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FOREWORD

A pilot study has been conducted with the aim of improving the linkage between diagnosis of rifampicin resistant tuberculosis (RR-TB) and subsequent care of affected patients. In this issue the importance of closing the gap between diagnosis and treatment as an essential tool for the reduction of TB induced morbidity and mortality is highlighted. Virus taxonomy comes under the microscope and in this issue it is shown that virus identification using morphological features is an important method of validating and guiding molecular approaches.

Surveillance reports for this issue include an introduction to the recently introduced clinic based surveillance for TB, HIV and other sexually transmitted infections (STIs) on the GERMS-SA platform. The incidence of non-viral causes of diarrhoea in children less than 5 years in South Africa is reported for the period April 2009 to December 2013. This is the first surveillance report of non-viral stool pathogens under the NICD coordinated rotavirus and other diarrhoeal pathogens surveillance programme and highlights a decrease in the incidence of bacterial and parasitic isolates detected after 2010. Lastly, trends in syphilis seroprevalence in women of reproductive age in the Northern Cape Province (NCP) between 2003 and 2012 are assessed and compared between data obtained from the NHLS Corporate Data Warehouse (CDW) and the National Antenatal Sentinel HIV & Syphilis Prevalence Surveys (ANSUR). Both data bases show that there was a significant decline in syphilis seroprevalence in the NCP during the period under review.

I trust you will find these diverse contributions interesting and useful, and thank all authors for their timely inputs.

Basil Brooke, Editor

CONTENTS

PUBLIC HEALTH ACTION TO REDUCE THE BURDEN OF RIFAMPICIN RESISTANT TUBERCULOSIS

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Introduction

The global burden of multidrug-resistant tuberculosis (MDR-TB) – specifically *Mycobacterium tuberculosis* resistant to rifampicin and isoniazid - remains high and was estimated at 450 000 incident cases in 2012 of which 170 000 cases were fatal.[1] In 2012, South Africa reported a total of 15 419 laboratory confirmed MDR-TB cases of which a treatment regimen was initiated in 6 494 (42%) cases.[1] This "gap" between diagnosis and treatment has subsequently increased with the expanded rollout of the Xpert MTB/Rif assay (GXP) and the increase in absolute numbers of rifampicin resistant cases detected.[2]

In an attempt to address this gap, the National Department of Health (NDoH) plans to appoint one linkage officer per district to enhance the tracing of patients diagnosed with rifampicin resistant tuberculosis (RR-TB) or MDR-TB and to ensure initiation of appropriate treatment. Although this process is still being rolled out, pre-existing tracer teams are already in place in some districts. In addition, the National Institute for Communicable Diseases (NICD) initiated the release of weekly alerts in January 2014. The primary objective of these alerts is to facilitate patient tracing and to improve the link between diagnosis and treatment. These alerts contain line listings of cases newly reported as RR-TB from those laboratories in South Africa using the Xpert MTB/Rif assay (GXP).[3] Alerts are sent to selected individuals in each provincial DoH and the line lists are broken down by province and district. However, this surveillance system is a one way process with a push of data and requires a feedback loop to assess effectiveness.

In order to strengthen this surveillance system, the introduction of community surveillance assistants (CSAs) has been proposed. The function of the CSAs will be to work with the existing tracer teams and follow up cases through patient tracing and interview. In addition, they will facilitate the collection of additional sputum specimens for genotyping and drug susceptibility testing at the NICD. These data will inform cluster monitoring and provide reasons for failure to initiate treatment, using a case record form (CRF). CRF forms are regularly submitted to the NICD and this information can be fed back to the NDoH in a bid to close the diagnosis – treatment gap.

A pilot study was initiated to evaluate how effectively these public health strategies improve linkage between diagnosis and care, and reduce initial loss to follow up among RR-TB cases diagnosed with GXP. Overall, it is envisaged that these strategies will decrease the burden of drug resistant tuberculosis in the selected districts.

Methods

Four districts were selected across South Africa: Francis Baard (FB - Northern Cape province), Dr Kenneth Kaunda (KK - North West Province), Nelson Mandela Metro (NMM- Eastern Cape Province) and Ehlanzeni (EZ-Mpumalanga Province). The target population included all patients diagnosed with RR-TB by GXP between 1 January and 31 March 2015. The study was nested into the existing GERMS-SA surveillance programme at the NICD. University ethics clearance as well as provincial and district approvals were obtained prior to initiation.

Community surveillance assistants (CSAs) were recruited in each district. Weekly case alerts as well as any new cases reported from the primary pathology laboratory in each district were used to regularly monitor and initiate early action. Cases in which the patient was known were followed-up at the primary diagnostic site to obtain their current treatment status. Known patients were contacted and a CRF for each patient was created. Subsequent sputum collections were taken following written informed consent from affected patients. If a patient was not known, a CSA would gather contact details from the relevant facility and either find the patient through the current tracing system or, if that failed, make an attempt to locate the patient directly and complete the process. All CRFs were sent to the NICD for data capture and further analysis. In addition, two field coordinators liaised with the field teams and performed an audit of their case tracing activities as part of the quality control process.

Results

Case numbers

A total of 286 RR-TB cases was identified via the notification system during the 3-month study period. Sixteen line listings representing exact duplicates from 8 patients were excluded. The specific reasons for exclusion were: repeated testing of patients at the same facility during the same week (N=2), repeated testing of patients at the same facility during different weeks (N=2) and repeated testing of patients at the different facilities during different weeks (N=4). One additional duplicate was identified (different 2nd name) and removed, bringing the unique line listing total to 276 cases.

Project sites

One CSA was appointed to each of the three selected sites and two CSAs were appointed to NMM-Eastern Cape based on an expected higher case burden.

The Francis Baard district revealed lower case numbers which could easily be managed by the CSA. However, patients were often scattered such that additional time and resources were required for follow-ups. Coordination with the MDR facility and the existing tracer team was very helpful for accessing patients located remotely from Kimberly.

The Dr Kenneth Kaunda district utilizes a centralized approach in which all RR-TB patients are referred to the MDR-TB unit at Tshepong hospital in Klerksdorp. This simplified the tracer requirements and thus the combined activities of the GERMS surveillance officer and the CSA were sufficient. Particular challenges for case finding in this district included referral of cases from neighbouring areas not relevant to the study and the presence of migrant workers at mines located within the district.

The Ehlanzeni district experienced a very high case load coupled to a wide geographic distribution with some cases more than 200km away. A total of 53 cases was excluded due to insufficient capacity to cover the large Bushbuckridge area using a single CSA.

The Nelson Mandela Metro has a well established and coordinated tracing system in place. In addition, the local MDR facility dedicates specific days for new MDR-TB case enrollments. Thus, coordination involving these mechanisms was used to achieve the tracing objectives. Challenges included cases being admitted outside of allocated days as well as delayed enrollment of cases into the MDR program pending confirmation of diagnosis and identification of a potentially high risk strain. These delays negatively affected the study objectives.

A total of 223 cases was thus available for inclusion in the study. The Nelson Mandela Metro experienced the highest case load compared to Francis Baard district which recorded the lowest (table 1).

Table 1: Distribution of rifampicin resistant tuberculosis (RR-TB) cases diagnosed by the Xpert MTB/RIF assay (GXP) and alerted by district during the period 1 January to 31 March, 2015.

District	Number GXP RR alerted cases	%
Dr Kenneth Kaunda	45	20
Ehlanzeni*	43	19
Francis Baard	16	7
Nelson Mandela Metro	119	52
All districts	223	100

*53 cases from the Bushbuckridge area were excluded – see text for details

Description of identified cases

The 223 cases identified were diagnosed at 103 different facilities of which of 20% of primary diagnoses came from a hospital as opposed to a clinic. The performance of each district, based on percentage of RR-TB patients diagnosed by GXP and subsequently placed on treatment, varied over the three months analyzed (figure 1). A general monthly increase in the proportion of cases placed on treatment was observed in Dr Kenneth Kaunda district while the reverse trend was evident in the other three districts. It should however be noted that the numbers for Francis Baard district are small, and that 2 of the 3 cases not placed on treatment were diagnosed on the last day of the study and were scheduled for follow up. On average, 69% of cases traced across the 4 districts were recorded as having started treatment (figure 1). Reasons for not having started treatment despite patient tracing efforts are presented in table 2.

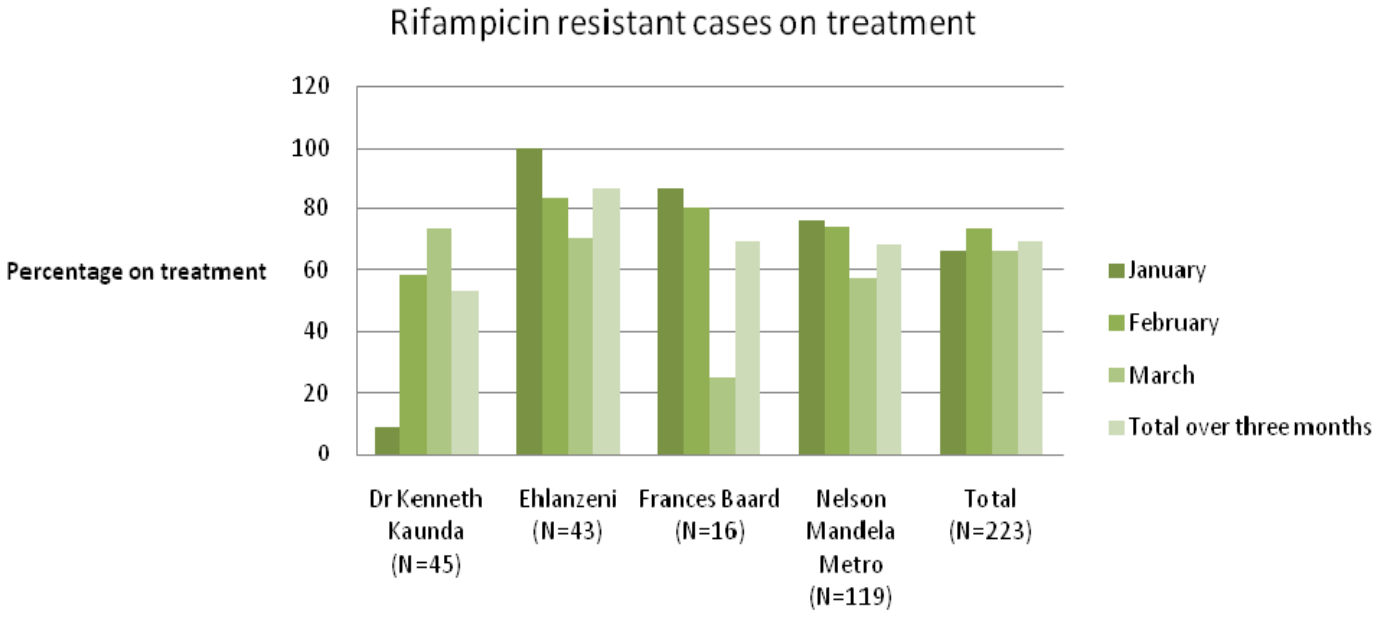

Figure 1: Percentages of rifampicin resistant tuberculosis (RR-TB) cases by district by month identified and subsequently started on treatment during the period 1 January to 31 March, 2015. Actual numbers are given in parentheses.

Table 2: Frequencies and proportions of common reasons for not initiating TB treatment despite patient tracing efforts across all districts in the study during the period 1 January to 31 March, 2015.

Reason	Number	%
Patient died	**3**	**4**
Patient refused treatment	**2**	**3**
Patient not found at address	**1**	**1**
Patient never returned	2	3
Patient moved	**1**	**1**
Patient from different district	**1**	**1**
Incorrect patient information	4	6
No tracing information	43	61
Migrant worker	3	4
Prisoner	**1**	**1**
Not done	8	11
Ongoing	**1**	**1**
Total	**70**	**100**

Numbers and percentages in bold indicate successful tracing but unsuccessful outcome (11%)

Discussion

This study provided important insights into the gap between the diagnosis of RR-TB and subsequent initiation of treatment and the issues that underlie the problem were highlighted.

Using rifampicin alerts, the majority of true RR-TB cases could be identified within a reasonable timeframe. The identification of duplicates assisted in understanding the extent and nature of the problem and improved tracing performance. An additional 29 cases that did not appear through the alerts were detected across the 4 districts. The reason(s) for this is/are unknown and should be investigated as this poses a potential problem in estimating the true case burden.

Operational challenges restricted a complete assessment of the Ehlanzeni district system and thus performance may be poorer in difficult-to-reach areas. Each district had a slightly different approach with a varying capability for patient tracing. Cases located further away from central sites required more resources to achieve follow-up. Similarly, differences in caseloads affected human resource distribution and logistical support.

The overall rate of 69% of cases notified and documented as having initiated treatment is low. This is because the GXP provides a rapid diagnosis and it therefore follows that a high rate of diagnosis should lead to a high rate of treatment initiation. No overall increase in cases on treatment by month was however observed in this study, but additional work is required to compare this data to a period preceding this project.

Other studies are being conducted to assess the impact of the GXP. Importantly, a recent report from the XTEND study showed that despite the increase in detection rate for TB (not specifically drug resistant TB), as well as rapid turnaround time of the test when

compared to smear microscopy, the GXP did not reduce initial loss to follow-up, did not increase the proportion of patients initiating treatment and did not reduce overall mortality.[4] Another feasibility study - TB-NEAT- showed that the GXP can produce rapid results in a clinic based setting with rapid initiation of treatment. However, these findings also did not translate into reduced TB-related morbidity.[5] Although the present findings were generated from a pilot study, the need to strengthen the current follow-up system is clearly evident. A feedback loop is required to close the gap between diagnosis and treatment which will enhance the impact of the GXP intervention.

Introducing the CSAs has provided a useful approach to monitoring the diagnosis-treatment gap by supporting existing follow-up structures through a supervised and a standardized reporting system that enables comparisons between sites. Of the cases that could not be traced and were not on treatment, 61% had no tracing information and 6% had incorrect tracing information. This is a major hindrance to the follow-up system and highlights several instances where documentation relating to patient contact details was lacking at the diagnosing facility or unreliable information was provided for subsequent patient tracing. These constitute areas requiring urgent attention. Of the remaining patients, 10 were successfully traced and found to have valid reasons for not being on treatment, implying that the overall rate of successful tracing was could be adjusted to 73%.

Conclusion

Diagnosing RR-TB rapidly and closing the gap between diagnosis and treatment initiation are essential tools for the reduction of TB induced morbidity and mortality. Various approaches including electronic notification systems and tracer teams are essential components of a TB control programme and resources should be allocated accordingly.

Acknowledgements

We thank the provincial and district Departments of Health for their assistance and their diligent work in tracing and managing patients in the program. We also thank the NHLS and Corporate Data Warehouse staff for their efforts in diagnosing patients, storing information and providing data for this study. Thanks are also also due to the alerts who are required to improve TB related health services. We are furthermore indebted to the community surveillance assistants (CSAs) who were employed through funding received from Clinton Health Access Initiative (CHAI).

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A MICROSCOPIC INTRODUCTION TO VIRUS TAXONOMY

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Introduction

The basis of current virus taxonomy originates from ultrastructural detail observed using transmission electron microscopy, and so the explosive development of virology in the second half of the last century was dependent initially on the advent and subsequent improvement of transmission electron microscopes (TEMs).[1-3] Despite the increasing focus on molecular characterisation, it is important to note that the expression of molecular characteristics results in taxonomically identifiable structure in most instances – so that certain ultrastructural features are indicative of specific molecular details. The approach to virus taxonomy presented below will illustrate this, as well as provide a tool for virus identification from an ultrastructural perspective. The emphasis is on medically important viruses, although some potentially zoonotic viruses from other hosts are also included as these pathogens do not always distinguish between humans and other animals, which explains why 60 - 70% of all human viruses are zoonotic in origin. [4,5] Virus nomenclature follows that of the 9th report of the International Committee on Taxonomy of Viruses (ICTV).[6]

Given the size of virus particles, it is possible to view them in their entirety using a TEM, with heavy metal staining enhancing the contrast between the components of the electron-lucent virions. This technique is known as 'negative staining' and although it is also possible to prepare virus particles for sectioning (by embedding them in an epoxy resin), it is the stained whole-mount that permits rapid virus identification to family level, and in some cases to sub-family and genus level.[3,5,7]

When identifying a negatively-stained virus from a micrograph, there are two crucial criteria to consider:

- Is the nucleocapsid naked or enveloped? (an envelope is a host-derived membrane through which viral glycoproteins extend; figure 1);
- What is the size range in diameter of the virions? If there is overlap between virus diameter ranges, the surface appearance of the virion must be used to differentiate between taxonomic families.

Application of these two criteria provides family distinctions in which neither the nature of the genomic material (RNA or DNA) nor the source of the specimen play a role (table 1).

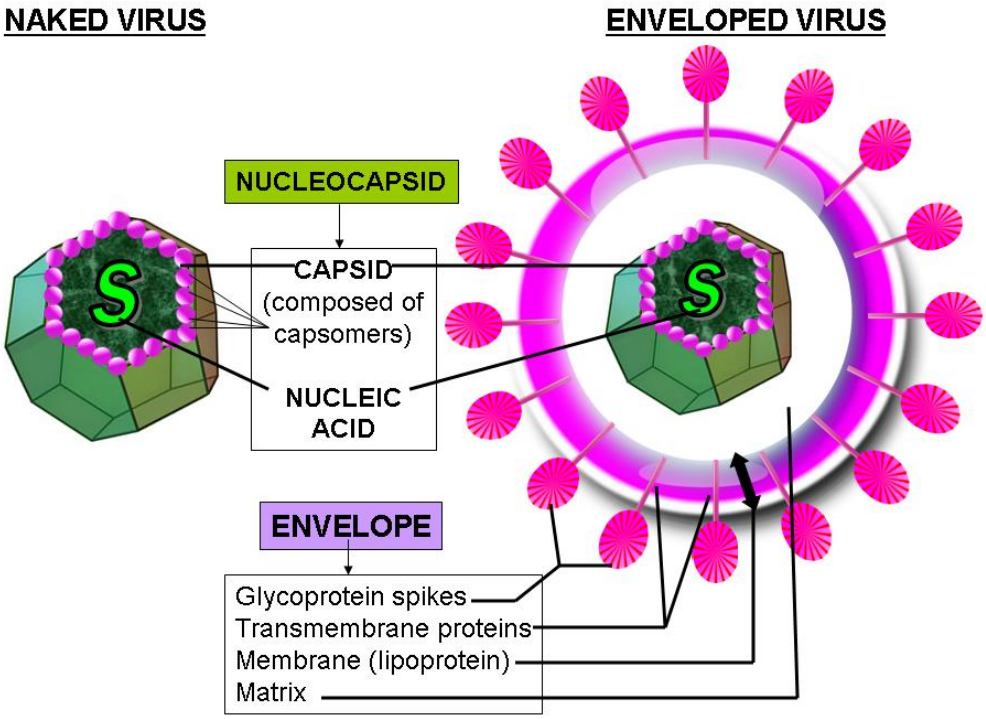

Figure 1: Schematic comparison between a naked, icosahedral virus and an enveloped virus with an icosahedrally symmetrical nucleocapsid. Enveloped viruses may contain a nucleocapsid that is icosahedrally symmetrical (as illustrated here), helically symmetrical (in which the capsomers are helically arranged around the nucleic acids) or lacking obvious symmetry (complex/uncertain nucleocapsid symmetry as is the case in the Poxviridae/Arenaviridae).

Table 1: Virion diameter range (nm) of selected virus families in which the nucleocapsid is either naked or enveloped.[6, 8] Family names in red indicate DNA- containing viruses.

Nucleocapsid phenotype	Diameter range of virion (nm)	Taxonomic family
Naked	17 – 21	Circoviridae
	21 – 26	Parvoviridae
	27 – 35	Hepeviridae
	27 – 40	Caliciviridae
	28 – 30	Picorna-; Astroviridae
	33 – 37	Picobirnaviridae
	40 – 45	Polyomaviridae
	52 – 55	Papillomaviridae
	60 – 80	Reoviridae
	70 – 90	Adenoviridae
Enveloped	40 – 60	Flaviviridae
	42 – 50	Hepadnaviridae
	45 – 100	Rhabdoviridae
	50 – 60	Bornaviridae
	50 – 120	Orthomyxoviridae
	50 – 300	Arenaviridae
	50 – 500	Paramyxoviridae
	70 – 75	Togaviridae
	75 - 160	Coronaviridae
	80 – 100	Retroviridae
	80 – 120	Bunyaviridae
	80 – 85	Filoviridae
	120 – 300	Herpesviridae
	140 – 450	Poxviridae
	450 – 750	Mimiviridae*

* The International Committee on Taxonomy of Viruses has not yet recognized the proposed family Megaviridae in which enveloped virions may be over 1000 nm.

The naked (non-enveloped) virus families

There is some overlap in the size ranges of the virion diameters, particularly with the smallest representatives. In these cases any taxonomic assignation should take into consideration the fact that there is a certain amount of virion shrinkage due to chemical fixation, heavy metal staining and subsequent viewing (typically with an electron beam of 80 000V). The use of cryotomography obviates these difficulties but requires specialised equipment not usually available to entry level TEM facilities.[9] Fortunately, it is still possible to distinguish between representatives of these families by negatively-stained surface details of the virions ie. the Hepeviridae typically have slightly lumpy, daisy-shaped virions (figure 2a), those of the Caliciviridae have cup-like indentations (figure 2b), whilst virus particles of the Picornaviridae typically have unremarkable surface ornamentation (figure 2c) in contrast to the star-like appearance of the Astroviridae (figure 2d). The Polyomaviridae and Papillomaviridae can be differentiated on the basis of size, as their surface structure is very similar (figures 2e & 2f). Representatives of the Adenoviridae are easily recognised as non-enveloped icosahedrons with spherical capsomers (hexons) symmetrically arranged on the faces of the icosahedron, thus high-lighting the angular symmetry of the virions (figures 2g & 2h). A protein fibre extends from each of the 12 pentagonal capsomers, giving the virion its characteristic 'sputnik' appearance, although the delicate nature of the fibres often precludes their visualisation in negatively-stained preparations. The remaining family of naked viruses, the Reoviridae, also display icosahedral symmetry, but generally appear spherical due to the fact that there are one to three concentric layers of capsid proteins surrounding the core of double-stranded RNA segments (figures 2i & 2j).

The enveloped virus families

Initial distinctions that can be made between enveloped virions is based on the appearance of the envelope itself. In several families, notably those containing vectors of arthropod-borne viruses (arboviruses) (Flaviviridae, Togaviridae, Bunyaviridae), the closely-fitting envelope resembles a fuzzy fringe around the nucleocapsid (figures 3a–3i). In these cases, it is often difficult to distinguish individual glycoprotein spikes, although some of the Bunyaviridae genera present a more structured particle surface, despite the actual virions being pleomorphic (figures 3h–3j). Pleomorphism may be a misleading feature in cultured isolates (figure 3k) as it may result from culturing conditions and the production of defective interfering particles.[10]

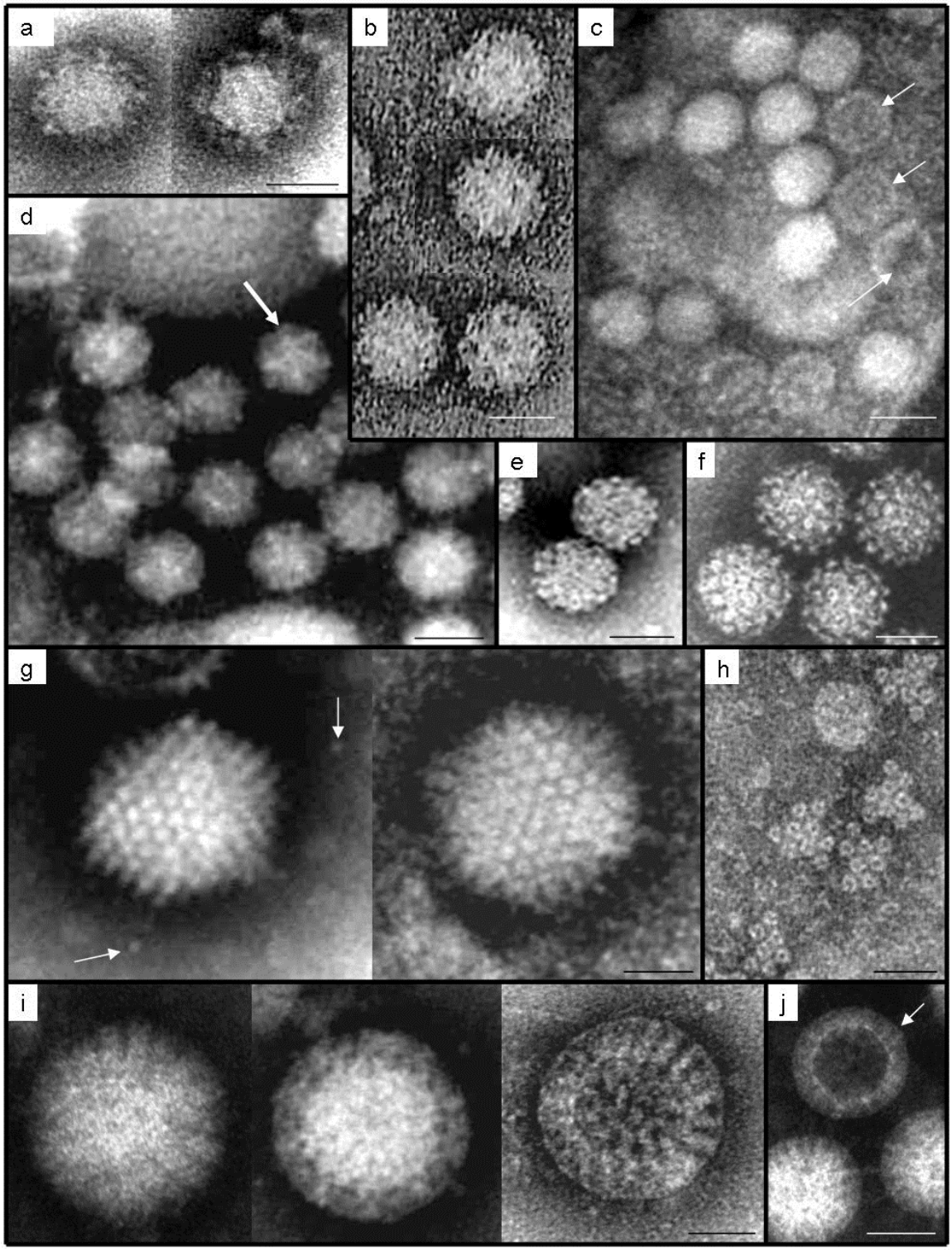

Figure 2: Naked virus particles representative of the following families: (a) Hepeviridae; (b) Caliciviridae; (c) Picornaviridae (*Enterovirus*) – dark-centred virions (arrows) are those in which the heavy metal stain has penetrated the capsid; (d) Astroviridae – only 10% of virions may present the star-like appearance (arrow) after which this family is named; (e) Polyomaviridae; (f) Papillomaviridae; (g) Adenoviridae (arrows indicate the distal knobs of two of the penton protein fibres; (h) clusters of spherical, non-vertex (hexagon) capsomers of the Adenoviridae (i) Reoviridae - from left to right: *Orthoreovirus* (2 capsid layers); *Orbivirus* (2 capsid layers); *Rotavirus* (3 capsid layers); (j) empty *Rotavirus* particle (arrow) with tri-layered capsid. Scale bars: (a) = 19 nm; (b) = 23 nm; (c) = 28 nm; (d) = 30 nm; (e) = 33 nm; (f) = 35 nm; (g) = 27 nm; (h) = 160 nm; (i) = 27 nm; (j) = 47 nm.

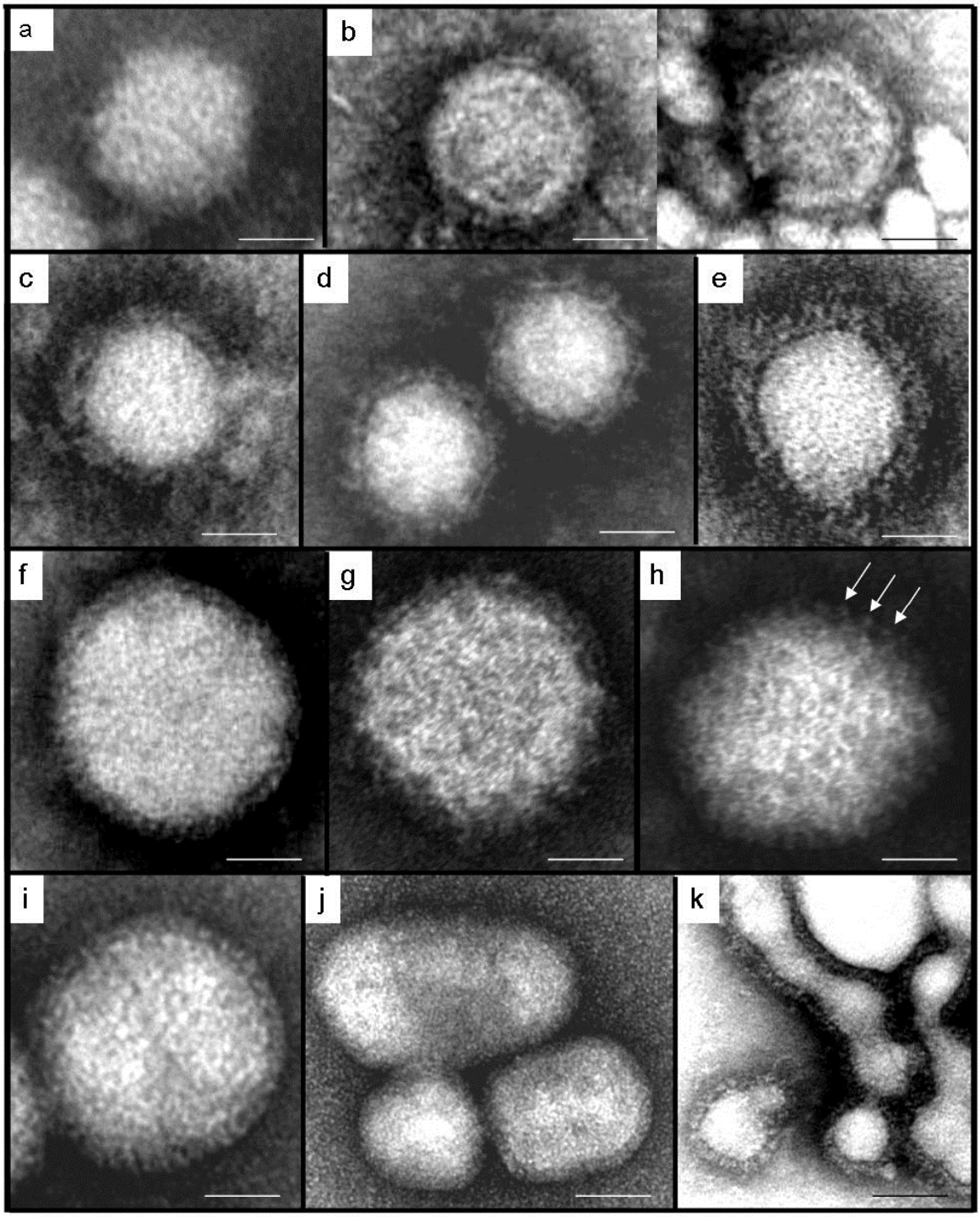

Figure 3: Tightly-enveloped virus particles representative of the following families: (a) Flaviviridae, *Flavivirus* – Portugal mosquito Marim virus; (b) Flaviviridae, *Flavivirus* – West Nile Virus. Note the icosahedral nucleocapsid filled with heavy metal stain; (c) Togaviridae, *Alphavirus* – Middleburg virus; (d) Togaviridae, *Alphavirus* – Ndumi virus; (e) Togaviridae, *Alphavirus* – Sindbis virus; (f) Bunyaviridae, *Nairovirus* – Crimean Congo Haemorrhagic Fever virus; (g) Bunyaviridae, *Orthobunyavirus*; (h) Bunyaviridae, *Phlebovirus* – Rift Valley Fever virus showing generic features of regularly spaced, cylindrical patterning (arrows); (i) Bunyaviridae – Gouleako virus with a structured surface appearance; (j) Bunyaviridae – pleomorphism obvious between clustered virions of Gouleako virus; (k) Togaviridae, *Alphavirus* – irregular viral profiles, all enveloped, in cultured isolate after over-passage and high, initial inoculum (Multiplicity Of Infection). Scale bars: (a) = 21 nm; (b) = 19 nm; (c) = 28 nm; (d) = 31 nm; (e) = 26 nm; (f) = 34 nm; (g) = 36 nm; (h) = 24 nm; (i) = 23 nm; (j) = 47 nm; (k) = 53 nm.

Another family in which individual envelope spikes are infrequently visible and virions are occasionally polymorphic, is the Herpesviridae, which differs from the Flavi-, Toga- and Bunyaviridae, in having a fairly loose envelope within which the icosahedral nucleocapsid is distinct, often seen free once the envelope is ruptured (figures. 4a-c).

Although the bur-like envelope of members of the Hepadnaviridae is not remarkable, and the size range overlaps with that of the Flaviviridae, virus particles assigned to this family are recognisable by the mixture of filamentous and spherical particles always present (figures 4d-f). In contrast, virions of the Retroviridae are consistently spherical and instantly identifiable by the apparent electron-lucent shroud present around mature particles (figure 4g). HIV-1 virions, in which the gag protein has not yet condensed, have distinct, evenly-spaced glycoprotein spikes (figure 4h). Coronaviridae virus particles also have distinctly ornamented envelopes, with the evenly spaced, spatulate spikes having pronounced terminal ends generally referred to as peplomers (figures 4i & 4j).

Filoviridae filamentous particles are easily recognisable by the consistent diameter of the finely studded filaments, the consistently visible, helical nucleocapsid and the phenomenal lengths of some of the virions, which may exceed 11 000 nm (figures 5a & 5b). Although the Orthomyxoviridae and Paramyxoviridae also have filamentous virions (figures 5c & 5d), the nucleocapsid is rarely apparent beneath the envelope of the filaments, and there are also spherical-to-pleomorphic particles which can be used to differentiate between the two families (table 1). As the envelopes are indistinguishable (compare figures 5c and 5e to 5f), the two features that can be used in identification are:

- Size range - the maximum size of the Orthomyxoviridae virions is far smaller than that of the Paramyxoviridae;
- Nucleocapsid structure - the 'herring-bone' structure of the Paramyxoviridae nucleocapsids is unmistakable (figure 5g).

It is interesting to note that with the exception of the Hepadnaviridae (which have a retroid, double stranded DNA genome replicating via single stranded RNA intermediates), all the enveloped, potentially filamentous virus families contain negative sense, single-stranded RNA.

The remaining two families of enveloped virus with a helically symmetrical nucleocapsid are the Rhabdoviridae and the Arenaviridae. The Rhabdoviridae are identified on the basis of the bullet- or cone-shaped particles (in the case of vertebrate pathogens), and the Arenaviridae are the most highly pleomorphic of all enveloped viruses (table 1).

The two families with the largest virions provide little challenge for taxonomic identification, and it is also possible to make ultrastructural distinctions between some of the genera of the Poxviridae, based on the patterning of the outer surfaces (figures 6a–6f). Virions of the largest and most complex of the virus families, the Mimiviridae, appear spherical due to the fine, fibrillar coat around the icosahedral body of the particles, within which lie two membranes, a protein coat, and two cores of double stranded DNA of more than a million base pairs (figures 6g & 6h). Clearly the ultrastructural complexity of the large enveloped viruses is a reflection of their double stranded DNA composition.

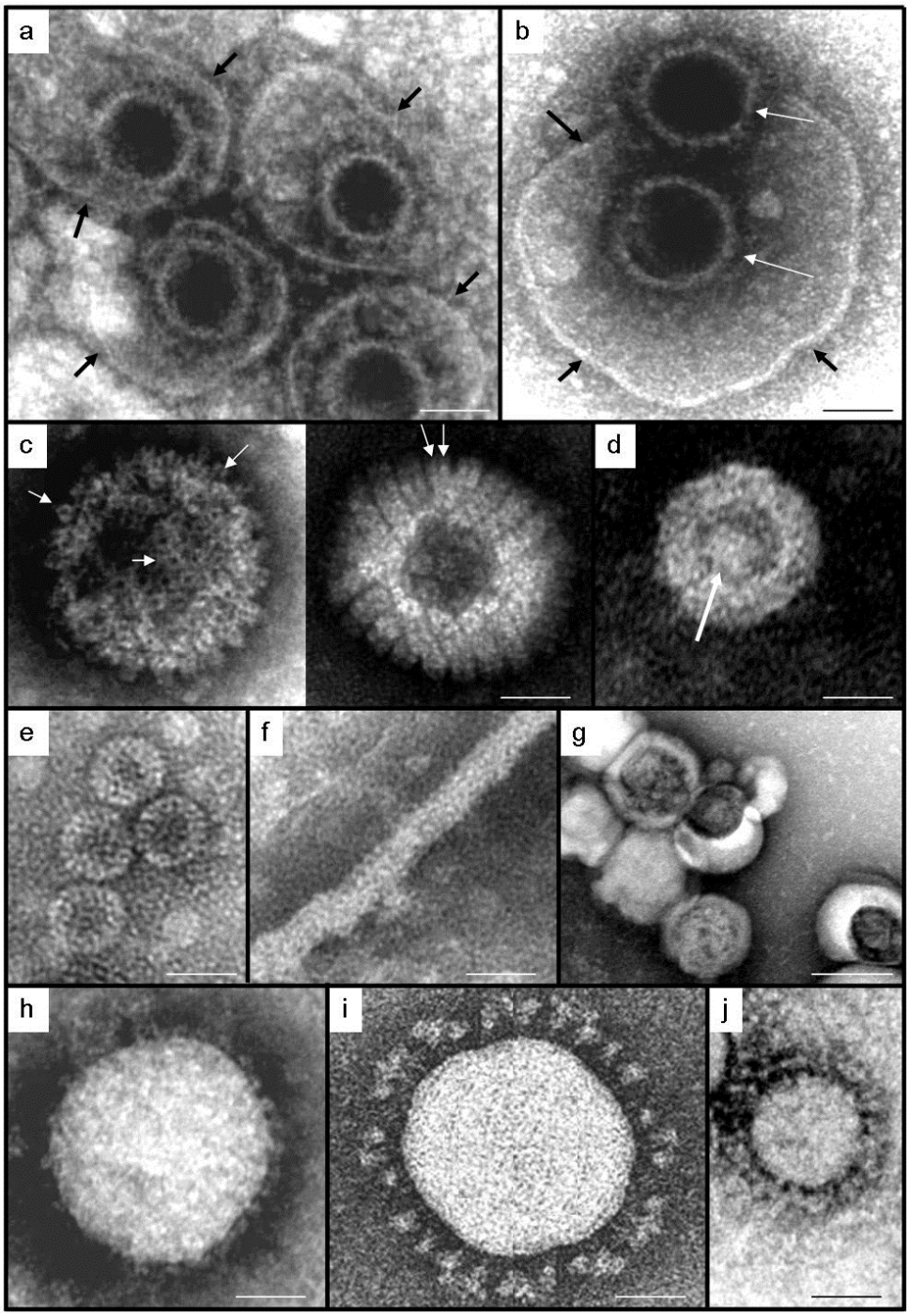

Figure 4: Enveloped virus particles representative of the following families: (a) Herpesviridae, *Simplexvirus* (the ''fried egg'' virus) with heavily stained nucleocapsids within loose envelopes (black arrows); (b) Herpesviridae, *Simplexvirus* with a nucleocapsid resting upon an entire virion (black arrows indicate envelope, white arrows the nucleocapsids); (c) Herpesviridae, *Cytomegalovirus* nucleocapsids showing the tubular capsomers (arrows) typical of the family; (d) Hepadnaviridae – infectious 'Dane' particle containing nucleocapsid (arrow); (e) Hepadnaviridae – non-infectious spherical particles with the spiky envelope typical of the family; (f) Hepadnaviridae – non-infectious filamentous fragment; (g) Retroviridae; (h) Retroviridae – sturdy glycoprotein spikes clearly evident around the spherical particle, prior to *gag* condensation; (i) Coronaviridae – roughly spherical particle with widely-spaced peplomers; (j) Coronaviridae – virions can be distinguished from those of the Togaviridae by the globular appearance of the envelope (in cases in which individual peplomers are not immediately apparent). Scale bars: (a) = 83 nm; (b) = 62 nm; (c) = 29 nm; (d) = 18 nm; (e) = 32 nm; (f) = 11 nm; (g) = 80 nm; (h) = 22 nm; (i) = 27 nm; (j) = 40 nm.

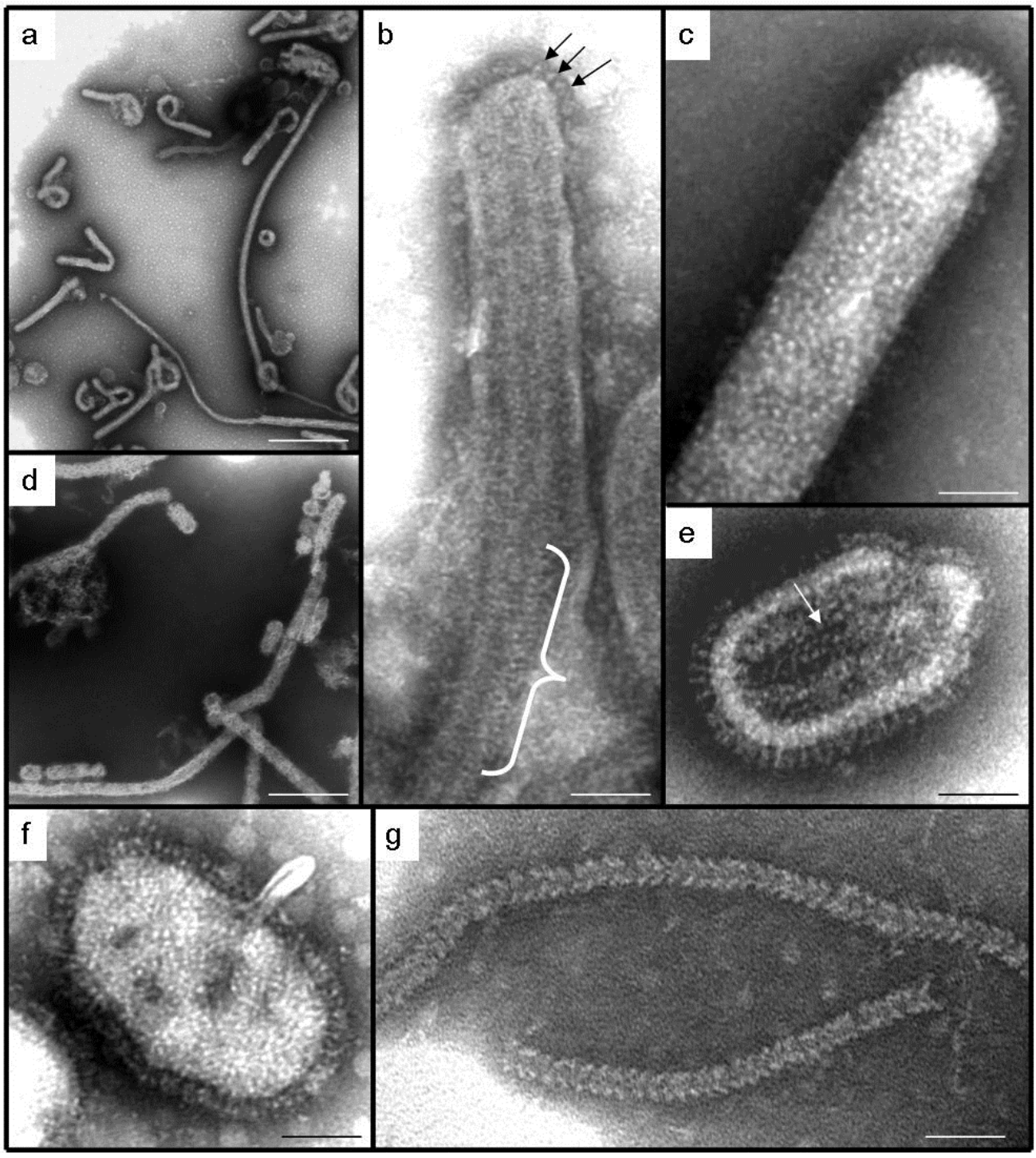

Figure 5: Filamentous/pleomorphic enveloped virus particles representative of the following families: (a) Filoviridae, *Ebolavirus* – the '6'-shaped particles are typical of cultured isolates; (b) Filoviridae, *Ebolavirus* with regular envelope spikes (black arrows) and helically symmetrical nucleocapsid (white bracket); (c) Orthomyxoviridae, *Influenzavirus A* filamentous virion with stippled envelope; (d) Orthomyxoviridae, *Influenzavirus A* cultured isolate (H3N2) with filamentous and spherical particles; (e) Orthomyxoviridae, *Influenzavirus B* truncated particle showing regular, straight spikes of the envelope and partially visible nucleocapsid (arrow); (f) Paramyxoviridae, *Morbillivirus* pleomorphic particle with stippled envelope; (g) Paramyxoviridae, *Morbillivirus* nucleocapsid released by envelope disruption. Dimensions of the nucleocapsid diameter and pitch can be used as sub-family determinants. Scale bars: (a) = 800 nm; (b) = 48 nm; (c) = 35 nm; (d) = 360 nm; (e) = 37 nm; (f) = 49 nm; (g) = 42 nm;

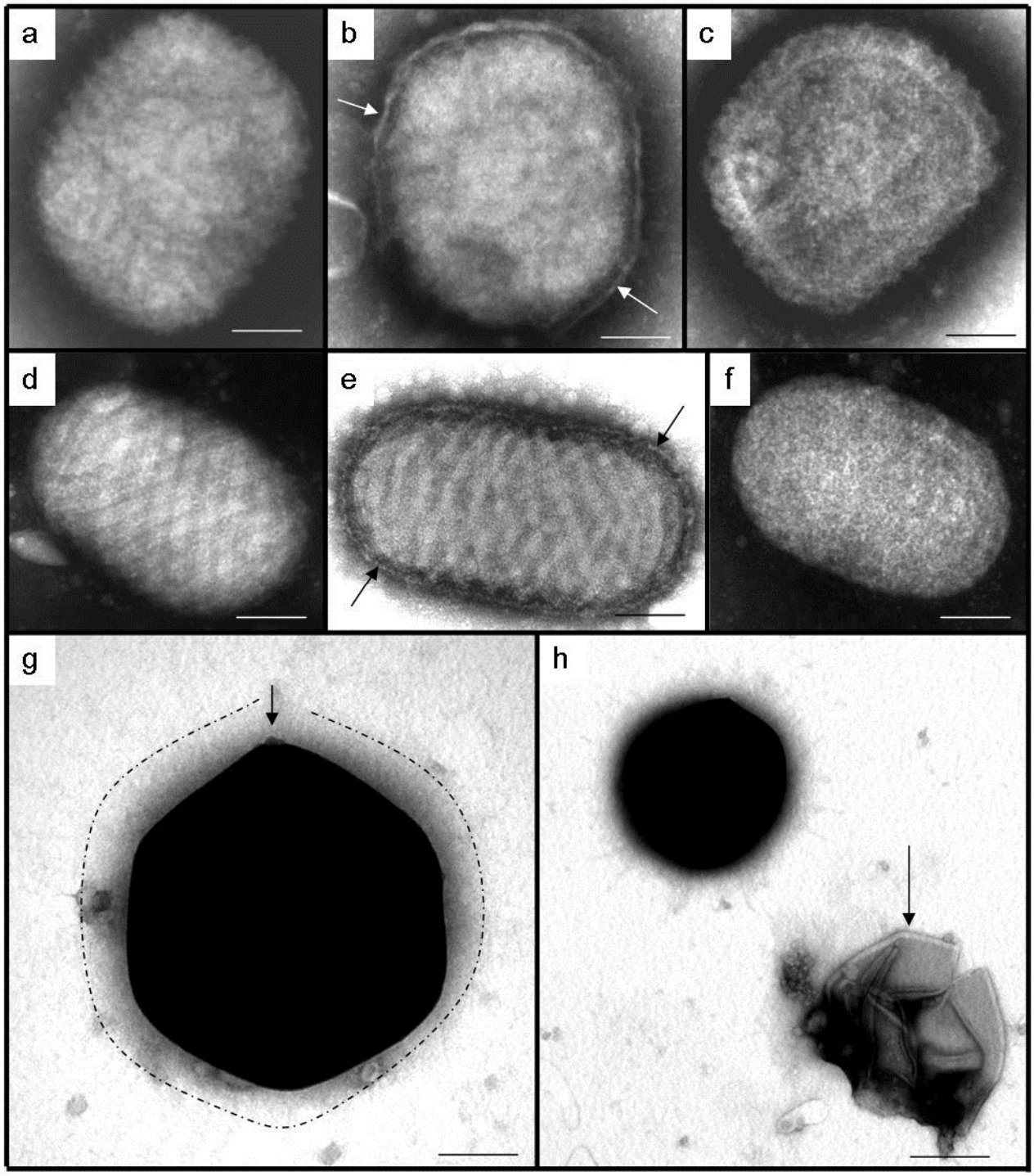

Figure 6: Large, complex enveloped virus particles representative of the following families: (a) Poxviridae, *Orthopoxvirus* a somewhat woolly appearance with thread-like ridges (b) Poxviridae, *Orthopoxvirus* extracellular enveloped virion (arrows indicate extra host-derived membrane); (c) Poxviridae, *Orthopoxvirus* stain-infiltrated virus showing complexity of layering; (d) Poxviridae, *Parapoxvirus* with spiral ornamentation creating a pinecone/basket weave effect; (e) Poxviridae, *Parapoxvirus* extracellular enveloped virion (arrows indicate host-derived membrane); (f) Poxviridae, *Parapoxvirus* mature virion; (g) Mimiviridae *Mimivirus* Icosahedral-shaped particle, with an apical stargate structure (arrow) and fibrillar coat (dotted line) of bacterial proteins which gives the virus a hairy appearance; (h) Mimiviridae *Mimivirus* intact virion and some membranous remnants (arrow) of the two layers that enclose the proteinaceous shell of the nucleic acid. Scale bars: (a) = 90 nm; (b) = 108 nm; (c) = 95 nm; (d) = 60 nm; (e) = 48 nm; (f) = 60 nm; (g) = 118 nm; (h) = 217 nm.

Conclusion

Nucleocapsid morphology and virion diameter ranges can be used to classify and identify virus taxa. Virus taxonomy and identification using morphological features is an important method of validating and guiding molecular approaches. Although deep sequencing may pre-empt the classical techniques of virology (such as virus isolation and electron microscopy) for identification of unknown viruses, all successful research - including the development of vaccines – relies on collaborative laboratory inputs from both traditional and molecular virologists.

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INTRODUCTION TO GERMS-SA CLINIC BASED SURVEILLANCE FOR TB, HIV AND OTHER STIS AND RELATED DRUG RESISTANCE

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Introduction

The NICD surveillance platform, GERMS-SA, has been in existence for more than a decade. Originally, GERMS included laboratory based surveillance where a surveillance officer (SO), alerted to a predetermined test result of a confirmed pathogen related disease (e.g. invasive pneumococcal disease) by NHLS laboratory staff, traced hospital based patients and gathered additional information on risk factors through patient interview and review of medical records. However, the platform did not include surveillance for tuberculosis (TB), human immunodeficiency virus (HIV) infection or sexually transmitted infections (STI). In 2012, following the introduction of Xpert MTB/RIF technology in South Africa, surveillance of rifampicin-resistant tuberculosis (TB) was included in the GERMS-SA programme and enhanced surveillance was expanded to include selected clinics as well as hospital based patients. Additional sputum samples were collected for drug susceptibility testing and genotyping at the National Institute for Communicable Diseases (NICD).

In 2014, a decision was made to further expand GERMS -SA to include clinic based surveillance, initially at one clinic per province in South Africa. The entry point for clinic based surveillance is patients presenting with a positive result for TB, HIV or STI which would then lead to further monitoring for related drug resistance. For this, protocols were developed and ethics approval obtained from the University of the Witwatersrand, local ethics boards and provincial approval bodies as required.

Integrated TB/HIV clinic based surveillance

Principal Investigators: Nazir Ismail, Centre for Tuberculosis, NICD and Gillian Hunt, Centre for HIV and STIs, NICD

In 2011, the National Department of Health began a phased implementation of Xpert MTB/RIF (Xpert) rapid testing for all TB suspects. This test is used to diagnose TB infection and assess rifampicin (Rif) resistance, but cannot test for isoniazid (INH) resistance. Isoniazid mono-resistance is more common than multi-drug resistant TB (MDR-TB) or rifampicin mono- resistant TB. The 2001/2002 South African Drug Resistance Tuberculosis survey reported INH resistance in 5.7% of new patients and in 11.8% of previously treated patients, with mono-resistance to INH present in 2.6% (new) and 2.9% (re-treatment) patients respectively. Thus, surveillance for INH and other first line resistance has been identified as a priority need in the national TB control program. In addition, understanding risk factors and microbiological characteristics (e.g. strain type, minimum inhibitory concentration changes etc.) related to this group are important for designing appropriate control strategies and are therefore also included in the surveillance programme.

South Africa has over 6 million HIV infected individuals with ~2.6 million people receiving antiretroviral therapy (ART), making this HIV control programme the largest in the world. Routine testing for HIV drug resistance (HIVDR) is offered to patients failing protease inhibitor-based ART only in terms of providing access to third-line antiretroviral options. Surveillance of HIVDR in patients

initiating therapy with or without prior exposure to ART is limited globally. However, as higher levels of HIVDR are expected in those with prior ARV exposure (for example women exposed to PMTCT, or patients returning to care after >3 months), these populations would be expected to contribute disproportionally to levels of observed resistance. It is necessary to differentiate these groups as changes in public health policy may warrant different first-line regimens for these different subgroups.

District and site/clinic selection for the surveillance programme was influenced by burden of disease, National Health Insurance status and in some instances, logistics as well as inputs from provincial Departments of Health. Surveillance officers have been placed at selected clinics and their brief is to work together with facility staff to identify eligible participants for surveillance. This includes all newly diagnosed rifampicin susceptible TB patients, all HIV positive patients older than 15 years of age initiating ART, a specified number of patients presenting with STIs and individuals presenting at family planning clinics. After obtaining informed consent, an SO administers a questionnaire including demographic features, risk factors for TB or HIV drug resistance and socio-economic status. Two blood specimens on ART initiators and one sputum specimen for TB patients are collected and sent to the NICD for HIV and TB drug resistance testing respectively. Clinic data regarding the numbers of patients tested for TB and HIV, rates of co-infection and other aspects of the TB-HIV treatment cascade are also monitored. Specimens are also collected for the STI component of the surveillance and a short questionnaire is completed.

This surveillance programme provides a unique national platform to understand, characterize and track the dual TB/HIV epidemic in an operational setting using a standardised approach across all provinces, and creates an opportunity to better understand the TB-HIV treatment cascade and associated challenges in a real life clinical setting. Both the HIV and TB treatment programmes use a public health approach with empiric regimens, which require on-going surveillance to monitor whether they remain appropriate over time. For TB, on-going enhanced surveillance will be needed to monitor emerging trends for INH resistance and resistance to other TB drugs, and will help to identify and study the risk factors for INH resistance. For HIVDR, the findings of this surveillance programme will inform the burden and extent of existing drug mutations (previous ART and unexposed to ART), support optimal regimen selection and inform strategic planning of ART programmes countrywide.

In addition, the objectives of this surveillance programme are to measure key components of the TB-HIV treatment cascade or continuum of care. This will help to better understand points of losses and determinants thereof within the South African TB-HIV continuum of care process. This data will inform strategies for strengthening current systems supporting TB-HIV care. Lessons learned during implementation and findings from this surveillance can potentially be used to inform larger representative cross-sectional studies where indicated.

Sentinel surveillance of sexually transmitted infection syndrome aetiologies, gonococcal antimicrobial resistance and HPV genotypes among patients attending public healthcare facilities in South Africa

Principal Investigator: Frans Radebe, Centre for HIV and STIs, NICD

Sexually transmitted infections (STIs) continue to be highly prevalent among individuals of reproductive age within South Africa. STIs have been treated using the syndromic management approach since the late 1990s.

The WHO recommends that periodic assessments of aetiologies of STI syndromes ((e.g. male urethritis syndrome (MUS), vaginal discharge syndrome (VDS), genital ulceration syndrome (GUS)) should be a core STI surveillance activity, especially in countries where STI syndromic management and case reporting are routinely undertaken. The numbers of total STI syndrome episodes and new episodes of MUS are recorded at all public sector primary health care facilities within South Africa. However, no data are routinely recorded for other STI syndromes, including VDS and GUS.

In the past five years, several antimicrobial resistance surveys and research studies undertaken in eastern and southern Africa have reported an unacceptably high prevalence of fluoroquinolone resistant *Neisseria gonorrhoeae* isolates. In response, South Africa has moved to replace fluoroquinolones with single dose oral cefixime. The first *N. gonorrhoeae* isolates demonstrating decreased susceptibility or resistance to oral extended spectrum cephalosporins (ESCs), in one case associated with cefixime treatment failure, were reported in South Africa among men-who-have-sex-with-men residing in Johannesburg in 2013.

HPV is the most common STI. Specific types of "high-risk" HPV can cause cervical cancer. The current vaccines, Cervarix and Gardasil, vaccinate against HPV-16 and HPV-18, the two major HPV types that cause cervical cancer, and also provide cross-protection against some of the other high-risk HPV types. In addition, Gardasil protects against HPV-6 and 11, which cause anogenital warts.

This surveillance programme will provide aetiological STI data on each of the three major STI syndromes (MUS, VDS and GUS) that present to South African primary healthcare clinics (PHC), at 9 sentinel public sector healthcare facility sites (1 site/province). The prevalence of HIV, HSV-2, hepatitis B and syphilis co-infections will be determined among patients with MUS, VDS and GUS. These data will additionally be analyzed by province and, for HSV-2, hepatitis and syphilis co-infections, by HIV status. Urethral discharge samples will be collected from a minimum of 150 men per province with MUS for gonococcal culture. All gonococcal strains that are isolated will be tested for antimicrobial susceptibility. In order to determine HPV prevalence among young sexually-active women, HPV surveillance will be conducted among 900 eighteen to twenty year old women attending family planning clinics within the same nine primary health care clinics. HPV prevalence and genotype data will provide important pre-vaccination data and will enable future monitoring of trends in both the prevalence of HPV detection and the relative prevalence of vaccine-related HPV genotypes once HPV vaccine is fully introduced into South Africa.

Progress to date

Clinic based surveillance sites have been established in the Eastern Cape (Nelson Mandela Bay) and North West provinces (Kenneth Kaunda) and in a small rural clinic in Mpumalanga Province. The KwaZulu-Natal (Umgungundlovu) and Mpumalanga (Ehlanzeni) sites are expected to be established by June or July 2015 and the Gauteng (City of Johannesburg) site before the end of 2015.

Reference

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NON-VIRAL CAUSES OF DIARRHOEA IN CHILDREN LESS THAN 5 YEARS FROM SENTINEL SITES IN SOUTH AFRICA, 2009 – 2013

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Introduction

Diarrhoeal disease is a major cause of morbidity and mortality in children less than 5 years old, particularly in Africa, where it is estimated to cause 12% of all childhood deaths.[1-3] In South African children between the ages of one month and one year, mortality rates in the poorest quintile are four times higher than in the wealthiest quintile. The majority of these deaths (82.6%) are caused by five conditions one of which is diarrhoeal disease, accounting for 20.7% of these deaths.[4]

While the major cause of childhood diarrhoea is rotavirus, the implementation and roll out of the rotavirus vaccination programme in 2009 has seen a significant decrease in the incidence of severe rotavirus gastroenteritis requiring hospitalisation in children less than one year.[5] There is therefore renewed interest in diarrhoeal diseases due to non-viral pathogens. Diarrhoeagenic *Escherichia coli, Salmonella, Shigella* and *Campylobacter* species are the most common bacterial pathogens[3,6,] while *Cryptosporidium* species and *Giardia lamblia* are the most common parasitic diarrhoeal pathogens in children less than 5 years.[7]

The findings from the diarrhoeal sentinel surveillance programme in South Africa, based on bacterial and parasitic isolates, are reported for the period April 2009 to December 2013. The data for 2014 are yet to be verified and will be presented in subsequent reports.

Methods

In 2009, national surveillance for rotavirus and other diarrhoeal pathogens in children less than 5 years old was set up by the National Institute for Communicable Diseases (NICD). Five sentinel sites were established in three of South Africa's provinces namely: Gauteng (Dr George Mukhari and Chris Hani Baragwanath Hospitals), Mpumalanga (Mapulaneng and Matikwana Hospitals) and KwaZulu-Natal (Edendale Hospital). Children less than 5 years of age, who were admitted to a sentinel site with symptoms of three or more loose stools in the past 24 hours, were enrolled into the programme after informed consent was obtained. A stool specimen was collected from each patient and subsequently tested for rotavirus and other enteric viruses at the Centre for Enteric Diseases (CED) Virology Laboratory at the NICD. If a stool sample was not available, nappy liners and/or rectal aspirates were collected. If a sufficient quantity of sample was left over, additional tests were conducted to identify bacterial and parasitic enteric pathogens. These additional tests were conducted at the CED Bacteriology Laboratory and the Centre for Opportunistic, Tropical and Hospital Infection's Parasitology Reference Laboratory respectively, following standard operating procedures.

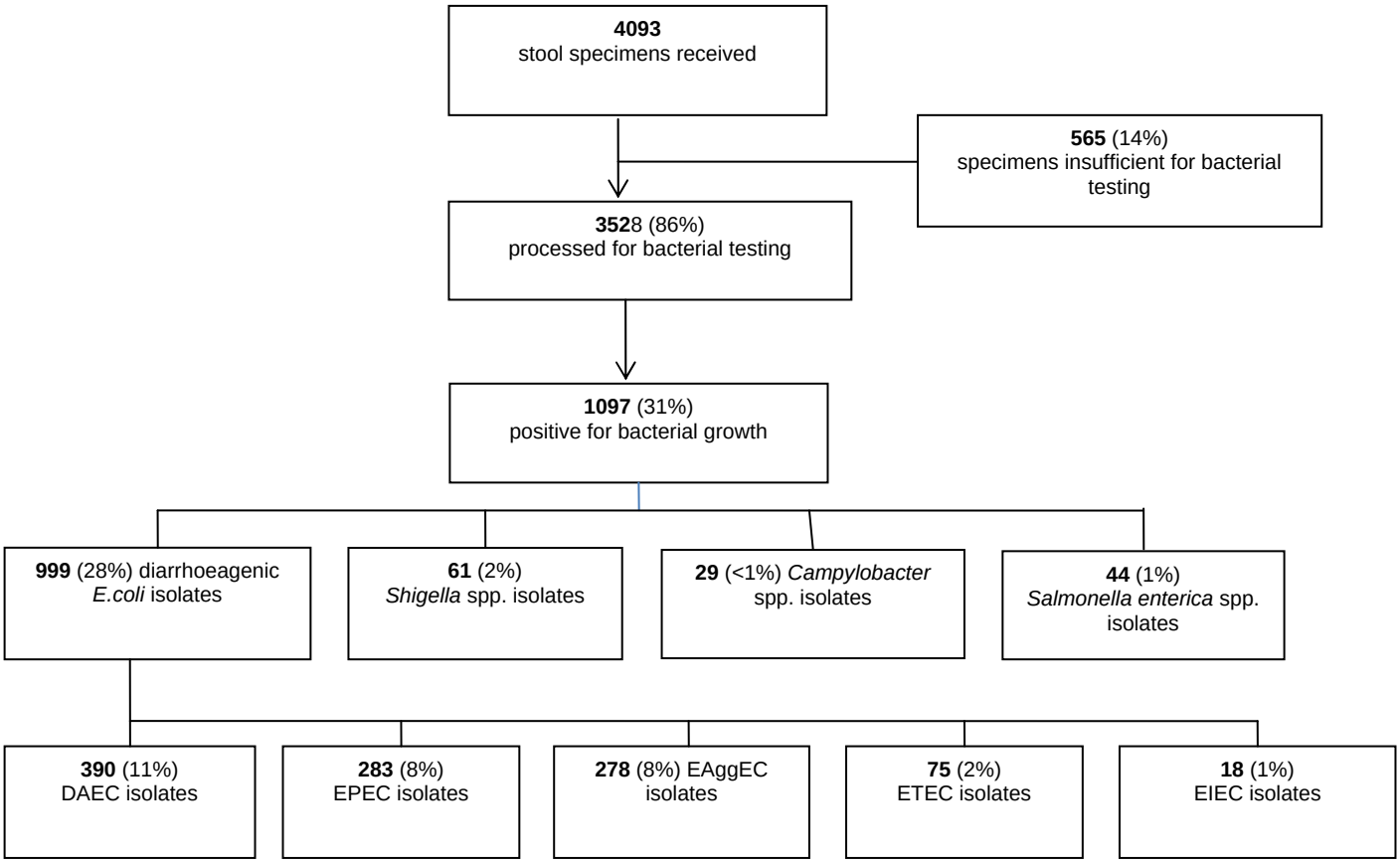
Results

Bacteria

The sentinel surveillance programme enrolled 4122 children fulfilling the case definition between April 2009 and December 2013, of which 4093 (99%) stool specimens were received by the CED Bacteriology Laboratory (figure 1). Thirty-one percent of all processed specimens were positive for bacterial growth. Twenty-

four percent (264/1097) of stool specimens tested for bacteria were co-infected with rotavirus. Co-infections between different strains of diarrhoeagenic *E.coli* (46/1097), between diarrhoeagenic *E.coli* and *Shigella* spp. (19/1097), between diarrhoeagenic *E.coli* and *Salmonella enterica* spp. (9/1097) and between diarrhoeagenic *E.coli* and *Campylobacter* (9/1097) were found amongst specimens positive for bacterial growth.

Figure 1: Flow of stool specimens received and the detection rate of bacterial pathogens amongst all specimens received during the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.



DAEC: Diffusely Adherent *E. coli*; EPEC: Enteropathogenic *E. coli*; EAggEC: Enteroaggregative *E. coli*; ETEC: Enterotoxigenic *E. coli*; EIEC: Enteroinvasive *E. coli*

The detection rate of pathogenic bacteria in stool was fairly constant throughout the year with a slight increase in the summer months. The detection rate of bacterial pathogens varied from 18-58% and there was an overall decrease in the detection rate after 2010 (figure 2). There was no clear seasonality in the trends by diarrhoeal pathogen although an increase in *Campylobacter* spp. isolates was observed in the latter half of 2013, following the introduction of new diagnostic protocols (figure 3).

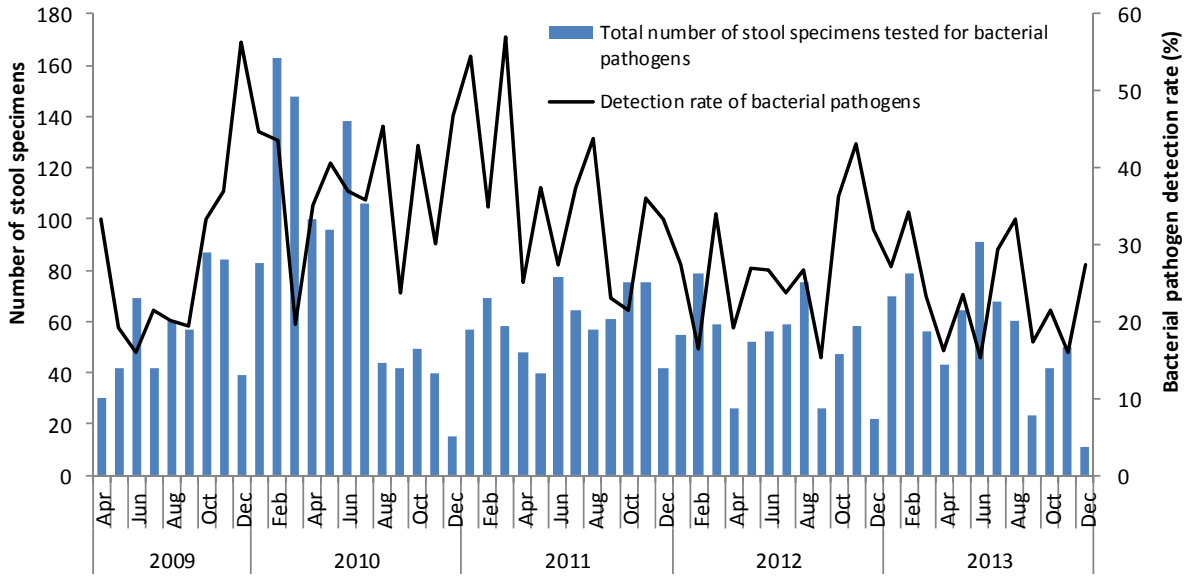
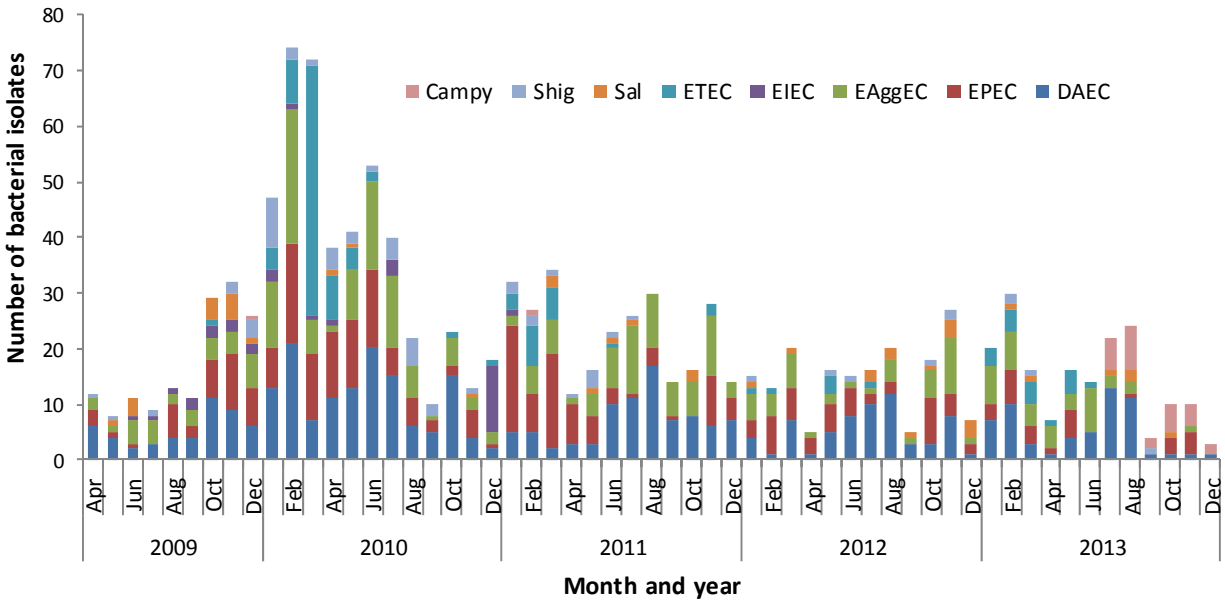


Figure 2: Numbers of stool specimens processed and the detection rate (%) of bacterial pathogens from five sentinel sites for the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Figure 3: Numbers of positive specimens by bacterial pathogen by month and year for the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.



Campy= *Campylobacter* spp., Shig= *Shigella* spp., Sal= *Salmonella enterica* spp., ETEC= enterotoxigenic *E. coli*, EIE=: enteroinvasive *E.coli*, EPEC= enteropathogenic *E. coli* and DAEC= diffusely adherent *E. coli*

The highest detection rate of bacterial cases was in the 6-11 months age group (30%) followed by the <6 months age group (table 1).The lowest detection rate was amongst the 36-59 months age group (4%). The largest proportion of isolates was received from Chris Hani Baragwanath Hospital which also had the highest detection rate of all the bacterial pathogens compared to the other hospitals. There was a lower detection rate of

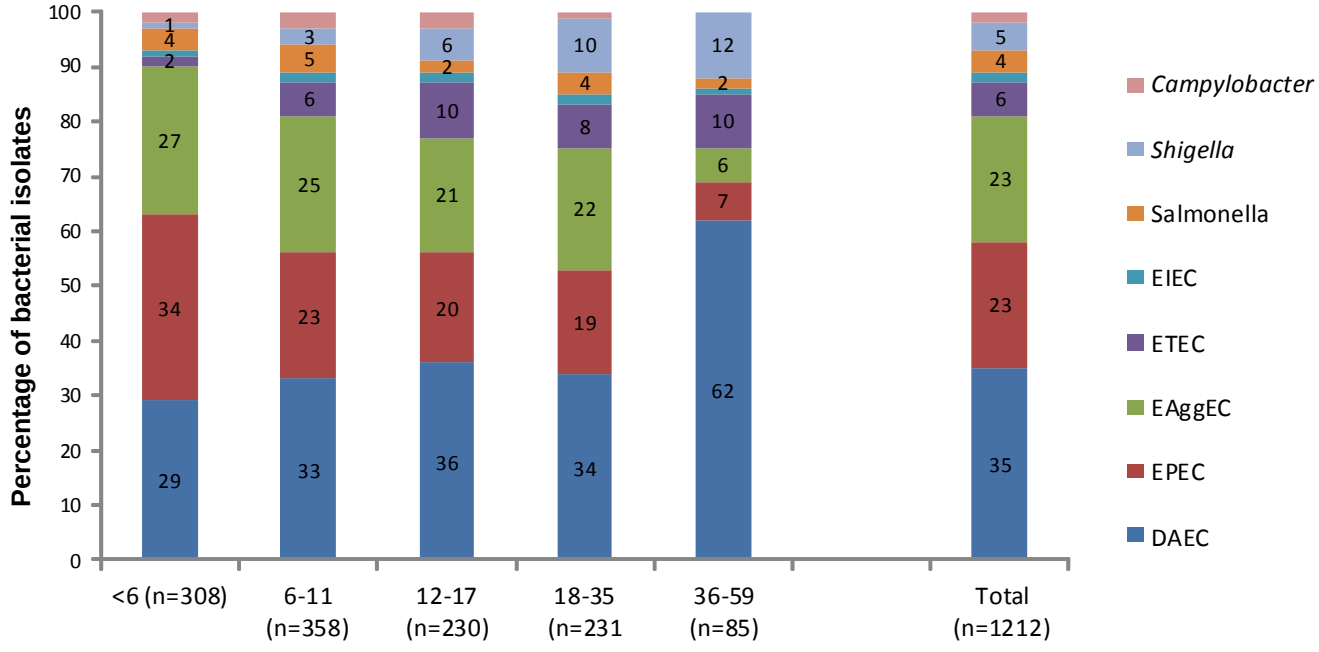
bacterial pathogens in females compared to males.

A large proportion of diarrhoeal cases were due to diffusely adherent *E. coli* (DAEC), followed by enteropathogenic *E.coli* (EPEC) and enteroaggregative *E.coli* (EAggEC) (figure 4). The largest proportion of DAEC occurred in the 36-59 months age group, whereas the proportion of EPEC, EAggEC and *Campylobacter* spp. decreased with increasing age. Enterotoxigenic *E.coli* (ETEC) and *Shigella* proportions increased with increasing age.

Table 1: Proportions of bacterial pathogens (including total) by age, gender and sentinel site in children less than 5 years admitted with diarrhoea, April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Variable	Total bacterial detection n/N (%)	Diarrhoeagenic *E. coli* n/N (%)	*Shigella* spp. n/N (%)	*Salmonella enterica* spp. n/N (%)	*Campylobacter* spp. n/N (%)
Age group (months)					
<6	293/1097 (27)	270/999 (27)	5/61 (8)	12/44 (27)	7/29 (24)
6-11	331/1097 (30)	304/999 (30)	11/61 (18)	17/44 (39)	12/29 (43)
12-17	213/1097 (19)	194/999 (19)	14/61 (23)	4/44 (9)	7/29 (24)
18-35	212/1097 (19)	193/999 (19)	21/61 (34)	9/44 (20)	3/29 (10)
36-59	48/1097 (4)	38/999 (4)	10/61 (16)	2/44 (<1)	0/29 (0)
Gender					
Female	480/1097 (44)	480/999 (48)	28/61 (46)	18/44 (41)	13/29 (45)
Site					
Chris Hani Baragwanath	478/1097 (44)	425/999 (43)	37/61 (61)	18/44 (41)	17/29 (59)
Mapulaneng	99/1097 (9)	90/999 (9)	3/61 (5)	4/44 (9)	4/29 (14)
Matikwane	244/1097 (22)	230/999 (23)	6/61 (10)	7/44 (16)	5/29 (17)
Dr George Mukhari	214/1097 (20)	200/999 (20)	11/61 (18)	13/44 (30)	0/29 (0)
Edendale	62/1097 (6)	54/999 (5)	4/61 (7)	2/44 (5)	3/29 (10)

Figure 4: Proportions of bacterial pathogens and strains isolated by age group during the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.



EIEC = enteroinvasive *E.coli*, ETEC = enterotoxigenic *E.coli*, EAggEC = enteroaggregative *E.coli*,

Parasites

Of the 4122 children enrolled into the sentinel surveillance programme, a total of 2225 (54%) stool specimens was processed for parasites. The detection rate of parasites was 12% (271) of which 266/271 (98%) were positive for *Cryptosporidium* spp. (figure 5).

Of 2072 specimens tested for both bacteria and parasites, 81 (4%) had co-infections where both bacteria and parasites were isolated. The co-infection rate between rotavirus and parasites was 6% (17/271) amongst specimens which were positive for parasites.

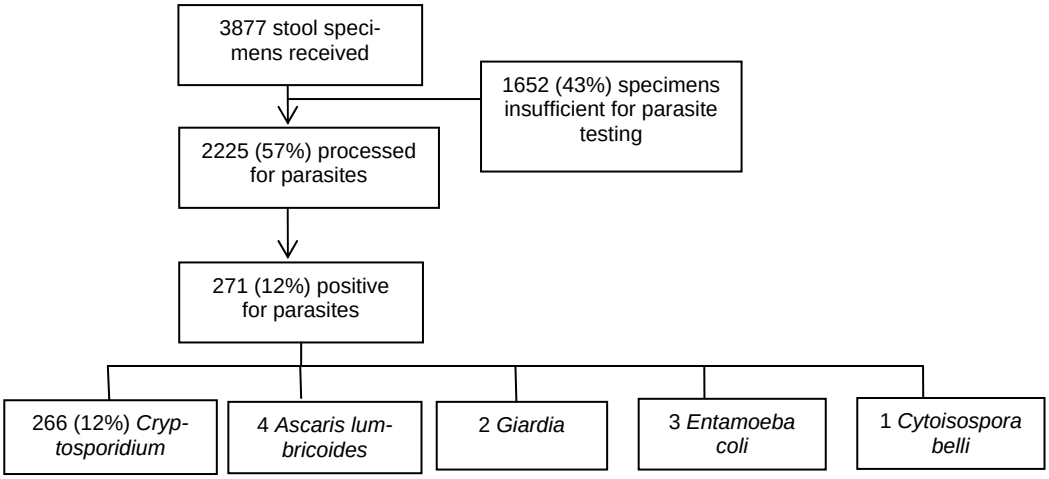


Figure 5: Flow of samples received and the numbers of parasites detected during the period April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

There was a peak in the detection rate of parasites towards the end of the summer months (figure 6) and an overall decrease in the number of stool specimens tested for parasites in the 2011-2013 period compared to 2010. *Cryptosporidium* spp. were most common in the older age groups (figure 7). The highest detection rate of *Cryptosporidium* spp. was at Chris Hani Baragwanath Hospital (14%) and most cases of *Cryptosporidium* spp. were seen in males (table 2).

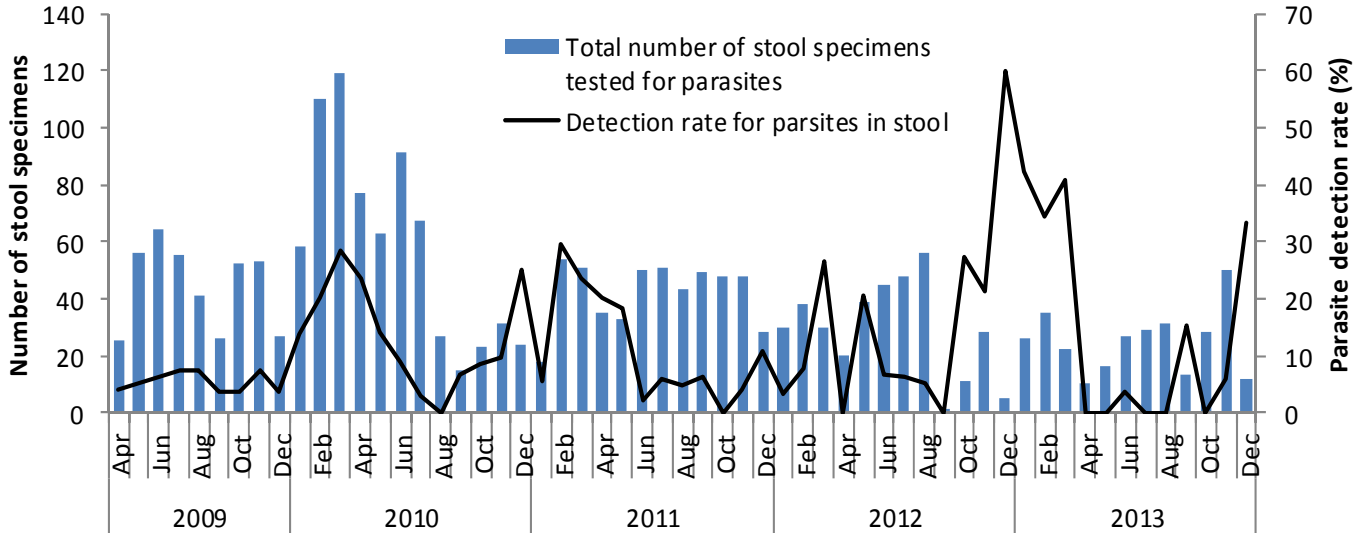


Figure 6: Numbers of stool specimens and the detection rates of parasites by month and year, April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

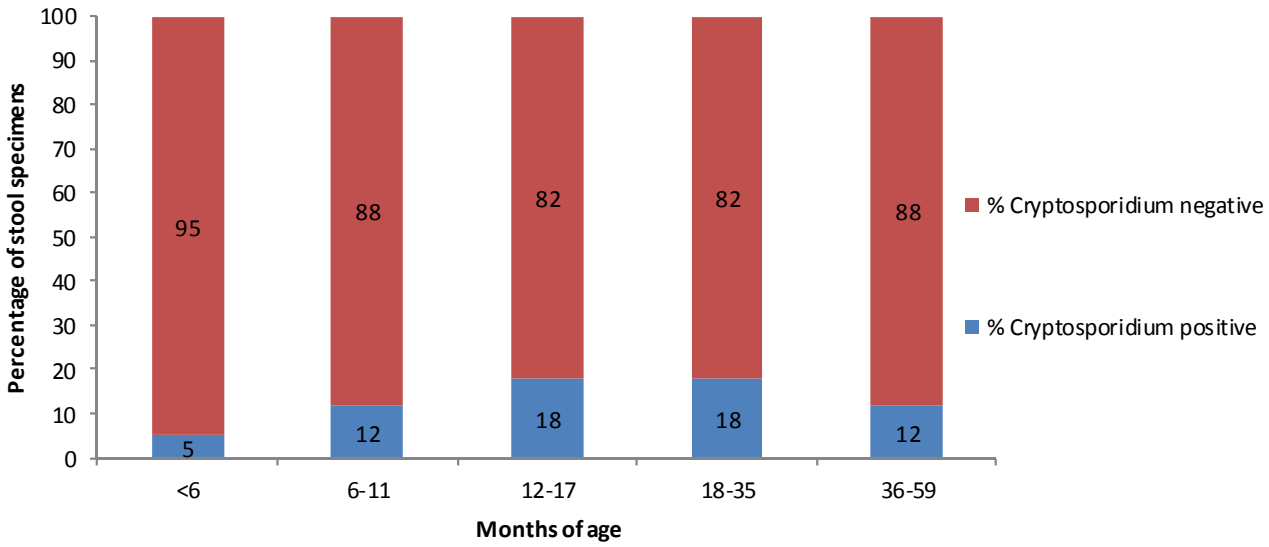


Figure 7: Detection rate of *Cryptosporidium* spp. by age (months), April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Table 2: Numbers of stool specimens collected and detection rate (%) by age, gender and sentinel site in children less than 5 years admitted with diarrhoea where *Cryptosporidium* spp. was detected, April 2009 to December 2013, rotavirus and other diarrhoeal pathogens surveillance programme, South Africa.

Variable	Total specimens	Number of *Cryptosporidium* spp. positive specimens	Detection rate of *Cryptosporidium* spp. (%)
Age group (months)			
<6	634	30	5
6-11	779	91	12
12-17	415	75	18
18-35	343	60	17
36-59	91	10	11
Gender			
Male	1270	165	13
Female	954	101	11
Site			
Chris Hani Baragwanath	800	115	14
Mapulaneng	183	13	7
Matikwane	378	40	11
Dr George Mukhari	740	82	11
Edendale	124	13	10

Discussion

The rotavirus and other diarrhoeal pathogens surveillance programme enrols moderate to severe cases of diarrhoea which require hospitalisation and therefore does not represent the true burden and causes of diarrhoeal disease in children less than 5 years. The decrease in bacterial and parasitic isolates detected after 2010 may be due to a combination of interventions such as the prevention of mother to child transmission (PMTCT) of HIV, the implementation of the

rotavirus vaccine and other hygiene initiatives. Diarrhoeagenic *E.coli* was the most commonly detected pathogen. There appeared to be increases in bacterial and parasitic cases during the summer months. This most likely coincides with water-related recreational activities, rainfall patterns and increased ambient temperatures (which aid the growth of bacteria) during the summer period. ETEC diarrhoeal infections have previously been shown to decrease in older children with the bulk of cases occurring in children <2 years, whereas the data presented here show that ETEC incidence increases in age groups older than 2 years.[8]

Genotyping of a subset of the isolates obtained during this surveillance programme showed that the *Cryptosporidium* spp. infections were mostly *C. hominis* and anthroponotic *C. parvum*, indicating that humans, rather than animals, were the major sources of infection.[9]

Chris Hani Baragwanath Hospital (CHBH) had the largest proportion of pathogenic bacteria and parasites isolated from stool, which may suggest case clustering. The close proximity of CHBH to NICD may result in faster specimen processing times and better bacterial isolation rates, which may be another reason for the increased proportion of bacterial cases seen.

A major limitation of this analysis is the occurrence of co-infections involving multiple viral, bacterial and parasitic diarrhoeal pathogens and discriminating between the likely pathogenic causative agents of the diarrhoea and non-pathogenic intestinal carriage. In addition, being a hospital-based study, children under five years with severe diarrhoea presenting to clinics or private practitioners would not be identified, precluding the generalisability of these results to less severe diarrhoeal disease. Further in-depth epidemiological analysis will need to be undertaken to address these issues.

This is the first report of non-viral stool pathogens from this diarrhoeal surveillance programme. Improvements in the surveillance programme have since been made to increase the number of healthcare facilities participating in the diarrhoeal surveillance and the incorporation of point-of-care diagnostic tests to bring the surveillance system closer to real-time is being planned. This will allow for prompt investigation of clusters and possible outbreaks so that enhanced interventions and programmes in affected communities can be implemented.

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- All patients and parents/guardians who have participated in this programme

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TEN YEARS OF SYPHILIS TRENDS IN THE NORTHERN CAPE PROVINCE, SOUTH AFRICA, UTILISING THE NHLS CORPORATE DATA WAREHOUSE

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Introduction

Syphilis continues to be a major global public health problem, with an estimated 12 million people infected each year.[1] Worldwide, nearly 2 million pregnant women are infected with syphilis annually, most of whom are not tested, while most of those tested are either not treated promptly or are not treated at all.[2] Approximately 80% of pregnant women with untreated syphilis will transmit the infection to their unborn children, resulting in early foetal loss or stillbirth, or adverse pregnancy outcomes such as low-birth-weight or congenital disease.[3,4]

In developing countries an estimated 3-15% of women of child-bearing age have syphilis, although the burden of congenital syphilis (CS) is likely underestimated.[5] About 30% of pregnant women with syphilis will have

stillbirths and another 30% will birth a live baby with CS, with subsequent mortality reaching 50%.[6]

The World Health Organization launched its initiative for the Global Elimination of Congenital Syphilis in 2007, with the goal that at least 90% of pregnant women are tested for syphilis and at least 90% of seropositive pregnant women receive adequate treatment by 2015.[1] In order to achieve these goals, strengthening of existing surveillance systems to track and monitor syphilis disease burden is important.

Since 1990, monitoring the syphilis seroprevalence amongst pregnant women in South Africa has been conducted annually using unlinked anonymous surveys in sentinel antenatal clinics (ANC) and the National Antenatal Sentinel HIV & Syphilis Prevalence Surveys (ANSUR). It is now also important to explore a complementary approach to understanding the syphilis burden in South Africa by utilising routinely-collected electronic laboratory data for surveillance, an approach that provides efficient access to data and has large-scale coverage.

The Northern Cape Province (NCP) is a region with a high syphilis burden compared with other provinces in South Africa.[7] The aim of this study was to determine whether the National Health Laboratory Service (NHLS) Corporate Data Warehouse (CDW), which contains electronically stored laboratory test records, can be employed as an appropriate surveillance instrument to track and monitor trends in syphilis seroprevalence. The specific objectives were to describe trends in syphilis seroprevalence in women of reproductive age in NCP between 2003 and 2012 using laboratory data routinely collected through the NHLS CDW and to compare the computed trends with findings from the ANSUR during the same period.

Methods

This cross-sectional study retrospectively analysed secondary data of longitudinally-collected laboratory measurements from the NHLS CDW. Data from the public sector health facilities of the NCP, where syphilis serology tests are conducted, were utilized. The study population was females of reproductive age, 12-49 years, identified for the NCP and included as a proxy to define or identify ANC attendees because the syphilis serologic tests done in public sector health facilities include people other than pregnant women, and the NHLS CDW dataset does not contain information on pregnancy status.

Criteria for inclusion in the study were females of reproductive age (12-49 years) who were tested for syphilis by serology (rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination assay (TPHA)). Exclusion criteria included females of the same reproductive age range with no syphilis test result. Data for this study were sourced from the NHLS CDW between 2003 and 2012. Variables included patient identifying information, demographic information, name of health facility where the test was performed, tests requested, date of test and test result. The standard algorithm for testing for syphilis infection includes screening using the non-treponemal test (RPR). The RPR test can distinguish between an active infection and a past infection. An RPR titre of >1:4 is indicative of active infection. RPR tests are not specific for syphilis and can produce false-positive results. Reactive RPR tests are confirmed using the treponemal (TPHA) test.[8]

A total of 8 471 425 syphilis tests covering South Africa's nine provinces for the period 2003-2012 was extracted. Analysis was then limited to the NCP from which 310 730 tests among females of reproductive age were identified. This dataset was then de-duplicated for each year to remove duplicate records. As a unique

identifier (master patient index) is not available in the CDW laboratory database, identification fields were used together and a probabilistic record linkage technique was applied. The Chi-square test for trend was used to determine whether there was a decreasing trend in syphilis seroprevalence from 2003 to 2012. The modified-Poisson regression to estimate prevalence ratios (PR) of syphilis seroprevalence (SSP) over time was used. Separate analyses by age group and geographical location were performed. All statistical analyses were conducted using STATA version 13 and a p-value of less than 0.05 was considered statistically significant.

Permission to analyse the data was obtained from the NHLS. Ethical clearance was obtained from the Faculty of Health Sciences Research Ethics Committee of the University of Pretoria.

Results

A total of 286 024 women was included in the study after de-duplication and exclusion of participants with missing data on key variables. The mean (SD) age ranged from 25.7 (6.9) years in 2003 to 27.9 (8.1) years in 2012. The majority of women were in the 26-49 years age group - ranging from 45.4% in 2003 to 55.4% in 2012. Out of 154 women for whom population group (race) was captured, 132 (86%) were black. The highest numbers of tests were performed in Frances Baard District (58.4% in 2012), whereas Namakwa District recorded the least number of tests (5.5% in 2012) (table 1).

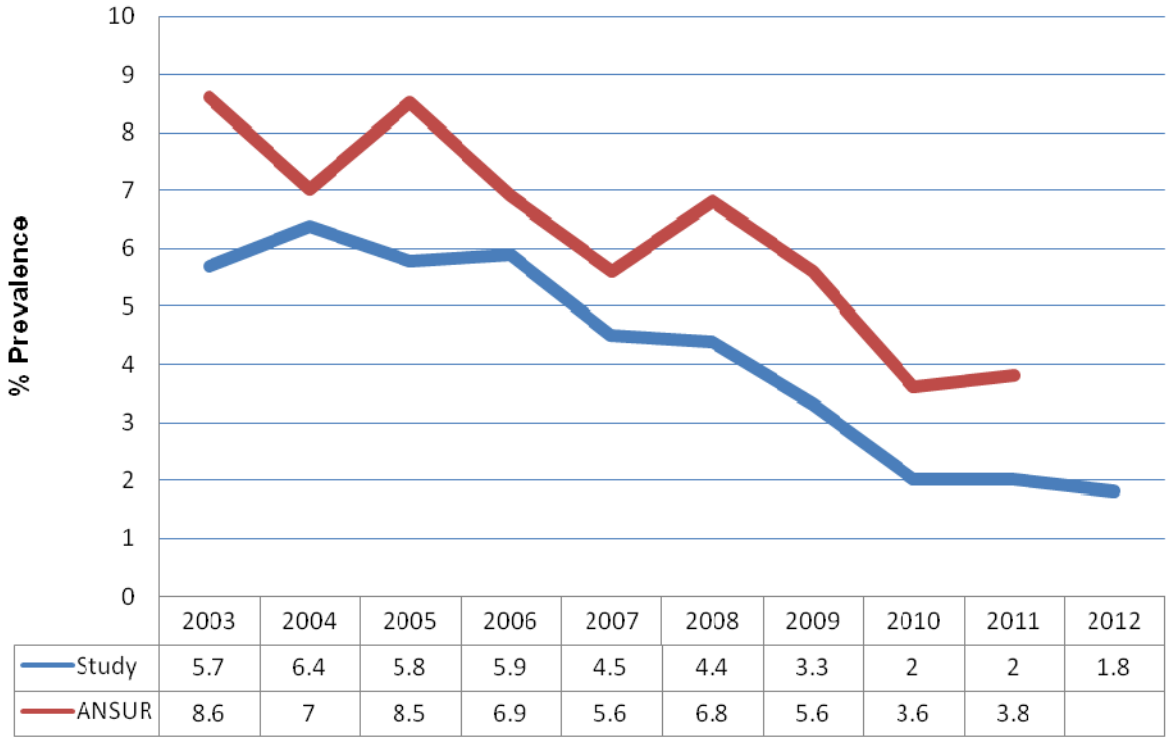
Table 1: Numbers of females of reproductive age (12-49 years) tested for syphilis by age, year and district in the Northern Cape Province, South Africa, 2003-2012.

	Year			
	2003 (N=14 514)	**2006 (N=31 006)**	**2009 (N=34 937)**	**2012 (N=19 792)**
Age category, n (%)				
12-17 yrs	1 312 (9.0)	2 742 (8.8)	2 897 (8.3)	1 455 (7.4)
18-25 yrs	6 624 (45.6)	12 777 (41.2)	13 261 (37.9)	7 363 (37.2)
26-49 yrs	6 578 (45.4)	15 487 (50.0)	18 779 (53.8)	10 974 (55.4)
Age, mean (SD)	25.7 (6.9)	26.7 (7.6)	27.5 (7.9)	27.9 (8.1)
District				
Frances Baard	5 248 (36.2)	11 623 (37.5)	12 267 (35.1)	11 565 (58.4)
John Taolo Gaetsewe	1 591 (11.0)	6 249 (20.1)	7 908 (22.6)	3 115 (15.7)
Namakwa	1 354 (9.3)	2 271 (7.3)	2 134 (6.1)	1 078 (5.5)
Pixley Ka Seme	2 859 (19.7)	5 130 (16.6)	6 342 (18.2)	1 427 (7.2)
Siyanda	3 462 (23.8)	5 733 (18.5)	6 286 (18.0)	2 607 (13.2)

Overall, there was a decline in SSP between 2003 (5.7%) and 2012 (1.8%) (p<0.01), which matches the downward trend reported in ANSUR from 2003 (8.6%) to 2011 (3.8%)[7] (figure 1). For every year between 2003 and 2012 there was a 14% reduction in the PR of syphilis seroprevalence (PR=0.86, 95% CI=0.85-0.87, p<0.01). There was also a decline in syphilis seroprevalence by age group from 2003 to 2012: 12-17 years (4.2% to 2.2%), 18-25 years (5.7% to 1.8%), and 26-49 years (5.9% to 1.7%) (p=0.001). Three of the five districts viz., Frances Baard (6.9% to 0.9%), John Taolo Gaetsewe (7.4% to 0.7%) and Namakwa (3.3% to 1.7%) showed significant decreases in syphilis seroprevalence over the 10 year period (p=0.001) (table 2).

Table 2: Syphilis seroprevalence obtained from the Corporate Data Warehouse and sorted by year, age and district among females of reproductive age tested for syphilis in the Northern Cape Province, South Africa, for the period 2003-2012.

	Year N (%)									
	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Overall seroprevalence	823 (5.7)	1 534 (6.4)	1 501 (5.8)	1 841 (5.9)	1 416 (4.5)	1 536 (4.4)	1 138 (3.3)	681 (2.0)	722 (2.0)	353 (1.8)
Age Group										
12-17 yrs	55 (4.2)	149 (6.2)	115 (4.6)	132 (4.8)	92 (3.4)	130 (4.4)	99 (3.4)	56 (2.0)	69 (2.4)	32 (2.2)
18-25 yrs	377 (5.7)	699 (6.5)	625 (5.5)	747 (5.9)	525 (4.2)	526 (3.9)	453 (3.4)	294 (2.2)	279 (2.1)	134 (1.8)
26-49 yrs	391 (5.9)	686 (6.4)	1 501 (6.4)	962 (6.2)	799 (4.9)	880 (4.9)	586 (3.1)	331 (1.8)	374 (2.0)	187 (1.7)
District										
Frances Baard	362 (6.9)	547 (6.5)	578 (6.4)	611 (5.3)	554 (4.8)	621 (4.8)	402 (3.3)	187 (1.6)	146 (1.2)	107 (0.9)
John Taolo Gaetsewe	117 (7.4)	205 (4.7)	225 (4.1)	226 (3.6)	166 (2.5)	184 (2.3)	123 (1.6)	50 (0.6)	75 (1.0)	23 (0.7)
Namakwa	44 (3.3)	70 (3.5)	61 (2.9)	82 (3.6)	58 (2.7)	40 (1.8)	36 (1.7)	44 (2.0)	28 (1.2)	18 (1.7)
Pixley Ka Seme	155 (5.4)	264 (6.4)	248 (6.1)	359 (7.0)	323 (6.3)	369 (6.5)	319 (5.0)	225 (3.6)	253 (4.3)	99 (6.9)
Siyanda	145 (4.2)	448 (9.0)	389 (7.8)	563 (9.8)	315 (5.5)	322 (5.4)	258 (4.1)	175 (2.8)	220 (3.4)	106 (4.1)



	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Study	5.7	6.4	5.8	5.9	4.5	4.4	3.3	2	2	1.8
ANSUR	8.6	7	8.5	6.9	5.6	6.8	5.6	3.6	3.8	

Figure 1: Syphilis seroprevalence by year for the Northern Cape Province, South Africa, using Corporate Data Warehouse data (Study) for the period 2003-2012 and National Antenatal Sentinel HIV & Syphilis Prevalence Surveys (ANSUR) for the period 2003-2011.

Discussion

Overall, syphilis seroprevalence in the NCP showed significant decreases between 2003 (5.7%) and 2012 (1.8%). The estimates given for data obtained from the CDW are consistent with those from ANSUR data which show a decline in prevalence from 8.6% in 2003 to 3.8% in 2011.[7] This study demonstrates that routinely collected electronic laboratory data can accurately portray trends in the prevalence of syphilis in South Africa, and is a useful and relatively cost-effective surveillance tool that can be used to inform the planning and allocation of health resources at national and provincial levels.

However, this study has certain limitations. Firstly, the use of 'secondary data' is invariably associated with a limited number of variables and incompleteness of information collected. Secondly, analysis based on the NHLS CDW dataset is limited by the variables that are collected at source and by the quality of the data collected, which in turn depends on accurate completion of the laboratory requisition form and data capturing. Since record linkage techniques were used, only data with complete information and therefore a greater chance of linkage were included in the study, leading to potential selection bias. Thirdly, data from only one of the nine provinces in South Africa were used and therefore cannot be used to generalize syphilis seroprevalence in the country as a whole. Lastly, the study population included only women who attended public health facilities and excluded those who attended private health facilities and those who did not access health services. This could lead to a potential overestimation of the syphilis burden and also precludes making population generalizations concerning syphilis seroprevalence in South Africa.

Conclusions

There was a significant decline in syphilis seroprevalence in the NCP during the period 2003 to 2012 and this trend is largely consistent with that obtained from the South African ANSUR data. Routinely collected electronic laboratory data can therefore accurately portray trends in syphilis seroprevalence because it provides stable prevalence estimates through broader geographical coverage and a larger sample size. It is therefore recommended that the NHLS CDW be considered for syphilis seroprevalence monitoring and that a more detailed analysis of trend data at national and sub-national levels be conducted in order to compare the findings with available data so as to confirm trends is SSP in South Africa. It is also recommended that there be improved data collection at facility level to better understand the determinants of risk of syphilis infection e.g. demographics, pregnancy status, HIV status and other STIs. These types of analysis are important in terms of reaching the elimination goals.

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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 2014/2015*

Disease/Organism	1 Jan to 31 Mar, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Botulism	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	1	0	0	0	0	0	0	1
Cryptococcus spp.	2014	121	41	313	277	25	52	8	36	160	1033
	2015	121	39	204	277	28	49	8	54	144	924
Haemophilus influenzae, invasive disease, all serotypes	2014	9	5	28	19	0	6	2	3	23	95
	2015	3	3	13	13	0	4	0	0	30	66
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2014	0	1	1	1	0	0	1	0	6	10
	2015	0	1	1	0	0	0	0	0	2	4
Serotypes a,c,d,e,f	2014	0	0	2	1	0	0	0	0	1	4
	2015	0	1	0	1	0	0	0	0	1	3
Non-typeable (unencapsulated)	2014	0	0	7	3	0	1	0	0	5	16
	2015	0	0	2	1	0	0	0	0	2	5
No isolate available for serotyping	2014	1	0	7	3	0	1	0	1	0	13
	2015	1	0	2	4	0	1	0	0	1	9
Measles	2014	0	0	1	1	0	1	0	0	1	4
	2015	0	0	0	0	0	0	3	1	1	5
Neisseria meningitidis, invasive disease	2014	11	2	7	2	0	0	0	0	9	31
	2015	6	2	2	4	0	0	0	1	6	21
Novel Influenza A virus infections	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Plague	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Rabies	2014	0	0	0	0	1	0	0	0	0	1
	2015	0	0	0	1	0	0	0	0	0	1
Salmonella typhi	2014	0	0	16	4	0	5	0	0	6	31
	2015	1	0	7	5	0	2	0	0	4	19
Streptococcus pneumoniae, invasive disease, all ages	2014	50	37	184	93	3	23	6	20	105	521
	2015	29	30	143	73	11	16	6	16	144	468
Streptococcus pneumoniae, invasive disease, < 5 years	2014	5	6	41	23	2	4	2	5	15	103
	2015	5	3	20	17	1	3	1	4	17	71
Vibrio cholerae O1	2014	0	0	0	0	0	0	0	0	0	0
	2015	0	0	0	0	0	0	0	0	0	0
Viral Haemorrhagic Fever (VHF)											
Crimean Congo Haemorrhagic Fever (CCHF)	2014	0	1	0	0	0	0	0	0	0	1
	2015	0	0	0	0	0	0	0	0	0	0
Other VHF (not CCHF)	2014	0	0	0	0	0	0	0	0	0	0
	2015	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.

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

0 = no cases reported

Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 31 March 2014/2015*

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	2014	27	15	41	42	16	20	7	10	14	192
	2015	27	9	18	27	10	15	1	5	6	118

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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