GENOTYPIC ANALYSIS OF MEASLES VIRUSES IN SOUTHERN AFRICA: IMPLICATIONS FOR REGIONAL AND GLOBAL ELIMINATION OF MEASLES

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DECLARATION

I, Sheilagh Smit, declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

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Sheilagh Brigitte Smit

_____ day of _____, 2012

To my parents, Lore and Gottfried Bautz,
And my daughter, Anastasia,

Thank you
for love, support, patience
and sacrificing so much family time for so many years

ABSTRACT

Measles is a vaccine-preventable disease. Implementation of global vaccination programmes has resulted in a dramatic decrease in measles-related deaths, from 2.6 million in 1980 to 164 000 in 2008. To support the global measles elimination goal, laboratories provide case-based surveillance which includes both serological diagnosis and viral characterisation using molecular platforms.

In this study, conventional hemi-nested amplification methods were developed to detect the nucleoprotein- and haemagglutinin-genes of measles virus, in specimens collected for rash-surveillance programmes in Africa. Viral characterisation involved amplicon sequencing and phylogenetic analyses of 1402 PCR-positive specimens (2655 specimens tested, 52.8% PCR-positive) with sequence submission to global databases. Only imported strains of genotypes B2, B3, D2, D4 and D8, have been detected in South Africa since 2002, demonstrating the successful interruption of transmission of endemic strains. Genotypic analyses of specimens from African countries provided information relating to strain origins, as well as temporal and spatial data relevant to the molecular epidemiology of measles in Southern Africa.

PUBLICATIONS AND PRESENTATIONS

Publications arising from MSc

1. **Smit SB**, Hardie D, Tiemessen CT.
   Measles virus genotype B2 is not inactive: evidence of continued circulation in Africa.
   J Med Virol. 2005 Dec;77(4):550-7.

2. Neverov AA, Riddell MA, Moss WJ, Volokhov DV, Rota PA, Lowe LE, Chibo D, **Smit SB**, Griffin DE, Chumakov KM, Chizhikov VE.
   Genotyping of measles virus in clinical specimens on the basis of oligonucleotide microarray hybridization patterns.
   J Clin Microbiol. 2006 Oct;44(10):3752-9.

3. Rota J, Lowe L, Rota P, Bellini W, Redd S, Dayan G, van Binnendijk R, Hahne S, Tipples G, Macey J, Espinoza R, Posey D, Plummer A, Bateman J, Gudino J, Cruz-Ramirez E, Lopez-Martinez I, Anaya-Lopez L, Holy Akwar T, Giffin S, Carrion V, de Filippis AM, Vicari A, Tan C, Wolf B, Wytovich K, Borus P, Mbugua F, Chege P, Kombich J, Akoua-Koffi C, **Smit S**, Bukenya H, Bwogi J, Baliraine FN, Kremer J, Muller C, Santibanez S.
   Identical genotype B3 sequences from measles patients in 4 countries, 2005.
   Emerg Infect Dis. 2006 Nov;12(11):1779-81.

4. Lemma E, **Smit SB**, Beyene B, Nigatu W, Babaniyi OA.
   Genetic characterization and progression of B3 measles genotype in Ethiopia: a study of five measles outbreak cases.
   Ethiop Med J. 2008 Jan;46(1):79-85.

5. Kremer JR, Nkwembe E, Oyefolu AOB, **Smit SB**, Pukuta E, Omilabu SA, Adu F, Muyembe Tamfum JJ, Muller CP.
   MV strain diversity reflects differences in measles epidemiology and control in Nigeria and the Democratic Republic of the Congo.
   Emerg Infect Dis. 2010 Nov;16(11):1724-30.

6. Adagba M, Akoua-Koffi C, Traore I, **Smit S**, Ekaza E, Kadjo H, Dosso M, Featherstone DA.
   Detection of viral RNA by RT-PCR from serum for molecular diagnosis of measles.
   Afr J Med Med Sci. 2010 Dec; 39(4):277-84.

7. Baliraine FN, Bwogi J, Bukenya H, Seguya R, Kabaliisa T, Kisakye A, Mbabazi W, **Smit SB.**
   Possible interruption of indigenous Measles virus transmission in Uganda, 2006-2009.
   Emerg Infect Dis. 2011 Jan;17(1):110-113.

8. Rota PA, Brown K, Mankertz A, Santibanez S, Shulga S, Muller C, Hübschen JM, Siqueira M, Beirnes J, Ahmed H, Triki H, Al-Busaidy S, Dosseh A, Byabamazima C, **Smit S**, Akoua-Koffi C, Bwogi J, Bukenya H, Wairagkar N, Ramamurty N, Incomserb P,

Pattamadilok S, Jee Y, Lim W, Xu W, Komase K, Takeda M, Tran T, Castillo-Solorzano C, Chenoweth P, Brown D, Mulders MN, Bellini WJ, Featherstone D.
Global distribution of measles genotypes and measles molecular epidemiology.
J Infect Dis. 2011 Jul; 204 Suppl 1:S514-23.

Publications arising from other work performed during MSc

1. Leroy EM, Rouquet P, Formenty P, Souquiere S, Kilbourne A, Froment JM, Bermejo M, **Smit S**, Karesh W, Swanepoel R, Zaki SR, Rollin PE.
   Multiple Ebola virus transmission events and rapid decline of central African wildlife.
   Science. 2004 Jan 16;303(5656):387-90. Erratum in: Science. 2004 Jan 20;303(5658):628.

2. Venter M, **Smit SB**, Leman P, Swanepoel R.
   Phylogenetic evidence of widespread distribution of genotype 3 JC virus in Africa and identification of a type 7 isolate in an African AIDS patient.
   J Gen Virol. 2004 Aug;85(Pt 8):2215-9.

3. Bausch DG, Nichol ST, Muyembe-Tamfum JJ, Borchert M, Rollin PE, Sleurs H, Campbell P, Tshioko FK, Roth C, Colebunders R, Pirard P, Mardel S, Olinda LA, Zeller H, Tshomba A, Kulidri A, Libande ML, Mulangu S, Formenty P, Grein T, Leirs H, Braack L, Ksiazek T, Zaki S, Bowen MD, **Smit SB**, Leman PA, Burt FJ, Kemp A, Swanepoel R; International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of the Congo.
   Marburg hemorrhagic fever associated with multiple genetic lineages of virus.
   N Engl J Med. 2006 Aug 31;355(9):909-19.

4. Swanepoel R, **Smit SB**, Rollin PE, Formenty P, Leman PA, Kemp A, Burt FJ, Grobbelaar AA, Croft J, Bausch DG, Zeller H, Leirs H, Braack LE, Libande ML, Zaki S, Nichol ST, Ksiazek TG, Paweska JT; International Scientific and Technical Committee for Marburg Hemorrhagic Fever Control in the Democratic Republic of Congo.
   Studies of reservoir hosts for Marburg virus.
   Emerg Infect Dis. 2007 Dec;13(12):1847-51.

5. Caidi H, Abernathy ES, Benjouad A, **Smit S**, Bwogi J, Nanyunja M, El Aouad R, Icenogle J.
   Phylogenetic analysis of rubella viruses found in Morocco, Uganda, Cote d'Ivoire and South Africa from 2001 to 2007.
   J Clin Virol. 2008 May;42(1):86-90.

6. Abernathy ES, Hübschen JM, Muller CP, Jin L, Brown D, Komase K, Mori Y, Xu W, Zhu Z, Siqueira MM, Shulga S, Tikhonova N, Pattamadilok S, Incomserb P, **Smit SB**, Akoua-Koffi C, Bwogi J, Lim WWL, Woo GKS, Triki H, Jee Y, Mulders MN, Bispo de Filippis AM, Ahmed H, Ramamurty N, Featherstone D, Icenogle JP.
   Status of global virologic surveillance for rubella viruses.
   J Infect Dis 2011 Jul; 204 Suppl 1:S524-32.

Presentations

Local Meetings attended:

1. Rubella control workshop: global, regional and national perspectives. 15-16 March 2004, NICD, South Africa. Oral presentation entitled “”Molecular characterization of South African rubella strains”.

2. Communicable Disease Co-ordinators meeting, 17 March 2004, NICD, South Africa. Oral presentation entitled “Measles outbreak 2003”.

3. Virology Africa congress, 8-11 November 2005, Cape Town. Oral presentation entitled “Molecular analysis of measles viruses in southern Africa”.

International WHO meetings attended:

1. Second WHO Global Measles and Rubella Laboratory Network Meeting, 13-14 October 2003, Cape Town. Oral presentation entitled “Detection of measles virus in clinical specimens”.

2. Southern Africa EPI Managers’ Meeting, 10-12 March 2004, Gaborone, Botswana. Oral presentation entitled “Molecular epidemiology of Measles Virus in southern Africa”.

3. First Measles and Yellow Fever Laboratory Directors Meeting, 2-4 August 2004, Dakar, Senegal. Two oral presentations entitled “Activity report of the measles regional reference laboratory for the WHO southern African region: NICD, South Africa” and “Genotypic analysis of Measles Virus from dried blood spots”.

4. Second Measles and Yellow Fever Laboratory Directors Meeting, 2-3 August 2005, Entebbe, Uganda. Oral presentation entitled “Southern Block report on measles diagnostics”.

5. Third WHO Global Measles and Rubella Laboratory Network Meeting, 25-26 August 2005, Geneva, Switzerland. Oral presentation entitled “Virological surveillance update: South Africa 2003-2005”.

6. Fourth WHO Global Measles and Rubella Laboratory Network Meeting, 28-30 August 2006, Geneva, Switzerland. Oral presentation entitled “Characterization of viruses identified in the African region and challenges in improving surveillance”.

7. Joint Measles and Polio Laboratory Directors Workshop, 24 – 26 July 2007, Accra, Ghana. Oral presentation entitled “progress report RRL NICD molecular aspects 2006-2007”.

8. Fifth WHO Global Measles and Rubella Laboratory Network Meeting, 26-28 September 2007, Geneva, Switzerland. Oral presentation entitled “Virological surveillance update: South Africa 2006-2007”.

9. Polio/Measles/Yellow Fever Laboratory Directors Workshop, 8-11 September 2008, Lusaka, Zambia. Oral presentation entitled “Update on Measles Virus genotypes in the African Region”.

10. Sixth WHO Global Measles and Rubella Laboratory Network Meeting, 23-25 September 2008, Geneva, Switzerland. Oral presentation entitled “Characterization of measles and rubella viruses identified in the African Region”.

11. Polio and Measles Laboratory Directors Meeting, 6-10 July 2009, Kampala, Uganda. Oral presentation entitled “Update of the genetic characteristics of recent measles and rubella viruses detected in the African region”.

12. The 7th Global Measles and Rubella Laboratory Network Meeting. 12-14 October 2009, WHO Headquarters, Geneva, Switzerland. Oral presentation entitled “Monitoring measles outbreaks in the African Region – southern African countries 2009”.

13. Polio and Measles Laboratory Directors Meeting, 15-16 July 2010, Harare, Zimbabwe. Oral presentation entitled “Update of the genetic characteristics of recent measles and rubella viruses detected in the African region”.

14. The 8th Global Measles and Rubella Laboratory Network Meeting. 20-22 September 2010, WHO Headquarters, Geneva, Switzerland. Oral presentation entitled “Molecular surveillance in the African Region”.

15. The 9th Global Measles and Rubella Laboratory Network Meeting. 19-21 September 2011, WHO Headquarters, Geneva, Switzerland. Oral presentation entitled “Molecular surveillance in the African Region”.

16. Measles and Yellow Fever Laboratory Directors Meeting, 20-21 October 2011, Harare, Zimbabwe. Oral presentation entitled “Measles molecular surveillance in the African Region”.

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Measles serology laboratory staff, NICD for IgM serology testing

Respiratory virus isolation laboratory staff, NICD for viral isolation

Clean laboratory staff, NICD for provision of flasks of Vero-hSLAM cultures

World Health Organization, Geneva

Measles section, Centers for Disease Control, Atlanta

World Health Organization, Measles Laboratory Network

World Health Organization, African Region

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Regional Reference Laboratory, Uganda Virus Research Institute, Entebbe, Uganda

National Laboratories of the southern African countries

National Laboratories of other African countries

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AA	amino acid
ADEM	acute disseminated encephalomyelitis, also known as acute post-infectious measles encephalomyelitis
B95-a	adherent Marmoset lymphoblastoid B cell line transformed with Epstein-Barr virus
BDX	BigDye® Xterminator
bp	base pair
CDC	Centres for Disease Control and Prevention, Atlanta, USA
CD46	also known as membrane cofactor protein, ubiquitously expressed on primate cells, protects against complement-mediated cell lysis; cellular receptor of laboratory-adapted strains of measles virus
CIF	case investigation form
CMI	cell-mediated immunity
CPE	cytopathic effect
cp/µl	copies per microlitre
CV-1	continuous cell line derived from the African green monkey kidney
DBS	dried blood spot
dNTP	deoxyribonucleotide triphosphate
DNA	deoxyribonucleic acid
DRC	Democratic Republic of the Congo
EDTA	ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
EIA	enzyme immunoassays also called enzyme-linked immunosorbent assays ELISA
EPI	Expanded Programme for Immunization
EPID	Epidemiological Country Code
EQA	external quality assurance
F	fusion protein
H	haemagglutinin protein
HIV-1	Human Immunodeficiency virus type 1
IgG	Immunoglobulin class G
IgM	Immunoglobulin class M
ISO3	Three letter country code specified by the International Organisation for Standardisation
L	large protein
LabNet	WHO measles Laboratory Network
M	matrix protein
MCV	measles containing vaccine

MCV1	first dose of measles containing vaccine
MCV2	second dose of measles containing vaccine
MIBE	measles inclusion body encephalitis
MVi	Measles virus sequence obtained from viral isolate
MVs	Measles virus sequence obtained from clinical specimen
mRNA	messenger RNA
µl	microlitre
mIU	milli-international units
MMR	vaccine containing attenuated strains of measles, mumps and rubella viruses
MR	vaccine containing attenuated strains of measles and rubella viruses
MV	measles virus
N	nucleoprotein
N-450	the 450 nucleotides coding for the carboxyl-terminal 150 amino acids of the MV nucleoprotein
ng	nanogram
NICD	National Institute for Communicable Diseases, a division of the National Health Laboratory Service
NL	National Laboratory
OF	Oral Fluid
ORI	Outbreak Response Immunisation
P	phosphoprotein
PCR	polymerase chain reaction
pmol	picomole
RRL	Regional Reference Laboratory of the WHO Measles and Rubella LabNet
RT-PCR	reverse transcriptase-polymerase chain reaction
RNA	ribonucleic acid
SIA	Supplementary Immunisation Activity
SLAM	Signaling Lymphocyte Activation Molecule, also known as CD150
SMC	suspected measles case
SSPE	Subacute sclerosing panencephalitis
$TCID_{50}$	Tissue Culture Infectious Dose causing infection in 50% of inoculated cell cultures
TBE	TRIS–borate-EDTA buffer
TE	TRIS buffer with EDTA
TS	Throat Swab
UV	ultraviolet
Vero	continuous cell line derived from the African green monkey kidney (derived from Verdant=green reno=kidney)
Vero-hSLAM	recombinant Vero cells expressing the human SLAM molecule

WHO	World Health Organisation
WT	wild-type virus