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FOREWORD

The NICD rotavirus surveillance programme has been in operation at five sentinel hospitals since the introduction of the rotavirus vaccine in 2009. Interestingly, the 2012 rotavirus season lasted only 15 weeks, three weeks shorter than the 2011 season and nine weeks shorter than the 2009 pre-vaccine season. The effect of the vaccine on the burden of diarrhoea in South Africa to date is described in this issue. Vaccines are the primary subject of this issue, which includes an update of polio eradication in Africa as well as an evaluation of the immunogenicity of HIV booster vaccines containing gp140 protein with MF59 adjuvant in the HVTN 073E vaccine trial. Also in this issue, the risk of human infection of the H5N2 and H7N1 Avian Influenza strains in South Africa is assessed based on a recent sero-survey. As always, contributors are thanked for their inputs, which I trust you too will find useful and interesting.

Basil Brooke, Editor

CONTENTS

ROTAVIRUS SURVEILLANCE IN SOUTH AFRICA, 2012

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Introduction

Rotavirus causes acute gastroenteritis accompanied by vomiting, fever and abdominal pain. The icosahedral rotavirus particle consists of 3 layers (core, inner capsid and outer capsid) and encapsulates a genome of eleven distinct segments of double stranded RNA. The outer capsid is made up of VP7 and VP4 proteins, which are able to independently elicit an immune response and were important epitopes during vaccine development. These proteins specify the G and P genotypes respectively, and to date 27 G and 35 P genotypes have been described. In human infections, five globally predominant (G1, G2, G3, G4 and G9) and two regionally pre-

dominant (G8 and G12) G genotypes circulate. For the VP4 protein, two globally predominant (P[8] and P[4]) and one regionally predominant (P[6]) P genotype tend to be associated with rotavirus infections.

Rotavirus infection is the most common cause of hospitalization as a consequence of dehydrating diarrhoea in children under 5 years.[1] The incidence of diarrhoeal disease in South Africa is estimated at 111.8 per 1000 children under the age of 5 years.[2] Based on WHO recommendations, South Africa incorporated the rotavirus vaccine into its expanded immunization programme (EPI) in 2009. Prior to the introduction of the vaccine, the National Institute for Communicable Diseases (NICD) introduced a sentinel surveillance programme for rotavirus at five sentinel hospitals including: Chris Hani Baragwanath in Gauteng Province, Dr. George Mukhari in Gauteng (serving Gauteng and North West provinces), Mapulaneng and Matikwana in Mpumalanga Province and Edendale in Kwazulu-Natal.

The main objectives of this surveillance programme are to describe the epidemiology of rotavirus infection and to monitor the effect of the rotavirus vaccine on the incidence of disease following its introduction in South Africa.

Methods

Children under 5 years of age who were admitted to any of the sentinel hospitals with symptoms of three or more loose stools within a 24 hour period were enrolled into the surveillance programme following informed consent. Case investigation forms were completed by a surveillance officer on which pertinent descriptors including patient identifying information, demographic and socioeconomic information, clinical information and patient outcomes were captured. A stool sample was collected from each case for subsequent rotavirus testing.

Testing of stool samples collected in 2012 was performed at the Virology Division, Center for Enteric Diseases (CEDv), NICD, and at the Diarrhoeal Pathogens Research Unit (DPRU), University of Limpopo Medunsa Campus. The stool samples were screened with the ProSpecT™ Rotavirus Microplate Assay (Oxoid, Basingstoke, UK).

Rotavirus positive samples were further characterized to determine the G and P genotype of each strain. Rotavirus dsRNA was extracted from each stool sample using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and genotyped using standardized RT-PCR methods and primers for G-specific (G1, G2, G3, G4, G8, G9, G10, G12) and P-specific (P[4], P[6], P[8], P[9], P[10], P[11], P[14]) genotypes.[3]

Results

A total of 632 cases of diarrhoea was reported to the rotavirus surveillance programme during 2012. Of these, 598 stool samples were collected of which 118 (20%) tested positive for rotavirus. Comparable detection rates were recorded in 2010 and 2011 (21% for both years). The start of the 2012 rotavirus season began in week 23 and the highest detection rate (75%) was recorded in week 26 with 6 out of 8 specimens testing positive for rotavirus. There was a steep decline in cases in week 38, signaling the end of the rotavirus season (figure 1). The 2012 rotavirus season started later (2 weeks) and finished earlier (2 weeks) than the 2011 season (figure 2). The introduction of the vaccine did not affect the age distribution of rotavirus infections, but instead resulted in an overall decrease in rotavirus disease in all age groups (figure 3).

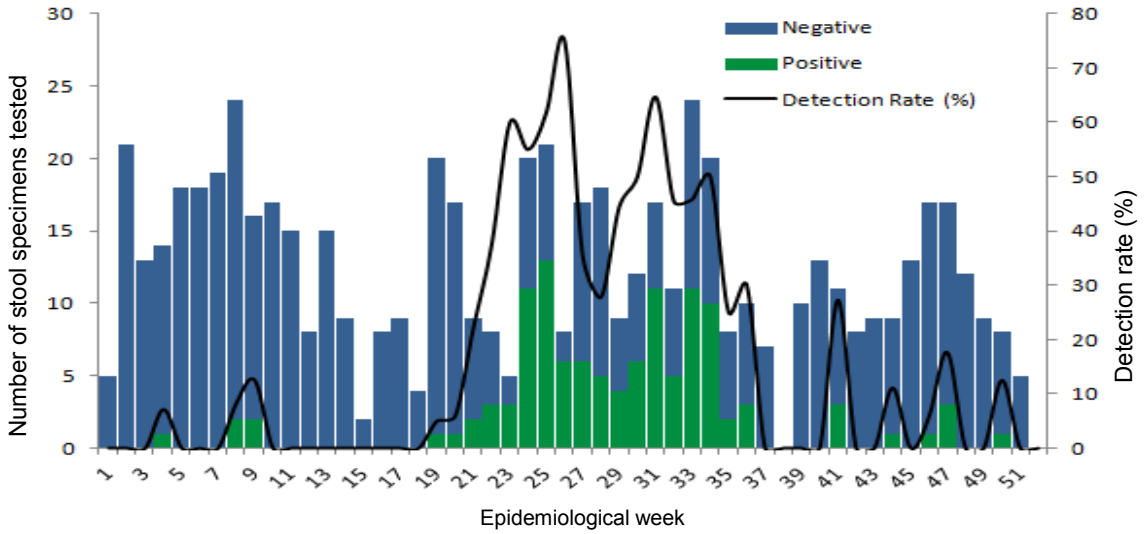

Figure 1: Number of stool samples tested and rotavirus detection rate (%) by week in children <5 years of age hospitalised for diarrhoea, South Africa, 2011.

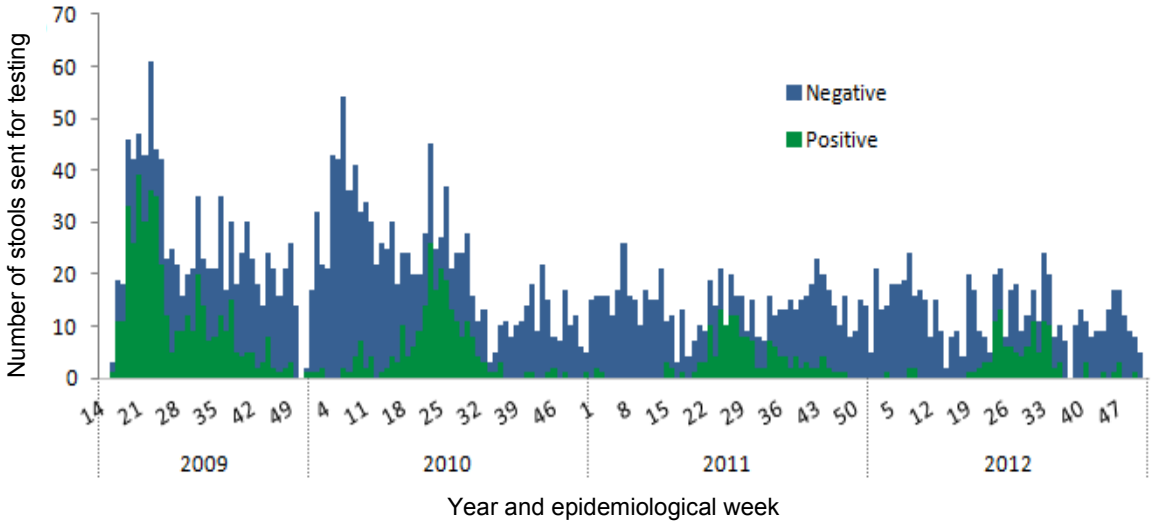

Figure 2: Number of rotavirus positive and negative stool samples tested by year for all sentinel surveillance sites, South Africa, 2009-2012.

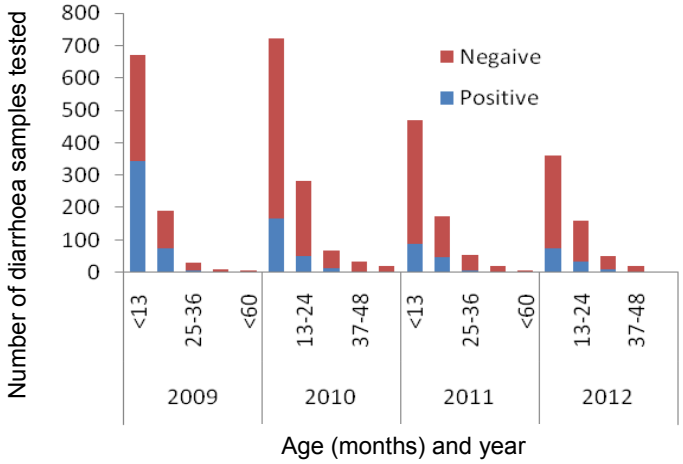

Figure 3: Age distribution of rotavirus positive and negative diarrhoea samples tested, South Africa, 2009-2012.

A total of 125 rotavirus strains was genotyped from the 2012 season to determine their G and P specificity. The results are summarized in table 1. For a second consecutive year, G12P[8] strains were predominant at all sentinel sites with G8P[4] common in the rural and coastal sites (Mapulaneng, Matikwana and Edendale) and the G2P[4] strains slightly more frequent in the Gauteng region (Chris Hani Baragwanath and Dr. George Mukhari).

Table 1. Summary of the G and P genotypes of rotavirus strains detected at sentinel hospitals in South Africa in 2012.

	CHBH		DGM		MP		MK		EdH		Total	
Genotype	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Rotavirus strains covered by the monovalent vaccine												
G1P[8]	1	3	0	0	1	6	0	0	0	0	2	2
G8P[8]	2	5	0	0	0	0	1	4	1	8	4	3
G12P[8]	11	28	18	56	4	25	16	64	5	38	54	43
G9P[8]	6	15	0	0	0	0	1	4	0	0	7	6
Total	20	51	18	56	5	31	18	72	6	46	67	54
Rotavirus strains not covered by the monovalent vaccine												
G2P[4]	7	18	6	19	1	6	2	8	0	0	16	13
G2P[6]	5	13	1	3	2	13	1	4	0	0	9	7
G3P[14]	1	3	0	0	0	0	0	0	0	0	1	1
G8P[4]	4	10	1	3	8	50	4	16	7	54	24	19
Total	17	44	8	25	11	69	7	28	7	54	50	40
Mixed and non-typeable rotavirus strains												
Mixed	0	0	4	13	0	0	0	0	0	0	4	3
Not yet typed	0	0	2	6	0	0	0	0	0	0	2	2
Negative	2	0	0	0	0	0	0	0	0	0	2	2
Total	2	5	6	19	0	0	0	0	0	0	8	6
Grand total	39		32		16		25		13		125	

CHBH = Chris Hani Baragwanath, DGM = Dr. George Mukhari, MP = Mapulaneng, MK = Matikwana, EdH = Edendale

Discussion

The proportion of diarrhoea cases positive for rotavirus has stabilized at 20 - 21% over the last 3 years following the introduction of rotavirus vaccine into the EPI schedule. The 2012 rotavirus season in South Africa started 2 weeks later than the 2011 season and 7 weeks later than the pre-vaccine 2009 season. The 2012 season lasted 15 weeks, three weeks shorter than the 2011 season which lasted 19 weeks and nine weeks shorter than the 2009 pre-vaccine season which lasted 24 weeks. The numbers of patient enrolments at sentinel hospitals have decreased substantially since 2010 with 1237 patients enrolled in 2010, 816 in 2011 and 632 in 2012. This represents an enrolment decrease of 51% between 2010 and 2012. Furthermore, despite the fact that 50% of the rotavirus strains detected are not present in the vaccine formulation, the total number of rotavirus infections recorded from the sentinel hospitals has dropped dramatically from 409 in 2009 to 238 in 2010, 151 in 2011 and 125 in 2012.

According to vaccine impact studies comparing pre-vaccination data (2008-2009) to post vaccination data (2010-2011) from the rotavirus surveillance system, there was a 54-58% reduction in the incidence of rotavi-

rus gastroenteritis in children under five years of age. Furthermore, the rotavirus vaccine resulted in an approximately 30% reduction in cases of severe (hospitalized) gastroenteritis in children less than 5 years of age.[4] This study, together with many other studies, has demonstrated the significant role that the rotavirus vaccine has played in reducing the burden of diarrhoea in South Africa.

Conclusion

The introduction of the rotavirus vaccine represents a significant public health achievement in South Africa. Ongoing surveillance is still required to track the epidemiology of rotavirus infection and to continue to monitor the effect of the introduction of the Rotarix® vaccine into the expanded programme on immunization.

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- Centre for Respiratory Diseases and Meningitis: Cheryl Cohen, Babatyi Malope-Kgokong, Jocelyn Moyes, Akhona Tshangela, Sibongile Walaza
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UPDATE ON POLIO ERADICATION IN AFRICA

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Background

The Global Poliomyelitis Eradication Initiative (GPEI) was initiated in 1988, after the declaration of the eradication of smallpox in 1980 by the World Health Assembly, which now aims to eradicate poliomyelitis globally by 2018.[1] The GPEI has been successful in reducing poliomyelitis cases: from 350,000 recorded in 1988 to fewer than 1000 cases recorded by the end of 2011. Pakistan, Afghanistan and Nigeria are the only remaining endemic countries.[2]

Monitoring for the presence of polio is based on acute flaccid paralysis (AFP) surveillance. In order to ensure that a polio case is not missed, AFP surveillance targets a symptom as opposed to a specific disease.[3] The clinical case definition of AFP is an acute onset of flaccid paralysis or paresis in any child under 15 years of age. Differential diagnoses for AFP include Guillain-Barre syndrome, transverse myelitis, enterovirus infections and traumatic neuritis. In general, AFP surveillance is a GPEI strategy designed to detect poliovirus circulation, re-importation of wild poliovirus into polio-free areas or regions and emerging vaccine derived polio viruses (VDPVs). As polio eradication is approaching, it is crucial to maintain high quality AFP surveillance (www.hpsc.ie/hpsc/A-Z/VaccinePreventable/Polio/.../File,2461,en.pdf).

Functions of the NICD national and regional reference laboratory

Since 1995, the molecular polio unit of the National Institute for Communicable Diseases (NICD) of South Africa has hosted a WHO-supported AFP surveillance network at both national and regional levels. At national level, the NICD serves seven countries including South Africa, Angola, Botswana, Lesotho, Mozambique, Namibia and Swaziland. The molecular polio unit also serves as a reference laboratory for many of the countries outside of the southern African region which fall under the WHO African Regional Office. Sequence analyses conducted at the molecular polio unit have been used to answer several epidemiological questions regarding the likely location of endemic poliovirus reservoirs and patterns of virus transmission. These analyses have also been used to determine if an unknown viral isolate is similar to endemic strains or has been introduced (imported).

AFP Surveillance in South Africa

The last wild polio virus in South Africa was detected in 1989. The criterion for adequate surveillance of AFP is 2 cases per 100 000 population of children less than 15 years of age coupled with an isolation rate of non-polio enteroviruses from AFP cases of greater than 10%. A total of 866 specimens from South African AFP cases was received in 2012. From this sample, adequate surveillance with an overall Non–Polio AFP rate of 2.0 per 100 000 children and a non polio enterovirus isolation rate of 12.5% was demonstrated (figure 1).

Wild-type polio viruses in Africa

During 2012, 352 poliovirus isolates were characterized as vaccine or wild-type. These isolates were sent to the NICD from National and Regional laboratories throughout Africa namely: Central African Republic, Ethiopia, Ghana, Kenya, Madagascar, Democratic Republic of Congo, Senegal, South Africa, Uganda and Zambia (figure 2).

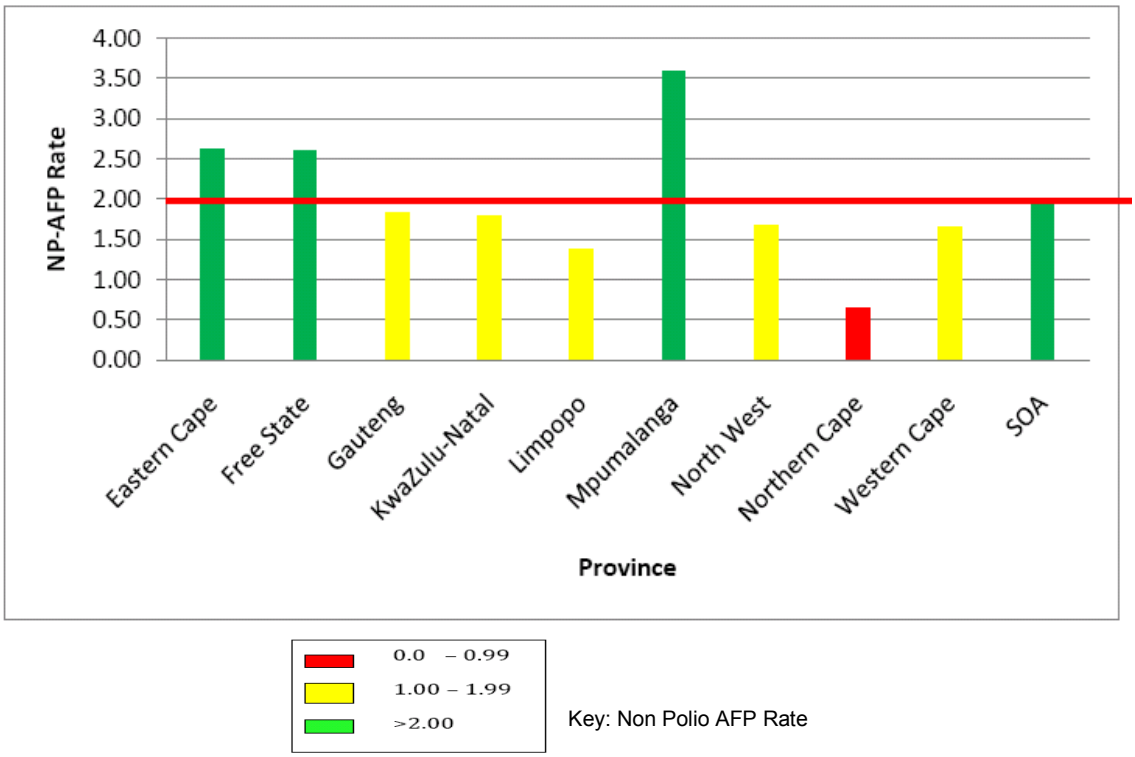

Figure 1: Annualised Non-Polio AFP rate per 100 000 children < 15 yrs of age by province, South Africa. Source: Directorate: Child and Youth Health; Sub-Directorate: Expanded Programme on Immunization (EPI; National Department of Health, South Africa). SOA = South Africa.

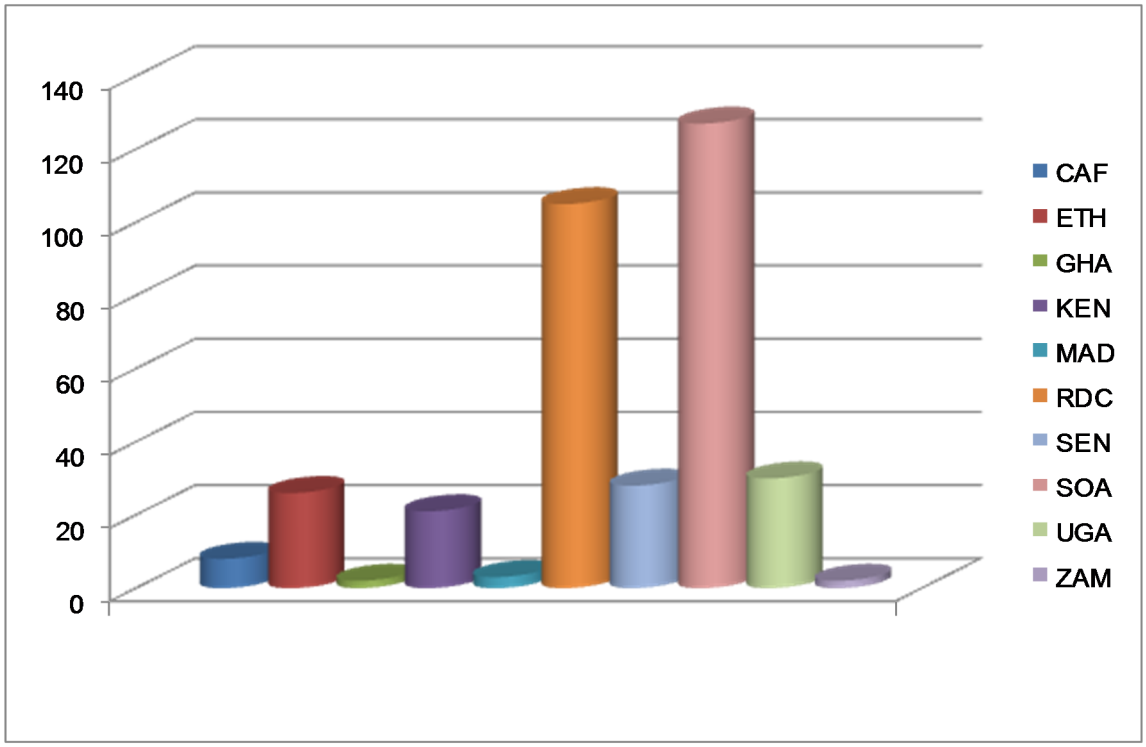

Figure 2: Number of poliovirus isolates from countries served by the AFP surveillance network of South Africa in 2012. Central African Republic (CAF), Ethiopia (ETH), Ghana (GHA), Kenya (KEN), Madagascar (MAD), Democratic Republic of Congo (DRC), Senegal (SEN), South Africa (SOA), Uganda (UGA) and Zambia (ZAM).

The total number of wild poliovirus cases with onset of paralysis in 2012 was 128. Nigeria still remains a highly endemic country with a total of 122 wild-type polioviruses from confirmed AFP cases and 15 from environmental samples (data not shown).

Polio virus type 1 wild-type isolates are distributed into three genotypes, India (SOAS), West African B1 (WEAF -B1) and West African B2 (WEAF-B2) (figure 3). The WEAF-B1 genotype is subdivided into 4 genetic clusters (N2, N5, N6 and N7) consisting of viruses from Nigeria, Niger and Chad. The SOAS genotype consists of those viruses from the DRC.

Of the identified Polio virus type 1 wild-types, 17 unique WEAF-B1 polioviruses were isolated from environmental specimens from Nigeria (data not shown). Orphan polioviruses (isolates not linked to any recently identified viruses) were also detected in Nigeria in 2012 suggesting an unidentified "silent" circulation or poor surveillance. Chad and Niger virus isolates typed to cluster N6 and the last cases reported had onset dates of 14 June 2012 and the 15 November 2012 respectively.

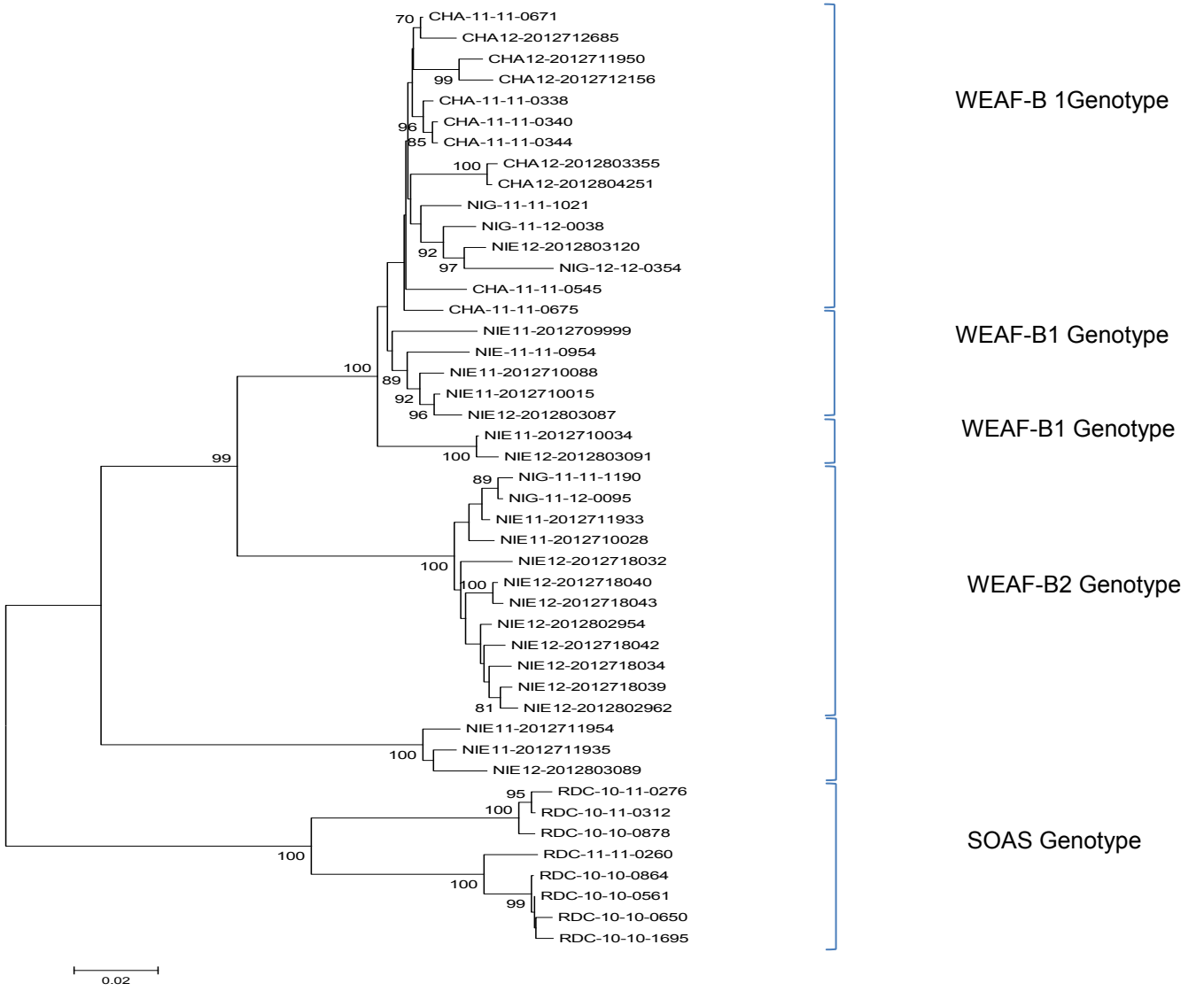

Figure 3: Neighbour-joining tree of WEAF-B1, WEAF-B2 and SOAS wild Poliovirus 1 representatives of isolates from Africa. Bootstrap values of greater than 70% are shown at the branch nodes. NIE = Nigeria, NIG = Niger, CHA = Chad and DRC = Democratic Republic of Congo.

For wild-type poliovirus 3 (PV3), only Nigeria reported the WEAF-B wild poliovirus type 3 in 2012, with a total of 19 cases compared to 13 cases in 2011. The genetic clusters circulating the most were F4 and F6 affecting seven Nigerian states namely: Bauchi, Borno, Jigawa, Kaduna, Kano, Taraba and Yobe. The F4 cluster was dominant in Borno state (data not shown) while F6 affected the rest of the states (figure 4). Serotype 3 polioviruses from environmental samples were also reported.

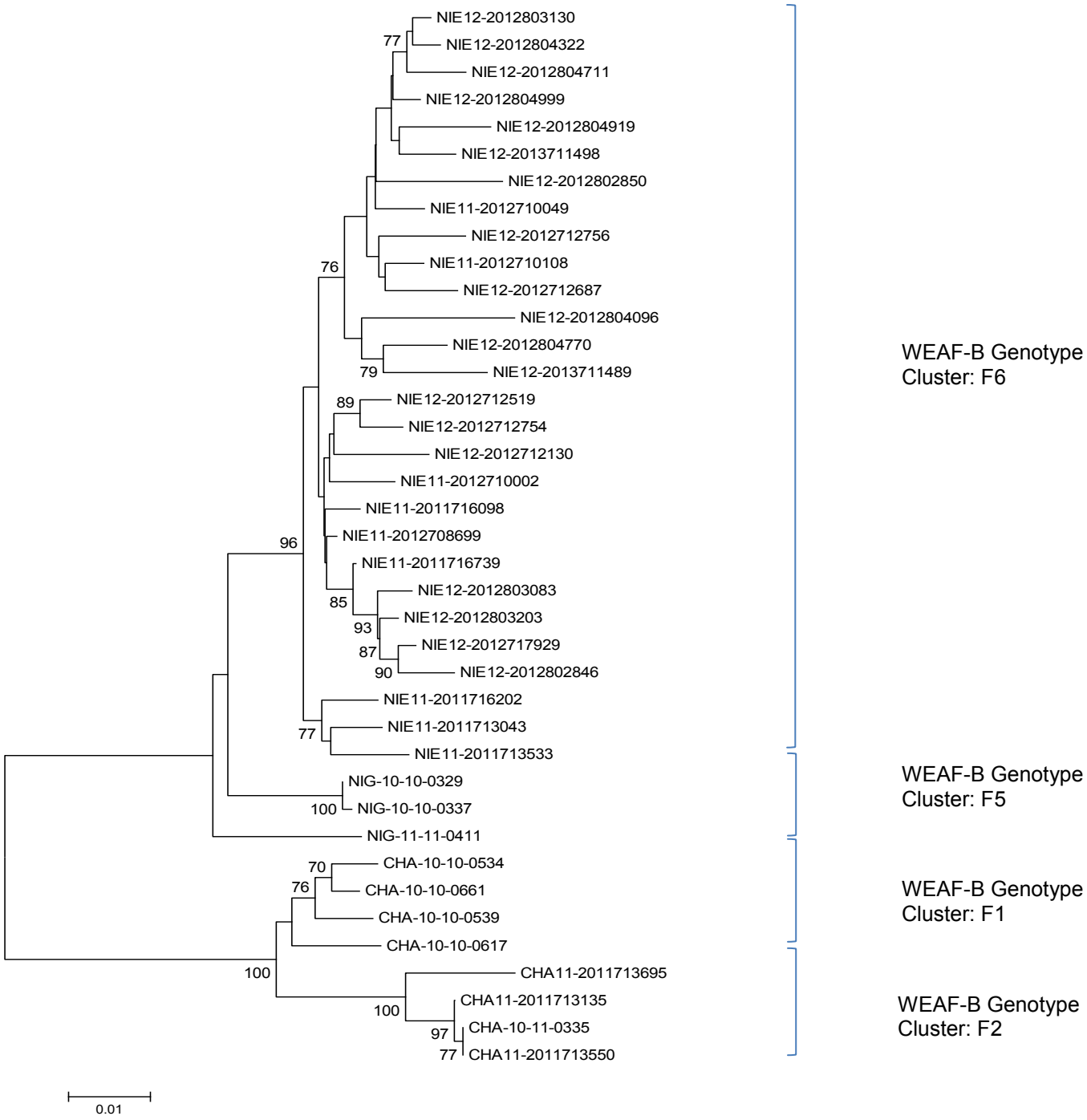

Figure 4: Neighbour-joining tree of a VP1 gene of WEAF-B wild PV3 representatives of isolates from Africa. Bootstrap values of greater than 70% are shown at the branch nodes. NIE = Nigeria, NIG = Niger, CHA = Chad.

Identification of wild poliovirus type 1 in Somalia in 2013

Only three countries reported wild polioviruses (WPV) in 2012 (Nigeria, Niger and Chad) compared to 13 countries in 2011. On 18th April 2013, a 32-month-old girl from Banadir region in Somalia presented with onset of paralysis. This case represents the first wild polio virus in Somalia since March 2007 and the first outbreak outside of an endemic country in 2013. In large areas of south-central Somalia, immunization campaigns have not been implemented since 2009 due to inaccessibility, affecting more than 500,000 children aged <5 years. Populations in this area are at high risk of polio as a consequence of this outbreak. This area is also affected by an ongoing circulating Vaccine Derived Poliovirus type 2 (cVDPV 2) outbreak, which has resulted in 18 cases in the country since 2009 (the most recent cVDPV2 case had onset of paralysis on 9th January 2013).

Characterisation of circulating vaccine–derived polioviruses in Africa

Live, attenuated oral poliovirus vaccine (OPV) is still the vaccine of choice for developing countries. However, reversion to virulence may occur during OPV replication in humans, resulting in person-to-person transmission and circulation of vaccine-derived polioviruses (cVDPV) in areas with low rates of vaccine coverage.[4] Vaccine-derived polioviruses show significant sequence drift (> 1% nucleotide difference in types 1 and 3, > 0.6% nucleotide difference in type 2), indicating prolonged replication of the vaccine strain in human populations and consequent changes in the phenotypic properties of neurovirulence and transmissibility.[4,5] Poliomyelitis outbreaks associated with cVDPVs have been reported in several countries including Egypt (1982-1993), Haiti (2000-2001), Dominican Republic (2000-2001), Philippines (2001), Madagascar (2002 and 2005), China (2004), Cambodia (2005-2006), Indonesia (2005) and Nigeria (2005-2010).[5-12] In 2012, five African countries reported cVDPV type 2 including Chad, Somalia, Nigeria, Kenya and the DRC. Seventeen cases were reported in the DRC in 2012 compared to eleven cases recorded in 2011. The DRC outbreak has been continuous since 2008. The cVDPVs detected in DRC are associated with the two most important biological properties of wild polioviruses namely (i) the capacity to cause paralytic disease in humans and (ii) the capacity for continuous person-to-person transmission.

As a result of accumulating evidence about the emergence and spread of cVDPV, there are plans for synchronized cessation of the use of OPV and the implementation of more widespread use of inactivated polio vaccine (IPV).[13-15] A better understanding of VDPV persistence and circulation is important for decision making about when and how to stop immunization with OPV after the global eradication of wild polioviruses.[16-18] South Africa is the first African country to introduce IPV into its routine immunization schedule.

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ANTIBODY RESPONSES IN THE HVTN 073E HIV VACCINE TRIAL

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Background

The HIV Vaccine Trials Network (HVTN) 073E Phase I trial, led by Prof. Glenda Gray, tested the ability of the South African AIDS vaccine initiative (SAAVI) DNA/MVA vaccines (developed by Prof. Anna-Lise Williamson and Prof. Carolyn Williamson, University of Cape Town) to prime antibody responses following two booster vaccinations containing gp140 protein with MF59 as an adjuvant.[1] This study enrolled HIV uninfected healthy adult participants in South Africa and the United States (US) who previously participated in the HVTN 073/ SAAVI 102 study.

Previous studies have shown that a protein vaccine boost to an HIV vaccine may improve antibody responses.[2] The aim of the trial extension was therefore to explore whether the SAAVI DNA-C2/SAAVI MVA-C vaccine regimen provided a good prime for antibody responses following protein boosting.

Methods

Participant cohort

The study enrolled 27 participants from the US and South Africa. There were 6 participants (5 from the vaccine group and 1 from the placebo group) from the US and 21 participants (17 vaccinees, 4 placebos) from South Africa. All enrolled participants received their first gp140/MF59 vaccination at visit 17 (day 0) and the second at visit 20 (day 84) (table 1). The time lag between the last vaccination in the HVTN073 trial and visit 17 (HVTN073E) was, on average, two years.

Study schema

All data are taken from HVTN 073 trial participants who rolled over to the extension study HVTN 073E. Participants received either DNA/MVA with a protein boost (T1/T2), DNA/MVA with a placebo boost (T1/C2), placebo with a protein boost (C1/T2), or double placebo (C1/C2) (table 1).

Table 1: The vaccination regimen for the HVTN 073 trial (visits 2 – 11) and the extension study HVTN 073E (visits 17 and 20).

Treatment Group (n=27)	**HVTN 073**					**HVTN 073E**	
Visit	**2 (Day 0)**	**4 (Day 28)**	**6 (Day 56)**	**8 (Day 112)**	**11 (Day 140)**	**17 (Day 0)**	**20 (Day 84)**
C1/C2 (n=4)	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo
C1/T2 (n=1)	Placebo	Placebo	Placebo	Placebo	Placebo	gp140/MF59	gp140/MF59
T1/C2 (n=6)	DNA-C2	DNA-C2	DNA-C2	MVA-C	MVA-C	Placebo	Placebo
T1/T2 (n=16)	DNA-C2	DNA-C2	DNA-C2	MVA-C	MVA-C	gp140/MF59	gp140/MF59

Neutralization Assays

Neutralizing antibodies against HIV-1 were measured as a function of the reduction in Tat-regulated luciferase (Luc) reporter gene expression in TZM-bl cells. This assay measures neutralization titers against a panel of heterologous Env-pseudotyped viruses that exhibit either a Tier 1A (Clade B: MN.3, Clade C: MW965.26) or a Tier 2 (Clade C: Du151.2, Clade B: TV1.21) neutralization phenotype in TZM-bl cells. Neutralization assays were performed at baseline (visit 17), 2 weeks after the 1st gp140/MF59 extension vaccination (visit 19), at the primary immunogenicity time point - 2 weeks after the 2nd gp140/MF59 (visit 22) and at 6.5 months after the 2nd protein boost (visit 24).

Binding Assays

Binding antibodies to ConS, TV1.21 and Du151 gp140 envelope glycoproteins and p24 Gag protein were assessed by a validated enzyme linked immunosorbent assay (ELISA). The ELISA was performed at the same time points as the neutralization assays.

Results

Figure 1 shows the percentage of responders by ELISA in the treatment arm (T1/T2) who received vaccines in both the HVTN 073 and HVTN 073E trials. At day 0 of the study extension, less than 30% of participants had positive responses to all antigens. More than 60% of the participants had binding antibody responses to Con S, TV1.21 and Du151 envelope proteins two weeks after the first gp140 extension boost (not shown) and reached their peak at 100% responders two weeks after the second protein boost (visit 22). As expected, there was a low percentage of responders for antigen p24 (gag) given that the protein boost in this regimen was an envelope protein. There was no positive response in the placebo group at any visit.

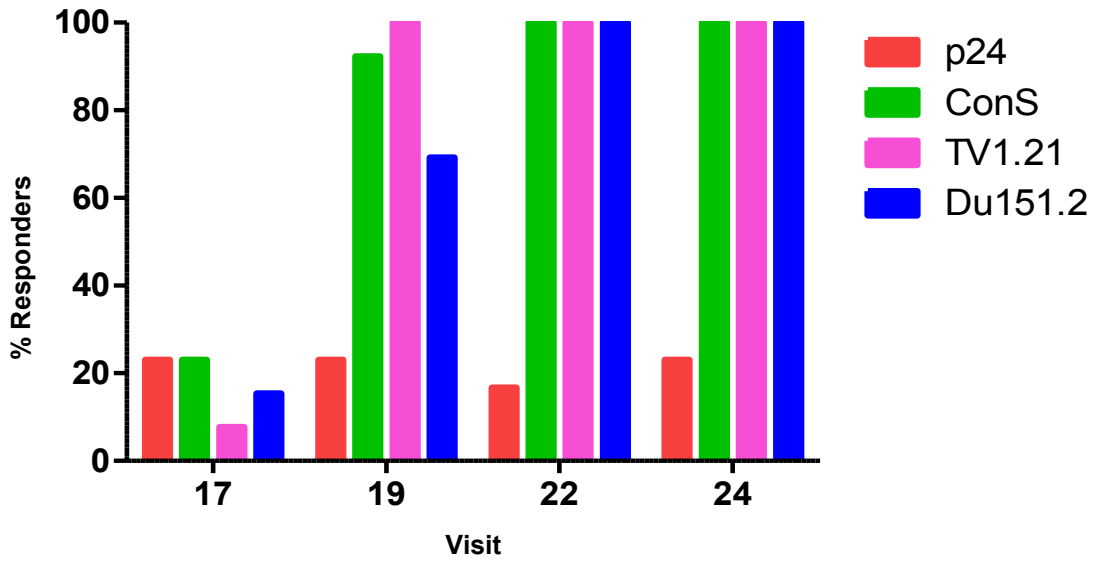

Figure 1: Percentages of individuals in the treatment arm who showed antibody responses to HIV-specific binding antibodies (p24, ConS, TV1.21, Du151.2) by visit in the HVTN073E vaccine trial.

Figure 2 shows the neutralizing antibody titer response rates by virus and visit. At visit 17 (day 0), none of the participants had HIV neutralizing antibodies against the viruses tested in their sera. However, the neutralizing antibody responses peaked at visit 22 (2 weeks after the 2nd protein boost), similar to the binding antibody data. Participants who received the vaccine in both HVTN 073 and HVTN 073E (T1/T2) had higher antibody

responses, in frequency and magnitude, than participants who received the vaccine in HVTN073 but not the protein boost in HVTN073E (T1/C2). However, by visit 24 (day 273), neutralization antibody titers began to wane (data not shown).

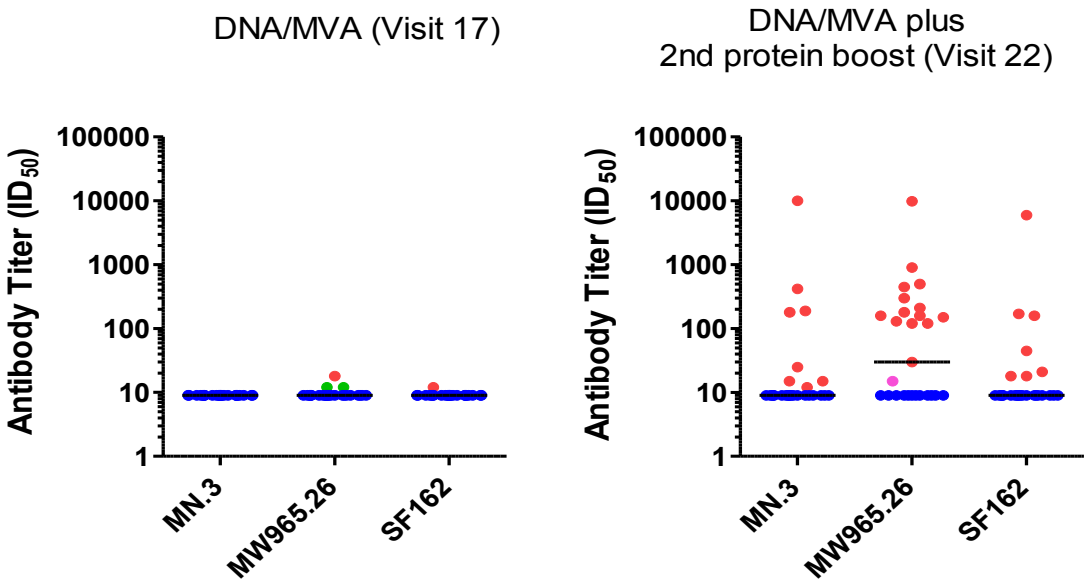

Figure 2: Neutralization of Tier 1 viruses in samples from the HVTN 073E trial. Each dot represents one individual and the black lines represent the median titer for each group. Red circles represent positive responders from the T1/T2 group, green circles represent T1/C2, pink circles represent C1/T2. Negative responders and the placebo group are in blue.

Conclusion

The first and second protein boosts elicited HIV-specific binding and neutralizing antibodies. We observed that antibody responses peaked after the second boost with most participants still showing detectable antibody responses 6 months later (visit 24). Responses were consistently stronger in the participants that received the DNA/MVA vaccine with protein boost providing strong evidence for the inclusion of a protein immunogen in the vaccine regimen. This is the first clinical trial of a subtype C vaccine showing measurable antibody responses and it is evident that the protein boost is necessary to elicit these responses. These data will provide a crucial step in the iterative process of developing an AIDS vaccine. Further studies aim to modify the immunogens, dosage, adjuvants and immunization schedules in order to stimulate primary virus (Tier 2) neutralization.

Acknowledgements

We thank the South African and US volunteers who participated in the HVTN 073 and HVTN 073E trials and the HIV Vaccine Trials Network for funding.

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AVIAN INFLUENZA AT THE ANIMAL-HUMAN INTERPHASE IN SOUTH AFRICA

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Introduction

On the 7th of April 2013 the State Veterinary Services, Department of Agriculture, Western Cape Government, reported actively circulating Influenza A virus. The virus was detected through the routine surveillance programme for Avian Influenza in ostriches. Specimens submitted to the Onderstepoort Veterinary Institute were confirmed as H7 positive in 3 ostrich chicks and preliminarily reported as NA1 positive in two chicks although the NA typing was inconclusive. DNA sequencing of the pathogenic marker indicated a low pathogenic genotype. Gastrointestinal symptoms were detected in some ostrich chicks on several farms but no symptoms were detected in older birds and there was no reported increase in deaths. An isolate was subsequently obtained by the Avian influenza laboratory of DELTAMUNE and sent to both the Onderstepoort Veterinary Institute (OVI) and National Institute for Communicable Diseases (NICD) for further investigation. This was done on request of the ostrich industry and the Department of Agriculture, Forestry and Fisheries in order to rule out the possibility of the isolate presenting as the new H7N9 virus from China. The isolate was shown by the NICD to be negative for the new H7N9 strain using a newly established real-time polymerase chain reaction (RT-PCR) assay which targets H7 and N9 gene fragments. The isolate was subsequently independently typed as H7N7 by the Centre for Respiratory Diseases and Meningitis (CRDM), NICD, using conventional RT-PCR and Sanger sequencing and by the University of Pretoria, Poultry research chair, Dr Celia Abolnik, using next generation sequencing.

Control measures

Western Cape Veterinary Services has instituted a 5 km buffer of movement control as well as quarantine of ostrich farms in the region and is conducting surveillance in that zone. The outbreak is confined to two farms within this radius although results from a further five farms are currently outstanding.

Background on Avian Influenza in South Africa

Several avian influenza (AI) outbreaks have been reported in ostriches over the last ten years in South Africa.[1] In the Western Cape the most recent are the highly pathogenic Influenza A H5N2 strain which was detected in 2011 in the Uniondale and Oudtshoorn areas, the low pathogenic (LPAI) H7N1 strain which was detected in the Heidelberg area in 2012 (http://www.elsenburg.com/vetepi/epireport_pdf/ November2012.pdf), and most recently the H7N7 strain which was detected in the Oudtshoorn district in 2013. No increase in ostrich deaths was reported in any of these outbreaks. Large scale culling of ostriches followed the 2011 H5N2 outbreak while movement control was implemented during the 2012 H7N1 outbreak. Both outbreaks resulted in a ban on the export of ostrich meat to the European Union which has proved devastating to South Africa's ostrich industry.

Notifiable Avian influenza (NAI), according to the guidelines of the OIE (Animal World Health Organisation), includes detection of the highly pathogenic (HP) Avian Influenza strains of the H5 and H7 types in any birds, and low pathogenic (LP) Avian Influenza in poultry

(http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm).

For trade purposes with the European Union ostriches are classified as poultry in South Africa and vigorous surveillance for AI is conducted on birds bred for slaughter and export. The Avian Influenza H5 and H7 strains have been detected in wild and migratory birds at a number of localities in South Africa but have so far been typed as low pathogenic (Unpublished data, Zoonoses Research unit, University of Pretoria and CRDM, NICD; Cummings et al 2011[2]). Wild birds appear to be the likely route for the spread of avian influenza across South Africa with aquatic bird reservoirs maintaining AI strains within southern Africa.[3] The highly pathogenic H5N1 strain, which has caused a high mortality rate in birds as well as in humans in several countries in Asia and in Egypt since 2004, has not been detected in South Africa.

Avian Influenza virology pathogenic types

Avian influenza strains are classified as highly pathogenic or low pathogenic based on their ability to kill six week old chicks inoculated intravenously. The HPNAI viruses cause at least 75 percent mortality in four to eight-week-old chicks. The presence of multiple basic amino acids at the cleavage site of the haemagglutinin molecule (HA0) similar to other HPNAI motifs is used as a genetic marker for HPNAI (http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm).

The haemagglutinin molecules (HAs) of low-pathogenicity AI (LPAI) viruses do not contain a series of basic amino acids at the protease cleavage site. This site is necessary for infection and is only cleaved by host proteases localized in the respiratory and intestinal organs of birds, resulting in mild localized infections in birds. Haemagglutinin molecules of high-pathogenicity AI (HPAI) viruses possess multiple basic amino acids at the cleavage site which is cleaved by ubiquitous proteases in a wide range of organs, resulting in lethal systemic infection in birds.

Avian Influenza strains do not normally kill wild birds, but highly pathogenic avian influenza strains may be highly infectious and pathogenic for poultry. Although Low pathogenic strains do not kill chickens, outbreaks of these strains may be difficult to control because they can spread undetected through populations and have the potential to revert to highly pathogenic phenotypes (reviewed by Alexander, 2007[4]).

Risk to humans in South Africa

Avian influenza subtypes H5, H7 and H9 have the ability to infect humans although human to human transmission is usually limited. Following the 2011 and 2012 H5N2 and H7N1 outbreaks in ostriches the CRDM, in collaboration with the Outbreak Response Unit, NICD, conducted sero-surveys in those high risk persons who were involved in the ostrich AI outbreaks. Screening of sera from 207 persons identified as high risk of exposure to AI types H5N2, as well as 66 sera from veterinarians, ostrich farmers, farmworkers and abattoir workers that were involved in the H7N1 outbreaks of 2011 and 2012, identified 4 people with significant HAI antibody titers greater than 1:40 for Influenza H5N2 or H7N1. These four cases included a veterinarian who was actively involved in postmortem investigations of culled ostriches, a farm worker and two abattoir workers. This survey suggests a low risk of infection for humans involved in the control of AI outbreaks with a seroconversion rate of 1.4% for Influenza A H5N2 and 1.6% for H7N1. Reported symptoms included conjunctivitis and flu-like illness although these were based on retrospective questionnaires completed at the time of specimen collection and could not be directly linked to the outbreak in question.

Pathogenicity of AI strains other than Highly Pathogenic H5N1 for humans

Human infections with Avian Influenza strains H5N2 and H7N1 have previously been detected in other countries. Several outbreaks of low pathogenic avian influenza (LPAI), highly pathogenic avian influenza (HPAI) H7N1 and LPAI H7N3 viruses occurred in poultry in Italy between 1999 and 2003. A serological survey of poultry workers found evidence of anti-H7 antibodies in 3.8% of serum samples collected during 2003 when LPAI H7N3 virus was circulating. Of 185 people tested, only one person showed symptoms of conjunctivitis during the outbreak.[5] A sero-survey conducted following the epizootic caused by H7N7 virus in the Netherlands in 2003 showed that approximately half of the individuals exposed to poultry as well as the household contacts of infected persons had anti-H7 antibodies. Symptoms included conjunctivitis without fever, upper respiratory tract symptoms, or both, and severity ranged from mild to fatal with one death reported.[5-7] Influenza H5N2 infection has been reported in humans in Japan although no symptoms were recorded.[8]

The possibility of human infection as demonstrated in this study emphasizes the need for biosecurity measures during culling procedures or during the handling of infected birds. Different strains and N types of avian influenza apparently have different infection rates in humans.

The emergence of a low pathogenicity H7N9 strain in China at the end of March 2013 resulted in severe pneumonia in 132 human cases by 14 May 2013, including 33 deaths. This outbreak serves as a reminder that the presentation of low pathogenic strains can be unpredictable in human infections. http://www.who.int/influenza/human_animal_interface/influenza_h7n9/Data_Reports/en/index.html.

Biosafety precautions

It is recommended that increased biosecurity precautions be practiced when dealing with Avian Influenza H7, H5 and H9 strains. People involved in the control of outbreaks should wear gloves, goggles and infection control masks (N95) when dealing with birds with suspected infections. Human and animal laboratories dealing with H7, H5 and H9 AI strains should only conduct molecular diagnoses in BSL-2 laboratories following the inactivation of specimens under BSL-3 conditions (BSL-2+). However, virus isolation and amplification of these strains should only occur under BSL-3 conditions because of the potential pathogenicity of these strains in humans. Furthermore, these precautions should mitigate the possibility of virus escape into bird populations and should prevent recombination between human and animal influenza strains in infected humans or in the laboratory.

Conclusion

From the sero-survey conducted in South Africa it is considered that the H5N2 and H7N1 AI strains pose a low risk of human infection and disease. Further investigation of the H7N7 strain is underway. Healthcare workers and animal handlers are encouraged to submit specimens from patients presenting with conjunctivitis, influenza-like illness or severe respiratory infections, who have a history of exposure to avian influenza positive ostriches or sick birds, to the CRDM, NICD, for investigation.

Acknowledgements

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Table 1: Provisional number of laboratory confirmed cases of diseases under surveillance reported to the NICD - South Africa, corresponding periods 1 January - 31 March 2012/2013*

Disease/Organism	1 Jan to 31 Mar, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Botulism	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2012	345	98	506	494	62	115	21	90	164	1895
	2013	179	64	504	449	34	93	11	63	163	1560
Haemophilus influenzae, invasive disease, all serotypes	2012	13	2	27	12	0	0	0	2	16	72
	2013	6	1	21	14	0	1	1	0	25	69
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2012	1	0	9	1	0	0	0	0	3	14
	2013	0	0	1	0	0	0	0	0	1	2
Serotypes a,c,d,e,f	2012	1	0	1	0	0	0	0	0	3	5
	2013	1	1	1	1	1	0	0	0	2	7
Non-typeable (unencapsulated)	2012	0	0	6	0	0	0	0	0	1	7
	2013	0	0	3	1	0	0	0	0	1	5
No isolate available for serotyping	2012	3	1	1	1	0	0	0	1	0	7
	2013	2	1	4	1	1	0	0	1	2	12
Measles	2012	0	1	1	0	0	0	0	1	1	4
	2013	0	0	1	0	0	0	0	0	0	1
Neisseria meningitidis, invasive disease	2012	6	0	15	8	1	0	0	2	9	41
	2013	4	2	5	7	1	1	1	0	8	29
Novel Influenza A virus infections	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Plague	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Rabies	2012	0	0	0	0	2	0	0	0	0	2
	2013	0	1	0	1	1	1	0	0	0	4
**Rubella	2012	101	8	26	39	8	18	11	15	55	281
	2013	12	5	19	12	1	11	5	9	6	80
Salmonella spp. (not typhi), invasive disease	2012	13	7	71	30	2	11	3	2	26	165
	2013	8	6	71	34	1	12	1	2	36	171
Salmonella spp. (not typhi), isolate from non-sterile site	2012	37	5	165	74	1	19	6	5	95	407
	2013	46	22	220	70	3	24	5	7	128	525
Salmonella typhi	2012	1	0	5	7	0	2	0	0	2	17
	2013	0	1	10	5	0	2	0	0	5	23
Shigella dysenteriae 1	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non Sd1)	2012	76	17	173	52	2	5	5	0	127	457
	2013	85	28	210	73	3	19	5	14	103	540
Streptococcus pneumoniae, invasive disease, all ages	2012	74	53	234	121	10	32	2	18	82	626
	2013	61	34	143	80	8	19	8	18	93	464
Streptococcus pneumoniae, invasive disease, < 5 years	2012	19	6	53	24	1	5	0	3	8	119
	2013	12	8	41	12	3	1	1	8	14	100
Vibrio cholerae O1	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	1	0	0	0	0	1
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	2	0	0	0	0	0	1	0	3
Other VHF (not CCHF)	2012	0	0	0	0	0	0	0	0	0	0
	2013	0	0	0	0	0	0	0	0	0	0

Footnotes

*Numbers are for cases of all ages unless otherwise specified. Data presented are provisional cases reported to date and are updated from figures reported in previous bulletins.

**Rubella cases were diagnosed from specimens submitted for suspected measles cases. Rubella testing was discountinued in mid March 2013.

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

U = unavailable, 0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 31 March 2012/2013*

Programme and Indicator	1 Jan to 31 Mar, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Acute Flaccid Paralysis Surveillance											
Cases < 15 years of age from whom specimens received	2012	17	14	21	21	6	11	2	11	10	113
	2013	14	5	16	18	5	9	4	1	6	78

Footnotes

*Numbers are for all ages unless otherwise specified. Data presented are provisional numbers reported to date and are updated from figures reported in previous bulletins.

Provinces of South Africa: EC – Eastern Cape, FS – Free State, GA – Gauteng, KZ – KwaZulu-Natal, LP – Limpopo, MP – Mpumalanga, NC – Northern Cape, NW – North West, WC – Western Cape

Monitoring for the presence of polio in a country is based on AFP (acute flaccid paralysis) surveillance – the hallmark clinical expression of paralytic poliomyelitis. The clinical case definition of AFP is an acute onset of flaccid paralysis or paresis in any child under 15 years of age. AFP is a statutory notifiable disease and requires that 2 adequate stool specimens are taken as soon as possible, 24 to 48 hours apart, but within 14 days after onset of paralysis, for isolation and characterisation of polio virus. The differential diagnosis of AFP is wide, the most common cause of which is Guillain-Barre Syndrome. The incidence of AFP in a population has been studied in a number of developing countries and WHO have determined, as a result of these studies, that the criterion for adequate surveillance of AFP is 2 cases per 100 000 population of children less than 15 years of age (it was formerly 1 per 100,000 but this was thought to be inadequately sensitive).

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