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FOREWORD

Foodborne disease is a common public health problem worldwide, but is generally under-reported and poorly investigated in South Africa. Although outbreaks of foodborne disease involving 2 or more persons are notifiable, such notification statistics are notoriously unreliable. The number of outbreak reports that reach the NICD via the communicable diseases reporting channels are almost certainly only the tip of a very large iceberg. The World Cup football event in June and July this year was a stimulus for improving the detection and management of food-related incidents, if only in the short term. As Lucille Blumberg's article in this issue describes, foodborne disease incidents featured among the communicable disease problems that the surveillance system picked up during the World Cup. Two other articles in this issue deal with recent foodborne disease incidents. Nyiko Hlungwani and colleagues describe an unfortunately frequent occurrence of a rural funeral linked to an outbreak of food poisoning. Morubula Manamela and colleagues illustrate the power of combining modern molecular diagnostics with 'shoe-leather' epidemiology, to associate cases of foodborne disease (typhoid in this case) with a probable common source. Typhoid fever is one of the exceptions to the rule that foodborne illness is not generally suited to laboratory-based surveillance, given its acute, sporadic and usually mild nature. Once any significant outbreak is recognized, however, the laboratory is an important element in the investigation. The shortage of laboratories that have this capacity is only one of several obstacles to improving the management of food-related outbreaks in South Africa.

John Frean, Acting Editor

CONTENTS

USE OF LABORATORY STATISTICS FOR MONITORING THE INTRODUCTION OF A NEW LINE PROBE ASSAY AND FOR SURVEILLANCE OF MDR- AND XDR-TUBERCULOSIS

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Routine diagnostic laboratories performing smear microscopy for acid-fast bacilli (AFB) and culture for *Mycobacterium tuberculosis* for the diagnosis of tuberculosis (TB) and treatment monitoring, and drug susceptibility testing (DST) for guidance on the choice anti-TB drugs, play an essential role in the clinical management of TB. Laboratories also perform an important role in support of the National Tuberculosis Control Programme (NTBCP) of South Africa, especially by providing data on the frequency of multidrug-resistant TB (MDR-TB) and extremely drug-resistant TB (XDR-TB) in the country. Laboratory-based surveillance of TB drug resistance in South Africa, including national surveys under the auspices of the World Health Organization/International Union Against Tuberculosis and Lung Disease (WHO/IUATLD)[1] is presently the responsibility of the National Tuberculosis Reference Laboratory (NTBRL).

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The advent of molecular techniques for the rapid detection of rifampicin (RIF) and isoniazid resistance enabled routine diagnostic laboratories to provide a presumptive diagnosis of MDR-TB to clinicians within 24-48 hours. The PCR-based GenotypeMTBDRplus line probe assay (Hain Lifescience GmbH, Nehren, Germany), designed to detect most *rpoB* mutations encoding resistance to RIF, as well as *katG* and *inhA* mutations responsible for a large percentage isoniazid resistance in *M. tuberculosis,* was shown in validation studies in South Africa to have excellent specificities, sensitivities and positive and negative predictive values for the detection of RIF and isoniazid resistance and MDR-TB.[2,3] These findings were confirmed in a large demonstration project conducted in South Africa and prompted WHO to recommend the use of this line probe assay (LPA) in developing countries. At the same time the South African Department of Health decided to adopt the LPA for the rapid detection of MDR-TB as a cornerstone of a revised NTBCP.

The introduction of LPA into the NTBCP resulted in major changes in the utilization of laboratory tests for patient management and this article will indicate the magnitude of the changes from data retrieved from the NHLS Corporate Data Warehouse. At the same time information on changes in the prevalence of MDR- and XDR-TB from 2004 to October 2010 will be provided.

Methodology of data retrieval

Comprehensive computerized data captured on the NHLS laboratory information management system (DISA) from 8 provinces have been available from the Corporate Data Warehouse (CDW) of the NHLS on laboratory tests performed for the diagnosis and treatment monitoring of TB for several years. Electronic transfer of historical data on laboratory tests from KwaZulu-Natal has however, as yet not been reliably achieved and statistics from this province will therefore not feature in this presentation.

For the purposes of this article, the numbers of investigations according to type of tests performed by NHLS laboratories (smear microscopy for acid-fast bacilli (AFB), culture for *M. tuberculosis* and DST by MGIT and LPA) were accessed from CDW and recorded, irrespective of the reasons why they had been performed or how they relate to patient management, and trends analysed in the 8 provinces over a period of approximately six years.

Extensive and meticulous “cleaning” of data was performed in order to provide as reliable MDR- and XDR-TB statistics as possible.

Trends and volumes of NTBCP-related tests

The volumes of TB management-related tests performed by NHLS laboratories (excluding KwaZulu-Natal) over the period 2004 to 15th October 2010 are shown in Table 1.

There was an increasing trend in the number of culture and smear microscopy tests performed between 2004 and 2009 reflecting the increased workloads associated with the introduction of LPS in 2007 in Western Cape and Gauteng and during 2008 and 2009 in other provinces.

Subsequent to the introduction of LPA there was a noticeable decrease in MGIT DST volumes, especially marked during 2009 and 2010. Examples of changes in volumes of LPA uptake in the NTBCP and subsequent decreases in DST performance in the Western Cape and Gauteng are illustrated in the accompanying Figure. Similar trends occurred in the other provinces but were less pronounced in those with a lower uptake of LPA (Limpopo, Mpumalanga, Free State and North-West Province) during 2009 and 2010 (see Table 2).

Table 1: Diagnostic and treatment monitoring tests performed at NHLS laboratories during 2004-2010*

Year	Culture MGIT**	Microscopy AFB***	LPA DST§	DST MGIT¶
2004	304708	1585778	-	34564
2005	397956	1981274	1	36903
2006	499540	2327811	5	48183
2007	603131	2442466	5963	65809
2008	774651	3117422	23128	60147
2009	837338	3284455	61575	40205
2010*	609072	2690949	57741	20230
2010‡	687642	3038081	65190	22840

* Tests performed up to 15th October 2010; ‡ Projected numbers of tests for whole year
** Culture for *M. tuberculosis* on MGIT 960 (Becton Dickinson)
*** Smear microscopy for acid-fast bacilli (AFB)
§ GenotypeMTBDRplus line probe assay (LPA) for rifampicin and isoniazid (MDR-TB) resistance
¶ Numbers of drug susceptibility tests (DST) by MGIT performed annually

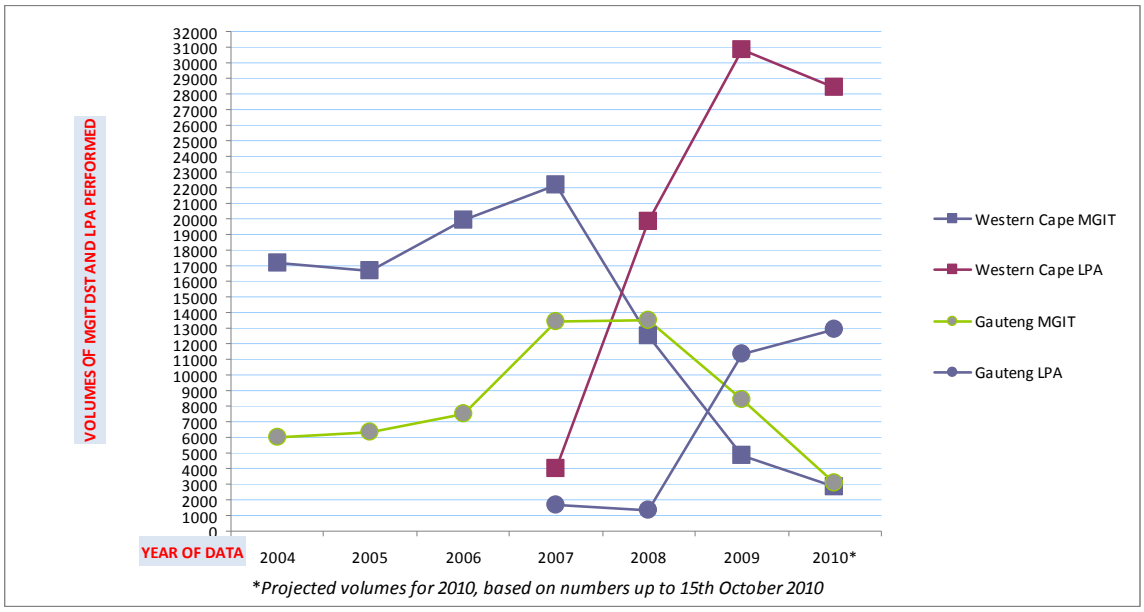

Figure: Effect of the introduction of the line probe assay on reducing the numbers of conventional long duration MGIT drug susceptibility testing

Table 2: Numbers of line probe assays introduced during 2007 to 2010*

Province	2007	2008	2009	2010*	Total
Eastern Cape	7	1488	7958	12006	21006
Free State	4	10	383	3547	3944
Gauteng	1736	1302	11260	11431	25734**
Limpopo	0	4	295	426	725
Mpumalanga	0	17	857	672	1546
North West	81	203	2696	1124	4104
Northern Cape	126	334	7316	6521	14297
Western Cape	4009	19770	30810	22014	76604***
Total	**5963**	**23128**	**61575**	**57741**	**148413**

* Line probe assay (LPA) tests performed up to 15th October 2010
** One and 4 LPA tests performed in 2005 and 2006 respectively
*** One LPA was performed in 2006

Prevalence and trends of MDR- and XDR-TB in South Africa over 6-year period

The numbers of MDR- and XDR-TB cases retrieved from the NHLS CDW over the period 2004 to 6th October 2010 and their respective mean annual rates per 100 000 and 1 000 000 persons in the various provinces during this period are presented in Table 3. Also given in the table are the ratios of XDR-TB relative to MDR-TB cases which may provide interesting information on adherence of MDR-TB patient management to the NTBCP but could also be influenced by unavailability or underutilization of laboratory facilities for DST on second-line anti-TB drugs.

The four provinces, excluding KwaZulu-Natal, with the largest numbers of MDR-TB cases are Western Cape (10166), Eastern Cape (7639), Gauteng (5933) and Mpumalanga (2366). Three of these provinces (Gauteng is the exception) also have the highest mean annual rates of MDR-TB. Of note is the high MDR-TB rate, as well as the high XDR-TB:MDR-TB ratio (5.7%) in the sparsely populated Northern Cape. This ratio was also very high in the Eastern Cape (8.1%). The four provinces with the largest numbers of XDR-TB patients (Eastern Cape [618], Western Cape [278], Gauteng [220] and Northern Cape [104]), also have the highest mean annual rates of XDR-TB with Northern Cape ranking first, followed by Eastern Cape, Western Cape and Gauteng.

Trends in the prevalence of MDR- and XDR-TB cases are shown in Table 4.

There was an increase in the numbers of MDR-TB and XDR-TB patients during the period 2004 to 2010 in all the provinces. The numbers of MDR-TB patients recorded during 2009 when LPA implementation was in full swing showed moderate increases in all the provinces except Mpumalanga and Free State, suggesting that the introduction of LPA did not affect the detection of MDR-TB cases adversely.

Table 3: MDR- and XDR-TB cases in South Africa, excluding KwaZulu-Natal, 2004-2010*

Province**	Population x10^6	MDR Cases	MDR Rates*** x10^{-5}	XDR Cases§	XDR Rates x10^{-6} (Rank)	X/M%¶
WC	5.2	10166	33 (1)	278	9.0 (3)	2.7
NC	1.1	1827	28 (2)	104	16.2 (1)	5.7
EC	6.7	7639	19 (3)	618	15.7 (2)	8.1
MP	3.6	2366	11 (4)	56	2.7 (6)	2.2
FSP	2.8	1563	9.5 (5)	28	1.7 (7)	1.8
NWP	3.2	1708	9.1 (6)	56	3.1 (5)	3.3
GP	11.2	5933	9.0 (7)	220	3.4 (4)	3.7
LP	5.4	826	2.6 (8)	18	0.6 (8)	2.2
National‡	**39.4**	**32038**	**13**	**1378**	**6.0**	**4.3**

* Numbers of cases and rates up to 6th October 2010
* * WC – Western Cape, NC – Northern Cape, EC –Eastern Cape, MP – Mpumalanga , GP – Gauteng Province, FSP – Free State Province, NWP – North West Province, LP – Limpopo Province
*** Mean annual MDR-TB rates per 100 000 persons. Ranks of MDR-TB rates per province are given in brackets
§ Mean annual XDR-TB rates per 1 000 000 persons. Ranks of XDR-TB rates per province are given in brackets
¶ Ratio of XDR-TB cases / MDR-TB cases expressed as a percentage
‡Excluding KwaZulu-Natal; population 10.6 x 10^6

Table 4: Trends in prevalence of MDR- and XDR-TB cases from 2004-2010*

Province** MDR/XDR	2004	2005	2006	2007	2008	2009	2010*	Total
WC: MDR	1158	1259	1222	1590	1681	1910	2346	10166
XDR	17	16	11	48	37	81	68	278
NC: MDR	164	163	182	161	203	472	482	1827
XDR	2	1	8	12	18	29	34	104
EC: MDR	394	579	875	1037	1320	1697	1737	7639
XDR	5	13	64	96	177	119	144	618
MP: MDR	156	137	150	422	689	411	401	2366
XDR	0	0	0	7	17	20	12	56
FSP: MDR	114	152	205	176	318	288	310	1563
XDR	1	4	2	5	5	7	4	28
NWP: MDR	111	180	181	359	285	427	165	1708
XDR	1	4	8	6	5	12	4	40
GP: MDR	520	707	679	992	937	1253	845	5933
XDR	3	3	13	58	24	64	55	220
LP: MDR	60	43	79	100	185	204	155	826
XDR	0	2	5	2	3	5	1	18
Total: MDR	**2677**	**3220**	**3573**	**4837**	**5618**	**6662**	**5441**	**32028**
XDR	**29**	**43**	**111**	**234**	**286**	**337**	**322**	**1362**

* Numbers of cases and rates up to 6th October 2010
* * WC – Western Cape, NC – Northern Cape, EC – Eastern Cape, MP – Mpumalanga, GP – Gauteng Province, FSP – Free State Province, NWP – North West Province, LP – Limpopo Province

Discussion

As illustrated in the preceding sections of this article, statistics generated by the CDW of the NHLS can provide useful information on laboratory-based management of patients and monitoring of aspects of the NTBCP, such as the implementation of new technology (e.g. LPA) into the programme, as well as ongoing surveillance of MDR- and XDR-TB in the country. Great care should however be exercised in the use and interpretation of CDW-derived statistics. For example, the volumes of smear microscopy and culture investigations related to the 2007-2010 period when LPA testing was introduced by the NTBCP, as indicated in Table 1, reflect overall laboratory activity while those of LPA and MGIT tests, being more directly involved in the management of drug-resistant TB, should be sufficiently reliable to provide useful information on laboratory-related costs of the revised NTBCP and progress made in the various provinces in the implementation of the new management algorithm.

The phased-in character of the introduction of the LPA into the NTBCP is reflected in Table 2. The main thrust of the LPA roll-out nationally was scheduled for January 2009 but in Western Cape and Gauteng provinces where implementation already started in 2007, the effect on volumes of MGIT DST performed was already evident in 2008 and continued to show a downward trend during 2009 and 2010 (See Figure). For the roll-out of LPA, 20 NHLS laboratories in 8 of the 9 provinces were identified for performance of LPA and suitable laboratory space and appropriate training of medical scientists for the task had to be in place before implementation and more time was required to achieve this in some of the provinces.

For surveillance of MDR- and XDR-TB, transition from specimen specific to patient specific data requires programming algorithms to identify unique patients, even with incorrectly spelt names and conflicting demographic data. Only after extensive and meticulous "cleaning" of data, could information on the number of MDR-/XDR-TB patients confirmed by NHLS laboratories could be extracted.

Not surprisingly, the rates of MDR-TB per 100 000 as indicated in Table 1 bear a good relationship to the overall rates of TB in the provinces with Western Cape and Northern Cape heading the list with figures of 1031 and 922 per 100 000 respectively in 2005 and Limpopo with the lowest rate of 287 per 100 000.[4,5] XDR-TB rates are highest in the Northern Cape, Eastern Cape and Western Cape and the high XDR-TB/MDR-TB ratios of 5.7% and 8.1% for the Eastern Cape and Northern Cape suggest deficiencies in the management of MDR-TB cases and possibly transmission of XDR-TB cases, as was experienced in the Tugela Ferry outbreak in KwaZulu-Natal.[6] Caution is required in the interpretation of XDR-TB/MDR-TB ratios: there are no criteria available for expected ratios in countries with different levels of MDR-TB management and low ratios may well relate to lack of laboratory facilities to perform DST to an appropriate fluoroquinolone and injectable second-line anti-TB drugs.

Further refinement of CDW statistics is required but the present system has already provided useful information for the NTBRL and NTBCP and is bound to be used more extensively in future.

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PHYLOGENETIC IDENTIFICATION OF HUMAN ENTEROVIRUSES ASSOCIATED WITH CLUSTERS OF MENINGITIS IN 2009 AND 2010

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Human enteroviruses (HEVs) are a diverse group of single-stranded positive-sense RNA viruses which are classified into 4 species, HEV-A to -D. Each HEV species consists of a number of serotypes with HEV-A comprising 21 serotypes, HEV-B, 59 serotypes, HEV-C, 19 serotypes including the three polioviruses and HEV-D, 3 serotypes. (http://www.picornaviridae.com). HEVs have a worldwide distribution and are responsible for both endemic and epidemic infections.

Enteroviral infections typically peak during the summer months of the year. While most infections are asymptomatic, these viruses are also responsible for a wide spectrum of illness including mild febrile illnesses, poliomyelitis, myocarditis and aseptic meningitis. The enteroviruses from species HEV-B are most commonly associated with aseptic meningitis in both adults and children.

Detection of HEV has traditionally been performed by isolation of virus in cell culture from CSF, throat swab or stool samples. However, PCR-based assays are increasingly being used to detect these viruses. Most screening assays target the conserved 5'-untranslated region (5'UTR). Although the discriminating power of this region for HEV typing is limited due to low genetic variability, it can nevertheless be used to type certain viruses.

From July 2009 to February 2010, 80 samples were received by the NHLS Virology diagnostic laboratory at Groote Schuur Hospital for HEV detection. Twenty-three were HEV positive by RT-PCR targeting the 5'UTR. The 5'UTR PCR amplicons were sequenced and a phylogenetic tree constructed using reference HEV sequences from the GenBank database.

During this period 2 community outbreaks of aseptic meningitis were reported. The first occurred in November 2009 in the Ladismith area of the Western Cape Province. Patients presented with a range of clinical symptoms, including features in keeping with aseptic meningitis, and samples were sent to determine the cause of the outbreak. Stools (n=4), throat swabs (n=4) and CSF (n=2) samples were submitted from 6 patients. In 5 cases the HEV nucleic acid that was amplified grouped with enterovirus 84 (EV 84) (Figure 1). In one stool sample poliovirus type 1 vaccine strain was identified. The second outbreak, which occurred in late January 2010, involved 5 members of the same family. All presented with symptoms in keeping with aseptic meningitis. An HEV was detected in two CSF samples and a stool sample from 3 cases. The HEV grouped with echovirus type 4 (E-4) (Figure 1). Interestingly a CSF sample from a 14-year-old child with meningitis, obtained on 10 December 2009, was also found to cluster with echovirus 4, suggesting that echovirus 4 was circulating in the community over this period.

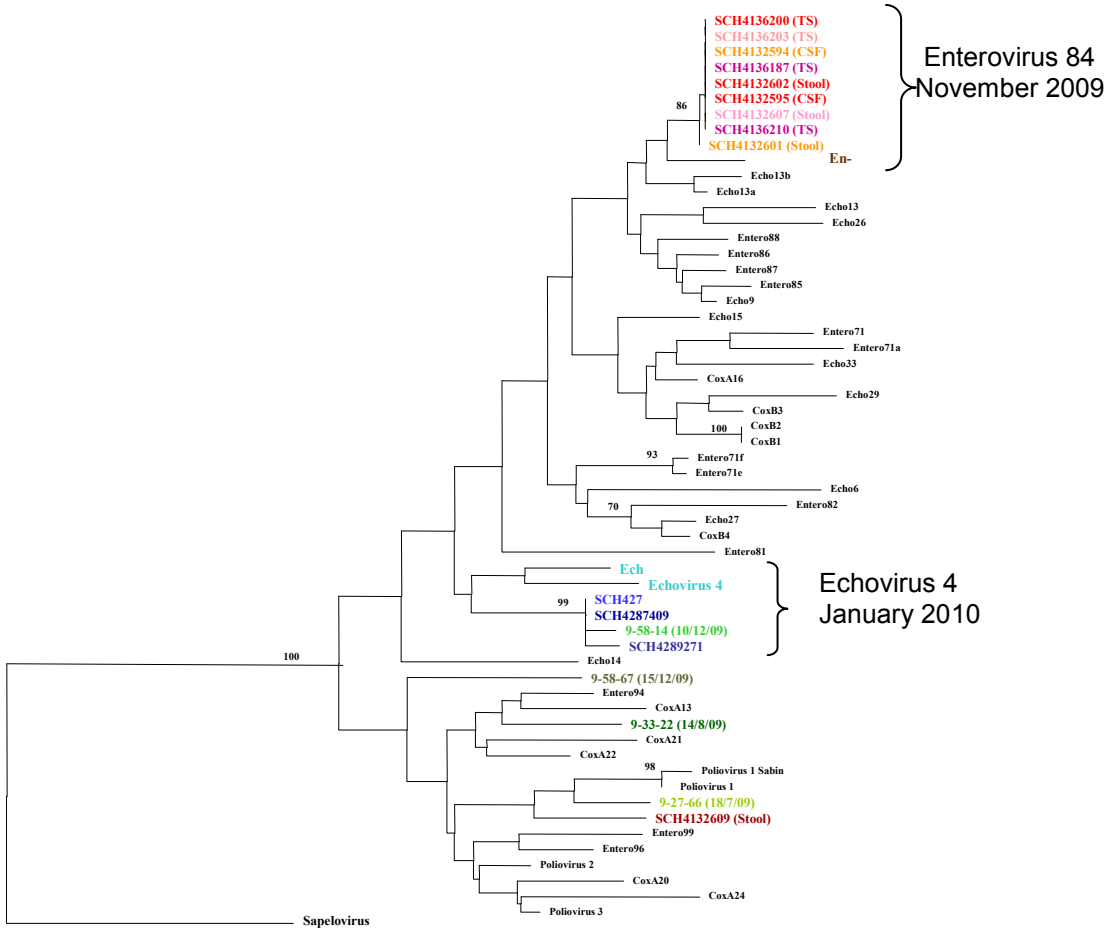

Figure 1: Phylogenetic tree of the 5'UTR depicting the relationship between HEVs from the 2 community outbreaks of aseptic meningitis and HEV sequences obtained from the GenBank database. The tree is rooted with Sapelovirus (accession number AY064708). The neighbour-joining tree was constructed using the Treecon software program.

These results provide evidence that HEV-B serotypes, EV-84 and E-4, were involved in the 2 different clusters of meningitis over a 3-month period.

The 5'UTR is highly conserved. This makes it a good region to target for diagnostic assays, but it is less useful for virus typing (low discriminatory power). However, analysis of this region was nevertheless sufficient to differentiate the two HEV-B serotypes associated these two meningitis outbreaks. For more in-depth analysis the VP1 region, which is more variable, would be a more suitable region to use.

There are very few published reports of the role that different HEVs play in aseptic meningitis in South Africa, specifically in the Western Cape Province. A report documenting the epidemiology of aseptic meningitis in Cape Town from 1981 to 1989 (McIntyre and Keen, 1993) described 5 major summer viral meningitis episodes, of which two were associated with echovirus 4. The first episode of 706 cases lasted 18 months from May 1981.[1] The second, smaller episode of 445 cases was of longer duration (34 months) in 1986/1987. Unfortunately there is no more recent epidemiological data as HEV detection is no longer routinely performed on patients with aseptic meningitis due to cost constraints. This is a pity, as confirming an aetiological diagnosis of viral meningitis can be valuable for patient management if the result is available quickly. It provides useful prognostic information, expensive antibiotics can be stopped, and hospitalised patients can be discharged sooner. Rapid molecular assays for virus detection in CSF are becoming available and use of such assays could provide greater understanding of the role these viruses play in our context.

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LABORATORY-CONFIRMED OUTBREAK OF TYPHOID FEVER IN PRETORIA, APRIL TO MAY 2010

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Introduction

Typhoid fever is caused by the bacterium *Salmonella* Typhi. This disease is an important public health problem in South Africa and other developing countries. Untreated, patients with typhoid fever may continue to be ill for weeks or months, and as many as 15% may die from complications of the infection.[1,2]

S. Typhi is transmitted primarily through the fecal-oral route, by the ingestion of contaminated food or water. The bacterium is commonly spread by ill or asymptomatic chronic *S.* Typhi carriers who fail to observe basic hygiene practices and proper food handling procedures.[1,3]

The incubation period of *S.* Typhi can range from 5 days to over 60 days, though most victims develop symptoms within 10-14 days. Without appropriate treatment, about 10% of typhoid fever patients excrete the bacteria for up to 3 months after the onset of symptoms and 2-5% of them become chronic carriers of the organism.[1, 3]

On 14 May 2010, the City of Tshwane Communicable Disease Control Co-coordinator (CDC) was notified of an increase in the number of culture-positive *S.* Typhi cases identified by a private laboratory in Pretoria; there had been five cases within a period of one month.

A team comprising Tshwane District Health members, environmental health practitioners from the local municipality and SAFELTP residents was assembled to investigate the possible source of the outbreak and to institute control and preventive measures.

Method

A descriptive cross-sectional study was conducted. A list was compiled of all the culture-confirmed *S.* Typhi cases that were reported during the period April-May 2010.

A case was defined as a person who ate or worked at restaurant A, located in a restaurant complex frequented by students from the University of Pretoria, between April and May 2010, and developed fever with headache, malaise or abdominal cramps, and had a laboratory test positive for *S.* Typhi.

We interviewed all the cases (telephonically or face-to-face) using the National Institute for Communicable Diseases (NICD) typhoid fever case investigation questionnaire. We also interviewed the management and staff of restaurant A, where the cases worked or had visited.

Environmental assessments were conducted in restaurant A and other establishments in the restaurant complex. Swabs from restaurant kitchen, water taps, food preparation areas and the hands of the kitchen staff at restaurant A were collected for laboratory testing. Rectal swabs were also collected from the kitchen staff on duty at restaurant A at the time of the investigation.

The five *S.* Typhi isolates from the private laboratory were sent to the Enteric Diseases Reference Unit (EDRU) of the NICD for further characterization, as were another three isolates of *S.* Typhi identified at NHLS laboratories in Tshwane during April-May 2010. Pulsed-field gel electrophoresis (PFGE) patterns were used to assess molecular relatedness of the isolates. The PFGE patterns were analyzed and compared using BioNumerics software (version 6.01). Antimicrobial susceptibility tests were performed on each isolate.

Results

A total of nine *S.* Typhi cases was identified and investigated. Eight were male and one was female. The median age was 23 years, ranging from 19 to 27 years.

The cases had dates of onset ranging from 16 April to 27 May 2010 (Figure 1).

Of the nine cases, six were students living around the student village in Pretoria, one case from Rustenburg (Northwest Province) and one from Nelspruit (Mpumalanga Province). All cases reported visiting the restaurant complex in Pretoria during April-May 2010.

The NICD was informed about a typhoid fever case diagnosed in Australia. The EDRU made contact with the relevant microbiology laboratory in Australia (through the PulseNet network) to investigate further. The Australian *S.* Typhi isolate was compared to the South African isolates and the isolates were noted to be indistinguishable by PFGE.

On 28 May 2010 another isolate with a similar PFGE pattern (the tenth case) was notified from Bloemfontein (Free State Province), through the laboratory-based surveillance system. Investigations revealed that the case was a Pakistani national working in Lesotho, and that he had travelled to Bloemfontein to seek medical treatment. There was no history of travelling to Pretoria and there was no apparent history of contact with any of the other *S.* Typhi cases under investigation.

Laboratory findings

Five isolates were confirmed as *S.* Typhi at EDRU, and their PFGE patterns were indistinguishable (Figure 2).

PFGE was performed on the Australian isolate (in Australia), and the pattern matched those of the South African cluster. The PFGE pattern of the isolate from the Pakistani national also matched the isolates from the Pretoria cluster (Figure 2).

The PFGE patterns of this *S.* Typhi outbreak cluster are different from *S.* Typhi strains previously characterized in South Africa. The isolates tested susceptible to a number of antimicrobial agents commonly used in South Africa, namely ampicillin, augmentin, trimethoprim, sulfamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin, tetracycline, kanamycin, streptomycin, imipenem, ceftriaxone and ceftazidime.

Environmental findings

Restaurant A had no hand-washing facilities for their employees. The employees were using public ablution facilities within the restaurant complex. This could have decreased the adherence to hand hygiene practice by the food handlers and waiters.

All the environmental specimens and the rectal swabs taken from the restaurant staff tested negative for *S.* Typhi.

Actions taken

The environmental health practitioners gave education on hand hygiene practice and food handling procedures to the staff working in restaurant A. Posters promoting correct

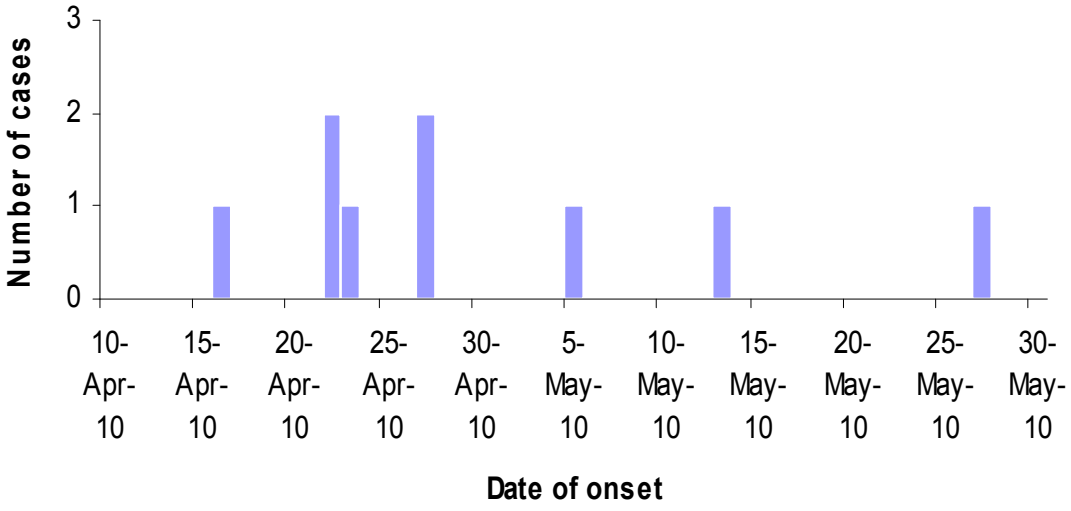

Figure 1: Distribution of laboratory confirmed *Salmonella* Typhi cases by date of onset, Tshwane District, April to May 2010.

hand hygiene practice were distributed to the restaurants in the complex. Typhoid management guidelines were distributed to the healthcare facilities in the surrounding areas. A memo was sent to all healthcare facilities and laboratories (both public and private sectors) around Tshwane to be on the alert for typhoid fever cases.

Following the environmental health assessment, restaurant A has made provisions for hand-washing facilities within the restaurant.

Conclusion

The typhoid outbreak in Pretoria was most likely linked to one restaurant in a restaurant complex. Lack of hand-washing facilities in the establishment could have contributed to the outbreak. We speculate that the S. Typhi strain that caused the outbreak may have originated from Lesotho, since both the Canadian national and the Pakistani national had a history of visiting Lesotho. The link could not be established as there were no strains from Lesotho reported on the Pulse-Net network for comparison.

Recommendations

All restaurants within the complex should provide hand-washing facilities for employees, and they should also receive ongoing health education regarding hand hygiene and safe food practice. The environmental health department should strengthen the monitoring of compliance with sanitary regulations by the restaurants.

Acknowledgements

We would like to thank the NICD Outbreak Response Unit and SAFELTP for assistance and guidance during the investigation. We also acknowledge the Enteric Diseases Reference Unit at the NICD for typing the isolates and for tracking the isolate from Australia through the PulseNet network. We also acknowledge the Tshwane District Health and Tshwane Local Municipality for leading the investigation.

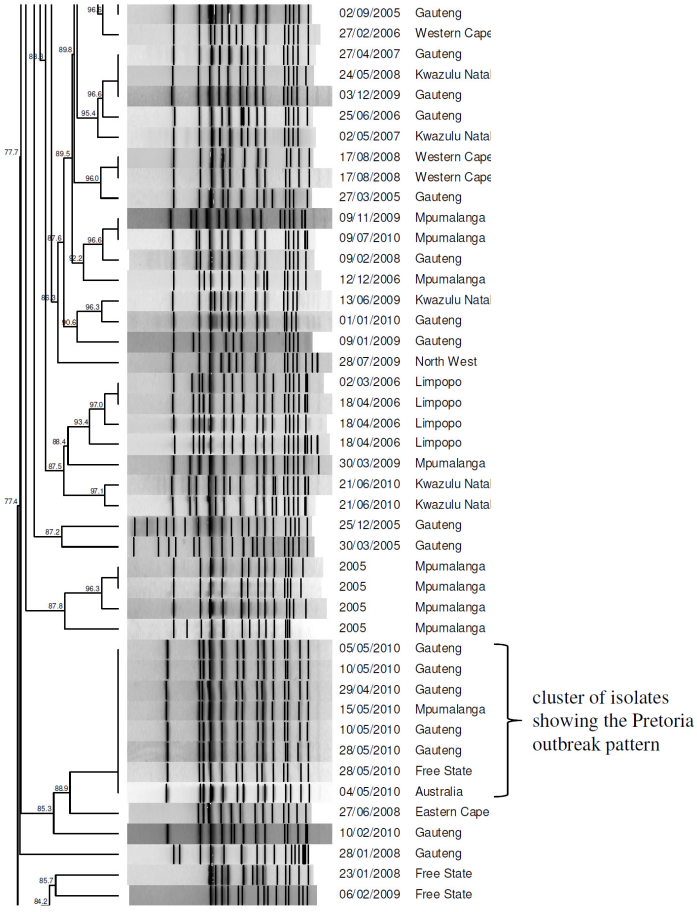

Figure 2: PFGE patterns of *Salmonella* Typhi isolated in South Africa. The cluster of isolates from the Pretoria outbreak pattern is shown in this figure together with the isolates from Free State (Bloemfontein) and Australia.

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FOOD POISONING OUTBREAK AMONG FUNERAL ATTENDEES IN TSHIVHILWI VILLAGE, VHEMBE DISTRICT, JUNE 2010

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Introduction

Food poisoning is an illness resulting from the consumption of food contaminated with an infectious or toxic agent. The toxic agents can be natural chemical toxins or bacterially-produced exotoxins, which can cause illness even if the bacterial agent is no longer present.[1,2]

Contamination of food is usually associated with improper handling, preparation, or storage.[3] Most food poisoning illnesses are mild and self-limiting; though occasionally they can result in permanent health problems or death, especially in high-risk populations like young children, pregnant women, and the elderly and other people with weakened immunity.[1]

Foodborne diseases are a major public health problem globally, especially in developing countries where they are associated with a high morbidity and mortality.[1]

On 19 June 2010, funeral attendees in Tshivhilwi village (Vhembe District, Limpopo Province) developed gastrointestinal symptoms after consuming food served at the funeral. A team comprising the Limpopo Department of Health outbreak response team members, the Vhembe District outbreak response team members and SAFELTP residents was assembled to investigate the outbreak to determine its source and to guide control measures.

Methods

We conducted a case-control study among the people who had attended the funeral.

A case was defined as a person who attended the funeral in Tshivhilwi Village on 19 June 2010 and developed diarrhea and at least one of the following symptoms: nausea, vomiting, fever (self-reported), headache and myalgia. A control was a person who attended the same funeral and did not develop gastrointestinal symptoms.

We interviewed 62 cases and 92 controls using a semi-structured questionnaire to collect information on demographic variables, clinical symptoms and food exposures during the funeral.

Three stool samples and food samples (beetroot, potato salad, cabbage, chicken, beef, gravy, and offal) were sent for laboratory analysis. We collected water and fruit juice samples for laboratory analysis but these could not be analyzed due to lack of funds. Epi Info (CDC) statistical software was used to analyze the quantitative data. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated to establish statistical associations between variables.

Results

Descriptive epidemiology

Out of the 400 people who attended the funeral, 195 (48%) reported illness. Food was served between 09:00 and 11:00; people started developing symptoms at 19:00, peaking at 22:00 and ending around 01:00 the following morning. The incubation period ranged from eight to sixteen hours.

The epidemic curve shows the distribution of the cases by the date and time of onset of illness. (Figure 1).

Common presenting symptoms included diarrhea, fever, arthralgia and headache (Table 1). Three patients were hospitalized and no fatalities were reported.

Table 1: Presenting symptoms in the food borne outbreak in Tshivhilwi Village, 19 June 2010

Symptoms and signs	Frequency (n=195)	%
Diarrhoea	195	100
Fever	195	100
Arthralgia	192	98.0
Headache	180	92.0
Vomiting	120	61.5
Rigors	83	42.6
Myalgia	72	37.0
Dizziness	67	34.0
Nausea	33	17.2
Oral sores	26	13.0

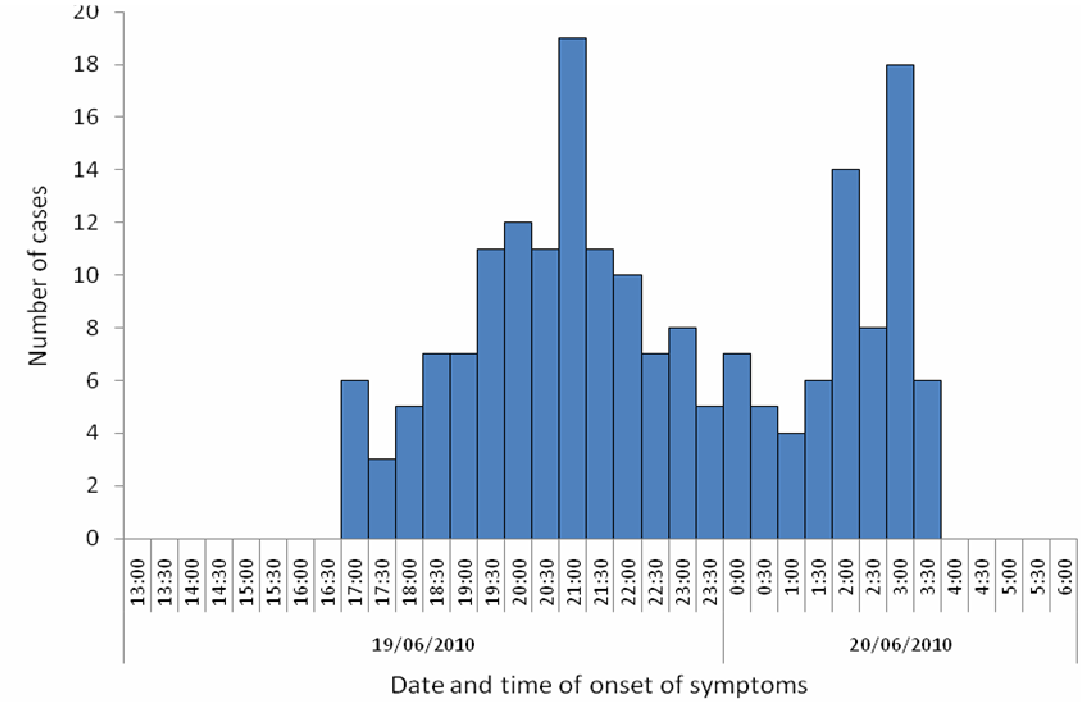

Figure 1: Epidemic curve of the food poisoning outbreak in Tshivhilwi Village, Vhembe District, 19 June 2010

Laboratory findings

Bacillus cereus was isolated from the potato salad and offal samples. No microbiological agents were isolated from the other food samples.

Laboratory cultures of the three rectal swab specimens were negative for salmonellae, shigellae, yersiniae and *Vibrio cholerae*.

Environmental findings

Drinking water was fetched from a nearby river the day before the funeral. The water was stored overnight in open containers under a tree (Figure 2). The same water was used for preparing the fruit juice the following morning (at around 04:00 on the morning of the funeral). The juice was prepared by adding juice concentrates (bought from the local shops) into the bulk water containers. The diluted juice was then stored under the trees and served with the food during the funeral.

Food was prepared in the family homestead in the early hours of the 19th of June (around 04:00). After preparation, the food was stored in covered bowls under the trees for about 5 hours, before being served to the mourners.

Analytical epidemiology

Drinking fruit juice (OR=32.1, 95%CI: 11.9-90.75) and water at the funeral (OR=4.8, 95%CI: 2.27-10.21) were statistically associated with illness (Table 2).

Table 2: Odds ratios and 95% confidence intervals for the association between food items and disease, Tshivhilwi Village, 19 June 2010

Food	Cases		Controls		Odds Ratio (95% CI)
	Eaten	Not eaten	Eaten	Not Eaten	
Fruit juice	54	8	16	76	32.1(11.9-90.75)
Water	42	20	28	64	4.8(2.27-10.21)
Chicken stew	24	38	16	76	3(1.34-6.8)
Porridge	46	16	57	35	1.8(0.83-3.85)
Beetroot	10	52	15	77	1(0.36-2.56)
Fried chicken	9	53	17	75	0.74(0.27-1.94)
Offal	12	50	26	66	0.61(0.25-1.40)
Rice	7	55	19	73	0.5(0.16-1.33)
Beef	28	34	54	38	0.5(0.29-1.17)
Gravy	18	44	41	51	0.5(0.24-1.06)
Cabbage	13	49	33	59	0.48(0.21-1.05)
Potato salad	6	56	32	60	0.2(0.064-0.54)

Figure 2: Drums that contained water used for drinking and preparing fruit juice

Actions taken
The remaining food items were destroyed. Affected patients were treated with oral rehydration fluids and the severely ill patients were given antibiotics.

Health education on good hygiene and food handling procedures was given to the community. The environmental health practitioners also emphasized the importance of good food and water storage.

Discussion and conclusions
Bacillus cereus was isolated from the potato salad and offal dishes, but these foods were not statistically associated with the illness. The food samples were sent for analysis more than 48 hours after the food preparation and it is possible that the *Bacillus cereus* grew after the food had been served. It is therefore possible that the *Bacillus cereus* was not the causative organism in this outbreak.

Water and fruit juice were statistically implicated as the cause of illness. Sweetened fruit juice probably provided a favourable medium for proliferation of bacteria, but microbiological analysis of water and juice was not able to be done. Untreated surface water is not safe for consumption or for preparation of unheated beverages.

About 13% of the cases presented with oral sores (in addition to other symptoms). Foodborne outbreaks caused by heavy metals have been associated with oral sores.[4] Since the water and the fruit juice were statistically associated with illness, it is possible that the water was contaminated by some heavy metals. Though heavy metal poisoning is unlikely because of the long incubation period (8-14hours), chemical analysis of the water could have assisted in excluding this diagnosis in this outbreak. Various forms of toxicity may occur if used industrial containers are recycled for domestic water storage.

Limitation
The provincial and district Outbreak Response Teams was notified about the outbreak more than 24 hours after the funeral and when the investigation was launched most of the funeral attendees had already left the village.

Recommendations
The Vhembe district local municipality should ensure that funds are available for the laboratory analysis of samples during outbreaks. The environmental health practitioners should strengthen the monitoring of food preparation, handling and storage during mass gatherings. Community health education on the preparation, handling and storage of food during mass gatherings should also be emphasized. Education on the health risks of untreated surface water and methods to ameliorate these (e.g. chlorination, heating) is also recommended.

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We acknowledge the Limpopo Provincial Department of Health and Social Development, the Vhembe District Department of Health and Social Development and the SAFELTP for their support during the investigation. We also thank the Motshekwane family for their cooperation and assistance during the investigation.

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2010 FIFA SOCCER WORLD CUP, SOUTH AFRICA: COMMUNICABLE DISEASE RISKS AND SURVEILLANCE

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The FIFA World Cup is the largest single sporting event in the world. An estimated three million spectators, including in excess of 350 000 foreign visitors, attended the 2010 event which took place in South Africa from 11th June to 11th July 2010, in 10 stadiums in 9 host cities across the country, with 64 matches being played. The event posed specific challenges given its size, diversity of attendees and the potential for transmission of communicable diseases.[1] These included endemic diseases such as TB and HIV, given the high prevalence of these in South Africa. Unless there were visits to neighbouring countries, the risks of malaria were considered low as the stadiums are outside of the malaria transmission areas and June would be considered low risk season. An ongoing countrywide measles outbreak that had started in Gauteng in 2009 did pose risks despite a mass vaccination campaign in May 2010. The risk of tourists acquiring disease related to the Rift Valley fever outbreak was considered remote, given that occupational exposure to infected animal tissue was the major mode of transmission rather than mosquito bites, and that few tourists were likely to visit the affected farms. The event coincided with the annual influenza season in South Africa and particular concerns were raised about transmission within a mass gathering-type setting, with influenza A H1N1 pandemic 2009 expected as the predominant strain. Food-related incidents, because of the increased capacity needs and bioterrorism potential, given the high profile of the event, were of particular concern.

A number of opportunities arose to reduce the risk of communicable diseases during the World Cup and included enhanced epidemic intelligence to timeously detect incidents and the provision of standard operating procedures for epidemic response.

Pre-travel advice included recommendations for yellow fever vaccination in accordance with IHR regulations, influenza vaccination as the event coincided with the southern hemisphere influenza season, hepatitis A vaccine because of the endemicity in South Africa, and measles vaccination for those considered non-immune, because of the measles outbreak in South Africa. Meningococcal vaccine was considered as the event coincided with the expected annual increase in sporadic cases and mass gatherings do pose specific risks, but the overall risk was considered low. A national influenza programme using WHO donated H1N1 2009 pandemic vaccine targeted front-line 2010 workers. Messages about safe sexual practices were highlighted.

Specific communicable diseases were prioritized for monitoring at private and public hospitals (see list). Routine surveillance systems were strengthened and supplemented with reporting from health facilities at the stadiums, and with real-time paper-based reporting to central points within the provinces and then to the National Health Operations Centre established in Tshwane, Gauteng. Laboratory support was provided by the National Health Laboratory Service, including the National Institute for Communicable Diseases, as well as private laboratory groups, and focussed on providing a diagnostic service for individual patients with suspected communicable diseases as well as any trauma-related incidents. Specialized diagnostic services for any potential imported diseases were provided by the NICD. All laboratories worked together to provide daily reporting of laboratory-confirmed cases with priority conditions. Surveillance programmes for meningococcal disease, influenza and measles provided important information on the intensity of transmission and circulating strains. International surveillance was supported by WHO and ECDC. A special Public Health Cluster, which included representatives from the national Departments for Communicable Diseases, Epidemiology and Surveillance, Port Health, Environmental Health and the National Institute for Communicable Diseases and NHLS, met daily to carry out risk assessments to determine the impact on the World Cup event. An emergency reporting system was also established. A risk assessment according to pre-established guidelines was carried out on each communicable diseases incident for possible impact on the World Cup, and any national or international impact.

Priority conditions for surveillance were:

- Anthrax
- Cholera
- Foodborne outbreaks
- Hepatitis A
- Meningococcal disease
- Measles
- Pandemic influenza A H1N1
- Rabies exposures
- Rift Valley fever
- SARS
- Smallpox
- Typhoid
- Viral haemorrhagic fevers
- Yellow fever

Thirty incidents were reported during the period of the World Cup and most of these were unrelated to the event itself. There were 5 foodborne outbreaks reported and investigated that were related to the World Cup. The majority of these affected volunteers. There was one confirmed case of cholera affecting a returning South African traveller from India. There was very little pandemic H1N1 activity, influenza A H3N2 and B predominated during the influenza season which was later and milder than usual, possibly because of school closure and the large number of attendees who were likely immune to influenza A H1N1 (2009) through previous exposure or vaccination. A number of measles cases involving World Cup attendees from other countries were confirmed, and some were further characterised and identified as genotype B3, the genotype currently circulating in South Africa.

Challenges included interpretation of data for decision-making given the lack of base-line data and a 'changing' population, and linking laboratory-confirmations with suspected cases. The use of well-established laboratory-based surveillance programmes for influenza, meningococcal disease and measles were particularly useful in identifying trends and disease activity. Other achievements included the establishment of the Public Health Cluster, the overall improvement in notifications, especially from the private health sector and an improved response to managing foodborne outbreaks. Sustaining many of these should be a legacy beyond the 2010 World Cup.

Acknowledgements
Grateful thanks are due to all the national (NDoH, provincial and local Departments of Health, NICD/NHLS), international (WHO, ECDC) and other partners who contributed to making the World Cup in South Africa a safe, healthy, and successful event.

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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 - 30 September 2009/2010*

Disease/Organism	Cumulative to 30 September, year	EC	FS	GA	KZ	LP	MP	NC	NW	WC	South Africa
Anthrax	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Botulism	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Cryptococcus spp.	2009	928	369	1659	1121	517	620	66	546	432	6258
	2010	1144	339	1600	766	447	595	50	445	435	5821
Haemophilus influenzae, invasive disease, all serotypes	2009	26	18	127	33	3	22	6	7	60	302
	2010	31	18	137	25	8	11	10	6	67	313
Haemophilus influenzae, invasive disease, < 5 years											
Serotype b	2009	3	6	15	14	1	1	2	0	15	57
	2010	4	6	20	4	2	7	4	1	12	60
Serotypes a,c,d,e,f	2009	0	1	16	2	0	2	0	1	6	28
	2010	0	2	6	0	1	1	0	0	8	18
Non-typeable (unencapsulated)	2009	1	0	23	7	1	2	0	0	9	43
	2010	1	1	38	3	0	0	1	1	11	56
No isolate available for serotyping	2009	4	3	18	3	1	5	2	3	1	40
	2010	9	3	15	2	2	1	1	1	4	38
Measles	2009	15	14	774	13	5	24	2	16	9	872
	2010	1303	646	1431	3784	288	1811	322	740	1764	12089
Neisseria meningitidis, invasive disease	2009	24	12	164	23	2	44	7	17	57	350
	2010	19	18	152	19	8	18	17	8	46	305
Novel influenza A virus infections***	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Plague	2009	0	0	0	0	0	0	0	0	0	0
	2010	0	0	0	0	0	0	0	0	0	0
Rabies	2009	7	0	0	3	1	1	0	0	0	12
	2010	2	0	1	3	3	1	0	0	0	10
**Rubella	2009	122	5	145	88	34	82	40	24	35	575
	2010	263	77	194	280	37	134	40	169	260	1454
Salmonella spp. (not Typhi), invasive disease	2009	57	25	312	82	4	32	10	32	71	625
	2010	39	18	285	65	11	21	12	9	62	522
Salmonella spp. (not Typhi), isolate from non-sterile site	2009	167	38	632	129	28	134	22	63	184	1397
	2010	156	38	501	167	12	67	6	30	128	1105
Salmonella Typhi	2009	7	1	19	2	0	5	0	1	10	45
	2010	7	2	23	8	1	8	0	0	8	57
Shigella dysenteriae 1	2009	0	0	0	0	0	1	0	0	0	1
	2010	0	0	0	0	0	0	0	0	0	0
Shigella spp. (Non-Sd1)	2009	169	59	481	118	5	49	10	12	321	1224
	2010	181	40	519	101	9	36	19	15	319	1239
Streptococcus pneumoniae, invasive disease, all ages	2009	295	229	1796	416	84	225	69	134	478	3726
	2010	297	228	1410	326	79	184	75	129	446	3174
Streptococcus pneumoniae, invasive disease, < 5 years	2009	85	53	499	130	16	75	35	20	162	1075
	2010	54	38	327	80	12	38	32	23	108	712
Vibrio cholerae O1	2009	2	0	47	0	618	310	0	28	4	1009
	2010	0	0	1	0	0	0	0	0	0	1
Viral haemorrhagic fever (VHF)											
Crimean-Congo haemorrhagic fever (CCHF)	2009	0	1	0	0	0	0	0	0	0	1
	2010	0	1	0	0	0	0	2	0	0	3
Other VHF (not CCHF)****	2009	0	0	0	5	0	0	0	0	0	5
	2010	17	123	0	0	0	0	76	9	11	236

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 are diagnosed from specimens submitted for suspected measles cases.

*** Confirmed cases. Excludes pandemic influenza H1N1. See weekly influenza reports on www.nicd.ac.za.

**** All Rift Valley fever . For 2010 the total includes 1 case from an unknown province.
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 - 30 September 2009/2010*

Programme and Indicator	Cumulative to 30 September, 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	2009	41	6	47	81	52	28	7	15	16	293
	2010	37	9	43	50	33	24	2	15	17	230
Laboratory Programme for the Comprehensive Care, Treatment and Management Programme for HIV and AIDS											
CD4 count tests											
Total CD4 count tests submitted	2009	283,872	99,849	482,474	616,415	161,122	179,607	40,341	173,095	163,400	2,200,175
	2010	292,410	140,259	575,420	715,141	191,966	220,853	47,317	187,552	181,640	2,552,558
Tests with CD4 count < 200/µl	2009	91,115	29,763	161,087	193,389	50,841	55,902	12,212	52,534	44,217	691,060
	2010	91,821	42,591	187,710	168,809	60,990	67,022	13,576	54,299	45,869	732,687
Viral load tests											
Total viral load tests submitted	2009	113,308	33,811	223,183	253,110	67,178	64,181	16,303	71,117	61,920	904,111
	2010	102,327	54,114	255,773	246,723	61,809	66,866	19,650	77,530	79,435	964,227
Tests with undetectable viral load	2009	67,786	25,765	150,465	176,064	42,155	41,854	10,213	48,905	50,884	614,091
	2010	64,052	35,903	176,661	181,074	43,488	50,729	11,942	51,068	60,475	675,392
Diagnostic HIV-1 PCR tests											
Total diagnostic HIV-1 PCR tests submitted	2009	22,306	8,029	48,413	55,573	12,189	12,972	2,714	12,902	12,882	187,980
	2010	22,010	10,446	45,339	56,933	15,860	17,105	3,536	13,734	13,073	198,036
Diagnostic HIV-1 PCR tests positive for HIV	2009	2,329	976	5,206	5,451	1,592	1,651	320	1,556	1,055	20,136
	2010	1,773	929	4,212	4,490	1,564	1,605	306	1,257	784	16,920

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

U = unavailable, 0 = no cases reported