninfected children.

Conclusion: Rotavirus, and the other viral enteric pathogens, was not significantly associated with the HIV status of young children presenting with diarrhoeal illness. Rotavirus infection was more commonly detected in children, whether HIV positive or negative, presenting with diarrhoea. No rotavirus strain was associated with HIV status. Rotavirus was detected as a common enteric infection in young children with diarrhoeal illness but there was no correlation with the HIV status of the children.

Clinical severity associated with rotavirus VP7 serotypes and strains

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Background: Human rotavirus is one of the commonest viral agent for severe childhood diarrhoea in Ghana contributing to a significant number of morbidity and mortality in children. Earlier studies have shown that rotavirus infection occurs as early 4 months after birth and repeated infections are common with varying disease outcome. Immunity to natural rotavirus infection is however known to be predominantly homotypic and usually the first infection is the most severe. The possible association between VP7 genotypes and disease outcome in a rural community over a 2 year study period is reported.

Methods: Diarrhoea stool specimens were collected from children under 2 years of age with diarrhoea brought to health facilities in the Upper East Regions of Ghana, a predominantly rural area. Demographic data were taken at recruitment and clinical data extracted from hospital records. All the stool samples were examined for rotavirus particles by commercial ELISA and the electropherotypes determined by PAGE. The VP7 genotypes of the rotavirus strains were determined by RT-PCR.

Results: A total of 2085 episodes of diarrhoea were recorded out of which 834 (40%) were found to be shedding rotaviruses. Severe disease outcome was recorded in 104 (12.5%) of children shedding rotavirus compared with 88 (7.0%) in non-rotavirus diarrhoea episodes. The relative risk of hospitalisation due to rotavirus infection was 1.88 (χ^2=19.6, p=0.0000). Severe diarrhoea was associated with rotavirus strains G2 (19%), G3 (20%) and G9 (21%) and predominantly in children between the ages of 5-12 months. No severe disease could be associated with G1 rotavirus strains.

Conclusion: Rotavirus strains with VP7 genotypes G2, G3 and G9 were associated with severe disease. The observance that conventional strains (G1and G4) were not significantly associated with severe disease, and that G4 strains were very uncommon highlights the need to continue surveillance of emerging/re-emerging strains especially in Africa. G9 strains may have to be considered for inclusion in future rotavirus vaccines

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Estimating the burden of rotavirus disease in Gauteng: Hospital utilization in the Ga-Rankwa/Madibeng district

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Introduction: Rotaviruses are the common cause of severe diarrhoea and dehydration among children in both developed and developed countries. Conservative estimates indicate that more than 600,000 young children will die each year from rotavirus infection with the majority in the developing world. An estimation of burden of rotavirus disease will help in the advocacy needs for a rotavirus vaccine as a public health intervention in developing communities. A study was conducted on health services utilization in the Ga-Rankuwa and Madibeng Districts to seek information on attitudes and practices towards gastroenteritis and its treatment, a vital input for the design of a burden of disease study.

Method: Eight hundred questionnaires were administered to mothers/caretakers of children less than 24 months of age in Lethabile, Oukasi, Soshunguve and Bertoni during the peak rotavirus season of April-May-June 2002. In all 200 questionnaires were administered in each locality. The data was double entered into a database using the Epi-info (CDC) programme. The data was cleaned and analysed with the STATA statistical package.

Results: Preliminary results show that 70% of respondents lived in shacks and only 26% in modern housing. Potable water was available to 73% of the population whilst 37% had flush toilets, 60% utilised pit latrines and more than 90% had electricity connected to their homes. Diarrhoea was a common ailment and more than 30% of children had had diarrhoea in the last 28 days. The majority of mothers/caretakers (>75%) preferred taking their children to the health clinics rather than to hospitals (6%). Money was not a major reason for non-use of health facilities as only 9% responded in the affirmative and respondents attributed it to other reasons. More than 90% of mother/caretakers interviewed had had either a primary education or had completed high school.

Conclusion: This study has shown that diarrhoea is a common ailment in this district with more than 30% of children less than 24 months of age experiencing at least one episode of diarrhoea during the previous month. Although access to health facilities was available, only 6% of mothers/caretakers took their children to the hospital during diarrhoeal episodes. Consequently children seen at hospitals with diarrhoea may not be the true reflection of the burden of diarrhoea disease in the Ga-Rankwa district. It may be important to include clinics in the district in the estimation of disease burdens due to diarrhoea in children. This information is important not only for this study but also for the implementation of ORS programmes in the community.

Rotavirus vaccines and intussusception. What it means for infants in Africa

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Introduction: Rotavirus is associated with 140-150 000 deaths in African children every year. The first licensed rotavirus vaccine (RotaShield®, Wyeth Lederle) was withdrawn after only one year due to a reported association with intussusception in the children who received the vaccine. In this study, a literature search was conducted to estimate the background rates of intussusception in young South African children and to examine the epidemiology of this disease in Africa.

Methods: A literature search through MEDLINE revealed that most studies investigating intussusception in children have been conducted in South Africa and Nigeria. These papers were extracted and examined to determine the background levels, the clinical presentation and the epidemiology of intussusception in Africa. A retrospective study of intussusception was performed at three large academic hospitals in Pretoria.

Results: A specific disease entity called "tropical intussusception" was described which occurs predominantly in adults in Africa. Amongst children, intussusception occurs predominantly in male infants and is observed between 3 to 11 months of age. The clinical differentiation of intussusception in Africa is compounded by clinical similarity to endemic syndromes such as amoebiasis and dysentery in young children. Associated problems with intussusception include the late presentation of the child, a delay in diagnosis and treatment. Surgical interventions were reported commonly.

Conclusion: Intussusception is a apparently rare event in Africa but is associated with relatively high mortality due to the associated problems with differential diagnosis and standard methods of treatment. However, the relative risks of rotavirus infection far outweigh these and constitute a major health problem to the young children of Africa. While new rotavirus vaccine candidates are under development, prospective studies on background levels of intussusception should be conducted in specific locations in Africa.

Human astroviruses in Africa: Is there a problem?

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Astroviruses (AstVs), which include both human and animal pathogens, are single-stranded RNA viruses classified within the family Astroviridae. AstV infection appears to be species specific and to date cross-infection between humans and animals has not been documented. Human AstVs (HastVs) were first detected by electron microscopy in diarrhoeal stool specimens from neonates during an outbreak in a maternity unit. Since the advent of enzyme immunoassays (EIA) and reverse transcriptase-polymerase chain reaction for the detection of HastVs these viruses have been identified as a common cause of sporadic episodes and outbreaks of diarrhoea in all age groups, with the young, the elderly and immuno compromised being at greatest risk. In the USA HastVs were identified as the most common cause of viral diarrhoea in human immunodeficiency virus (HIV)-infected patients. In general HastV infection has been reported in 2% to 16% of children hospitalized with diarrhoea and in 5% to 17% of diarrhoea in the community. There are limited data on astrovirus infection in Africa. In children in Malawi early electron microscopy-based investigations revealed a prevalence of 1.2% while subsequent EIA-based investigations showed a prevalence of 1.9% in hospitalized patients and 2.3% in outpatients. Disease in patients co-infected with HIV appeared to be more severe. A prevalence of 5% to 7% was demonstrated in two independent studies in South Africa. In one of these studies HastVs were identified as the second most important viral diarrhoeal pathogen, surpassed only by human rotaviruses. HastVs have also been associated with an outbreak of gastroenteritis in a South African child care centre. Early seroprevalence studies in a Zulu population demonstrated a similar antibody acquisition pattern to that of the United Kingdom. In Nigeria and Ghana EIA-based investigations on paediatric diarrhoeal specimens revealed prevalences of 6.7% and 9% respectively. A study in rural Egyptian children HastV infection, often associated with severe dehydration, was found to be as common as rotavirus infection. A large percentage (38%) of infections were in children <6 months of age. HastVs are transmitted by the faecal-oral route with person-to-person spread being the most important route of infection. Outbreaks have however been associated with faecally contaminated food and water. The detection of HastVs in surface water used for domestic and recreational purposes in South Africa has identified faecally contaminated water as another possible source and resevoir of infection in the African setting.

To date 8 serotypes of human astvs (hastvs), which correlate with genotypes, have been described. the distribution of hAstVs differs by year and location. In general, hAstV type 1 (hastv-1) is the most commonly identified serotype worldwide. In Europe and North America, hAstV-2 to hAstV-5 seem to be less common and hAstV-6,7 and 8 are rarely detected. Limited molecular epidemiological studies in Africa have identified hAstV-1 as the most frequent serotype while hAstV-8 appears to be more common than in the rest of the world. It has yet to be established whether this observation is of clinical significance. The initial data suggest that hAstVs are an important viral diarrhoeal pathogen in Africa and that there are differences in the epidemiology to that in the rest of the world. Further epidemiological studies in different geographical and clinical settings are required to determine the true burden of astrovirus disease.

Molecular characterisation and seroprevalence of caliciviruses in Africa

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Gastroenteritis remains a public health problem in both developing and developed countries. Rotavirus infection is particularly responsible for extensive morbidity and mortality in children. The caliciviruses are another group of enteric viruses forming an extensive family that is divided into 3 genogroups. They are known to cause both acute and sporadic outbreaks of gastroenteritis in both children and adults and may account for a number of previously unknown or unidentified causes of diarrhoeal illness in Africa. A number of methods have been used to determine calicivirus infection. Electron microscopy has allowed the visualisation of the small (33-35 nm) caliciviruses, as exemplified by (SV). The use of RT-PCR and direct amplicon sequencing can further characterise/identify caliciviruses as belonging to genogroup I or II noroviruses (with Norwalk virus (NV) and Snow Mountain virus (SM) as prototypic examples) or as being a genogroup III sapovirus. Recombinant viral antigen has become available to specifically detect caliciviral antigen or antibodies in enzyme immunoassays. Research has linked SM, NV, Mexico virus (MxV), Hawaii virus (HV) or Sapporovirus (SV) to various outbreaks of gastroenteritis in Africa. Use of EIA to show the humoral response to caliciviruses has indicated that children in Africa are commonly infected with caliciviruses soon after birth. It appears that MxV infection precedes that of NV and that by 4 years of age, the children show an antibody response to these viruses at similar titres to that found in adults. Unfortunately, neutralising antibodies do not appear to be elicited against repeat calicivirus infection. Similar immune responses were reported in children from Mexico and from other developing countries in south-east Asia. Examination of African stool samples by EM detected the presence of virus in between 2,6% and 9,2% of cases. Stools were also examined using EIA and RT-PCR to detect calicivirus. This indicated that 2,2% of Kenyan patients were excreting SV with only 0,1% excreting NV. Likewise, in South African outbreaks, NV was found to infect between 0,26% and 1,8% of patients, while MxV was more commonly detected, at an incidence between 2,7% and 4,3%.

Epidemiology of enteric adenoviruses in Africa

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Human adenoviruses are classified into 51 serotypes and six subgenera (A to F) with different tropisms. Adenoviruses are often recovered from the stools of young children with gastroenteritis and range from 3-14% of cases of paediatric diarrhoea in developed countries. The adenoviruses most commonly associated with infantile gastroenteritis include those of subgenus F (types 40 and 41) and to a lesser extent subgenus A (types 12, 18, and 31). Although several studies indicate the enteric adenoviruses as the second most import viral agent as a cause of infantile gastroenteritis, the epidemiology of these viruses is relatively unknown in Africa. Limited studies have been performed in African countries such as Malawi, Tanzania, and Algeria. In South Africa, enteric adenoviruses have been reported in 6.5% to 13.2% of paediatric diarrhoeal cases. The level of detection of adenoviruses in diarrhoeal stools is, however, probably under reported. A recent study has indicated the possibility that either previously unidentified or newly emerging adenovirus types are not detected by commercial diagnostic tests. In this paper, the importance of enteric adenoviruses associated with gastroenteritis in Africa will be reviewed.

Group C rotavirus infections in Africa

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Introduction: Rotaviruses are well known to cause acute infantile gastroenteritis. Group A rotavirus infections remain a major cause of severe diarrhoea in infants and young children and have been well studied. Group C rotavirus infections cause gastro-enteritis in children and adults and have been shown to have a worldwide distribution. In this study we reviewed published and unpublished data and articles pertaining to group C rotavirus infections in Africa.

Materials and methods: Diarrhoeal stool specimens were available from over 10 000 young children in 19 different African countries. The strains were screened by polyacrylamide gel electrophoresis of the viral RNA to detect the presence of rotavirus RNA typical of group A, B or C strains. Furthermore, specific stool collections were examined by a recombinant VP6 ELISA utilising a human group C rotavirus strain (Bristol strain) developed by Southampton Medical School.

Results: Group C rotavirus infections have been identified in six African countries where the stool samples from children between the ages of 0 to 60 months were screened using PAGE, ELISA and RT-PCR. Group C rotaviruses were identified in Cameroon (9%), Malawi (3,3%), Nigeria (2,7%), Kenya (1,7%), Ghana (0,6%) and South Africa (4,9%). In a current study in South Africa involving infantile and adult diarrhoeal stools, it was found that 6.6% of the screened stool samples were positive for group C rotavirus infections by ELISA. A sero–epidemiological study conducted in South Africa revealed that 34.4% of the study population were positive for group C rotavirus infections.

Discussion: The role of Group C rotavirus infections in humans has been considered to be insignificant in the global picture of diarrhoeal illness due to the apparent sporadic nature of the disease. In this study, Group C rotaviruses have been recovered from significant numbers of African children. With the development of more sensitive techniques and simpler ELISA-based assays, larger epidemiological surveys could be conducted to diagnose diarrhoeal disease of unknown aetiology.
