The 2012 winter influenza season in South Africa is being carefully monitored by three influenza surveillance programmes coordinated at the NICD. Last year, the season was characterized by two distinct virus detection peaks. The predominant strains identified in 2011 and their genetic sequence characteristics are described in detail in this issue. Fortunately, no neuraminidase inhibitor resistant H1N1 samples were detected in 2011 and it is hoped that this scenario will continue through 2012. Also in this issue, the scientific basis (or lack thereof) for treating chronic fatigue syndrome with doxycycline is under scrutiny, outbreaks of the mosquito borne Sindbis virus during the period 2006 to 2010 in South Africa are described and a method for diagnosing malaria infections using a multiplex polymerase chain reaction assay is evaluated, with very promising results. I trust you will find these contributions interesting and useful, and thank all authors for their timely inputs.

Basil Brooke, Editor

FOREWORD

COMMENTARY: IS THERE A SCIENTIFIC BASIS FOR TREATING PATIENTS WITH CHRONIC FATIGUE SYNDROME WITH PROLONGED COURSES OF DOXYCYCLINE?

John Frean
Centre for Opportunistic, Tropical and Hospital Infections, NICD, NHLS

Introduction
The chronic fatigue syndrome is a prolonged illness characterised by disabling fatigue, musculoskeletal pain, neurocognitive problems, and mood disturbance. As a result many patients experience substantially diminished quality of life, productivity, and/or income. Since conventional medicine can do little for those with chronic fatigue syndrome, they may seek alternative or unconventional therapy, including antibiotic treatment for alleged chronic rickettsial or other infections. The scientific evidence for this practice is the subject of this brief review.

Definition of chronic fatigue syndrome (CFS)
Chronic fatigue syndrome is defined as persistent or relapsing fatigue that cannot be explained by other medical or psychiatric conditions, has been present for at least six months, is not improved by rest, and causes significant reduction in daily activities.1,2 CFS is a heterogeneous condition that encompasses a variety of clinical entities and also, probably, a variety of causes.1 There are a number of other terms applied to the same condition, among them myalgic encephalomyelitis (ME). Gulf War veterans’ illnesses (GWVI), while epidemiologically limited to a particular risk group, is a symptom complex largely clinically indistinguishable from CFS.3 Suggested aetiologies of CFS include infections, psychiatric functional disorders, immunological disorders, endocrinopathies and metabolic disorders.2,4
Evidence for infectious causes of CFS

Some of the common features of CFS, such as fatigue, muscle and joint pain, sore throat, and swollen lymph glands, suggest an infectious disease. The onset of CFS is often described as sudden or ‘flu-like’, additionally indicating a possible infectious aetiology. Certain infectious agents and diseases can lead to chronic illness; these include Epstein-Barr virus (EBV, glandular fever), cytomegalovirus, human herpes types 6 and 7, brucellosis, Lyme disease, Ross River virus, enteroviral meningitis, mycoplasmas (particularly in the case of GWVI), and chlamydial infections. Q fever (Coxiella burnetii infection) is associated with a post-infection fatigue syndrome (QFS), thought to be related to immune system dysfunction in the form of cytokine abnormalities. However, identification of a single infectious agent (or related group of agents) specifically associated with all cases of CFS has not been possible with normal laboratory tests.

Association of particular infections with CFS

Extensive case-control seroepidemiologic surveys to detect antibody responses to numerous known viral, bacterial, and rickettsial agents have failed to show a consistent difference between CFS cases and normal controls. A prospective study in Australia suggested that CFS is a quite common sequel of a number of different infections. In this study, Ross River, EBV, and Q fever were strongly implicated. The authors suggest that it is the host response (but not chronic inflammation), rather than the pathogens themselves, that causes the associated CFS symptoms, which merge into a common clinical picture, and that infectious causes form only a subset of a larger group of aetiologies.

Molecular techniques applied to CFS

As antibodies are an indirect indicator of prior exposure to an organism, another approach has been to seek evidence of infection in the form of pathogen nucleic acid in the blood of patients by molecular means, such as DNA amplification by the polymerase chain reaction (PCR). DNA of Coxiiella burnetii and an Australian spotted fever rickettsial species have been found in the blood of QFS and CFS patients, respectively. In the latter study the proportion of PCR-positive CFS patients was small (14/526, 3%) and these findings need to be confirmed by cohort and prospective studies, as the authors point out. Another weakness of this approach is that organism-specific PCR is intentionally designed to not detect other pathogens present that may have stronger associations with CFS.

In view of the fact that appropriate lesion or tissue specimens may not be available for many infections potentially associated with CFS, Vernon et al looked at cell-free circulating DNA concentrations and applied broad-range amplification to detect conserved 16s ribosomal sequences (16s rDNA) in the plasma of 34 CFS patients and 55 non-fatigued controls. The 55 non-fatigued subjects had higher plasma DNA concentrations than those with CFS and more CFS subjects (6/34, 18%) had no detectable plasma DNA than non-fatigued subjects (2/55, 4%), but these differences were not significant. Bacterial sequences were detected in 23 (26%) of 89 subjects. Only 4 (14%) CFS subjects had 16S rDNA sequences amplified from plasma compared with 17 (32%) of the non-fatigued (P = 0.03). All but 1 of the 23 16S rDNA amplicon-positive subjects had five or more unique sequences present. Numerous different bacterial 16S rDNA sequences were detected (none of which were from known rickettsial species), but none were unique, previously uncharacterised or predominant in either CFS or non-fatigued subjects. In summary, the CFS patients appeared to be at less risk for occult infection than the normal controls. This supports the caveat that applies to all apparent associations between an organism and a disease: more than just the demonstration of presence of the organism (in the form of an isolate, antibodies, or DNA) is required to establish the fact that it causes disease.

Evidence-based treatment

The term ‘evidence-based’ is widely used in medicine as the ideal or ‘best practice’. Evidence-based medicine represents the application of published research findings to a clinical problem, such as the best treatment or the best diagnostic test for a disease. In this context ‘published’ means dissemination in a journal that is indexed (eg, in PubMed) and peer-reviewed (i.e. by experts in the field). Unpublished anecdotes are not considered as evidence. Accepted levels of evidence are, from highest to lowest:
Published evidence of antibiotic efficacy in treating CFS

PubMed, Google Scholar, the Cochrane reviews, and NIH Clinical Trials Register were searched for relevant clinical trials of antibiotics in chronic fatigue syndrome. At a high evidence level, a randomized, double-blind, placebo-controlled study of long-term (12 months) doxycycline was carried out in 491 Gulf War veterans who had *Mycoplasma* DNA in their blood. There was no detectable effect of treatment on symptoms or mental or physical function, but treated subjects had more adverse side effects and adherence at 6 months was poor. Two small, open-label, uncontrolled trials of minocycline in QFS, and minocycline or doxycycline in QFS and CFS were found (table 1). The CFS patients in the latter were a subset of a larger group of patients with markers of Q fever infection, who met the study's criteria for CFS. The rest, although symptomatic, did not, and were therefore designated QFS. Both papers point out that they are reporting pilot studies and that larger randomized controlled trials are required. The level of evidence for these trials is therefore low, at level 6 on the above scale.

<table>
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<td>Clinical improvement, Q fever DNA cleared (PCR), Q fever antibody titres reduced</td>
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<td>Minocycline or doxycycline, 3 months</td>
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<td>Minocycline or doxycycline*, 3 months</td>
<td>Clinical improvement, Q fever DNA cleared (PCR), Q fever antibody titres reduced</td>
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</table>

*3 patients received levofloxacin because of intolerance or allergy

Table 1: Published trials of antibiotic treatment of Q fever fatigue syndrome (QFS) and chronic fatigue syndrome (CFS)

Summary of literature review

- CFS is the outcome of the neurobiological response to unknown insults or triggers, in some cases mediated by immune components such as cytokines.
- In a small proportion of cases, the trigger may be an infection.
- Q fever and possibly some viral infections (Ross River, EBV) are associated with a post-infection fatigue syndrome. In the case of Q fever, the association seems to be well accepted and therefore these QFS patients do not have CFS, by definition.
- In most cases the cause or association is not evident, either by conventional or molecular laboratory methods.
- There is no high-level evidence (i.e. of high enough level to represent generally accepted, recommended, or best practice) for the use of antibiotics to treat CFS.
- There is a small amount of low-level evidence to suggest that some patients with QFS may benefit clinically from a 3-month course of appropriate antibiotic treatment, but this benefit does not extend to patients with CFS.

Comment

My literature review showed that conventional medicine’s attitude to the treatment of CFS with antibiotics has not substantially changed since the South African Medical Journal’s editorial on the subject was published in 1992.
The author stated then that:

‘...it seems unacceptable that medical practitioners should administer unproven treatments to ME sufferers on anything but a strictly controlled experimental basis. This is especially so when the agents are associated with potentially serious adverse effects or when their side-effect profiles are unknown. An example of the latter is the current use of high-dose, intermittent, alternate tetracycline/doxycycline/minocycline therapy for ME in South Africa, about which the Department of Pharmacology at the University of Cape Town has received numerous queries recently. This regimen is based on the hypothesis that rickettsiae may be causative in ME. However, extensive literature searches and consultation with experts in the field have failed to provide scientific evidence to support such an idea. Further, there are no clinical trials suggesting efficacy of tetracyclines in ME. Hence it seems that at present the postulated benefits of tetracyclines in ME are speculative and anecdotal.’

References


2. The severe acute respiratory illness (SARI) programme
3. The respiratory morbidity surveillance system

The principal findings of each programme for the year 2011 are given below:

**Viral Watch and Enhanced Viral Watch surveillance programme**

The Viral Watch sentinel influenza-like-illness (ILI) surveillance programme was initiated in 1984. It aims to provide information on the geographic spread and timing of influenza virus circulation as well as the type and distribution of circulating influenza viruses each year. During 2011, 202 registered practitioners in South Africa submitted specimens from patients with ILI. Of these, 133 submitted specimens to the NICD, 9 to the virology laboratory at the University of the Free State (UFS), 12 to the Department of Virology at Inkosi Albert Luthuli Central Hospital/University of KwaZulu-Natal (UKZN), and 48 to the NHLS Tygerberg Hospital laboratory in the Western Cape (Tygerberg). Under normal circumstances, positive specimens from these sites are sent to the NICD for confirmation, serotyping and sequencing. The databases of all specimens received are sent to the NICD on a weekly basis.

A total of 3,131 specimens was submitted to the NICD during 2011 ((UFS 62 (2%), UKZN 161 (5%)), NICD 2,581 (82%) and Tygerberg 327 (10%)). Of these 1,156 (37%) were positive for influenza. Dual influenza virus infections were detected in 33 (3%) patients [15 A(H1N1)pdm09 & A(H3N2), 12 A(H1N1)pdm09 & B, and 6 A(H3N2) & B]. The remaining 1,123 were characterized as 860 (74%) A(H1N1)pdm09, 139 (12%) A(H3N2), 110 (10%) B, and 14 (1%) influenza A unsubtyped.

The start of the influenza season is defined as the first week the influenza case detection rate (calculated using specimens tested at the NICD only) rises above 10% and then either stays at this level or continues to rise. The end of the season is defined as the week before the detection rate drops below 10% (figure 1). The first influenza infection of the season was detected from a specimen collected on 3rd May (week 18), and the last from a specimen collected on 26th July (week 29). Sporadic cases were detected both before and after the season.

In 2009, in response to the emergence of the influenza pandemic, Enhanced Viral Watch centres at 12 public hospitals were enrolled to detect influenza strains in hospitalized patients presenting with lower respiratory tract infection. During 2011, 144 specimens were received from eight of these centres. Of these, 68 (47%) came from the Northern Cape. Influenza was detected in the specimens of 14/144 (10%) patients ([9 A(H1N1)pdm09, 3 A(H3N2), 1 A(H1N1)pdm09 and A(H3N2), and 1 B]) (figure 1). Other respiratory viruses were detected in a further 20 patients of which 12 (60%) were respiratory syncytial virus.
Severe acute respiratory illness (SARI) surveillance programme

The SARI sentinel surveillance programme was initiated in April 2009 and is presently functioning at six public hospitals in four provinces. The primary aims of the programme are to describe trends in the numbers of SARI cases at sentinel sites and to determine the relative contribution of influenza and other respiratory viruses to the syndrome of SARI. The SARI sites include Chris Hani Baragwanath Hospital (CHBH) in Gauteng, Matikwana and Mapulaneng hospitals, which form the Agincourt site in Mpumalanga, Klerksdorp-Tshepong hospital complex in the Northwest Province and Edendale Hospital in KwaZulu-Natal.

Hospitalised patients meeting the clinical case definition of acute respiratory illness are prospectively enrolled. Clinical and epidemiological data are collected using standardized questionnaires. Information on in-hospital management and outcome is also collected. Upper respiratory tract samples (oropharyngeal and nasopharyngeal swabs in patients ≥5 years old or nasopharyngeal aspirates in patients <5 years of age) are collected and tested at the NICD for the presence of influenza and other respiratory viruses using real-time reverse transcriptase polymerase chain reaction (RT-PCR). Blood specimens are tested for the presence of pneumococcal DNA using quantitative real-time PCR for the lytA target. The SARI case definition has been expanded at two enhanced surveillance sites (Edendale and Klerksdorp-Tshepong) to include patients admitted with respiratory illness for >7 days and patients with suspected tuberculosis.

During 2011, 5377 patients were enrolled into the SARI programme. The majority (2932/5377 55%) were from CHBH. Children under 5 years accounted for 2436/5371 (45%) of patients and 2759/5373 (51%) were female. Influenza results were available for 5210/5377 (97%) of enrolled patients and 448 (9%) were positive for influenza using RT-PCR. Of these, 174 (39%) were positive for influenza A(H1N1)pdm09, 155 (35%) for influenza B, 113 (26%) for influenza A(H3N2), 5 (1%) for influenza A(H3N2) and influenza B and 1 (0.22%) for influenza A(H1N1)pdm09 and influenza A(H3N2).

During week 20 (week starting 16th May 2011) the influenza detection rate rose above 10% and remained above 10% until
week 31. The peak detection rate occurred in week 24 (28%). During the first influenza peak of 2011 the predominant virus isolated was A(H1N1)pdm09, accounting for 175/448 (39%) of annual cases. This was shortly followed by a second peak during which the detection rate rose above 10% in week 34 and remained above 10% until week 43. During this second peak influenza A (H3N2) and influenza B dominated, (273/448, 61% of annual cases) (figure 2).

During 2011 the respiratory syncytial virus season preceded the influenza season. The detection rate for RSV remained above 10% from week 5 until week 38 and reached a peak of 41% in week 15. Of the 5377 patients enrolled into SARI, 4619 (86%) had blood specimens tested for the presence of pneumococcal DNA. Of these, 261 (6%) were positive for pneumococcus and 18 of these patients (7%) were co-infected with influenza (figure 4).

### Figure 2: Influenza detection rate and numbers of positive specimens by viral subtype - Severe Acute Respiratory Illness (SARI) Surveillance Programme 2011.

Amongst patients enrolled into the SARI programme, testing for additional respiratory viruses identified respiratory syncytial virus (RSV) in 14% (721/5210), adenovirus in 16% (832/5210), rhinovirus in 26% (1380/5210), enterovirus in 6% (324/5210), human metapneumovirus in 3% (173/5210), parainfluenza 3 in 4% (226/5210), parainfluenza 1 in 1% (72/5210) and parainfluenza 2 in 1% (62/5210) of samples (figure 3).
Figure 3: Numbers of specimens received and detection rate of respiratory viruses by epidemiologic week - Severe Acute Respiratory Illness (SARI) Surveillance Programme 2011.

Figure 4: Detection rate for influenza (INF), respiratory syncytial virus (RSV) and pneumococcus (SP) by epidemiologic week - Severe Acute Respiratory Illness (SARI) Surveillance Programme 2011.
Respiratory Morbidity Surveillance

In order to describe the influence of the influenza season on the number of pneumonia and influenza hospitalizations, the NICD reviews anonymized data from a private hospital group. The numbers of hospitalizations for pneumonia and influenza during the influenza season are compared to those for the periods preceding and following the season. During 2011 there were 975,767 consultations reported to the NICD through the respiratory morbidity data mining surveillance system. Of these, 30,072 (3%) were due to pneumonia or influenza (P&I) (International Classification of Diseases 10 codes J10-18). There were 20,340 (68%) inpatients and 9,732 (32%) outpatients with P&I discharge data.

An increase in P&I consultations and admission was observed during the period with a higher number of seasonal influenza virus isolations reported to ‘Viral Watch’ and SARI surveillance programmes respectively (figures 5 and 6). A second lower peak was seen preceding the influenza season, corresponding to the circulation of respiratory syncytial virus.

Figure 5: Numbers of private hospital admissions with a discharge diagnosis of pneumonia and influenza (P&I) and viral isolates by epidemiological week - Severe Acute Respiratory Illness (SARI) Surveillance Programme 2011.

Figure 6: Numbers of private hospital outpatient consultations with a diagnosis of pneumonia and influenza (P&I) and viral isolations by epidemiological week - Viral Watch Surveillance Programme 2011.
Molecular characterization of influenza virus strains

Twenty-two influenza A(H3N2) strains were selected for sequencing from throughout the 2011 season. The 2011 strains proved genetically similar to those identified in the previous influenza season. P-distance analysis of the amino acid sequences indicated a range of 0.003%-0.02% differences between the 2011 strains and the A/Perth/16/2009 vaccine strain. All 2011 strains clustered together with the 2010 strains within the A/Victoria/208/2009 clade (figure 7). A small sub-cluster of four strains from three provinces contained two amino acid changes, N145S and I223V, representing one of the three genetic lineages within the A/Victoria/208/2009 clade. The predominant strain in South Africa contained the amino acid changes K62E, K144N and T212A, representing the A/Victoria/208/2009 strain.

Fifty-nine influenza A(H1N1)pdm09 strains were sequenced from the 2011 season of which 41 were selected from the ILI and SARI surveillance programs and 18 were collected during an outbreak investigation at the Red Cross Children’s Hospital in the Western Cape. Two of the six genetic lineages which dominated the northern hemisphere 2010/2011 influenza season were identified in South Africa. The first lineage, characterized by two amino acid changes, D97N and S185T, was identified in seven strains. The second lineage, characterized by four amino acid changes, D97N, R205K, I216V and V249L, was the predominant lineage in 2011 (figure 8). Within the predominant lineage, an additional amino acid change, H138Q, was acquired. This sub-lineage may be associated with severe illness. P-distance analysis indicated a range of 0.006%-0.03% amino acid differences in the HA protein of the 2011 strains compared to the vaccine strain.

Phylogenetic analysis of those strains identified from the nosocomial outbreak at the Red Cross Children’s Hospital in weeks 22/23 revealed the presence of 3 lineages one of which clustered with the predominant lineage of the 2011 strains. A second lineage, characterized by three amino acid changes, S143G, K163I and A197T, was detected only in these samples.

The HA gene of 31 influenza B specimens was sequenced in the 2011 influenza season of which 30 were included for phylogenetic analysis. Eight specimens clustered with the B/Brisbane/60/2008 vaccine strain and 22 clustered with B/Yamagata/16/1988-like strains.

Antigenic characterisation of influenza virus strains

In 2011 a total of 208 influenza virus strains were isolated in embryonated eggs or Madin-Darby canine kidney cell cultures. Of these 95 ((52 influenza A(H1N1)pdm09, 9 influenza A(H3N2), and 34 influenza B strains)) could be characterised by hemagglutination inhibition (HAI) for reactivity against reference antisera. Ten influenza B virus isolates were antigenically similar to the B/Victoria-like lineage (B/Brisbane/60/2008-like) and 24 showed low reactivity (titers = 8 to 64) to the B/Florida/4/2006-like strains (B/Yamagata-like lineage). Of the 9 influenza A(H3N2), one showed low reactivity (titer of 32) and the other eight showed high titers, indicating that they are antigenically similar to the vaccine strain, A/Perth/16/2009. Fifty of the 52 influenza A(H1N1)pdm09 isolates produced HAI titers similar to the A/California/7/2009 reference and two showed low reactivity. Representative cell culture and egg isolates as well as clinical samples were sent to the WHO Collaborating Centres in London and Melbourne for further characterization.

Resistance testing of influenza virus strains

Virus isolates and clinical samples positive for influenza A were also tested for sensitivity to neuraminidase inhibitors. No drug resistance was identified in any of the virus strains tested which included influenza A(H3N2) isolates from 2007 to 2010 as well as A(H1N1)pdm09 isolates from 2009 to 2011. All influenza A (H1N1)pdm09 and influenza A(H3N2) viruses tested for the H274Y and E119V [A(H3N2)] oseltamivir drug resistance mutations by allelic discrimination or sequencing presented as wild type confirming their sensitivity to the neuraminidase inhibitors.

Discussion

The influenza season of 2011 was characterized by two distinct virus detection peaks. During the first peak influenza A(H1N1)pdm09 predominated, while influenza A(H3N2) and influenza B predominated during the second peak. The second peak of virus circulation was significantly more obvious amongst patients enrolled in the SARI surveillance programme compared to the Viral Watch programme. This discrepancy could reflect a bias in specimen submission practices amongst clinicians participating in the Viral Watch programme as they may have
stopped sending specimens once they felt that the influenza season was over. Alternately, this could reflect true differences in influenza virus circulation between different population subgroups or different clinical syndromes. Systematic surveillance for ILI will be established at outpatient clinics close to two of the SARI enhanced surveillance sites in 2012, which will hopefully provide useful information in light of the above considerations.

Molecular analysis showed that limited genetic drift occurred in the influenza A(H1N1)pdm09 and influenza A(H3N2) strains relative to the 2010 vaccine strains. However, only a few low reactors were detected using HAI tests for influenza A(H1N1)pdm09 or A(H3N2) strains and no seasonal influenza A(H1N1) strains were detected during the 2011 season, which was dominated by influenza A(H1N1)pdm09 infections. Few influenza B strains could be characterized. However, the identification of B/Yamagata/16/1988-like strains amongst those should be noted as this lineage is not included in the 2012 influenza vaccine formulation.

Figure 7: Maximum likelihood tree of the A(H3N2) HA1 region (923bp). The vaccine strain is indicated in red and the 2011 South African isolates are indicated in green. Amino acid changes corresponding to different lineages are indicated.
Figure 8: Maximum likelihood tree of the HA1 region of A(H1N1)pdm09 strains. The vaccine strain is indicated in red. The South African 2011 strains are indicated in green and purple (nosocomial outbreak).
Introduction

Sindbis virus (SINV) is a mosquito-borne virus which mainly occurs in birds and is occasionally transmitted to humans. The virus is native to Africa, Asia and Australia as well as the Scandinavian and former Soviet Union countries. Epidemics usually occur following periods of high rainfall, when mosquito vector populations are greatly increased. Despite the wide distribution of SINV and evidence of seropositivity in humans, symptomatic cases and epidemics have only been reported from restricted geographic areas in Finland, Sweden, Russia and South Africa\(^1\). This may be due in part to under-reporting and under-diagnosis of SINV and similar alphaviruses because the disease often presents in subclinical and self-limiting forms. In its clinical form, Sindbis (SIN) fever is characterised by fever, maculopapular rash, arthritis, muscle pain and fatigue. These symptoms have been shown to persist for months and even years in several SIN cases.\(^1,2\) South African SINV strains have been found to be similar to those found in Finland and Sweden, possibly due to the translocation of these strains by migratory birds.\(^3\)

Studies conducted by McIntosh et al\(^4\) in the 1970s and Jupp et al\(^5\) in the 1980s showed that SINV infections occur infrequently during the summer in South Africa, mostly in the Gauteng, Free State and Northern Cape provinces. A large epidemic involving thousands of cases in the Karoo and Northern Cape occurred in South Africa in 1974 due to unexpectedly high temperatures and rainfall.\(^4,5\) A second epidemic occurred in the Pretoria/Witwatersrand region during 1984, involving hundreds of cases.\(^5\) In 2010 an outbreak of Rift Valley fever (RVF), after a quiescence of nearly 30 years, was accompanied by co-circulation of other mosquito borne fevers, namely West Nile (WN) and SIN. In addition to these outbreaks, WN and SIN fevers are laboratory confirmed annually in patients from South Africa. Nevertheless, little is known about the epidemiology of and the risk factors for SINV infection in South Africa. Here we briefly report the epidemiological characteristics of SINV infection in humans in South Africa based on a retrospective study of suspected arbovirus cases submitted for laboratory verification.

Sindbis fever in South Africa, 2006-2010

Specimens from suspected Sindbis cases are subjected to serological screening using the haemagglutination inhibition assay after which recent infections may be indicated by specific IgM ELISA testing. Reverse transcription PCR and virus isolation may also be diagnostically helpful for acute cases. From 2006 through 2010, a total of 3 631 specimens from patients with suspected arboviral infections were submitted to the Center for Emerging and Zoonotic Diseases, NICD, for laboratory investigation. The specimens revealed the presence of SINV IgM antibodies in 21 out of 1 606 samples for the period 2006 to 2009, giving a detection ratio of 1.3%. In 2010 the specimen submission and detection ratio of 10% (208/2 025) was markedly higher ((odds ratio (OR): 8.64; confidence interval (CI) 95%: 5.39 – 13.99; P < 0.001)) compared to the preceding period (figure 1).
Of the 3,631 specimens that were submitted from 2006 through 2010, almost twice the number of specimens received were from men (64%) as compared to women (35%). The SINV IgM antibody detection rate was higher amongst men than women ((men: 167/2,334 (7%); women: 62/1,265 (5%); OR for gender: 1.49; CI 95%: 1.09 – 2.03; P=0.009)). This is possibly due to an increased frequency of mosquito bites among men as a consequence of employment in the farming sector and other outdoor-associated labour. However, it should be noted that the results obtained for gender in this study may be biased as most of the specimens tested for SINV were originally submitted from farmers and farm workers (who are at the highest risk of RVFV infection) for RVFV investigation.

The majority of specimens submitted for SINV between 2006 and 2010 were received from persons aged between 20 and 49 years (2,279/3,537 specimens for which age data were available), while the least number of specimens were received from persons younger than ten years (176/3,537) and older than 70 years (86/3,537). Only 7% (15/229) of persons infected with SINV were under the age of 18. The risk for acquiring a SINV infection increased linearly with age ((under 10 years: 3/176, 2%; 10 – 19 years: 16/291 (5%); 20 – 29 years: 32/716 (4%); 30 – 39 years: 35/845 (4%); 40 - 49 years: 64/718 (8%); 50 – 59 years: 42/497 (8%); 60 – 69 years: 16/208 (8%); over 70 years: 12/86 (14%); P < 0.001), while the average age of persons infected with SINV was 42 years (range: 7 – 85 years). This is comparable to data given by Kurkela et al, following studies conducted in Finland, which showed that the average age of persons infected with SINV was 41 years.

Figure 1: Number of samples tested for Sindbis fever, as well as the Sindbis IgM detection rate, cumulative by month for 2006 - 2009 and by month for 2010.

*Sindbis detection rate = Number of Sindbis IgM positives/Number of samples tested x 100
** Cumulative number
The period during which the majority of SINV infections were diagnosed (March and April) corresponds to the period during which *Culex* mosquitoes (important vectors for arboviral diseases) are abundant in South Africa.\(^7\) The majority of specimens submitted to the diagnostic arbovirus laboratory and for which the geographic data was available (2197/3631), originated from the Gauteng Province (709/2 197; 32%), followed by specimens sent from the Free State and the Northern Cape provinces (572/2 197; 26% and 251/2197; 11%, respectively). The remaining 31% of specimens were sent from the other six provinces of South Africa, namely the Eastern Cape (153/2 197; 7%), North West (153/2 197; 7%), Western Cape (208/2 197; 9%), KwaZulu Natal (75/2 197; 3%), Limpopo and Mpumalanga (76/2 197; 3%) provinces. During the study period (2006 to 2010), the detection rate of SINV was higher in the Free State (102/572, 18%) compared to the detection rates of the other provinces (Eastern Cape: 5/153, 3%; Gauteng: 40/709, 6%; Northern Cape: 36/251, 14%; North West: 8/153, 5%; Limpopo: 0/16, 0%; KwaZulu Natal: 2/75, 3%; Mpumalanga: 3/60, 5% and Western Cape: 11/208, 5%). The high number of cases observed in the Free State and Northern Cape is likely attributable to high rainfall and corresponding increases in mosquito populations in these provinces during this period.

Based on an analysis of questionnaires concerning SINV laboratory confirmed cases, which were sent out to clinicians retrospectively, the most frequently observed symptoms for SINV infection were fever (39/58; 67%), myalgia (45/58; 78%), arthralgia (20/58; 35%), headache (40/58; 69%) and fatigue (20/58; 35%). These findings are consistent with studies conducted in Finland on the major symptoms associated with SINV infection.\(^{1,2,8,9}\)

**Conclusion**

Limited epidemiological data exist on SINV infections in South Africa and elsewhere in the world. This study was based on a retrospective analysis of suspected arbovirus cases for which specimens were submitted for laboratory testing. More systematic epidemiological and surveillance studies should inform on the burden and morbidity attributed to Sindbis fever in South Africa. Although SINV infection is generally considered to cause mild and self-limiting disease, it is noteworthy that the patients in this study did seek medical consultation and testing. It is suggested that SINV infection be considered as a differential diagnosis for mild flu-like illness especially during periods of increased mosquito activity.

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**References**


Introduction
Malaria kills nearly three-quarters of a million people each year, most of whom are children under 5, and almost 90% of whom live in sub-Saharan Africa. Each year there are over 200 million clinical cases of malaria, five times the combined number of TB, AIDS, measles and leprosy cases. Malaria is responsible for one out of every four childhood deaths in Africa. Therefore rapid diagnosis and early treatment of clinical cases is important in terms of reducing mortality. Light microscopy is the gold standard for the laboratory diagnosis of malaria, but unfortunately requires highly-trained personnel, is labour-intensive and time-consuming. Rapid diagnostic tests for the identification of malaria are available in most laboratories in South Africa, but the sensitivities of these tests are generally lower than that of the microscopic examination of thick blood films, and their usefulness for identifying the infective Plasmodium species is limited. The purpose of this study was to validate a multiplex polymerase chain reaction (PCR) assay for malaria confirmation and Plasmodium species identification, even in cases of mixed infections. This method is considered more sensitive than light microscopy and has the capability to detect very low parasitaemias (0.02 parasites/µl). It is based on a single round of amplification and is less prone to cross-contamination than nested PCR protocols.

Methods
This procedure was based on a single-round, multiplex PCR that targets species-specific sequences in the conserved 18S small-subunit RNA gene of four of the Plasmodium species that infect humans. The validation was performed on samples available to the Parasitology Reference Laboratory (PRL), NICD, from either proficiency testing schemes (PTS) or routine samples sent to the PRL for malaria species identification. A single reverse primer, conserved in all four species, was used with four species-specific forward primers to produce species diagnostic products of different sizes. The products were visualised on a 2% agarose gel and interpreted by comparing test products to positive controls for each Plasmodium species.

Reproducibility of the assay
The aim of this experiment was to compare the results of the multiplex PCR against those of an identical and validated PCR from another laboratory that offers this test routinely for clinical samples. Dried blood spots of randomly selected samples were sent to London’s Hospital for Tropical Diseases and were tested by the Department of Clinical Parasitology for the presence of malarial parasites. This PCR was developed in the laboratory by Padley et al. In addition, four samples were sent to the Drug Resistance and Diagnostics (DRD) section, Army Malaria Institute, Australia, to test the inter-laboratory reproducibility of the multiplex PCR assay. Each sample was labeled only with a file number; no other information was given to the participating laboratories. The external test results were withheld from those personnel performing the multiplex PCR in the PRL until all samples were processed.

Sample types and extraction methods
To validate other commonly-encountered sample types for the multiplex PCR assay, various samples were randomly selected from those available for testing. DNA extraction from whole blood was compared to extraction from dried blood spots with the Qiagen DNA mini kit. In addition, extraction of whole blood with the Qiagen DNA isolation kit was compared to automated extraction of DNA with the Roche MagNA Pure Compact.

Comparison between microscopy and PCR
The purpose of this experiment was to compare malaria diagnostic microscopy results to those from the multiplex PCR. A total of 114 samples was analysed with the multiplex PCR and compared to microscopic examination of the same samples using thick and thin blood films. Species identification was performed on thin films by experienced microscopists working in the PRL.
Results
There was 100% agreement between the results reported for the reproducibility experiments from the Hospital for Tropical Diseases, London; the Army Malaria Institute, DRD section, Australia and the Parasitology Reference Laboratory. Of 23 samples tested, 17 were identified as *Plasmodium falciparum*, one as *P. ovale*, one as *P. vivax*, one as a mixed *P. falciparum/P. malariae* infection and two did not yield a PCR product. By testing the reproducibility of the assay, dried blood spots as a sample type for the multiplex PCR assay was indirectly validated.

There was also 100% agreement between the results from the dried blood spots and those from the whole blood samples following DNA extraction using the Qiagen DNA mini isolation kit. Of the 12 samples tested, 11 were identified as *P. falciparum* and one as *P. vivax*.

The comparison between DNA samples extracted using either the Qiagen DNA mini kit or the Roche MagNA Pure Compact methods showed 100% agreement between the two extraction techniques. Of the 15 samples tested, 11 were identified as *P. falciparum*, two as *P. vivax*, one as *P. ovale* and one sample did not yield a PCR product.

The results of the diagnostic comparison between light microscopy and the multiplex PCR are shown in figure 1. There were 10/114 (9%) non-concordant results. Two samples (<2%) were positive by PCR and negative by microscopy. Five samples (4%) were identified as *P. vivax* by PCR as opposed to *P. ovale* by microscopy. One sample (<1%) identified by microscopy as being a relapsing species (either *P. ovale* or *P. vivax*) was found to be *P. vivax* by PCR. Two samples identified as mixed infections by microscopy were shown to be single species infections.

![Bar chart showing the comparison between samples identified using multiplex PCR and light microscopy. NPS = no parasites seen.](chart.jpg)

Discussion
The results show that the multiplex PCR is reproducible because three laboratories were able to run the same assay and get the same results. Dried blood spots and whole blood were validated as legitimate sample types for this assay. Two different DNA extraction methods, one manual (Qiagen DNA mini kit), the other automated (Roche MagNA Pure Compact), have both been validated for use on whole blood.

There was nine percent non-concordance between *Plasmodium* identification by microscopy (the gold standard) and the results from the multiplex PCR. Of these disparate results, two
were positive by PCR and negative on microscopy. This was not unexpected as PCR is generally more sensitive than microscopy. In both cases malaria was clinically suspected by the attending physician. Most non-conformant results were seen when the two relapsing species (*P. ovale* and *P. vivax*) were identified by microscopy. It is sometimes difficult to distinguish between the two relapsing species, and 100% certainty by microscopy is not always possible. One microscopic result of ‘relapsing species’ was identified by PCR as *P. vivax*. Two samples that were identified by microscopy to be possible mixed infections were identified by PCR as single infections. Two other samples were determined by both microscopy and PCR to be indeed cases of mixed infection, which shows that the multiplex PCR can identify mixed infections.

We conclude that the malaria multiplex PCR has proved to be sensitive and specific for malaria identification. It is capable of identifying mixed malarial infections as well as very low parasitaemias. Following this validation, the multiplex PCR assay will be added to the schedule of routine tests offered by the PRL.

**Acknowledgements**

We would like to thank those laboratories that sent samples to the PRL for our proficiency testing schemes as well as the staff of the PRL.

**References**

**Rubella cases are diagnosed from specimens submitted for suspected measles cases**

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

### Viral Haemorrhagic Fever (VHF)

**Other VHF (not CCHF)**

**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**

**All cases for 2011 were confirmed as Rift Valley Fever**


0 = no cases reported

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Table 2: Provisional laboratory indicators for NHLS and NICD, South Africa, corresponding periods 1 January - 31 March 2011/2012*

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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.