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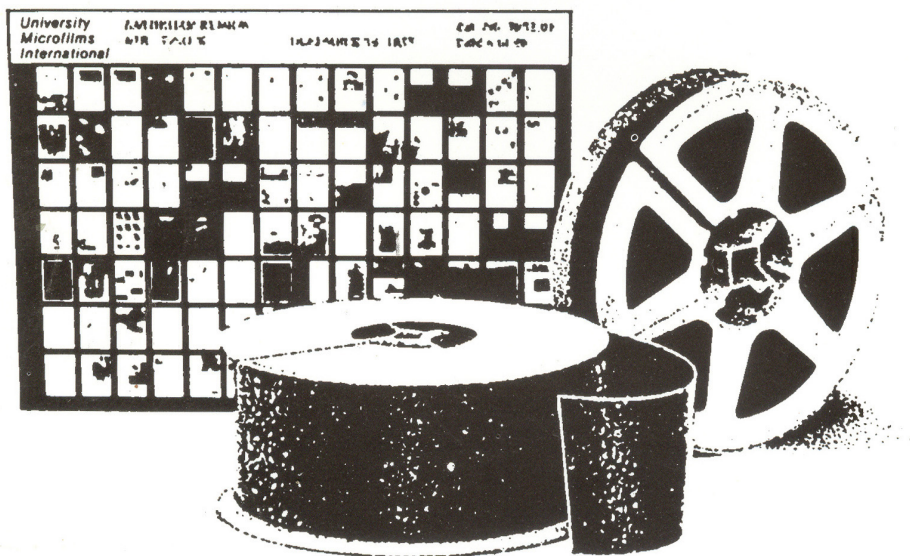
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CONTENTS   FOCUS ISSUE ON PNEUMONIA RESEARCH

EDITORIAL

Pneumonia in Papua New Guinea: lessons learnt for the way forward

In August 2010, the National Department of Health (NDoH), World Health Organization (WHO) and the Papua New Guinea Institute of Medical Research (PNGIMR) convened a colloquium entitled 'Action against pneumonia: a celebration of 40 years of pneumonia research and finding the best way forward'. The colloquium was opened by the Minister for Health and HIV/AIDS, Honourable Sasa Zibe, and closed by the Minister for Education, Honourable James Marape. More than 300 people attended the colloquium, including more than 20 overseas guests from Australia, Switzerland, England and the United States. Despite pneumonia being treatable and preventable, it continues to be the main cause of hospitalization and death in children in PNG; and indeed throughout the world. In addition, research on pneumonia, both locally and globally, is hampered by the relative difficulty in attracting funds compared to research into diseases with lower burden in children, such as human immunodeficiency virus (HIV) infection, malaria and tuberculosis.

To commemorate 40 years of pneumonia research and to advocate for the importance of pneumonia, this focus issue of the Journal comprises a series of articles on research related to respiratory infections conducted over the past 4 decades, while also highlighting future research and policy needs in PNG.

The editorial by Michael Alpers (1) provides an overview of the complexity of factors that contribute to the high burden of pneumonia in PNG and outlines the need for an integrated and holistic approach by communities and government agencies to improve health systems. In particular, communities can play a major role in improving health care delivery through a variety of mechanisms. Two issues highlighted by Alpers that contribute to the high mortality due to pneumonia are firstly that parents and carers may not recognize signs of severe disease and secondly that there is an acceptance, particularly in areas where there is poor access to health care, that people commonly die of pneumonia.

Nevertheless, people are pragmatic and will use health services if available. The government's intention to deliver life-saving treatment and immunizations as near people's homes as possible would have a significant impact on mortality due to pneumonia. According to Alpers, a change in attitudes and practices, as well as community participation in planning, operating and maintaining community health care facilities, is required to lower the burden of pneumonia in PNG.

Decreasing the burden of pneumonia requires an understanding of the aetiology of the disease, a fact that was not lost on two of the pioneers of research on acute respiratory infections (ARIs) in PNG, Bob Douglas and Ian Riley. Soon after his arrival in the then Territory of Papua and New Guinea in 1967, Douglas noted that severe pneumonia was the main cause of hospitalization in young men in Lae. In addition to describing the clinical features of pneumonia in adults, Douglas and Riley set about determining the aetiology of the disease, and found that the most commonly isolated pathogens were *Streptococcus pneumoniae* (the pneumococcus) and *Haemophilus influenzae* (2,3). In this focus issue both Douglas and Riley provide historical accounts of the early ARI research conducted in PNG (4,5).

A few years later two astute doctors working in Port Moresby, Isi Kevau and Adolf Saweri, noted that young men from Goilala in Central Province were commonly presenting with severe pneumonia. In this issue Kevau and Saweri describe their early observations and discuss possible mechanisms for the change in clinical presentation of lobar pneumonia from a severe to a more moderate presentation in the past 40 years (6). In the 1980s researchers found *S. pneumoniae* and *H. influenzae* to be the most important causes of moderate and severe pneumonia in children in the highlands of PNG (7,8), similar to the findings of studies conducted in adults some 20 years earlier in lowland PNG.

In this issue Kim Hare and colleagues review the bacteriology of acute and chronic lower respiratory infections in Indigenous Australian and Papua New Guinean children, who suffer similar high rates of respiratory infections and early onset of dense upper respiratory tract carriage (9). The authors suggest that, as in Indigenous Australians, Papua New Guinean children who suffer severe or recurrent pneumonia are likely to be at increased risk of developing chronic suppurative lung disease or bronchiectasis. Hare et al. also highlight the importance of nontypeable *H. influenzae* (NTHi) in chronic lung disease (CLD) (9). While the prevalence of CLD in Papua New Guinean children is unknown Robert Clancy points out that CLD is a major cause of death in adults aged >30 years in the highlands of PNG (10). Trials in PNG and overseas suggest that oral immunotherapy with inactivated NTHi reduces acute on chronic exacerbations of CLD in adults and the density of bacteria in sputum. Oral immunotherapy may reduce household transmission and hence disease in children. However, a formula for use in children should also be considered (10).

Upper respiratory tract colonization is a necessary precursor to pneumonia and otitis media. Bacteriological methods established in PNG have formed the basis of methods now used in carriage studies worldwide. Eileen Dunne and colleagues report in this issue the findings of a study in which investigators systematically documented colony morphology to determine whether colony morphology could be used to identify particular pneumococcal serogroups (11). Given that morphology was generally not useful in identifying particular serotypes and in view of the expense and time involved in serotyping using the Quellung reaction, it is now necessary to move to molecular serotyping methods in PNG, which have the added advantage of improving detection of multiple serotypes (12).

With the introduction of the *H. influenzae* type b (Hib) vaccine into the routine childhood immunization schedule in PNG in 2008 and the proposed introduction of a pneumococcal conjugate vaccine in 2013, surveillance needs to be conducted to determine the impact of these vaccines on morbidity, mortality and serotype distribution of disease and carriage. Multisite surveillance of meningitis is being conducted by the NDoH, but there are no data available for aetiology

of pneumonia. In this issue Lea-Ann Kirkham and colleagues (13) summarize the findings of aetiological studies conducted over the past 40 years in PNG, and discuss molecular methodology that could be adopted in PNG to supplement bacterial culture. Although blood culture remains the gold standard for diagnosis of bacterial pneumonia in young children, it lacks sensitivity (at best 30%), which is further reduced when children have already been administered antibiotics prior to presentation to hospital outpatients. Allan Cripps notes that a combination of blood culture, trans-thoracic fine-needle aspiration and screened sputum can assist in determining aetiology, though needle aspiration cannot be used routinely as it is an invasive procedure and furthermore it can only be done in the presence of consolidation (14). Molecular-based diagnostic methods are being used more and more frequently in industrialized countries and the technology is gradually becoming cheaper and more robust. As such, the application of robust molecular detection methods for bacteraemic pneumonia should be considered at sentinel sites in PNG in the future.

In addition to *H. influenzae* and the pneumococcus, other pathogens, both bacterial and viral, may play an important role in the aetiology of respiratory infections. Previous studies detected fastidious bacteria such as *Chlamydia trachomatis* (15) in young infants with respiratory illness. Studies conducted in Western Province have demonstrated a region of endemicity of *Burkholderia pseudomallei* (16), the causative agent of melioidosis, which is an important cause of community-acquired pneumonia in tropical areas. In this issue Jeff Warner and colleagues provide an overview of melioidosis in PNG, and state that there are likely to be other areas of endemicity within PNG where melioidosis remains undiagnosed (17).

The role of viruses in the aetiology of moderate and severe pneumonia has become a much debated topic in recent years. However, no detailed virological studies have been conducted in PNG since the development of improved molecular detection methods, which are generally more sensitive and cost-effective than viral culture. Respiratory viruses predispose individuals to secondary bacterial infection. However, many respiratory viruses can cause severe ARI in their own right. Perhaps the best

example is influenza virus, which has been the cause of multiple pandemics in the past century, though high mortality may be the result of secondary bacterial pneumonia (5,18). It was the high mortality during the 1969 pandemic of Hongkong influenza (H3N2) that prompted the establishment of the Pneumonia Research Unit in Tari, Southern Highlands Province (5). Seasonal and pandemic influenza is a major health concern worldwide, and surveillance is crucial for understanding the epidemiology of influenza and guiding vaccine formulation globally. In this issue Anne Kelso and Patrick Reading provide an historical account of influenza in the region and outline the importance of local laboratory-based influenza surveillance in the Pacific region, particularly in the Pacific Island Countries and Territories (19).

In addition to coinfection with respiratory viral and bacterial pathogens, other factors may predispose individuals to developing moderate or severe pneumonia, not least HIV infection. HIV has a direct pathological effect on lung cells, making HIV-positive people prone to respiratory infections. Moreover, their reduced immune function makes HIV-positive people more susceptible to infection by recognized and opportunistic pathogens. Despite HIV research being well funded in PNG relative to other diseases, little is known about the aetiology and epidemiology of respiratory infections in HIV-positive people in PNG, though a study is currently underway to address this. John McBride and Andrew Greenhill have reviewed respiratory infections in HIV-positive people in the PNG context, drawing on findings from other low-income countries until data become available locally (20).

Vaccination is an important public health strategy for prevention of pneumonia. Studies of safety and immunogenicity of Hib vaccine in PNG contributed to the inclusion of Hib vaccine into the routine immunization schedules in 2008. Pneumococcal vaccine trials have been ongoing with the seminal studies demonstrating efficacy of pneumococcal polysaccharide vaccine not only in adults but also in infants (5,21,22) and the recent pneumococcal conjugate vaccine (PCV) trial that has provided evidence of safety – including immunological safety (23) – and immunogenicity of PCV given at birth or early infancy followed by a pneumococcal polysaccharide vaccine booster at age 9 months. Suparat Phuanukoonnon and

colleagues describe in detail the design of the neonatal 7-valent PCV trial, the population characteristics and the challenges faced in conducting such a trial in a resource-poor setting (24). This was a complex trial, but was completed successfully largely as a result of strong community engagement and mutual good will. The completion of the neonatal PCV study is indicative of the capacity to conduct complex vaccine trials in PNG. With assistance from the Global Alliance for Vaccines and Immunization (GAVI), pneumococcal vaccine may at last be available to children who need it most.

Vaccines offering protection against other potential respiratory pathogens should also be considered. The introduction of the Hib vaccine is likely to prevent episodes of meningitis and pneumonia, but only protects against one serotype of the pathogen (albeit the most important). While invasive disease is due primarily to capsular serotypes of *S. pneumoniae* and *H. influenzae*, the role of NTHi, commonly isolated from the upper respiratory tract and lung aspirate, has yet to be elucidated (14). This is important given that the recently licensed 10-valent pneumococcal conjugate vaccine has *H. influenzae* protein D as the protein conjugate and may therefore offer protection against lower respiratory infections (and otitis media) due to NTHi (14).

Other preventive measures are also required, as vaccines alone will not eliminate disease. Introduction of pneumococcal vaccine will no doubt decrease the burden of pneumococcal pneumonia and meningitis but will not eliminate it, given the broad range of serotypes causing invasive disease in PNG, the limited coverage afforded by even the higher valency PCVs (10-valent Synflorix and 13-valent Prevenar13) and the complex epidemiology including early dense carriage of up to 50 of the >90 known serotypes (25). It is important also to address environmental factors such as indoor air pollution, personal hygiene (nose blowing and hand washing) and crowded living conditions that contribute to the high burden of pneumonia in PNG and other low-income settings.

Timely and appropriate treatment can also significantly decrease the burden of infectious diseases. Improving health-care-seeking behaviour is important (1) but then patients must be referred to higher-level health care facilities as appropriate and in a timely manner,

where adequate treatment must be available, including appropriate antibiotics. Ongoing monitoring of antibiotic resistance in major bacterial pathogens is required in PNG to provide treatment guidelines. Antibiotics alone are not enough to treat respiratory infections, particularly when the cause of infection is viral. The administration of oxygen to patients with respiratory illness saves lives; indeed death rates due to pneumonia in children can be decreased by as much as 35% (26). As such, a cost-effective and reliable supply of oxygen is imperative for all regional and district hospitals. Trevor Duke and colleagues provide a cost comparison for three possible methods of oxygen supply in hospitals in PNG, and conclude that in many cases oxygen concentrators may be a suitable method of supplying oxygen in the health care setting in PNG (26).

The outcomes of the pneumonia colloquium are reflected in this special issue of the PNG Medical Journal. In PNG we must continue to focus on pneumonia in an effort to decrease the unacceptably high childhood mortality rates. Previous studies have shown that the burden of respiratory diseases is also high in adults in PNG; however, most interventions that improve outcomes for children will also improve outcomes in adults. As pointed out by Douglas in this issue (4), globally significant ARI research has been conducted in PNG, but the population has not benefited from that research in the form of the availability of a vaccine or a significant decrease in the burden of ARI. Over 30 years ago studies conducted in PNG demonstrated that the pneumococcal polysaccharide vaccine saved children's lives, but it was never introduced because the cost was more than PNG could afford and subsidies were not available. A pneumococcal vaccine must be introduced in the near future. While GAVI support makes the conjugate vaccines viable in the short-term, their high cost and the potential for replacement disease due to non-PCV serotypes are a concern. As such, the potential use of the 23-valent pneumococcal polysaccharide vaccine should not be ignored, particularly in combination with conjugate vaccines. Environmental and social factors that contribute to the burden of disease need to be addressed: interventions need to be evaluated and effective measures need to become part of national health policy. Oxygen should be widely available and be seen as a cost-effective life-saving intervention. Researchers need to work closely with policy makers to ensure that the outcomes of studies do influence policy. Finally, communities need to be proactive in addressing and overcoming health issues. The fight against pneumonia is far from over, but past studies conducted in PNG and elsewhere have taught us much. We need to build on these foundations to improve health outcomes in the future.

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EDITORIAL

Some general factors to be considered when implementing a program to control pneumonia

In implementing any contemporary health program it is essential to look beyond health and address issues such as community engagement and intersectoral collaboration and take into account wide-ranging social, political and ecological considerations. In research there has to be a defined and solvable problem, which necessarily determines a narrow focus and a relatively short time frame, even if an understanding of the broader picture helps to identify the most burning research questions. When it comes to implementation, however, a broad approach and a set of goals extending over a long time frame are required. Implementation takes place within a theatre of operations – and this is a military not a surgical analogy. Operations require goals, objectives and targets and take place within systems – a health system, a political system, a socioeconomic system and an ecosystem. Operational research or health systems research can help evaluate systematic ways, entrenched or innovative, of improving people's health, but most operations will be conducted using conventional means and first principles. Strong and stable leadership makes a crucial difference – good generals, in our military analogy, win campaigns. However, often the outcome of success or failure is determined by the system. A systems failure or a bottleneck can wreck the whole operation. All this is well known – indeed, so well known that it can easily be taken for granted. The principal purpose of this editorial is to jog our memories of these essential operational truths in the context of pneumonia.

To counter failures and bottlenecks in the system, we need the combination of creative and adaptable leadership and a broad understanding of all the relevant factors involved. Bottlenecks can be released but not if their location is unknown, and a hidden systems failure will suggest no remedy. However, if the leaders' vision is broad enough nothing will escape their attention. Even if the critical problem lies outside their control, to identify the problem and draw attention to it is the beginning of its solution.

If the health centre has drugs to treat pneumonia but mothers are not bringing their sick children in for treatment in time this is a problem that first must be addressed in the community. If a new vaccine has been tried and tested and is in good supply but vaccination coverage is low this may be a question of a shortage of trained staff, poor staff morale, poor understanding in the community, the appalling state of the roads, lack of transport – or all of these. To address – and solve – this problem has higher immediate priority than evaluating a better vaccine or a potentially better immunization schedule. The difficulty here is that the research and operational components of the system have their own leaders, sources of funding and constraints, and cross-linking their activities is not easy. For this reason a high-level think-tank is needed that will incorporate the best minds in the country within the overall health system and charge them to discuss, criticize, advise, innovate, plan, identify gaps, formulate key research questions and promote action to improve the health of the nation.

In August 2010 the Colloquium which is the inspiration for this focus issue of the Journal was held in Goroka to celebrate 40 years of research on pneumonia in Papua New Guinea (PNG). The final session was devoted to the task of finding the best way forward. The discussion was led by William Pomat, who provided a domain of discourse to encourage participants to think broadly as they considered the question and contributed to the discussion. The philosophy and purpose behind this list of factors to enable a meaningful discussion about the way forward are the same as those that underpin this editorial. It therefore makes sense to include this domain of discourse here: with his permission, it is provided in Table 1. Furthermore, I want to pick up and expand a little on some remarks made in the Magazine of the Colloquium (1). I suggested that the contemporary context demands innovative approaches to pneumonia control, and the research required to validate them, but also raised the possibility that "the wealth from the

TABLE 1

A DOMAIN OF DISCOURSE FOR A PANEL DISCUSSION: FACTORS TO BE CONSIDERED IN FINDING THE WAY FORWARD FOR PNEUMONIA IN PAPUA NEW GUINEA*

Community demand, attitudes, consultation, education, participation

District health services and access to treatment

Provincial hospitals

Communication – roads, transport, mobile phones, internet

Clinical training and supervision, at all levels

Laboratory training, capability and supervision

Management training and problem-solving

Selection and supply of drugs for standard treatment and of laboratory reagents

Oxygen – demand and supply

Vaccines – selection and delivery

Good nutrition

Environmental health – housing, air quality, hygiene, water supply, hand washing: Healthy Islands Concept

Special needs of women, the fetus and newborn, children, men, young adults, the aged

Influence of HIV infection, malaria and other diseases

Integration with improvements in health care delivery for other diseases

Health information

International information networks

Research, surveillance and monitoring – clinical, epidemiological, microbiological, immunological, social and behavioural, health services/health care delivery

Outbreak response capacity

Policy and politics

Finance

Commitment at all levels of society

Creative use of the media

Drive and leadership

*Drafted by MPA (later slightly modified) and used by William Pomat at the 40th Anniversary Pneumonia Research Colloquium in Goroka to lead the panel discussion of the best way forward to reduce the burden of pneumonia in Papua New Guinea
HIV = human immunodeficiency virus

minerals boom will pour into district services to the benefit of every rural and squatter community in PNG" and, on a different issue, emphasized the need to take into account the impending global crisis due to climate change. These ideas were not developed in the space of the magazine article but they were not merely throwaway remarks.

The wealth generated by the minerals boom, and in particular the liquid natural gas (LNG) project, will be enormous. It could benefit everybody – or it could destroy the social fabric of the nation, as has happened in Nigeria. It is essential that all the people and communities of PNG receive their rightful benefit from the exploitation of their national natural resources, and not let the mining/ petroleum companies, local landowners and self-promoting politicians of every stamp and hue take it all. To achieve the right outcome requires coordinated sector-wide and community action now. The health sector needs to develop a specific action program, in collaboration with members of the community and other sectors such as education, to ensure that the nation's health will benefit from this influx of wealth. An action committee of determined people needs to monitor every move, make personal contact with all stakeholders, lobby, protest as necessary, make skilful use of the media and ensure that health is never forgotten and always remains high on the priority list as the mining wealth is distributed.

Climate change, encompassing the often unpredictable outcomes of global warming, is a fact of contemporary life and no planning agenda can ignore it. Though specific outcomes may be uncertain, some long-term trends have already become clear and must be monitored and taken into account in planning. For example, mountains are becoming warmer, permanent snows and glaciers are disappearing and ecological zones are changing, with lowland plants, birds, animals and insects slowly extending their distribution upwards, often displacing other species. We all have a duty, not yet acknowledged by governments, to take local action for the global good. Further, though no local action will by itself turn back global trends, adaptive action is required to mitigate any local deleterious effects and exploit new opportunities created by climate change – for example, to prevent vector-borne diseases as their range expands or to plant nutritious crops in areas where they did not flourish

before. There is a lot being published on climate change, including its effect on health (2,3), and the health sector needs to keep abreast of all this new information, relate it to the changing environmental characteristics of different parts of PNG and seize on all the possible connections to health, positive and negative. Moreover, measures to mitigate climate change itself can have health co-benefits (4), including the reduction of respiratory disease, and health policy-makers should be active participants and drivers of national climate change mitigation programs. In the tropics the prevalence of many diseases is rising because of climate change, in particular through global warming, changing rainfall patterns and threats to the food supply, and pneumonia, though often forgotten about (as usual), should be included in this list (5).

Improvements in health care delivery apply to health generally, not specifically to pneumonia, and health operations must adopt sector-wide not disease-specific strategies. However, pneumonia is a major disease problem and therefore any operational improvements in community awareness, the structure and function of health facilities or outreach services will necessarily have a major impact on pneumonia. Specific problems that relate to pneumonia include the widespread acceptance of death from pneumonia, in the young infant and the elderly, as a fact of life. This attitude is common in rural communities and precludes action being taken when an acute respiratory tract infection is recognized. Indeed this attitude is global and pervades health professions, health policy-makers and funding agencies, to the detriment of pneumonia control programs world-wide. This glaring deficiency has been addressed by a Global Action Plan against Pneumonia (GAPP), which was initiated at the Fifth International Symposium on Pneumococci and Pneumococcal Diseases held in Alice Springs (organized by Allan Cripps, Amanda Leach and Deborah Lehmann) in 2006 and has since been taken up by other agencies (6,7). Further to pneumonia in the local community, it is also worth noting that cough is a common sign/symptom for people everywhere and all cultures have a term for it. Since it is often associated with mild or chronic disease mothers must be taught to recognize signs of severity, which indicate that their child should be taken as soon as possible to a health facility: whether you call

it 'strong cough' or 'pneumonia' the main thing is for the signs to be linked to immediate action by the child's carers.

Cultural differences are important and we all know the extraordinary cultural diversity found in PNG. However, there are also striking commonalities (8) and these should be exploited in developing health programs. In some places it may be possible to pursue focused ethnographic enquiry or use other rapid cultural assessment procedures (9), which can be designed or adapted explicitly for respiratory illness. Such studies will not always be feasible or rewarding, but since the success of primary health care is dependent on how carefully cultural factors are taken into account, in most cases this will have to be achieved through strong community participation in the locally implemented health plan. The local use of traditional medicine and patterns of treatment-seeking behaviour should also be understood and may merit investigation by educated members of the community using well-tested techniques (10).

Changing people's attitudes and increasing local knowledge and awareness are hard tasks and usually require cultural adjustments over a long time. In this context one thing is essential: that change is a collaborative process based on dialogue and not conceived of as the imposition of superior health knowledge on an ignorant population. Getting behavioural change may be even more difficult; however, since behaviour is changed on the basis of a different (if overlapping) set of factors from those influencing attitude, awareness and knowledge, health-related behaviour may be induced to change independently of the others. For example, when people in a village community recognize that effective treatment is conveniently available at a local health facility they make use of it on pragmatic grounds without changing their belief system. In the past a network of widely distributed aid posts well supplied with injectable procaine penicillin had a profound effect on mortality, leading to a rapid reduction in infant mortality rates (11). Similar effects have been seen at the other end of the human lifespan. When in 1962 I first came to live in the village of Waisa in the Okapa District of the Eastern Highlands the oldest members of the community were aged in their mid-40s (with the exception of 2 much older women who had been old for as long as anyone else in the community could remember). In the past the major cause of death was pneumonia (often called by the name of a powerful sorcery) and this was now being treated at the local clinic or aid post. Over the next 30 years most of this elderly cohort stayed alive, and the demography of the village changed dramatically. Eventually the members of this cohort died, but only aged in their late 60s and 70s. These examples illustrate the powerful social and demographic changes that can occur when pneumonia is controlled. In these examples pneumonia was not prevented but the use of locally available antibiotic treatment stopped people dying of it; with the advent of appropriate vaccines the effects of controlling pneumonia through prevention can be expected to be even more profound.

The current National Health Plan gives high priority to the upgrading of district health services and making life-saving treatment available as close as possible to where people live (12). Community health posts will replace the aid posts of the old system; the emphasis on 'community' indicates the determination of PNG's health leaders and policy-makers to bring the community into the health system. This, we all hope, will soon become reality and from this priority establishment many systematic health benefits will flow. For no disease is this system change more relevant than for pneumonia. Moreover, pneumonia is not only life-threatening but also universally prevalent. Therefore we cannot discuss pneumonia without giving fervent support to the new policy to improve health services at the district and community levels throughout the nation. To reduce pneumonia mortality and morbidity, the Papua New Guinea Child Health Policy and Plan 2009-2020 (13) has as its first policy strategy "Improvements in the quality of services at community health (aid) posts, to include immunization services and IMCI [Integrated Management of Childhood Illness] case management and standard treatment". We must all contribute, in every way that we can, to ensure that this policy is fully implemented.

The infrastructure of district health services in many districts has disintegrated. Many previously well-functioning aid posts are closed and health centres struggle with poorly maintained structures and equipment, low numbers of appropriately trained staff and drugs in short supply. It is no wonder that

staff morale is low – though it must be acknowledged that the resilience of some health workers is amazing. Firstly these structural and staffing problems must be addressed, but there should be no delay in involving the community in this process of rehabilitation. The sooner a working collaboration between the health services and the community is established the better. This is a two-way process and will not happen overnight. Often there are barriers of ignorance and prejudice to be broken down. There is, in general, little community demand for better health, and this will have to be slowly created through patient and sympathetic community liaison work. Even those members of the community keen to make use of health services such as antenatal clinics have often been discouraged by the attitude of health workers (14), and this is a widespread problem that needs to be sympathetically but firmly addressed.

A District Health Committee and health liaison officers in each community are needed. The District Health Committee must go beyond 'management': it should create an interface between the health services and the community and foster cooperation and collaboration between the two. This should be primarily a proactive, creative collaboration, with engagement and goodwill on both sides, and the Committee will be able to deal with problems and complaints – from either party – if they arise. A good Committee will see health in the broader context of an enhanced quality of life, and will work closely with the education sector to increase the education of women, for example, and with other sectors to improve law and order, upgrade and maintain roads and other forms of communication, and encourage economic advancement through village-based cash crops and local small businesses. The Committee also has an advocacy role and should raise high the community voice for health: better services, better access, greater utilization, higher vaccination coverage, better nutrition. Only through this process can we expect to achieve a sustained improvement in health generally and, in particular, a steep and permanent decline in severe pneumonia and in death from pneumonia, at all ages. The medical and technical means of achieving this decline are discussed in detail in the excellent set of papers that make up this focus issue of the Journal. However, these powerful means will on their own have limited effect without due attention being paid to the general factors considered and briefly discussed in this editorial.

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Pneumonia in Papua New Guinea, from the past to the future

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SUMMARY

This paper briefly describes a journey with pneumonia and the pneumococcus that began in partnership with Ian Riley at the Lae Hospital in 1967 and continues 43 years later. It is a journey that signalled the global emergence of penicillin-resistant pneumococci and played an important role in the licensure of pneumococcal polysaccharide vaccine for use in adults around the world. The journey involved many other people whose experience began in Papua New Guinea (PNG), playing lead roles in the global program to reduce pneumonia deaths in developing countries. But none of this has benefitted Papua New Guineans as it could and should have done. In this paper I assert that substantial benefits could now follow from widespread use of the 23-valent polysaccharide vaccine in PNG adults not suffering from HIV and that there is also good scientific reason why children over the age of 9 months should be offered the potential benefits from use of this vaccine that were demonstrated in PNG in the 1980s. Indeed there are very good medical and economic reasons why it should happen.

The Lae and Moresby adult pneumonia wards, 1967-1970

To my surprise, when I undertook my first ward round at the Lae Hospital in Papua New Guinea (PNG, then called the Territory of Papua and New Guinea) in May 1967, I discovered that the dominant cause of hospitalization was severe pneumonia in young men. In six years of hospital training in Australia I had never seen anything like the severity of this disease, which in Western adult hospital wards was largely a disease of the elderly. Within weeks of arriving in Lae, I made a visit to Port Moresby to discuss with the Director of Public Health, Dr Roy Scragg, my firm interest in initiating a research program into adult pneumonia. Scragg gave me unconditional support to initiate research in this field, pointing out that pneumonia was the leading cause of both hospitalization and death in both adults and children and that there was no work going on to elucidate its control in PNG at that time.

Returning to Lae, I consulted with Dr Ian Riley, who was working with me as a medical registrar, and we agreed together to establish a separate ward in the hospital which would become the pneumonia research ward. We began a systematic study of the microbiology of pneumonia cases that were being admitted to our ward. We also undertook a trial comparing crystalline and procaine penicillin in treating the condition (1). Both of us began reading avidly about pneumococcal pneumonia.

One day Ian discovered that there was a move in the United States to license pneumococcal vaccine, a process that had already been well advanced at the time of the discovery of penicillin. The preliminary work that Ian and I carried out in Lae provided a strong starting point for me to embark on a major study of the microbiology of pneumonia in the adult medical wards in Port Moresby, where I moved in 1968. A microbiologist, Lorraine Devitt, joined me in an investigation of the microbiology of 632 of my patients and in serotyping all bacterial isolates from sputa, lung aspirates and blood cultures. These were shown to be predominantly pneumococci and non-encapsulated *Haemophilus influenzae*. A limited number of pneumococcal serotypes were involved in serious disease (2-4).

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Angugunak, 1969

At the annual medical symposium in Papua New Guinea in 1968, I met up with John Sturt from Angugunak in the West Sepik District, who had established a small hospital in the jungle which he had been running for about ten years and kept very careful medical records of all his work. We agreed that the pneumonia problem in his area was so serious that we should consider testing an approach that was then in vogue for preventing recurrences of rheumatic fever: the use of regular doses of prophylactic penicillin to inhibit acquisition and carriage of bacteria in the upper respiratory tract.

We planned a study in which we offered all the residents of two villages close to John's hospital one of two different preventive treatments, with the object of comparing the incidence of pneumonia in the two villages. All the residents of one village would receive a monthly injection of iron to benefit their chronic anaemic status as a result of high levels of hookworm disease and all the residents of the other village would receive a monthly injection of long-acting penicillin. John explained our idea to the leaders of the villages of Brugap and Angugunak, each of about 250 people, and they agreed to our plan. For four months, most of the villagers from each village attended John's clinic for their monthly injection, when Heatherbell Glasgow, a laboratory assistant employed on some funds I had received from the Wellcome Trust, also swabbed and cultured the organisms that were being carried in their throats. As well as monitoring the impact of these treatments on the incidence of admissions to hospital with pneumonia in the two villages, we hoped that we would also reduce the colonization by bacteria of the throats of healthy people in the penicillin-treated village.

This was a bold but rather poorly conceived experiment and it had to be aborted four months after we began it, mainly because it became an impossible drain on John Sturt's time. But in the four months of the study we discovered something that contributed to international understanding and clinical practice. We were sending the bacteria that were cultured from the throat swabs of the healthy villagers to David Hansman, a microbiologist in Sydney, for him to check on the accuracy of our laboratory methods. David isolated a number of strains from the throats of healthy Angugunak people that were quite resistant to penicillin. We were able to demonstrate from Sturt's records that both populations had been heavily exposed to penicillin in the previous 10 years, but that the Angugunak population, to whom we were giving monthly additional doses of penicillin, were carrying more penicillin-resistant pneumococci than their counterparts in the iron-treated village. In fact, resistant pneumococci had also been grown from a child in the iron-treated village on the first visit before the trial began, but the administration of regular doses of penicillin in Angugunak had apparently preferentially promoted the transmission of resistant organisms in that village (5).

Our reports in *Nature* (6) and the *New England Journal of Medicine* (7) were the earliest reports of significant numbers of penicillin-resistant pneumococci in the world. Many had not believed that this would occur. When it was looked for systematically, there was found to be a significant incidence of penicillin-resistant pneumococci in populations right across PNG. Penicillin had been in widespread use for treatment of many ailments for about twenty years (8,9).

United States licensure of pneumococcal vaccine

Late in 1969 I contacted Dr Robert Austrian in Philadelphia to enquire how his pneumococcal vaccine program was developing and to tell him of our research and our belief, on the basis of our Moresby findings, that the vaccine he was developing for use in elderly patients in the United States (US) could play a vital role in developing countries like PNG. This led Austrian to invite me to join the team in Philadelphia.

Ian Riley had recently returned from his studies in the United Kingdom and it was agreed with Roy Scragg that Ian would establish a new pneumonia research unit in Madang, as well as a field research unit in the Tari Basin in the Southern Highlands Province where death rates in the 1969 flu epidemic had been massive. We considered that Tari would be an excellent site to evaluate the new vaccine when it became available and Ian instituted systematic demographic and health surveillance in the basin. He also recruited a microbiologist, Helen Miles, to assist him in establishing these two units, while I went to Philadelphia

for what I thought would be a year (and became three) to work with Austrian.

In Philadelphia I was put in charge of the volunteer studies and the development of field trials to assess the impact of the vaccine on disease. But I also learned from Jerry Schiffmann, the immunologist on the team, the rudiments of pneumococcal immunology and the application of the radioimmunoassay to measure antibodies to the vaccine (10). I travelled to many parts of the US, establishing field study sites in Chicago, San Francisco, North Carolina, Maryland and Boston.

It rapidly became clear that it would be difficult to demonstrate unequivocal efficacy of the vaccine in United States populations where the overall incidence of invasive disease by the serotypes against which the vaccine was being developed was relatively low, even though pneumonia mortality was high in some restricted populations. We also knew that mortality from pneumococcal disease was very high in PNG and also in the South African gold mines. I was particularly keen to develop an efficacy study in PNG and an arrangement was made between the National Institutes of Health and the South African authorities for exploratory work to begin among South African gold miners.

I helped to establish baseline information for two American studies before leaving the US to return to Australia. Both studies were subsequently to provide evidence that was supportive of the licensure of the vaccine. But without the strong evidence of efficacy provided by the South African and Papua New Guinean studies in adults, the case for licensure would have been relatively unconvincing (11).

The Papua New Guinea pneumococcal vaccine studies

Back in Papua New Guinea, Ian Riley was gearing up for a trial of the vaccine in adults. A new vaccine manufacturer, Merck, entered the field and their vice-president, Maurice Hillemann, helped us establish a trial amongst 10,000 adults in the Tari Basin.

In the event, the PNG trial proved decisively the efficacy of pneumococcal polysaccharide vaccine in reducing disease and death rates in adults (12).

At the same time as he was managing the trial in adults, Ian was assembling evidence that pneumococci were responsible in PNG for even higher illness and death rates in children under two years than adults. Working under great logistic difficulty, he and Helen Miles undertook careful bacteriological studies which established the importance of a restricted number of pneumococcal serotypes in causing severe pneumonia in children in the Tari Basin.

But in the US the evidence was accumulating that pneumococcal vaccine produced relatively poor antibody responses to some serotypes in children under the age of two years and there was uncertainty whether the Food and Drug Administration would sanction their use in trials in children.

We had several hundred doses of vaccine left over from the adult study. Ian successfully put the case to the national Medical Research Advisory Committee in PNG that we should use the available vaccine to test its effect on prevention of morbidity and mortality in children as well in the Tari Basin. For the children, we halved the dose that was being used in adults. With only 840 children randomly assigned to the vaccine or placebo arms of the trial, we obtained quite stunning results. There were eight deaths in the group of children who had received the placebo and only one in the group of children who had received the vaccine (13).

When we reported these findings to the authorities in the US they were received with some consternation. We had not followed the usual protocol and had behaved irregularly from the US regulatory perspective. Of course we had done so because we knew that if we had asked for permission to proceed, it would have been denied. We reasoned that the imperatives in PNG, where death was so common, were different from those in the US and that the data that Ian had collected prior to the trial had fully justified the attempt.

World Health Organization and the Program for Control of Acute Respiratory Infections, 1977-1989

In 1977 I received an invitation from the World Health Organization (WHO) in Manila to undertake a consultancy on the development of a program to control respiratory infections in young children in the developing world. It transpired that I had been identified to undertake this task because of my

experience in PNG and involvement in the US pneumococcal vaccine program. The upshot of this was that for the next ten years I was frequently on a plane to Manila or Geneva, as first the Western Pacific Region and then the global body began to act on my recommendation for development of a global program on control of acute respiratory infections (ARIs), which was built on principles which emanated primarily from PNG (14-17). In my advice to WHO I was drawing extensively on the work carried out by my colleagues in PNG, Ian Riley and Frank Shann. Frank had developed simple algorithms for the management of pneumonia in early childhood that could be easily taught to mothers and primary health care workers.

International Meeting on Acute Respiratory Infections in Childhood, Sydney, 1984

In August 1984 it was estimated that at least 6 million children died each year from ARIs, especially in developing countries around the world. We held in Sydney the first international conference on acute respiratory infections in childhood, which was attended by 120 delegates from 18 nations. The meeting was supported by WHO, United Nations Children's Fund (UNICEF), the Australian Development Assistance Bureau and the Postgraduate Committee of Medicine at the University of Sydney. The communiqué of that meeting concluded: "Enough is already known to systematically introduce control measures in a phased manner, evaluating their effects in changing morbidity and mortality as the program proceeds." (18).

Further PNG report of vaccine efficacy in childhood, 1986

In 1986 Ian Riley, Michael Alpers and Deborah Lehmann in PNG, with support from WHO, reported the results of the further larger studies of pneumococcal polysaccharide vaccine in early childhood which I had recommended during my 1979 consultancy. These new studies in Tari and Goroka confirmed the convincing protective effect on mortality in children when given over the age of 6 months (19,20). Furthermore, in Tari there was a protective effect against severe ARI morbidity (21).

In 1988 I was invited to Washington to discuss the feasibility of the US Agency for International Development (USAID) introducing pneumococcal polysaccharide vaccine into a trial in early childhood in The Gambia. The view in PNG and Australia was quite clear. This was now proven to be a highly efficacious intervention in PNG and should be replicated in other parts of the developing world. That was what the meeting in Washington concluded and I left Washington believing that it would happen.

But the forces favouring a new and different approach to pneumococcal immunization in early childhood prevailed – mainly, I suspect, because a different approach was clearly needed to deal with middle ear infections in affluent countries like Australia (22-26) and the US. The Gambia was reserved for a later trial of the new and much more expensive conjugate vaccine. The proposed Gambian trial, using the original, and potentially relatively cheap, polysaccharide vaccine, never eventuated and so the PNG findings have never been replicated; this has meant that, because of cost, they have not even been applied in PNG. The only time there was a program of universal pneumococcal polysaccharide vaccination in PNG was during an effectiveness study of the vaccine conducted between 1989 and 1995 in the Tari Basin. In that study protection against mortality was consistent with the earlier efficacy trial (27).

The new approach to childhood immunization using conjugate vaccines in children has now been adopted for use in developed countries, where it has been shown to be highly efficacious in reducing serious disease in childhood and providing herd immunity (28-31). The new vaccine when used over a decade later in The Gambia produced about the same impact on child mortality as the original vaccine had done in PNG, with additional benefit in children aged less than 6 months (32).

International Meeting on Respiratory Infections, Canberra, 1997 and Fifth International Symposium on Pneumococci and Pneumococcal Diseases, Alice Springs, 2006

In 1997 we hosted an international conference on acute respiratory infections in Canberra that was attended by 300 of the world's experts in this area (33). A highlight of the meeting was the production of a communiqué and a set of workshop reports which identified the international public health challenges in this field. A key focus of the

meeting was on the use of evidence and the development of a Cochrane Acute Respiratory Infections Group to review treatment and prevention trials of respiratory infections. We held 9 plenary sessions that were addressed by international experts and 33 workshops which reported their consensus on what we know and what we need to know and do.

At the Fifth International Symposium on Pneumococci and Pneumococcal Diseases held in Alice Springs in 2006 a number of Australians proposed a new global initiative to tackle childhood pneumonia. Ian Riley and I have been working with Allan Cripps, Kim Mullholland, Michael Alpers, Deborah Lehmann and many other colleagues in recent years to activate this program. In September 2008 Australia 21 undertook a review of the role of pneumococcal vaccines in the reduction of pneumonia in developing countries (34) and in July 2009 we hosted a meeting of representatives from Australia, PNG and Indonesia to attempt to regain some of the international momentum on pneumonia that was lost in the early 1990s (35).

Where next?

So where is my story leading? I have argued here that what began 43 years ago in PNG has been quite influential in what has happened around the world on the management of pneumonia. Penicillin resistance became known around the world because of the findings of the Angugunak study. Frank Shann's work on diagnosis of pneumonia in early childhood profoundly influenced international thinking on this issue. The first two international meetings on pneumonia control and the Cochrane Acute Respiratory Infections Group grew from the PNG research. A series of trials carried out in Tari and in Goroka demonstrated that the pneumococcal polysaccharide vaccine was highly efficacious in preventing both disease and death in both adults and children. The adult work played a key role in the licensure of the pneumococcal polysaccharide vaccine. A great deal of work has been done on the issue of maternal immunization with the vaccine both in the seventies and more recently (36).

And yet, Papua New Guineans have not reaped the benefits of much of this research.

The agenda has been set elsewhere and the benefits have flowed especially to developed countries, much less to developing countries and very little to the people of Papua New Guinea.

Largely in response to the needs of developed countries, the science of pneumococcal immunization has moved on and a vaccine that is more effective in inducing antibodies in young infants has been developed. It is still not as polyvalent as the polysaccharide vaccine and it is certainly a great deal more expensive. Furthermore, there have been reports of increased incidence of invasive disease due to serotypes not included in the pneumococcal conjugate vaccine (37-39).

We held an Australia 21 Roundtable in 2008 to discuss the current status of the polysaccharide vaccine. We agreed in that discussion that it would be highly desirable for Papua New Guineans to be able to reap some of the benefits from the work that has been done in PNG in the last 40 years (34). It is my assertion that Papua New Guinea could benefit enormously from mass immunization of adults and especially prospective mothers who do not have HIV infection – it has been reported that there is a risk in giving pneumococcal vaccine to people with HIV infection although the evidence for this is still somewhat equivocal.

The evidence obtained from the PNG studies in the 1980s suggested that the polysaccharide vaccine could be profoundly effective in reducing mortality in children over the age of nine months. The work that emerged from the early studies in Tari and elsewhere since that time also suggests that immunization of young mothers with pneumococcal polysaccharide vaccine either before or just after the birth of their child can potentially offer significant protection against disease caused by many of the serotypes in the vaccine in the early months after birth (36).

There are major logistic difficulties in distributing vaccine in Papua New Guinea. Nevertheless, I hope that one of the issues that still is worthy of discussion is the possibility of making broad use of the polysaccharide vaccine pending clearer specification of the benefits of the new, more expensive and less polyvalent conjugate vaccines.

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Pneumonia research in Papua New Guinea: 1967-1986

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SUMMARY

Between 1967 and 1985 research on pneumonia in Papua New Guinea (PNG) was fundamental not only to standard treatments of disease in PNG, but also to the establishment of the World Health Organization's global Program for Control of Acute Respiratory Infections. Pneumonia was the leading cause of death in both population-based and hospital studies. Research that began in 1967 revealed a pattern of disease in adults reminiscent of that seen in industrialized countries in the early 20th century. *Streptococcus pneumoniae* (pneumococcus) was the predominant causative organism. Pneumococci were commensals of the upper respiratory tract that invaded first the lungs and then the blood stream. Some serotypes were more invasive than others and case fatality increased with deeper levels of invasion. The pandemic of Hong Kong (H3N2) influenza spread to the Southern Highlands in 1969 resulting in 2000 deaths. The conclusion that pneumococcal pneumonia had been the principal cause of death led to the establishment of a pneumonia research unit in Tari. A field trial of pneumococcal polysaccharide vaccine showed the vaccine to be most effective in preventing invasive disease. Vaccination reduced pneumonia mortality by 44% in previously healthy adults. The epidemiological situation was more complex in children than in adults because many different species and serotypes of bacteria could be isolated from lung aspirate. Although many of these organisms would normally have been regarded as non-pathogenic, *S. pneumoniae* and *Haemophilus influenzae*, recognized pathogens, were the principal causes of severe morbidity and mortality. The same principles of carriage of and invasion by upper respiratory commensals applied as much to children as they did to adults, and the rank order of invasive serotypes of *S. pneumoniae* and *H. influenzae* was the same in different age groups. Slow maturation of a child's immune system meant, however, that children could be susceptible to invasion by particular serotypes. Infants were frequently colonized by pathogenic bacteria within days of birth. Nasal discharge, which was extremely common, was most probably a result of domestic smoke pollution and low standards of hygiene. Aspiration of infected secretions was a likely explanation for the variety of organisms isolated from lung aspirate. A trial of pneumococcal polysaccharide vaccine showed the vaccine to be effective in preventing death from pneumonia in children 6-9 months of age provided pneumonia was not associated with other causes of death; this result was shown to be consistent with the principles of infection and invasion described above. Principles of antibiotic therapy for child pneumonia were also established at this time.

Introduction

In 1967 Bob Douglas and I commenced research on the adult pneumonias in Lae. We were aware of high admission rates of young adults with lobar pneumonia to the medical wards and a pattern of disease quite different from what we had experienced in Australia. We had no real idea of why pneumonia was the leading cause of admission to and death in hospital.

By 1986 we had a differentiated understanding of the adult and child

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pneumonias and a model which gave basic explanation for the high incidence and mortality in Papua New Guinea (PNG). By then research in PNG had made a major contribution to the early development of the global Program for Control of Acute Respiratory Infections (ARIs) of the World Health Organization (WHO). This is an account of the research over that twenty-year period and of the associated development of ideas. It is based on a presentation at the colloquium held in Goroka in 2010 to celebrate 40 years of pneumonia research and, as such, is as much a personal account of those developments as it is a comprehensive review. To those whose work I may have inadvertently omitted, I apologize.

Research on pneumonia before World War 2

Pneumonia had been a leading cause of death and a major public health problem in industrialized countries, particularly the USA, from the late nineteenth century (1). *Streptococcus pneumoniae* (pneumococcus) was recognized as the dominant pathogen, with bacteraemic cases having a case fatality rate 4-5 times higher than non-bacteraemic cases (2). In the 1920s only about 17% of adults with bacteraemic pneumococcal pneumonia survived. By the early 1930s survival had improved to 53% due to the development of treatment with type-specific antiserum (3). By that time 32 different serotypes had been identified and sophisticated bacteriological techniques had been developed for their rapid isolation in patients. Penicillin, first used to treat pneumococcal pneumonia in 1944, had dramatic impact. By the 1960s survival in bacteraemic pneumococcal pneumonia in adults had further improved to about 90% (3).

From the earliest days, health authorities in what is now Papua New Guinea had become aware of high mortality from recurrent epidemics of respiratory disease. In 1895 MacGregor noted that pneumonia had appeared in epidemic form in Papua (4,5). A report from New Guinea in 1929 (6) stated:

"Epidemics of influenza with pneumonia sweep across the country (especially the inland portion of the mainland) like a flame varying in severity in different years, and moving so fast that by the time … the epidemic is reported it is impossible to

catch up with it in rough country."

Demand for labour for the plantations meant that men were being moved from one part of the country to another thus creating a workforce that was susceptible to pneumonia and dysentery. In a 1925 report from New Guinea (7) it was stated that:

"Pneumonia is the most prolific cause of death in the Territory. Natives show very little resistance to the pneumococcus and are very prone on being moved from one locality to another, even under circumstances which produce no such disorder in those natives who are resident in the locality in question."

A bacteriology laboratory was established near Rabaul in the early 1920s. In 1940 the doctor in charge, T.C. Backhouse, was able to review an autopsy series that extended from 1922 to 1939 (8). Leading causes of death by the number of autopsies were:

Pneumococcal infections – 386 (26.9%)

Tuberculosis – 282 (19.6%)

Bacillary dysentery – 183 (12.7%)

Enteric fever – 30 (2.1%)

Other – 555 (38.6%)

Total – 1436 (100%)

In a series of 412 cases between 1925 and 1940, Backhouse showed Type 1 pneumococcus to be responsible for over 60% of pneumococcal isolates (9). By the late 1930s he was evaluating one of the new sulphonamides, M&B 693, had reported its side-effects, and had observed a reduction in case fatality rate from 20-30% to about 7% (10).

Only slowly did Bob Douglas and I become aware of this history. In 1978, I contacted Backhouse by telephone in Sydney and described the work we were doing. "Ah," he said, "I thought we had solved that problem in 1939." He was not alone in thinking like this.

There has been a strong tendency to criticize the efforts of the pre-war health services (5:237-240). The authorities were definitely aware, however, of the public health

problems posed by epidemic respiratory disease and of pneumonia in recruits to the workforce. What they lacked were effective interventions. An indication, though, of the limitations of these early health services is that they do not seem to have left a description of a third problem, that of paediatric pneumonia.

Carriage of bacteria in the upper respiratory tract

S. pneumoniae and *Haemophilus influenzae* possess polysaccharide capsules that appear to swell in the presence of specific antibody. This capsule enables resistance to phagocytosis and determines an organism's immunogenicity and invasiveness. At the present time over 90 distinct serotypes of pneumococcus and 6 serotypes of *H. influenzae* have been described. There are also non-capsulated strains of each.

The common invasive serotypes of *S. pneumoniae* differ between adults and children. The 'adult' serotypes were the first identified historically and are the most invasive; immune competence is acquired comparatively early in life. The 'paediatric' serotypes are less invasive but acquisition of immune competence is delayed (11). *H. influenzae* type b (Hib) is generally considered to be the most invasive and hence the most lethal of the *H. influenzae* serotypes; it is the target for the current conjugate vaccine. Other serotypes and all non-capsulated strains invade much less frequently and are often regarded as opportunistic pathogens.

An account of epidemic pneumococcal pneumonia in a US Army Air Force (USAF) technical school between 1942 and 1945 provides the classic description of the relationship between pneumococcal carriage in healthy persons and invasion which causes disease (12). During this period the school became a reservoir for numerous serotypes of pneumococcus, which was associated with an extremely high attack rate of pneumococcal pneumonia. The total population of the school was between 8000 and 17,000 men with an average duration of stay of 16-24 weeks. New arrivals carried relatively few invasive serotypes but the carrier rate built up rapidly during the first 4 to 6 weeks of their stay on the post to about 65%, which probably represented 'saturation'

with pneumococcus. The peak incidence of pneumonia coincided with the acquisition of new serotypes. The authors concluded that the attack rate of pneumococcal pneumonia could be "expressed with reasonable certainty by the formula: Pneumonia rate = (pneumococcus carrier rate) x (nonbacterial respiratory disease rate) x (K). Factors likely to influence the value of K were: (a) the infectivity of the types of pneumococci involved; (b) the type-specific resistance to pneumococcal infection of the individuals composing the population; and (c) possibly the nature of the nonbacterial respiratory disease involved." This equation can be applied equally to *H. influenzae* which, unlike pneumococcus, induces an effective immune response during childhood.

Studies in PNG in the early 1970s demonstrated carriage rates of pneumococcus which were unremarkable by US pre-war standards but high by post-war standards. For example, pneumococcal carriage in healthy villagers in Madang and Tari were 57% and 67% respectively in children and 32% and 23% respectively in adults. Studies in Kiriwina and Port Moresby gave similar results. The Beon Corrective Institute near Madang had a high turnover of prisoners but there was no evidence either of epidemic pneumonia or epidemic pneumococcal carriage. The mean rate of carriage was 19%, less than for healthy villagers. Although the 'paediatric' serotypes were commonly carried it was most unusual to identify an 'adult' invasive serotype in a healthy person. The circumstances that gave rise to the epidemic of pneumonia in the USAF technical school were never found in PNG.

PNG research on the adult pneumonias

Health services development after World War 2

The present structure of rural health services in PNG was established during the immediate post-war period. New and powerful chemotherapeutic agents had become available. Policy under John Gunther, the first post-war Director of Public Health, was to make treatment for the major killing diseases widely available. His aim was "to place 1000 assistants in 1000 villages" with the ability to treat pneumonia, malaria, dysentery, meningitis and tuberculosis. Control programs were established for malaria,

tuberculosis and leprosy, and maternal and child health clinics were established through the Christian missions. On the advice of Macfarlane Burnet, who visited the Territories in 1956, a Division of Medical Research was established within the Department of Health. This was actively supported by the second post-war Director, Roy Scragg. Through small population mortality surveys, a national morbidity survey, and strengthening of health information systems, he was concerned with the definition of what we would now call the burden of disease as a basis for developing cost-effective interventions. The role of the hospital specialist, he said, was to define each major disease problem so that most admissions could be handled without reference to medical practitioners (13).

Pneumonia proved to be the leading cause of death in the studies of the cause structure of mortality in small populations (14). It was the leading cause of death in hospital, being responsible in 1961 for one-third of such deaths (15): pneumonia mortality had not responded to making antibiotics widely available. This was the context for Scragg's support of the research in Lae in 1967 (16).

Clinical research, 1967-1970

About one-third of all admissions to the adult medical wards in Lae were for pneumonia. Typically, the patient was a young man with lobar pneumonia. Tenderness on percussion was a useful sign of underlying consolidation; jaundice (17) and abnormalities of renal function were common. The condition was often associated with Gram-positive bacteraemic shock which could lead to death within hours of admission (18). *S. pneumoniae* was exquisitely sensitive to penicillin. We demonstrated in a controlled trial that the response to daily injections of procaine penicillin aluminium monostearate was as good as the response to six-hourly injections of crystalline penicillin G, ie uncomplicated pneumonia could be treated as well in the village aid post as it could be in hospital (19).

This was quite different from anything we had experienced in Australia. We concluded that the pattern of disease was "reminiscent of that described by Osler and others in the pre-antibiotic era" (19). We learnt of the epidemic of pneumococcal pneumonia in the USAF technical school that is described above and of a similar South African

experience in the gold mines since the beginning of the 20th century where young male recruits had experienced, and continued to experience, an extremely high attack rate of pneumococcal pneumonia which declined rapidly after about 9 months, a phenomenon known as seasoning (20). We thought it probable that young adult migrants to urban areas in PNG were involved in multiple micro-epidemics where they encountered new serotypes of pneumococcus, although we were never able to identify common source outbreaks.

In Port Moresby Bob Douglas teamed up with Lorraine Devitt, who introduced new standards of respiratory bacteriology. She was to become the first in a series of bacteriologists who, through their careful attention to technique and their scientific interest in the microorganisms, made subsequent research possible. They were to include Heatherbell Glasgow, Helen Miles, Marion Andrew, Margaret Pfeiffer, Mike Gratten, Janet Montgomery, Tony Lupiwa and Audrey Michael, and David Hansman from the Adelaide Children's Hospital.

Douglas and Devitt went on to study over 600 cases of pneumonia in adults (21,22). They not only cultured sputum and blood but also lung aspirates. *S. pneumoniae* was cultured from 58% of sputum specimens, 62% of lung aspirates and 10% of blood cultures. *H. influenzae* was cultured from 44% of sputum specimens, 12% of lung aspirates and 0.4% of blood cultures. They serotyped both pneumococcus and *H. influenzae* – work which laid the foundation for the introduction of vaccines (21). They raised the question of the role of *H. influenzae*, and of non-capsulated strains in particular, in the pathogenesis of pneumonia and hence about the invasiveness of these pathogens vis-à-vis the susceptibility of the human host (22) – questions which were to appear more acutely in the 1980s in relationship to paediatric pneumonia.

Subsequent work in Madang from 1969 to 1972 served mainly to confirm the earlier findings. Of interest, however, was an epidemic of type 46 pneumococcus in hospital patients. This serotype had been described in South Africa. Type 46 had never been identified in the USA. It was highly invasive, was only identified in carriers on a few occasions, and was highly lethal to mice. Its presence in PNG and South Africa

suggested common epidemiological circumstances in these countries (23).

Unexpectedly in 1969 a new direction was given to the research by the pandemic of Hong Kong (H3N2) influenza which reached PNG that year, and was a reminder of the epidemics of respiratory infection that had plagued PNG in the early years of the territories. It cost 2000 deaths in the Southern Highlands and, as is common in influenza epidemics, was associated with high mortality in young adults. The initial effects were paralysis of services and communications. At the height of the epidemic the Administration called for support from the Australian Army, which provided 700 troops and 25 aircraft. Patrols provided vaccination against influenza in non-affected areas and treated cases of pneumonia with penicillin (24). Phillip Challands, then with the Papuan Infantry Regiment and later with the Tari Research Unit, told me of breaking into huts in Margarima to remove the bodies of people who had died of influenza. This was at an altitude of 9000 feet. At the end of the epidemic it was concluded that pneumococcal pneumonia was the leading cause of death although the definitive isolations of pneumococcus were never made.

Population-based research, 1970-1976

In the aftermath of the epidemic the Department of Health decided to take advantage of recent research into pneumonia. A departmental subcommittee was appointed to examine the possibility of a trial of pneumococcal polysaccharide vaccine. After intense debate Tari in the Southern Highlands was selected as the field site for a trial. Additionally, Scragg was keen to extend the small population studies of mortality to a larger highlands population. The Tari Research Unit, established at this time, was to be active from 1971 until 1995, when it was closed for security reasons. Its output included detailed studies of the epidemiology of acute respiratory infections and pneumonia, pneumococcal vaccine trials first in adults and then in children, and studies of demography. Its presence stimulated research into land use and agricultural practice, child nutrition and medical anthropology.

In 1964 in the United States, Robert Austrian had pointed out that despite the effectiveness of antimicrobial therapy, bacteraemic pneumococcal pneumonia still carried a 10% case fatality rate and that mortality in the first 6 days of the disease was unchanged since the 1930s (3). Despite a successful trial of a pneumococcal polysaccharide (Pnc PS) vaccine in the US military towards the end of World War 2, the licence for the vaccine had been revoked without prejudice. There was simply no interest given the success of antibiotics (3). In 1970, Bob Douglas linked up with the American group.

That same year we started household surveillance for respiratory infections in the population of the Tari Basin, and in 1973 commenced a randomized placebo-controlled trial of a 14-valent Pnc PS vaccine in 12,000 persons 10 years of age and over (25). The vaccine was well-matched to the distribution of invasive serotypes, covering 75% of blood culture isolates in a series of 460 confirmed invasive isolates from Port Moresby, Madang and Tari (23). It was specially formulated to include type 46.

The effects of the vaccine upon morbidity are summarized in Table 1. The more severe the pneumonia, the greater was the effectiveness of the vaccine. Thus, bacteraemia was reduced by 86%; major consolidation was reduced by 63%, but minor consolidation by only 43% (23,25). Bronchial breathing was found in 11 placebo patients but not in any patients who had received vaccine. Pneumonia, diagnosed either clinically or radiologically, was reduced by 29%. The effects of the vaccine upon mortality are summarized in Table 2 (23,25). Death from all causes was reduced by 22%. Deaths from pneumonia were reduced by 32%. The vaccine was 44% effective when pneumonia was the sole cause of death but only 20% effective when the death was associated with chronic (nontuberculous) lung disease (CLD) (25-27). The vaccine was far more effective in persons under the age of 40 than it was in persons aged 40 years or above.

We concluded that the vaccine reduced the multiplication of pneumococcus in the alveoli and its invasion of the bloodstream, but had little effect on invasion of the lower respiratory tract. This was consistent with an effect mediated through the stimulation of IgG (25,28).

Over the years there has been much debate

TABLE 1

Effects of a polyvalent pneumococcal vaccine upon pneumonia morbidity in the first 16 months after immunization in persons over the age of 10 years in Tari, 1973-1974

Diagnosis	Vaccine	Placebo	Vaccine efficacy
Proven invasive pneumococcal disease (blood culture and/or lung aspirate)	2	14	85.7%
Multisegmental X-ray	6	16	62.5%
Unisegmental X-ray	13	23	43.5%
Bronchopneumonia	8	1	Negative
Clinical and radiological	44	62	29.0%
All ALRI	114	138	17.4%

ALRI = acute lower respiratory infection
From Riley et al. (25)

TABLE 2

Vaccine efficacy (VE) of a polyvalent pneumococcal vaccine upon pneumonia mortality in the first 3 years after immunization in persons over the age of 10 years in Tari, 1973-1976

Age (years)	Pneumonia as sole cause			CLD-associated			Total		
	Vaccine	Placebo	VE	Vaccine	Placebo	VE	Vaccine	Placebo	VE
<40	0	6	100%	1	4	75%	1	10	90%
≥40	23	35	34%	31	36	14%	54	71	24%
All	23	41	44%	32	40	20%	55	81	32%

CLD = chronic lung disease
From Riley (23) and Riley et al. (25)

about the efficacy of Pnc PS in adults. This was the only trial to demonstrate the effectiveness of the vaccine in an open (ie non-institutionalized) population. The other major trials demonstrating efficacy of Pnc PS were in young men living in institutions (20,29). The first was in the US technical school that had been subject to epidemic pneumococcal disease and the second in recruits to a gold mine near Johannesburg. Both groups were subject to extremely high attack rates of pneumococcal pneumonia – as high as 150/1000 in the USA and 90/1000 in South Africa. It was concluded after the

US trial that the vaccine had reduced carriage. In PNG we could not demonstrate an effect of the vaccine on carriage but, then again, were not dealing with an epidemic of invasive serotypes. Nor were we dealing with a migrant group. The vaccine was most effective in young adults and those with uncompromised pulmonary defences.

At the end of these studies Scragg asked whether the focus should be on prevention with vaccines or on treatment with antibiotics. There was no simple answer to his question. The two interventions were complementary.

Vaccination had many advantages but was necessarily of limited spectrum. Treatment had broader spectrum but not only placed far greater demands on families to bring a sick person to health services but also placed organisms under selective pressure for antibiotic resistance. In 1976 the management of pneumonia research formally passed from the Department of Health to the PNG Institute of Medical Research.

PNG research on the paediatric pneumonias

Population-based research, 1970-1986

In Tari, both children and adults were placed under household surveillance for respiratory events. Consistent with later WHO definitions, paediatric pneumonia was defined first in terms of a rapid respiratory rate, and second in terms of intercostal indrawing. Attack rates in infancy and early childhood were many times higher than in adults. The attack rate for pneumonia in children 0-4 years was 290 cases/1000 and declined very rapidly as the child matured – from 730 cases in children under one year to 50/1000 in children aged 4 years. Many children suffered multiple episodes. In comparison, the attack rate in adults over the age of 50 was just 36/1000. It needs to be borne in mind, however, that the cumulative incidence in adults is higher than in young children (28).

It had not been possible to exclude women in the early stages of pregnancy from the adult trial. As a result Pnc PS was given to more than 400 pregnant women. There were no deleterious effects on the fetus. Through household surveillance it was possible to monitor the infants. The vaccine not only prevented pneumonia among infants who were in utero at the time of maternal immunization but also infants aged 1-17 months when their mothers were immunized (Table 3) – a finding that suggested transfer of antibodies in breast milk and protection through breastfeeding (28,30).

Pneumococcal pneumonia in adults is usually a dramatic event clearly demarcated from any associated upper respiratory tract infection. The transition from upper to lower respiratory infection is more subtle in the child. Rapid respiratory rate in the child with cough is a sign with an arbitrary threshold defining the child at risk of death requiring antimicrobial therapy. Hospital studies in Tari demonstrated radiological evidence of pneumonia in 35% of over 400 cases of pneumonia admitted to hospital with fast breathing. Bacteria were isolated from 8 of 18 lung aspirates (44%): *S. pneumoniae* was isolated from 7 cases and *H. influenzae* from 1 case (23,31).

This evidence of the protective effect of maternal immunization with Pnc PS together with the small lung aspirate series encouraged us to carry out a trial of Pnc PS in Tari in children aged between 6 months and 5 years from 1974 to 1977. There were 8 respiratory deaths in the placebo group but only 1 in the vaccinated group; this difference was considered statistically significant (p = 0.03). We argued that a larger trial in children was

TABLE 3

Vaccine efficacy (VE) in 1973 following immunization of mothers in Tari, measured in terms of the incidence of ALRI in their children

Age of child at maternal immunization	Duration of follow-up	Maternal Vaccine		Maternal Placebo		VE	p
		ALRI episodes	Number of mothers	ALRI episodes	Number of mothers		
In utero	3 years	57	84	73	93	14%	0.1
1-17 months	1-5 months	84	286	133	310	32%	0.003
1-17 months	3 years	218	286	284	310	17%	0.02

ALRI = acute lower respiratory infection
From Riley and Douglas (28), Lehmann et al. (30)

needed (32).

The protocol for this trial needed to be approved by the WHO Committee for Research and Investigation in Human Subjects in Geneva. Ironically, the Committee was so impressed with our arguments in favour of pneumococcal immunization it was reluctant to grant ethical approval. It only did so with the proviso that deaths be analysed using sequential analysis charts, a process which would stop the trial once a significance level of 0.05 was reached. With the results of the adult trial in mind we chose acute lower respiratory infections (ALRIs) as sole cause of death as the primary end point. The study was conducted from 1981 to 1985 and had arms in Tari and the Asaro Valley. Results from the 1974-1977 trial were included in the analysis as a third arm to add power to the study. All told, about 7000 children were involved. The trial commenced with cross-sectional immunization of children aged 6-59 months followed by continuous entry of children from age 4 months . The vaccine was 59% efficacious in children under 5 years (p <0.01) and 50% efficacious in children under 2 years (p <0.05) (Table 4). The vaccine was 19% effective against mortality from all causes in children under five years (p = 0.18) (33). The vaccine was 28% effective against morbidity from moderate/severe ALRI; this effect was consistent with the effect on mortality but not statistically significant (34). However, it is noteworthy that the vaccine was efficacious in children immunized at a young age just prior to an epidemic of ALRI (34). We were unsuccessful in our attempts to reintroduce respiratory bacteriology into Tari. We were

left with an effect of the vaccine upon mortality greater than we would have predicted at the outset but no internal evidence of how the vaccine might have worked.

Clinical studies in PNG

Awareness of the extent of the problem of child pneumonia in PNG can be traced back to hospital statistics published in 1961 which, for the first time, were classified by age. Two years later (1963-1964) these statistics showed that in children aged from one month to four years, pneumonia was responsible for 17% of all admissions to and 25% of all deaths in hospital.

I first worked in the Goroka Hospital in Eastern Highlands Province in 1964 and for a period was responsible for the paediatric ward. From my Australian training I had a good grasp of the principles of diagnosis of infant pneumonia and practical experience of its management but was quite unprepared for the devastatingly high case fatality rates I encountered. The experience had a lasting impact on me. I recall anxious consultations with Brenda Paine, who was a paediatrician in Mount Hagen, seeking reassurance from her and trying to work out the best treatment regimens.

The publication of the Manual for the Standard Treatment of Common Illnesses of Children in Papua New Guinea in 1974 was a major step forward in providing guidance for junior doctors and allied health workers. This first edition classified pneumonia into mild, moderate and severe forms which were to be

TABLE 4

Effects of iimmunization with polyvalent pneumococcal polysaccharide in children under the age of 5 years in Tari, 1981-1985

	ALRI sole cause		All causes	
	All ages	<2 years at vaccination	All ages	<2 years at vaccination
Vaccine efficacy	59%	50%	19%	25%
Limits of confidence	19-79%	1-75%	−10-40%	−6-47%
p value (level of association)	0.008	0.043	0.179	0.099

ALRI = acute lower respiratory infection
From Riley et al. (33)

treated with procaine penicillin, crystalline penicillin and penicillin plus chloramphenicol respectively. Although the nomenclature was to be revised the concept of these three levels of disease was to remain the basis for all subsequent classifications of ALRI. From the late 1970s research in the Goroka Hospital, led first by Frank Shann, later by Jane Barker and subsequently by Trevor Duke, addressed questions of the combination of signs and symptoms to be used in the disease classification (35), the aetiology of ALRI and the most appropriate first-line antibiotic therapy.

In 1984, Shann and others published an account of a study of 83 children with pneumonia investigated by lung aspirate and blood culture in the Goroka Hospital between 1978 and 1981 (36). Bacteria were isolated from blood culture in 35% of cases and from lung and/or blood in 61%. *H. influenzae* was recovered from 40% of cases – type b in 6%, other serotypes in 25%, and non-serotypeable strains in 56%. Pneumococcus was identified in 34% of cases. There were multiple isolates of organisms considered to be non-pathogenic (*Branhamella catarrhalis*, *Staphylococcus epidermidis*, *Streptococcus viridans*). Respiratory viruses were cultured from 29% of cases. The case fatality rate for the series was 11%.

The interpretation of these results led to much discussion at the time. The study gave unequivocal support to the concept of the overwhelming importance of bacteria in the aetiology of paediatric pneumonia. Such variety of organisms was not unheard of (37) but what was the aetiological significance of each? Clinical series in the antibiotic era had been biased against pneumococcus through prior administration of antibiotics but these patients had been very carefully selected. The frequency of bacteraemia was extremely high for a paediatric series. Was this due to the selection of cases with large areas of consolidation suitable for lung aspirate, a process that had taken well over two years? Were the non-serotypeable strains of *H. influenzae* truly pathogenic? If so, the success of the pneumococcal vaccine was difficult to explain. It could be argued on the one hand that the isolation of an organism from the lung was sufficient to incriminate an organism as a pathogen but on the other hand that a bacterium from a bolus of material aspirated from the upper respiratory tract could multiply without contributing to disease caused by other coincidentally aspirated bacteria. Certainly, the organisms considered to be least virulent were more commonly isolated in combination with known pathogens than alone.

Some of these questions were answered in two later studies: a series of 155 children with culture-positive meningitis (38) and a series of 253 cases of childhood pneumonia investigated by blood culture but not lung aspirate (39). Patients in the latter series were admitted on the basis of the WHO clinical criteria. *S. pneumoniae* or *H. influenzae* were isolated from blood culture in 25% of cases. The case fatality rate in bacteraemic cases was 17% and in non-bacteraemic cases it was 4%. If the odds of blood invasion were compared with upper respiratory carriage the 'adult' serotypes of *S. pneumoniae* were the most invasive, and type b by far the most invasive *H. influenzae* serotype. Hib constituted less than 5% of upper respiratory carriage strains of *H. influenzae*, 67% of blood culture isolates, and 82% of cerebrospinal fluid (CSF) isolates. Isolation of non-serotypeable strains of *H. influenzae* was approximately the converse of these percentages. These results confirmed the principles of invasion established by Hodges and MacLeod in the 1940s (12). High rates of invasive disease could be attributed to high levels of carriage and to immunological susceptibility in children in the PNG highlands.

Towards a model of the aetiology of the high incidence of pneumonia in the PNG highlands

A study of the acquisition of pneumonia in neonates published in 1986 shed new light on the reasons for the high incidence of severe disease in PNG infants and children (40). All infants acquired both *H. influenzae* and *S. pneumoniae* within the first 3 months of life and 60% were colonized by 25 and 15 days respectively. Carriage occurred as early as 3 days for *H. influenzae* and 1 day for *S. pneumoniae*. Colonization densities were high. These organisms were likely to have been acquired from young family members: at the time of acquisition few mothers carried the same serotype as their infants. In a number of cases infants appeared to have infected their mothers. This could be contrasted with a study in the USA where the mean age of acquisition was 6 months; spread of infection usually occurred within one month

of acquisition of a new type and was seldom associated with prolonged carriage; 15% of acquisitions of new strains resulted in disease (41).

Thus, in the PNG highlands, infants are exposed to bacterial pathogens at extremely young ages when the immune system is immature. Too young to mount an effective response, they are dependent on maternal antibody for protection but acquire organisms from other family members. Fresh recruits to the extra-uterine world, they resemble, epidemiologically, unseasoned recruits to gold mines and the military. High rates of acquisition lead to a high incidence of bacteraemia and hence mortality.

For the unseasoned foreigner, the concentration of wood smoke in highlands houses is almost unbearable. Domestic smoke pollution almost certainly plays a major aetiological role although no research was done during this period. Visible, purulent nasal discharge is so common as to be unremarkable. In one study in Lufa in the Eastern Highlands the prevalence lay between 20% and 25% although, unexpectedly, there was no association with housing type (42). Inhalation of nasal secretions would promote the transport of organisms into the lungs and may be the explanation for the range of organisms identified from lung aspirate.

In one study of family groups none of the children had respiratory complaints although the majority had a nasal discharge: 83% of children carried pneumococcus as did 33% of mothers. Among mother-infant pairs 20% carried the same serotype and among sibling-infant pairs 43% carried the same serotype; 14% of hand swabs were positive for pneumococcus, the majority being the same serotype as was obtained from the nose. This study emphasized firstly the importance of siblings in transmission of respiratory bacteria and secondly that low standards of personal hygiene also favour transmission (43).

Antibiotic resistance and the development of new treatment regimens

The identification of *S. pneumoniae* showing relative resistance to penicillin in PNG was a sharp reminder of the inexorable spread of antibiotic resistance to new species

of bacteria (44-46). Subsequent studies demonstrated the spread of relative resistance to penicillin among an increasing number of serotypes of *S. pneumoniae* (47-50). Relative resistance, which develops step by step in bacteria, was defined either as a minimum inhibitory concentration (MIC) to penicillin G in the range 0.1-2.0 µg/ml (or 0.12 -<2 µg/ml). This approximates the level of resistance in non-beta-lactamase-producing *H. influenzae*. High-level resistance was not described in *S. pneumoniae* in PNG.

As resistance was acquired relatively quickly by the 'paediatric' pneumococcal serotypes which were common commensals, but only slowly, if at all, by 'adult' serotypes, it appeared that resistance was acquired by exposure to penicillin of organisms in the upper respiratory tract and not in the lungs. It was the general, widespread use of penicillin and not simply its use in the treatment of respiratory infections that had created the problem.

For an antibiotic to be effective peak concentrations needed to be four to eight times the MIC for a particular pathogen; also, concentrations needed to be higher than the MIC between doses. First-line therapeutic regimens for pneumonia needed to take the possibility of concomitant meningitis into account. Although the pulmonary concentration of penicillin could be regarded as the equivalent of the serum concentration, the concentration in CSF in meningitis was only about 10% of serum concentration.

The clinical problems created by antibiotic resistance were more acute in paediatric than they were in adult patients. Chloramphenicol, with good penetration into CSF, was antagonistic to penicillin. These considerations led to systematic investigation of the efficacy of different antibiotic regimens in the paediatric wards of the Goroka Hospital (51-54). Chloramphenicol alone rather than chloramphenicol plus penicillin was recommended for severe pneumonia, and aqueous procaine penicillin was substituted for oily procaine penicillin in the third edition of the Standard Treatment Manual.

WHO Program for the Control of Acute Respiratory Infections, 1979-1986

In 1973 an article in the *Bulletin of the World Health Organization* on public health priorities (55) commented that:

"Respiratory infections are not considered as major health problems in either the developing or the developed countries, but in both groups of countries they are high on the list of the principal causes of mortality."

This stimulated awareness within WHO that the pneumonias were indeed a public health problem, not simply a clinical one. It was apparent that pneumonia and diarrhoea were the leading causes of child death worldwide and the programmatic approach which had been taken in the control of diarrhoeal disease should also be taken for acute respiratory infections.

The early lead was taken by the Western Pacific Regional Office of WHO (WHO/WPRO). Recognizing that acute respiratory infections were "dominant in producing death and disease in the region", the Office called a meeting in Goroka in January 1979 to "design a sentinel unit methodology" with the aim of establishing a sentinel unit for acute respiratory infections at the Papua New Guinea Institute of Medical Research (IMR), thus marking a new phase of research and program development. The meeting approved a clinical algorithm for the diagnosis of pneumonia and defined methods for population surveillance which were largely based on the Tari experience (56). WHO/WPRO then gave support for research in Goroka and began to promote research on ARI in the Western Pacific. These initiatives were in advance of developments in Geneva.

A WHO global Program for the Control of Acute Respiratory Infections was established in 1982 by resolution of the Thirty-fifth World Health Assembly. Meetings in Geneva in 1983 formulated a medium-term program for the period 1984-1989. By then PNG had well-established clinical guidelines for the case management of ARI as well as the research program described above. Researchers with PNG experience were thus in a position to make major contributions to the development of the global program.

The program aimed "to reduce the morbidity, severity, and mortality from acute respiratory infections". An ARI program was to be integrated into health services and not to become yet another vertical program. Vigorous debate, early in the life of the program, concerned the relative importance of viruses and bacteria in the pathogenesis of severe ALRI. Having settled that debate in favour of the overwhelming importance of *S. pneumoniae* and *H. influenzae*, the program moved to the development and validation of technical guidelines for the management of pneumonia at first-level health facilities. This was followed by the demonstration in a number of countries that the introduction of appropriate case management could reduce population mortality from pneumonia by 50%. The therapeutic principles established for pneumonia and meningitis were integral to the development of treatment regimens for the subsequent WHO Program for the Integrated Management of Childhood Illness.

PNG contribution to world research on the pneumonias

It would be fair to say that for much of the period under discussion PNG was leading the world in research on acute respiratory infections in developing countries and this research was seminal in the development of the WHO global program. A disease paradigm that had been developed in the first half of the 20th century for a workforce in industrialized North America and the South African gold mines was reworked and reapplied to children in the New Guinea highlands and shown to have global application (57). It is easy to forget that in the early 1980s the bacterial aetiology of childhood pneumonia and the emphasis on the roles of *S. pneumoniae* and *H. influenzae* in global mortality were still controversial subjects among the small group of epidemiologists and paediatricians working with WHO. The PNG clinical classification of the pneumonias and research in Goroka Hospital were important contributions to the development of international treatment regimens, although controlled trials with population mortality as an outcome were not possible given Papua New Guinea's adoption of a standardized approach to antimicrobial therapy in the early 1970s.

The trial of Pnc PS in adults in Tari was critical to licensure of the vaccine in the USA and subsequent utilization for the prevention of pneumonia in the elderly in many countries. The vaccine has also been utilized in the prevention of adult pneumonia in Aboriginal communities in Australia. It would have been preferable, perhaps, if the lesson from Tari – that it was most effective in previously healthy young adults – had been better understood. The vaccine's potential for

the prevention of respiratory mortality during influenza epidemics has never been put to the test.

The major disappointment was that the trial of Pnc PS in children never really led anywhere and no further trials were ever conducted. This was partly because we were initially unable to demonstrate the mechanisms by which the vaccine prevented pneumonia and also because of widespread disbelief, in the face of the evidence, that Pnc PS was immunogenic in children under the age of two years. Further evidence did accumulate and was summarized by myself and others in 1991 (58). Shann argued the case for a further controlled trial in a *Lancet* editorial in 1998 (59). These arguments were ignored. Altogether, two decades were lost while the international public health community waited on the arrival of the conjugate vaccines. The good news is that the Hib conjugate vaccine is now widely available in PNG and the pneumococcal conjugate vaccine should become available within a few years.

The challenge for PNG today is one of coverage: to ensure that children have access to potentially life-saving antibiotics within 24 hours of the onset of fast breathing and that they are fully immunized by the age of one year. Fundamental change will depend on improvements to child nutrition, to the domestic environment, and to personal hygiene. The risk of epidemic respiratory disease will never go away.

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Pneumonia in Goilala

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SUMMARY

The clinical syndrome of pneumonia in adults in Port Moresby, the capital city of Papua New Guinea, has changed from the 1970s to the present. The severe lobar pneumonia commonly diagnosed in young adult men, characteristically from Goilala and living in settlements in Port Moresby, is no longer seen. Today pneumonia in adults is likely to be milder and bronchopneumonic in type. Possible explanations for the change include changes in immunity and in the bacteria found in the environment and carried in the nasopharynx of recent immigrants to the city. A change in treatment-seeking behaviour together with the wide availability of oral antibiotics is considered to be the most likely cause of the altered clinical syndrome that we have observed.

Introduction

The title of this paper, as written in the program of the colloquium held in Goroka in 2010 to celebrate 40 years of pneumonia research, could mean that the authors either worked in health centres in Goilala or that they originated from there; of course, the truth is that neither of them worked there nor are they descendants of this ethnic group in Central Province.

The presentation was actually based on the experience of the first two national physicians working in the 1970s, either as resident medical officer (IHK) or as a junior consultant (AS) at the Taurama Native Hospital, which later became the Port Moresby General Hospital. Both are natives of New Guinea, one (IHK) a Motuan and from Port Moresby, and the other (AS) from West Papua; as such, both had the ability to identify patients according to their district of origin. A more appropriate title of the talk would have been 'Bedside diagnosis of pneumonia in adult patients admitted to the medical wards of Taurama Native Hospital in the 1970s'.

Clinical manifestations of pneumonia in the Goilala patients

The patients were young adult men from Goilala, a Subdistrict in the Central District – as the areas were then called – living in squatter settlements and working in the emerging town, Port Moresby. The typical presentation was that of an acute febrile illness with high fever and rigors, cough productive of rusty sputum, pleuritic chest pain and dyspnoea. On examination, the patients appeared toxic with temperature usually around 38-40°C with cyanosis, tachypnoea and tachycardia with or without icteric sclerae. The usual clinical signs of lobar consolidation comprising dullness to percussion, bronchial breath sounds and even percussion tenderness would be elicited and a chest X-ray confirmed the consolidation by the presence of a homogeneous opacity in the involved lobe.

In the presence of the constellation of symptoms and signs outlined above with radiological support, the provisional diagnosis of lobar pneumonia would be made

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and treatment instituted immediately as soon as blood for culture, haematology (full blood examination) and biochemistry (urea, electrolytes and creatinine) had been collected.

Treatment

The usual treatment was intravenous crystalline penicillin 2 million units statum followed by 1 million units 6 hourly. Oxygen administration using a nasal prong, pain relief with codeine compound containing aspirin and codeine phosphate, and intravenous fluid for rehydration were also instituted.

Progress

Within the first 24 hours of admission and initiation of antibiotic treatment, a dramatic response with a drop in the temperature to 36°C was observed and in the ensuing 2-3 days the patients' feeling of wellness returned; many stayed on for the rest of the week until completion of treatment while several absconded.

Assertion

Based on these observations, the authors assert that there is a difference in the syndrome of clinical pneumococcal pneumonia of the 1970s from what is being seen these days in the same hospital. The impression now is that pneumonia is of a mild form and is bronchopneumonic in pattern with a low-grade fever; many have had prior antibiotic treatment either at the clinics or from the many pharmacies in the city.

Assumption and possible explanations

We assume that the organism causing pneumonia in the two different periods is *Streptococcus pneumoniae* and propose three possible explanations for the change in the clinical presentation of pneumonia.

It is extremely difficult to discuss meaningfully the two different pictures based just on clinical observations; nevertheless, we are game enough to put forward a few theories.

Firstly, it is asserted that the patient population, namely young adult men that left their villages and went to a new and temporary home, were exposed to a totally different environment. They were forced by circumstance to all reside in the same squatter settlement with poor ventilation and unhygienic surroundings. In this new home, they would by necessity be sharing everything ranging from clothes to cooking utensils, including the microorganisms carried in the upper respiratory tract, the genital tract and elsewhere. According to Ian Riley, this picture is similar to what others had described in the past, particularly in young US soldiers [1]. Raphael Cilento in the 1920s made similar observations about young adults in New Guinea. The socioeconomic arrangements of the settlements are in fact little 'boot camps' bringing together people with low herd immunity. The same phenomenon is seen in donovanosis, which was rife in the 1950s and 1960s, but is slowly decreasing now. The settlements are still there and the trade commodities (grog, sex and betelnut) are as rampant as ever. Thus, either the Goilalas' herd immunity increased or there has been a dilutional effect of admixture with other ethnic groups.

The second theory is that the more liberal availability of broad-spectrum oral antibiotics such as amoxycillin, cotrimoxazole and doxycycline in the clinics and streets of Port Moresby has now prevented development of the 'full-blown' picture of lobar pneumonia/pneumococcal pneumonia as was seen in yesteryears. Community-acquired pneumococcal pneumonia is preceded by cough, fever and pleuritic chest pain for 2-3 days before the toxaemic state (lobar consolidation). The theory is that the pleuritic chest pain is so irritating and debilitating that the patient seeks relief quickly. By word of mouth or from previous experience, the person knows that a few tablets of paracetamol will fix the pain and fever, and that a few amoxycillin capsules will relieve the cough. By contrast, in other febrile illnesses such as malaria, typhoid (enteric fever) and meningitis treatment-seeking is delayed until the toxaemic or neurological symptoms or complications supervene. These patients still present to the accident and emergency department of the Port Moresby General Hospital.

Our third theory is that the pattern of pneumococcal nasopharyngeal carriage has changed among the Goilala community living in the settlement. The growing population in the city and the admixture of groups from other parts of the country may have introduced less invasive pneumococcal

serotypes into the community and hence fewer people come down with the severe invasive disease.

In conclusion, we share here our experience of the changing pattern of adult pneumonia in Port Moresby and we offer three possible causes. The most likely one would be the change in treatment-seeking behaviour and the liberal availability of oral broad-spectrum antibiotics.

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Collaborative studies in mucosal immunology in Goroka

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SUMMARY

A collaborative program between the Papua New Guinea (PNG) Institute of Medical Research and the Hunter Mucosal Group has completed studies relevant to protection of the airways against bacterial infection. Specifically, these studies addressed the mucosal capacity to produce local immunoglobulins and the capacity of the airways to respond to an oral vaccine containing inactivated nontypeable *Haemophilus influenzae (*NTHi). The mucosal IgA response to NTHi antigens was blunted in both children and adults in PNG compared with that found in Australian children and adults, whose airways are colonized only intermittently. Despite this, when oral NTHi is given to Papua New Guinean adults with chronic airways disease, it is followed by a significant (50%) reduction in incidence of acute bronchitic episodes, and a 3-log reduction in density of colonization, which persisted about 10 months. The implications of these key findings are discussed with respect to both mechanism and wider control of pathology emanating from abnormal airways colonization in a PNG environment.

Introduction

The Hunter Mucosal Immunology Group has had two major objectives: first, to understand the mechanisms whereby host-parasite relationships at mucosal sites determine health and disease outcomes; and second, to develop therapeutic strategies that shift the balance of these relationships towards protection against disease. One particular focus has been the airways. When our collaborative work with the Papua New Guinea Institute of Medical Research (PNGIMR) began, the dogma regarding airways immunity was as follows:

i Protection of the gas exchange apparatus (eg, from *Streptococcus pneumoniae*) was a systemic IgG antibody response.

ii Protection against chronic parenchymal infection (eg, tuberculosis) was mediated by a systemic T lymphocyte response.

iii Protection against endobronchial infection (eg, nontypeable *Haemophilus*

influenzae (NTHi)) was mediated by a local IgA antibody response (1).

Two collaborative studies in the PNG highlands provided important information, taking forward our understanding of mucosal immunology in relation to both the PNG and western environments.

The Studies

1. **Clancy R, Cripps A, Yeung S, Standish-White S, Pang G, Gratten H, Koki G, Smith D, Alpers M.** Salivary and serum antibody responses to *Haemophilus influenzae* infection in Papua New Guinea. *PNG Med J* 1987;30:271-276. (2)

The clinical pattern of airways disease in PNG reflects environmental conditions from birth, with smoking an additional threat in many adults. Children are colonized soon after birth by both NTHi and *S. pneumoniae*, with all children colonized by 3 months (3). The commonest cause of death in the first year of life is bacterial pneumonia (4,5), usually caused by the biotype and/or serotype of the dominant colonizing bacterial

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species. In adults aged ≥30 years living in the highlands, 16%-25% of deaths are due to chronic lung disease, with a further 18% caused by acute lower respiratory tract infections (4,5). NTHi and *S. pneumoniae* can be grown, respectively, from 90%-100% and 30%-40% of subjects with chronic lung disease (6).

This study found that the local salivary IgA antibody response to colonizing NTHi in the PNG highlands is blunted, with downregulation most apparent in adults. This suppression of antibody response in the mucosal compartment was selective, as a vigorous serum IgG and IgA response to NTHi antigens was seen in serum samples of adult Papua New Guineans.

2. **Lehmann D, Coakley KJ, Coakley CA, Spooner V, Montgomery JM, Michael A, Riley ID, Smith T, Clancy RL, Cripps AW, Alpers MP.** Reduction in the incidence of acute bronchitis by an oral *Haemophilus influenzae* vaccine in patients with chronic bronchitis in the highlands of Papua New Guinea. *Am Rev Resp Dis* 1991;144:324-330. (7)

This was a clinical study examining the effect of an oral NTHi vaccine in adults with chronic airways disease. An oral vaccine comprising inactivated NTHi bacteria had been developed to reduce morbidity from endobronchial infection and was also used to assess mechanisms of control of colonization of damaged airways in chronic obstructive pulmonary disease (COPD). In Australia it had been shown to reduce the incidence of acute exacerbations, with an associated reduction in frequency of isolates of NTHi from sputum, without an increase in specific IgA antibody in airways secretions (8). The oral vaccine was given monthly for 3 consecutive months, each cycle with 6×10^{11} orally administered NTHi. The PNG study was in adults with chronic airways disease (70%-80% with a history of smoking). The study followed recognition that acute inflammatory episodes in the lower airways at all ages were a major health problem. A major reason for the study was as a precursor to studies in the first year of life, where early universal colonization with NTHi (and *S. pneumoniae*) was a determinant of lower respiratory tract infections and middle ear infections. A successful study in Papua New Guinean adults would extend proof of concept to include NTHi isolates in PNG, providing a framework to assess the vaccine's

value in early life. A double-blind study was conducted in PNG in which 30 subjects received active tablets and 32 subjects placebo tablets monthly for 3 consecutive months, and they were all followed up for 12 months. The major outcomes were:

i Episodes of acute bronchitis were reduced by 50% (p <0.05) over the 12-month period.

ii Colonization density of NTHi was reduced by 3 logs for about 10 months (p <0.05). *S. pneumoniae* carriage density was also reduced over a similar period.

iii No reduction in pneumonia was observed.

Significance

There are two outcomes of these studies – specific information related to the therapeutic value of oral NTHi therapy in PNG, and a contribution to a broader understanding of mucosal immunity in humans and the mechanism of protection following oral immunotherapy with NTHi. The major outcome relevant to PNG is that oral immunotherapy is an apparently safe way to downregulate bronchial inflammation in adults with chronic bronchitis and COPD. NTHi isolates cross-react (perhaps due to highly conserved outer membrane antigens) with the isolate used effectively in Australian trials and thus a single vaccine is relevant to different geographical areas. By extension it is likely that a reduction in morbidity in infants with respect to lower airways infection could occur. Studies to control abnormal colonization in the upper airways – targeting otitis media and its sequelae (an original aim of the PNG trial) – are appropriate, but require an infant formulation. The clear demonstration that the effector mechanism (phagocytosis) is non-specific with significant impact on both NTHi and *S. pneumoniae* in sputum reinforces the potential for NTHi immunotherapy in PNG populations, where a range of pathogens occur. The key requirement for a successful clinical outcome is sensitisation to NTHi.

The results of the two PNG studies had a profound effect on understanding the mechanism of action and subsequent development of NTHi immunotherapy. First, the positive clinical results confirmed the

findings of the initial Newcastle study (8), broadening the geographical value of immunotherapy. Second, clinical benefit could be demonstrated in the most difficult microbiological climate – the natural history in PNG is a lifelong pattern, often involving high-density polybacterial colonization. Third, downregulation of IgA antibody responses in airways secretions was consistent with the absence of a local IgA antibody response following oral NTHi immunotherapy. Further studies in rodents showed that only thoracic duct T cells from immune animals could transfer immunity and antigen-specific T cells were detected following oral immunization with NTHi in subjects with COPD (9). Recently, the critical role of gut-derived Th17 cells in respiratory tract immunity has been demonstrated, and we have detected IL-17 in bronchial washings following oral immunization (unpublished observations). Fourth, the most significant observation in understanding the sequence of events following oral immunization with NTHi – a reduced antigen mass within bronchi – was the 3-log reduction in colonization density of NTHi and the significant reduction in heavy growth of *S. pneumoniae*. This observation became a basic tenet of the hypothesis subsequently developed, that acute exacerbations in patients with COPD represent a hypersensitivity response involving Th17 cells to colonizing bacteria. Reduction in the antigen load after oral NTHi 'buffers' against both bacterial- and viral-initiated episodes (ie, the resident bacterial population is a common denominator for most acute episodes, irrespective of initiating organism). Co-infection of mice with NTHi and influenza virus is followed by an increase in the titre of both microbes; prior oral immunization with NTHi abrogates these changes (10). These observations challenged the existing idea that NTHi descended the airways following intercurrent virus infection. Fifth, the duration of protection was best defined by the PNG study, where both quantitative NTHi and *S. pneumoniae* data in the active group merged with placebo at about 10 months. Thus annual re-immunization is now recommended. Sixth, an increase in adult serum IgG antibody in PNG is consistent with recent data obtained in Newcastle and the USA, where a specific IgG increase in serum has been linked to unprotected exposure to NTHi in those with damaged airways (10). In the Australian study, this increase in IgG antibody is seen as a surrogate parameter of mucosal protection (11,12).

Conclusion

Review of the impact of studies in mucosal immunology highlight the value of collaborative research between PNG and western countries, with obvious benefits to both the societies involved, in progressing understanding and in defining future objectives. Studies with oral NTHi immunotherapy have continued – a recent Phase 2 study in Australia confirms protection, with a 90% reduction in admission into hospital in those with most severe disease (13). That is being followed in 2011 with a multisite study across Australia in 340 subjects, using a commercial-quality highly characterized product (HI-164OV). Work with oral NTHi immunotherapy has substantially added to a current recognition of the importance of bacteria in driving acute (and possibly chronic) manifestations of COPD. It is time to return to PNG populations, in particular to explore the value of oral NTHi in infants with respect to lower and upper airways infections. Furthermore, earlier studies in Goroka suggesting that infants are infected with NTHi and *S. pneumoniae* from the adults with COPD who share their living space (R Grimley, unpublished manuscript) can now be tested using oral NTHi immunotherapy. The work done in Goroka has had a substantive influence on changing our understanding of airways protection, with a primary non-antibody role of T cells in controlling endobronchial colonization, and on the value of quantitative bacteriology in understanding these mechanisms where local antibody secretion has been suppressed.

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Oxygen supplies for hospitals in Papua New Guinea: a comparison of the feasibility and cost-effectiveness of methods for different settings

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SUMMARY

Oxygen therapy is essential in all wards, emergency departments and operating theatres of hospitals at all levels, and oxygen is life-saving. In Papua New Guinea (PNG), an effective oxygen system that improved the detection and treatment of hypoxaemia in provincial and district hospitals reduced death rates from pneumonia in children by as much as 35%. The methods for providing oxygen in PNG are reviewed. A busy provincial hospital will use on average about 38,000 l of oxygen each day. Over 2 years the cost of this amount of oxygen being provided by cylinders (at least K555,000) or an oxygen generator (about K1 million) is significantly more than the cost of setting up and maintaining a comprehensive system of bedside oxygen concentrators (K223,000). A district hospital will use 17,000 l per day. The full costs of this over 2 years are K33,000 if supplied by bedside concentrators, or K333,000 plus transport costs if the oxygen source is cylinders. In provincial and district hospitals bedside oxygen concentrators will be the most cost-effective, simple and reliable sources of oxygen. In large hospitals where there are existing oxygen pipelines, or in newly designed hospitals, an oxygen generator will be effective but currently much more expensive than bedside concentrators that provide the same volume of oxygen generation. There are options for oxygen concentrator use in hospitals and health centres that do not have reliable power. These include battery storage of power or solar power. While these considerably add to the establishment cost when changing from cylinders to concentrators, a battery-powered system should repay its capital costs in less than one year, though this has not yet been proven in the field. Bedside oxygen concentrators are currently the 'best-buy' in supplying oxygen in most hospitals in PNG, where cylinder oxygen is the largest single item in their drug budget. Oxygen concentrators should not be seen as an expensive intervention that has to rely on donor support, but as a cost-saving intervention for all hospitals.

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Introduction

Oxygen is essential for the care of many seriously ill patients with respiratory and non-respiratory conditions. It is required in all wards, emergency departments and operating theatres of hospitals at all levels. For decades, in Papua New Guinea (PNG), as in many developing countries, oxygen supplies have proved expensive and unreliable because of geographical remoteness, poor road conditions, logistics of transport, and the monopoly of supply through a private company. Oxygen, purchased in cylinders, is the single largest drug expense by the government health sector in PNG. In recent years, potentially more reliable options for supplying oxygen – oxygen concentrators and oxygen generators – have been trialled, and the resultant improvements in the detection and management of hypoxaemia have reduced mortality from childhood pneumonia by up to 35% (1,2). It is essential that choices made about oxygen systems are informed by the best available evidence on reliability, cost-effectiveness and safety. Fortunately there is direct evidence from PNG over the last decade that can guide these choices.

This review estimates the oxygen requirements in a typical regional hospital in the highlands. Outside of Port Moresby General Hospital, these hospitals have the highest oxygen requirements in the country. The costs of different methods of providing oxygen in these settings are estimated. In light of the findings of estimated oxygen use and costs, the factors determining the choice of oxygen supplies for different hospitals are discussed.

Current methods of supplying oxygen

Oxygen cylinders

Cylinders filled with oxygen are purchased centrally by the National Department of Health (NDoH) from BOC, a private gas company, and can be collected from Area Medical Stores by staff from hospitals, health centres and provincial health authorities. The cylinders remain the property of BOC, so rent on the cylinder is also charged. The transport costs are met by the hospital, and this can exceed the cost of the gas if vehicle hire is required. There are thus three costs attached to the use of oxygen from cylinders: the cost of the gas, rent for the cylinder and the cost of cylinder transport.

A large G-size cylinder holds about 7000 litres of oxygen at a filling pressure of 13000 kPa (130 bar). Pressure regulators and flow-meters are needed to deliver oxygen from cylinders to patients.

Oxygen concentrators

Oxygen concentrators are small machines which can deliver either 5 or 10 l/min of gas with 85%-95% oxygen concentration continuously. When used together with a multiple flow-meter device, depending on the flow rates and delivery means used, they can deliver individually controlled flows to up to 5 children or 1-4 adults at the same time. The 5 l/min concentrator generates the equivalent of 1 G-size cylinder (7000 litres) in 24 hours. Oxygen concentrators need continuous 240 V, 50 Hz electrical power, which in most provincial hospitals comes primarily from a mains supply. Weekly changing of the coarse-particle filter is the only maintenance needed on a regular basis, and this can be done by nursing staff. Internal maintenance is required eventually, but some manufacturers guarantee a maintenance-free 5-year period. In PNG all internal repairs have been provided by specially trained NDoH engineers. There are now close to 50 concentrators in use at 17 provincial and district hospitals in a program which started in 2005.

Oxygen generators

This name has recently been given to large oxygen concentrators that are designed to supply the oxygen requirements of an entire hospital. They come in various sizes, with capacities to produce oxygen equivalent to 2-100 cylinders per day. They use the same principle as the small bedside concentrators. They often require a new building to house them. The product gas can be fed into a hospital gas pipeline, or used to fill cylinders which can be stored and then transferred to the wards and operating theatres. Oxygen generators need reliable mains electricity. Power must be continuous if oxygen is fed into pipelines. Where supplies of power are intermittent, generators can be used to fill oxygen cylinders which can be used at times of power interruption.

Oxygen generators are individually constructed to agreed specifications for each

hospital; they are one-off products, as compared to the mass-produced bedside concentrators. Training for engineering staff on maintenance of these machines is essential to maintain continuity of supply.

An oxygen generator with a capacity of almost 60,000 litres (just over eight 7000 l cylinders) of oxygen per day has been running in Goroka General Hospital since September 2009.

Methods

Estimating oxygen requirements

We estimated the patient needs for oxygen in each ward per day in a busy provincial/ regional hospital and district hospital. In the highlands, around 50% of children hospitalized with severe pneumonia will require oxygen (3), at a starting flow rate of around 2 litres per minute (4). The average duration a child with severe pneumonia requires oxygen exceeds 2 days (5). Hypoxaemia also occurs in children with serious non-respiratory illnesses, and the prevalence of hypoxaemia in these critical conditions has been previously estimated at 30% (6). In addition, around 40% of sick neonates will be hypoxaemic at admission (6). Daily case loads for pneumonia, non-pneumonia and neonatal illnesses were derived by taking the average number of admissions in 3 highlands hospitals (Mt Hagen, Mendi and Kundiawa) between September 2006 and September 2007 (1).

Estimates of oxygen demands for adult wards, emergency departments and theatres in provincial hospitals were based on clinical experience of case loads and oxygen use in these settings in several provincial hospitals. We estimated daily oxygen needs in a district hospital based on average estimated case load.

Estimating cost

We did a cost comparison between the three methods (cylinders, concentrators and large generators) of providing the estimated daily oxygen usage of a large provincial or regional hospital. To do this, direct costs were sought from the manufacturers for concentrators (Airsep, USA) and cylinders (BOC, PNG), from the records of cylinder usage by one provincial hospital (Mt Hagen General Hospital), and from the usage of the

generator installed in another provincial hospital (Goroka General Hospital). Indirect costs, including installation and training costs, were estimated based on the programs for implementation of bedside oxygen concentrators in hospitals in PNG (1) and from the installation of the large oxygen generator in Goroka. Transport costs for oxygen cylinders could not be reliably estimated as these vary significantly according to remoteness, availability and modes of transport to the oxygen plant. However, transport adds considerably to the overall cost of providing oxygen in cylinders, and the estimate proposed here for cylinders is therefore lower than the actual cost. In the bedside oxygen concentrator model it was assumed that 9 concentrators would be needed for a large provincial hospital (2 in the children's ward, 1 in the adult medical ward, 1 in the adult surgical ward, 1 in the emergency department, 1 in the special care nursery, 1 in the surgical recovery area/ intensive care unit and 2 spare), since it would be logistically important to provide oxygen in all patient care areas where hypoxic patients may be managed.

A similar cost estimate was done for district hospitals. Given the sharing of patient care areas between patient groups in these facilities, it was assumed that 3 concentrators would be sufficient: 1 with 5 l/min capacity, 1 with 10 l/min capacity and 1 spare.

Results

Oxygen requirements for a large provincial or regional hospital on an average day are shown in Table 1. The estimated total number of litres required per day – about 38,000 litres – is equivalent to 5 large 7000-litre oxygen cylinders per day.

The estimated costs of supplying in excess of 38,000 litres per day over two years are outlined in Table 2. Oxygen cylinders cost about K555,000, bedside concentrators cost about K223,000 and an oxygen generator costs K1 million over two years. The annual recurring costs (excluding the initial outlay and one-off building costs) of using a generator amount to K210,000, and this is similar to the cost of setting up and maintaining a system of bedside concentrators for the provision of oxygen on all the wards and the running of an anaesthetic machine and ventilator.

Oxygen requirements for a small provincial

TABLE 1

OXYGEN REQUIREMENTS FOR AN AVERAGE DAY IN A REGIONAL HOSPITAL IN THE HIGHLANDS

Hospital admissions/procedures	Average number of daily admissions	Average number hypoxaemic	Flow rate (l/minute)	Oxygen requirements (l/day)
Children with pneumonia or bronchiolitis	2.2	1.1	2	3,168
Children with pneumonia admitted the previous day, still hypoxaemic	1	1	1	1,440
Non-pneumonia admissions	3	1	1	1,440
Neonates with respiratory distress	2-3	1	1	1,440
A moderately busy surgical list requiring an anaesthetic machine to run 6 hours, assuming only one theatre is running	-	4 surgical operations (other procedures may not require oxygen)	10	3,600
Adults with COPD, pneumonia or heart failure in the emergency department and medical wards	-	3	4	17,280
Emergency adult trauma patients in surgical ward	-	1	6	8,640
Out of hours obstetric emergency requiring 2 hours of anaesthetic machine	-	1 surgical operation	10	1,200
Total				**38,208**

COPD = chronic obstructive pulmonary disease

hospital or a district hospital are shown in Table 3, and costs over 2 years are outlined in Table 4. The estimated cost of supplying around 17,000 litres of oxygen per day over 2 years using cylinders is K333,000 plus transport costs and for bedside oxygen concentrators is K33,000.

Discussion

More than 38,000 litres of oxygen are needed on an average day for new admissions in a busy provincial hospital, and more than 17,000 litres a day in a district hospital. This estimate of overall needs may be conservative, as some patients will require more than one day of oxygen therapy. We have factored in that children with severe pneumonia will require on average 2 days of oxygen therapy, but this is also likely to apply to adults with chronic lung or heart disease and to neonates with respiratory disease. While in resource-poor settings the necessity of oxygen has been recognized in anaesthetics and increasingly

TABLE 2

ALTERNATIVE MODELS FOR REGIONAL HOSPITALS: COSTS OF PRODUCING ABOUT 38,000 LITRES PER DAY FOR 2 YEARS

	Cost per unit (kina)	Total cost for 2 years (kina)
Oxygen cylinders (5 x 7000 l cylinders = 35,000 litres)		
Gas cost for one 7000 l cylinder (G-size)	140	511,000
Transport costs[a]	Depend on hospital requirements, location, vehicle hire rate	
Deposit for cylinders (x 45)[b]	800	36,000
Rent for 1 cylinder per year (x 45)[b]	96	8,640
Total for cylinders		**K555,640**
Oxygen generator system (capable of producing about 60,000 litres per day)		
Oxygen generator with 10 cylinders (including delivery)	565,000	565,000
Building costs	120,000	120,000
Existing manifold servicing and repair of leaks in gas pipeline	70,000	70,000
Electricity for 2 years (kW hours) 28kW x 24 x 0.65 x 365 x 2 x K0.75 (per kW hour)	65% usage[c] of 28 kW per day, at a cost of 0.75K per kW hour[d]	239,148
Additional cylinder purchase (x 17)[e]	700	11,900
Maintenance costs (estimated)	25,000 per year	50,000
Total for oxygen generator system		**K1,056,048**
Bedside oxygen concentrators producing approximately 64,000 litres per day (plus 2 spare concentrators)[f]		
Airsep Elite (5 l/min) (x 3 + 1 spare)[f]	1,490	5,960
Airsep Intensity (10 l/min) (x 4 + 1 spare)[f]	3,388	16,940
Flow-metre devices (x 8)	1,220	9,760
Concentrator, anaesthetic machine and ventilator x 2 (US$20,000 each)	54,200	108,400
Delivery costs	15,000	15,000

	Cost per unit (kina)	Total cost for 2 years (kina)
Indirect costs: installation, training, supervisory visits and maintenance	10,000	20,000
Electricity costs[f] Elite: 3 x 350 W = 1050 W 1.05kW x 24 x 365 x 2 x 0.75K/kW hour	13,797	
Intensity: 4 x 590 W = 2360 W 2.36kW x 24 x 365 x 2 x 0.75K/kW hour	31,010	
Anaesthetic machines: 1 x 590 W = 590 W 0.59kW x 8 x 365 x 2 x 0.75K/kW hour[g]	2,584	47,391
Total for bedside oxygen concentrators		**K223,451**

[a] Note that cylinder transport costs are not included as they vary widely between hospitals, but should be taken into consideration when evaluating the economics of using oxygen cylinders: estimates range from K50,000 upwards per year depending on the location of the hospital and the frequency of trips to the depot

[b] The cost estimate budgets for cylinder deposits and rental are for 45 oxygen cylinders where these are used as the main source of oxygen, which allows for 5 cylinders in use at any one time, 5 full in reserve and 35 either full or available for refilling

[c] Electricity use can be reduced by running the generator intermittently to fill oxygen cylinders, where oxygen can be stored; we therefore budgeted for 65% usage per day, required to produce 39,000 litres per day

[d] Electricity costs assume 24-hour operation of the concentrators at K0.75/kWhr

[e] The generator can be used to fill oxygen cylinders, which allows power to be used intermittently rather than continuously, and excess cylinders can supply surrounding small hospitals or health centres/clinics

[f] Oxygen output of bedside concentrators exceeds demand, but this number is needed to supply all patient care areas of the hospital and have 2 spare in case of malfunction; the electricity costing for bedside concentrators assumes that 7 concentrators are working 24 hours a day, which is unlikely, and therefore the overall cost is likely to be lower; a similar relationship holds between the theoretical and realistic daily oxygen outputs

[g] Electricity costs for anaesthetic machines assume 1 machine working 8 hours each day of the year

in the management of childhood pneumonia, it is equally important in many conditions for all age groups (7). Hospital oxygen demands therefore need to take into account the routine requirements of adult and paediatric wards as well as scheduled and emergency surgery. Oxygen needs will fluctuate depending on seasonal changes in the incidence of acute respiratory infections, disease outbreaks, emergencies and surgical activity, and the use of equipment with a high oxygen requirement such as ventilators.

Oxygen cylinders have been used for decades to deliver oxygen to the least accessible locations. They require hardly any maintenance and minimal pre-existing infrastructure, and are universally acceptable by health workers. However, the estimates produced in this paper, along with previous studies (8), confirm that this option is very expensive. Previous studies in PNG show that using cylinders is unreliable and leads to unavailability of oxygen on 20% of occasions

(3). The estimate that we have made on the 2-yearly cost of oxygen cylinders (K555,000) is conservative as we have not included transport costs, which will be particularly high in locations remote from the manufacturing plant, and especially where vehicle hire is needed. Though no estimates are available of the overall cost of transport of cylinders to hospitals, even without that the PNG Health Department spends more money on cylinder oxygen than any other drug.

The estimate we made of the costs of bedside oxygen concentrators is comprehensive: it includes a number of machines in excess of the oxygen requirements of a provincial hospital because of the need to have oxygen in multiple patient care areas and a spare concentrator in case of malfunction, and includes the costs of training and maintenance. In district hospitals, fewer oxygen concentrators are needed as the numbers of patient care areas and patients treated are fewer than in provincial or regional

TABLE 3

Estimated daily oxygen requirements for a district hospital

Hospital admissions/procedures	Average number of daily admissions	Average number hypoxaemic	Flow rate (l/minute)	Oxygen requirements (l/day)
Children with pneumonia	2	1	2	2,880
Non-pneumonia admissions	2	1	1	1,440
Neonates with respiratory distress	2	1	1	1,440
Surgical procedures under ketamine taking 1 hour each, including recovery	-	2 surgical operations	4	480
Adults with COPD, pneumonia or heart failure in medical wards	-	1	4	5,760
Emergency adult trauma patients in surgical ward needing oxygen for first 12 hours	-	1	6	4,320
Out of hours obstetric emergency requiring 2 hours of anaesthetic machine	-	1 surgical operation	10	1,200
Total				**17,520**

COPD = chronic obstructive pulmonary disease

hospitals. Therefore, only 3 concentrators are needed, including one spare, making it possible to set up an oxygen concentrator system in a district hospital and run it for two years for around K33,000.

Bedside concentrators are significantly cheaper than both cylinders and generators. However, they do require a continuous power source, which is limiting in remote health facilities. They also require regular maintenance by the health workers using them (cleaning of external pore filter) and periodic checks every 12 months by a technician or engineer. It is necessary to have a skilled engineer who can provide timely repairs if faults occur. If these services are not in place, a health facility may be without oxygen until repairs can be completed. A back-up oxygen cylinder should be available in all health facilities using concentrators to cope with faults that occur from time to time, while repairs are being done, and to cope with power interruptions. The use of concentrators needs well-functioning systems for communication between health facility and biomedical support. Oxygen concentrators are now being used in 17 provincial hospitals in PNG, and there is good experience in what is required to sustain them.

One disadvantage of oxygen concentrators is that the maximum pressure generated (140 kPa) is not sufficient to run standard anaesthesia equipment, which typically requires 400 kPa. This problem can be overcome by using anaesthesia machines which have been specifically designed to be used with an oxygen concentrator (see www.diamedica.com). The model we have

TABLE 4

ALTERNATIVE MODELS FOR A DISTRICT HOSPITAL OR SMALL PROVINCIAL HOSPITAL: COSTS OF PRODUCING ABOUT 17,000 LITRES PER DAY FOR 2 YEARS

	Cost per unit (kina)	Total cost for 2 years (kina)
Oxygen cylinders (3 x 7000 l cylinders = 21,000 litres)		
Gas cost for one 7000 l cylinder (G-size)	140	306,600
Transport costs[a]	Depend on hospital requirements, location, vehicle hire rate	
Deposit for cylinders (x 27)[b]	800	21,600
Rent for 1 cylinder per year (x 27)[b]	96	5,184
Total for cylinders		**K333,384**
Bedside oxygen concentrators producing approximately 21,000 litres per day (plus 1 spare concentrator)[c]		
Airsep Elite (5 l/minute) (x 1 + 1 spare)[c]	1,490	2,980
Airsep Intensity (10 l/minute) (x 1)[c]	3,388	3,388
Flow-meter devices (x 3)	1,220	3,660
Delivery costs	7,500	7,500
Indirect costs: installation, training, supervisory visits and maintenance	2,000	4,000
Electricity costs Elite: 1 x 350 W 0.35kW x 24 x 365 x 2 x 0.75K/kW hour[d]	4,599	
Intensity: 1 x 590W 0.59kW x 24 x 365 x 2 x 0.75K/kW hour[d]	7,753	12,352
Total for bedside oxygen concentrators		**K33,880**

[a] Note that cylinder transport costs are not included as they vary widely between hospitals, but should be taken into consideration when evaluating the economics of using oxygen cylinders

[b] The cost estimate budgets for cylinder deposit and rental are for 27 oxygen cylinders where these are used as the main source of oxygen, which assumes that refilling cylinders would occur once every week or two and allows for 3 cylinders in use at any one time, 3 full and ready for use and 21 available for refilling

[c] Oxygen output is 5 l/min x 60 x 24 x 1 = 7,200 (Elite) and 10 l/min x 60 x 24 x 1 = 14,400 (Intensity), total per day = 21,600 l

[d] Electricity costs assume 24-hour operation of the concentrators at K0.75/kWhr

proposed also includes anaesthesia equipment for surgery independent of cylinder oxygen, a cost that may not be necessary in all hospitals. The top of the range machine costs about US$20,000 and a simpler version without a ventilator costs about US$10,000. A busy provincial hospital will have 2 operating rooms, so the costs for 2 top of the range anaesthetic machines have been included in the oxygen concentrator model. This amounts to about 50% of the total cost of supplying oxygen using concentrators. Smaller hospitals where only minor surgery is done would not need this equipment, making oxygen concentrator systems even cheaper in district hospitals. If an anaesthetic machine is needed in district hospitals the additional cost is US$10,000 (K27,000).

The bedside oxygen concentrators currently used in PNG were all purchased from the same manufacturer and have proved reliable in tropical conditions. They are made in very large numbers (total unit numbers exceed one million), which leads to low unit cost, and they receive good product support from the manufacturer. Expertise in bedside oxygen concentrator maintenance and repair and the provision of spare parts are needed in each of the four regions of the country. Currently such expertise is not sufficiently spread throughout the provinces where concentrators are used. Work is required on all concentrators at intervals of about 10,000 hours of use (more than a year of continuous use). Measures need to be in place to carry out this essential maintenance locally at minimal cost.

The experience with large oxygen generators in PNG is so far positive, but limited to one centre (Goroka Hospital). Oxygen generators have some advantages over bedside concentrators: they can generate sufficient pressure (400 kPa) to run conventional anaesthetic machines, and by filling transportable cylinders there is the potential for producing enough oxygen to supply surrounding rural hospitals and smaller clinics if oxygen generation exceeds hospital usage. However, this excess is likely to be less than predicted, especially if there is leakage from pipelines, and it is currently unclear whether the Goroka generator will produce enough oxygen to meet the oxygen needs of all health facilities in the Eastern Highlands Province.

Compared to bedside oxygen concentrators, a generator requires a very large initial outlay: more than 4 times the cost of establishing a comprehensive oxygen system to all hospital clinical areas based on bedside oxygen concentrators. Oxygen generators require significant technical expertise and infrastructure, and malfunctions will result in shortage throughout the whole hospital if there is no back-up available. Bedside concentrators, being portable, allow the use of a concentrator from another ward or a spare, while repairs are done. If generators are supplying oxygen to patient care areas through gas pipelines wastage of oxygen can occur if there are leakages in gas piping systems (9).

When compared to the high cost of cylinder oxygen from a private supplier, the initial capital cost of the oxygen generator system at Goroka General Hospital should eventually be recovered, for the provision of an equivalent amount of oxygen. However, infrastructure and ongoing costs – a new building to house the generator, repair and maintenance of gas pipelines and manifold, and the capital cost of cylinders if the system is used to refill cylinders – substantially add to the initial capital costs of the generator, and mean that the total cost would take more than 3 years to be recovered. The oxygen generator in Goroka needs to be monitored for performance, maintenance requirements and costs for the first 5 years to evaluate if it is a successful prototype for similar hospitals.

Considerations when choosing oxygen systems in different hospitals

Three types of hospital situations will be considered:

- Hospitals with pre-existing medical gas pipelines and mains electricity

- Hospitals and health centres with 24-hour mains electricity but no pre-existing pipeline system

- Hospitals and health centres with limited or no mains electricity.

Large hospitals with pre-existing medical gas pipelines

Port Moresby General Hospital and some provincial hospitals in PNG have existing gas pipelines, but many of these are in need of maintenance. These hospitals generally have

adequate electricity supplies from mains power, with an electricity generator as a back-up supply. Although this is often assumed, when considering the use of oxygen concentrators or generators the electricity supply should be checked and upgraded as needed.

In addition to cost, a major consideration in choosing a method for supplying oxygen in these hospitals is the condition of the pre-existing gas pipelines: whether they would deliver gas from an oxygen generator, or a bank of cylinders connected to a manifold, effectively, safely and without substantial leakage. Several questions regarding pipelines which arise from consideration of the relevant International Standards Organization (ISO) Standards for medical gas pipeline systems (10) are listed in Table 5. If pipelines are in poor condition, oxygen generators can be used to fill oxygen cylinders, which are transported to areas of patient care when needed, though, given the large oxygen requirements of provincial hospitals, this exercise is time-consuming and labour-intensive.

Repair or replacement of copper pipelines should be carried out by specialist subcontractors, and is an expensive exercise. Once a pipeline has been upgraded and is ready for testing to the specified requirements, a third party expert assessor should certify that the system complies with the specification and the safety aspects of the relevant ISO Standards.

The more widespread adoption of pipelines and oxygen generators into PNG hospitals will require either the development of specialist engineers qualified in this complex field, or the use of specialist subcontractors with its attendant costs. If repair, replacement or maintenance of gas pipelines is considered too costly or complex in an individual institution, or the set-up costs of a generator are unaffordable, use of bedside oxygen concentrators in all hospital clinical areas where oxygen is required will cost less than a quarter of the cost of an oxygen generator, and currently is the preferred option.

Hospitals and health centres with 24-hour mains electricity but no pre-existing pipeline system

This situation applies to most provincial hospitals and many district hospitals in PNG, where there is a combination of mains electricity with a back-up generator delivering 240 V, 50 Hz AC with adequate wiring throughout the hospital. In these hospitals, the use of bedside oxygen concentrators (5 l or 10 l per minute) in designated high-dependency areas within wards (Figure 1) will be far cheaper than installing an oxygen generator and a piped system, or buying oxygen in cylinders (Table 2).

Most provincial hospitals employ engineers who are capable of extending the electrical wiring system if required at low cost, and such engineers have installed the short, plastic-

TABLE 5

QUESTIONS OUTLINED IN INTERNATIONAL STANDARDS ORGANIZATION ISO 7396-1:2007 (10), ESSENTIAL IF GENERATORS OR A BANK OF CYLINDERS ARE BEING CONSIDERED AS THE OXYGEN SOURCE

Are the pipelines leaking?

What is the condition of the terminal units (wall outlets) where connections are made?

Are there sufficient shut-off valves?

Does the manifold work and is it usable for the intended higher throughput?

Is there a reserve manifold capable of supplying the pipeline at 100% flow?

Is the pipeline of adequate diameter for the intended flows? If the copper pipe is too small in diameter, larger pipes may be required to be installed.
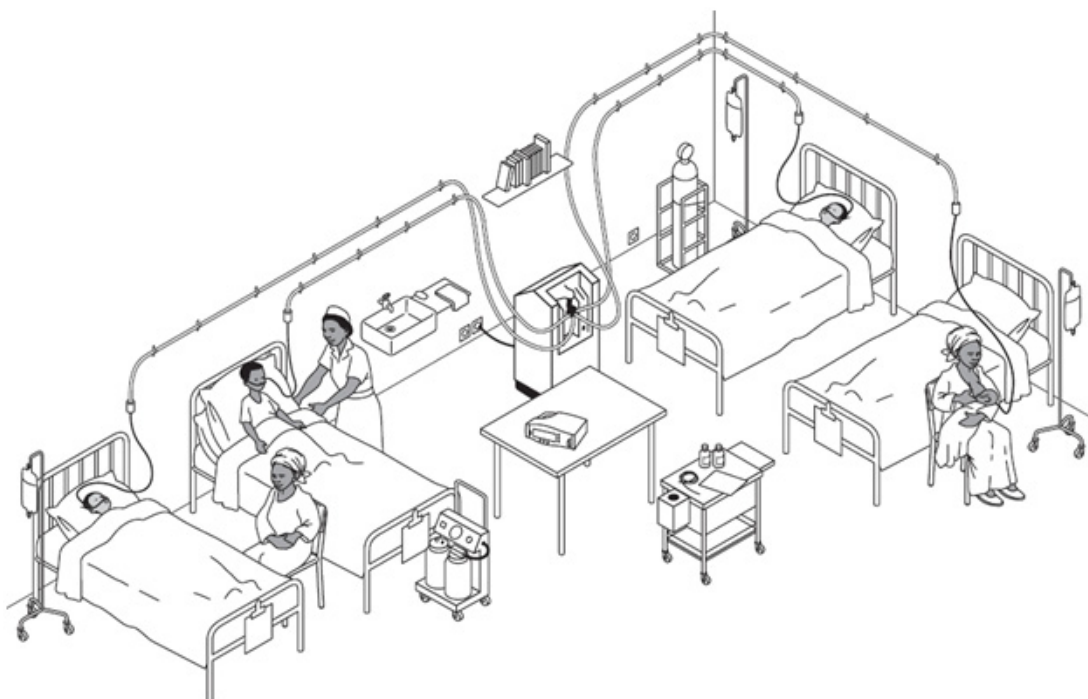
Figure 1. A high-dependency area in a children's ward that is supplied with oxygen by an oxygen concentrator. Figure by David Woodroffe of David Woodroffe Digital Illustration, United Kingdom, reproduced from The Clinical Use of Oxygen (11).

tubing systems used to deliver oxygen from oxygen concentrators to several beds in a high-dependency area, in compliance with ISO 8359 (12).

Hospitals and health centres with limited or no mains electricity

For hospitals without a reliable source of 240 V, 50 Hz electrical power the only ways to provide oxygen are (a) the transportation of cylinders, often over large distances, (b) to provide an alternative power source for 24 hours per day, or (c) to use small oxygen generators which can fill cylinders when power is available and provide a reservoir for when power is not available. Currently the third option is only a theoretical possibility and has not been tested in any field setting. Whether it would be feasible depends on the number of hours of power available, whether the power when available is continuous or intermittent, how much power is required to refill cylinders and whether this would exhaust the power needed for other requirements of the hospital. Different options for providing alternative energy sources to run concentrators (b, above) are

described below.

Storing power to run oxygen concentrators

For hospitals with between 4 and 23 hours of mains electricity per day it is possible to charge up to eight batteries with DC power at 6 volts per battery and then to use an inverter to provide AC power at 230 V and 50 Hz to drive a single 5 l/min concentrator when the mains power is not available. Such a system should cost between K8000 and K23,000 depending on the number of batteries needed and will be significantly cheaper than currently available solar power systems.

Solar power systems to run oxygen concentrators

Solar power has been used to run concentrators, but there are cost and logistical barriers (13). In general a solar panel will produce an average of 6 hours of DC power at the maximum rated output per day. This power therefore needs to be stored in batteries and connected to an inverter to give the 24-hour AC output needed to run a concentrator. There

are known systems which can be purchased to do this.

Unfortunately a capital cost of about US$20,000 for the solar power system is needed to run concentrators costing only around $550 each. However, the solar panels have a long life if not vandalized or stolen (about 25 years) and other components have a life of at least 5 years. Therefore the annual amortisation cost of the complete solar power system is about US$1500 (about K4100). The capital cost then becomes about US$2000 per year for each 5 litre/min concentrator over a life of at least 5 years. The annual cost of an equivalent quantity of oxygen (1 cylinder per day) from cylinders would be about US$18,900 (K140 x 365 divided by 2.71) (see Table 2). There are new building designs which provide extensive areas for solar panels sufficient for driving a 350 watt concentrator for 24 hours a day. Appropriate solar and battery power systems are available from a company which claims to have supplied 70% of the world market for solar refrigerators to WHO specifications (see www.dulas.com).

Intermittent mains or electricity generator power

The same kind of batteries as those used for a solar power system can be used to store power from intermittent mains or an electricity generator and thus can deliver the 24-hour continuous AC power needed by an oxygen concentrator. The battery system costs up to US$8500 depending on the number of hours of mains power available and therefore the number of batteries needed. This system will also produce the equivalent of 1 cylinder per day, with an annual cost of US$18,900, and therefore would repay its cost in less than a year.

Direct current concentrators

Concentrators that can be powered by DC power, thereby removing the need for an inverter when alternative DC power sources are used, are currently made for single-patient use. These portable machines are smaller (maximum of 0.5-1 l/min) and more expensive than the standard 5 or 10 l/min concentrators that have been used in PNG. However, concentrators powered by a DC motor are about 20% more efficient on energy consumption than the normal AC concentrators. There are hopes that a 5 l/min concentrator that runs off DC may be developed in the next few years at a reasonable price.

Pulse oximeters

Pulse oximeters are essential for the accurate detection of hypoxaemia (11,14). Regardless of the source of oxygen used, pulse oximeters should be purchased for all hospitals. Pulse oximeters cost about $1000 (K2700) and reusable sensors cost about $150-200 (K470). Reusable sensors can be expected to last a year if properly used. A provincial hospital needs 4-5 pulse oximeters and a 5-year supply of sensors, adding K23,000 to the cost of any oxygen system over 5 years. A district hospital needs 2 oximeters and a 5-year supply of sensors, adding K10,000 to the cost of an oxygen system over 5 years.

Conclusion

Oxygen supply systems based on bedside oxygen concentrators are currently the 'best-buy' for supplying oxygen in provincial and district hospitals in PNG, being much cheaper than the alternative sources. The choice of method for supplying oxygen needs to take into account pre-existing infrastructure including availability of electricity, and accessibility of technical and engineering expertise. In large hospitals where there are existing oxygen pipelines in good condition, or in newly designed hospitals, a large oxygen generator may be a long-term infrastructure investment, but still much more expensive than bedside concentrators for the same volume of oxygen generation. For a functioning national oxygen concentrator or generator program more biomedical engineering capacity is urgently needed in each region of the country. Further training is needed for the existing engineering staff.

There are some options for oxygen concentrator use in remote health centres that do not have reliable power, including battery storage of mains or solar power. Although these add considerably to the establishment costs when changing from cylinders to concentrators, a battery-powered system should repay its capital costs in less than one year; however, this has not yet been evaluated under field conditions.

The PNG Health Department and hospital chief executive officers (CEOs) should not wait for external agencies or donors to support

oxygen concentrator programs. Changing to oxygen concentrators as the primary source for oxygen should be seen as an urgent, cost-saving intervention for the Health Department and for individual hospitals. Pulse oximeters are an essential part of managing hypoxaemia and should be included in any program of improved oxygen systems.

COMPETING INTERESTS

The authors declare that they have no contracts, consultancies, shares or other financial interest in any company which manufactures oxygen equipment or other medical devices.

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Improving the aetiological diagnosis of bacterial pneumonia and meningitis in Papua New Guinea

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SUMMARY

Bacterial pneumonia and meningitis are major causes of childhood mortality in Papua New Guinea (PNG). Laboratory techniques for detection of bacterial pathogens have improved in the last decade, particularly molecular techniques that can be applied to culture-negative samples. With adequate training and support, a number of these techniques are readily available to research staff in PNG. In this article we summarize previous studies on the aetiology of pneumonia and meningitis in PNG, describe current diagnostic approaches and discuss available diagnostic tools to enhance surveillance of bacterial pneumonia and meningitis.

Introduction

Pneumonia is a major cause of mortality in Papua New Guinea (PNG), with the burden of disease being greatest in children less than 5 years of age. In children less than 1 year of age pneumonia is second only to perinatal conditions as a cause of death, and accounts for over 20% of inpatient mortality in this age group (1). Meningitis, although not as common as pneumonia, was found to be one of the five most common causes of death in children up to 12 years of age at Goroka Hospital between 1998 and 2000 (2). Studies conducted in the Eastern Highlands Province of PNG have repeatedly demonstrated the importance of *Streptococcus pneumoniae* (the pneumococcus) and *Haemophilus influenzae* in the aetiology of acute lower respiratory tract infection (ALRI) and meningitis (3-6). There are no recent data on the aetiology of ALRI in PNG, but recent studies of meningitis have shown that *S. pneumoniae* and *H. influenzae* remain the most important causes of meningitis in Papua New Guinean children (7, M. Laman and colleagues, unpublished data). In the current era of pneumococcal conjugate vaccine (PCV) and its imminent introduction to PNG in 2013, along with the introduction of *H. influenzae* type b (Hib) conjugate vaccine in 2008, it is crucial that the aetiology of bacterial pneumonia and meningitis is correctly assessed. Detailed aetiological data will inform policy-makers on the appropriate choice of pneumococcal vaccines for PNG and provide a baseline for ongoing monitoring of invasive pneumococcal disease (IPD) following PCV introduction.

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An historical overview of the aetiology of bacterial pneumonia and meningitis in PNG

Upon arriving in PNG in the 1960s as a physician, Bob Douglas soon noticed the high burden of pneumonia, particularly amongst young adults (8). This led to two early investigations into the aetiology of pneumonia in PNG, which found the pneumococcus and *H. influenzae* to be important pathogens (9,10). One of the earliest studies conducted in Port Moresby on the aetiology of meningitis in adults identified *S. pneumoniae* and *Neisseria meningitidis* to be the most common causes of culture-confirmed bacterial meningitis (11).

Other early work on the aetiology of pneumonia in children was by Frank Shann and colleagues (6), where lung aspirates and blood samples were cultured from 83 children with X-ray-confirmed pneumonia in Goroka Hospital between 1978 and 1981. Bacteriological culture of the lung aspirates identified *H. influenzae* and *S. pneumoniae* as the most common pathogens, with one or both organisms isolated from 52% of lung aspirates. Nontypeable *H. influenzae* (NTHi) was the predominant pathogen isolated, although it was usually found in combination with other bacterial pathogens, while capsular *H. influenzae* strains including *H. influenzae* type b were less common (6).

Barker and colleagues (3) also demonstrated the importance of *S. pneumoniae* and *H. influenzae* in the aetiology of ALRI in children in PNG. These two organisms accounted for 87% of all clinically significant isolates grown from blood culture from children admitted to Goroka Hospital between 1983 and 1984 with moderate/severe ALRI. Other researchers have also reported on the importance of ALRI and meningitis as causes of childhood illness in the highlands of PNG, and noted the importance of *S. pneumoniae* and *H. influenzae* in the aetiology of these diseases (2,4,5,12). In a study of bacterial meningitis in Goroka from 1980 to 1984, *H. influenzae* (49% of positive samples) and *S. pneumoniae* (43% of positive samples) accounted for a total of 92% of 155 culture-positive cerebrospinal fluid (CSF) samples (4). More recent surveillance has demonstrated a similar pattern of meningitis aetiology, with *S. pneumoniae* and *H. influenzae* accounting for 89% of bacterial

pathogens isolated from 384 culture-positive CSF samples between 1996 and 2005 in Goroka (ARG and colleagues, unpublished data) and 89% of culture-confirmed bacterial meningitis cases in Port Moresby (7). Previous studies have reported pneumococcal serogroups 6, 7, 14, 19 and 23 to be the most commonly isolated from blood and lung tissue, while those most commonly isolated from CSF were 2, 5, 7, 12, 23, 45 and 46 (4).

Routine diagnosis of pneumonia and meningitis in PNG – current methodology

In PNG, diagnosis of pneumonia and meningitis is primarily based on clinical manifestations. The criteria used to diagnose pneumonia in children in PNG are: cough and raised respiratory rate (>60/minute at age <1 month and >40/minute from age 1 month onwards), with chest indrawing as well in the case of moderate pneumonia. A diagnosis of severe pneumonia is made if one or more of the following are also present: heart failure (pulse rate above 160/minute with an enlarged liver), difficulty feeding, cyanosis or restlessness (13). Chest X-ray is also conducted at hospitals with the capacity for radiography. Symptoms used to diagnose meningitis include neck stiffness, Kernig's sign, Brudzinski's sign, bulging fontanelle, impaired consciousness and a history of convulsions (13). These and other symptoms may be non-specific and individually they are poor indicators of proven or probable acute bacterial meningitis (5). Lumbar puncture is strongly recommended for febrile children presenting with symptoms of meningitis where the diagnosis is uncertain, despite the lack of laboratory facilities in rural PNG (M. Laman, personal communication, 2010). It is recommended that all children with symptoms of pneumonia or meningitis be administered antibiotics.

Laboratory diagnosis of pneumonia and meningitis is not routinely conducted in PNG. Port Moresby General Hospital Pathology Laboratory is the only service laboratory in PNG that is currently conducting blood culture for clinically diagnosed pneumonia (and thus is the only such laboratory with the capacity to diagnose the aetiology of bacteraemic pneumonia in children). The diagnosis of meningitis has improved in recent years, largely on account of the ongoing Hib surveillance facilitated by the

National Department of Health. Eight sentinel sites have been established throughout PNG to monitor the impact of the Hib vaccine since its introduction in 2008. Bacterial meningitis is diagnosed using a combination of direct microscopy, bacteriological culture and latex agglutination for *S. pneumoniae*, *H. influenzae* and *Cryptococcus neoformans* on CSF samples. All CSF samples are cultured at some of the eight sentinel sites, while at other sites such as the PNG Institute of Medical Research (PNGIMR) in Goroka only CSF samples with leukocytes present are cultured. With culture-positive blood and CSF samples, bacterial species can be identified and antibiotic susceptibility tested to guide treatment. In addition, the subcultured isolate can be stored for subsequent analysis, including serotyping, to assess the impact of vaccination on the population of invasive pneumococci and to assist in the development of new vaccines.

The lack of routine culture conducted at pathology laboratories impedes our understanding of the aetiology of ALRI and meningitis and trends in antimicrobial susceptibility in PNG. Furthermore, the lack of laboratory diagnostic capacity may contribute to the widespread use of antibiotics due to the need for empirical treatment, thus increasing the likelihood of emergence of resistant strains. This impedes targeted interventions and appropriate prescription of antimicrobials. PNG has a long history of bacteria resistant to antibiotics, and some of the first penicillin-resistant strains of *S. pneumoniae* were identified in this country (14). A study at three PNG hospitals from 1997 to 2000 found that 21% of *H. influenzae* type b isolated from CSF were resistant to chloramphenicol, which is a standard treatment for meningitis in PNG (15). Recent data indicate that there is a rise in antibiotic resistance in *S. pneumoniae* and *H. influenzae* strains causing meningitis in PNG (15), and a similar trend is plausible in strains causing ALRI though data to support this are lacking.

Limitations of current methodology for laboratory diagnoses

Although blood culture is a highly specific technique, the major limitation is its poor sensitivity. It is estimated that the sensitivity of blood culture for the diagnosis of bacterial pneumonia is <25% (16), and often considerably lower in paediatric cases at ~10% (17). Historically the rates of positive blood culture from children were high in PNG (25% and 37%) (3,6), possibly due firstly to the severity of illness (potentially reflecting higher bacterial loads), as all recruits were hospital inpatients with moderate or severe pneumonia, and secondly to the fact that children who had received antibiotics in the previous 3-7 days were excluded from these studies. Subsequent studies have had isolation rates of 5%-7% in blood samples from children with community-acquired pneumonia and other bacterial infections (4,5), but this, at least in part, is probably due to the very broad inclusion criteria for these studies (18). Lung aspirates are more likely to identify the cause of severe pneumonia but are intrusive and difficult to collect in an already sick child. They are rarely performed in current medical practice primarily because of the risk of the procedure. Thus, despite their lack of sensitivity, blood cultures continue to be considered the 'gold standard' for aetiological diagnosis of childhood bacteraemic pneumonia, and offer the benefit of enabling antibiotic susceptibility testing and epidemiological studies to be conducted on the isolated bacteria.

The conventional laboratory diagnosis of bacterial meningitis is based on microscopy and bacterial culture of CSF; however, like blood culture it has limitations. Detection is time-consuming and there is moderate sensitivity by culture. Additionally, the likelihood of isolating a bacterial pathogen decreases when there is a delay in processing the sample (19) and in cases where there has been prior use of antibiotics (20).

Antigen detection assays for the identification of *S. pneumoniae* and *H. influenzae* in patients with pneumonia and meningitis have been evaluated in numerous studies over the past 40 years. The data repeatedly show that these assays have poor specificity in the diagnosis of pneumonia in children using urine and serum samples due to false-positive reactions resulting from nasal carriage (21,22). Antigen detection assays appear to have some utility in the diagnosis of meningitis in children using CSF, with slightly improved sensitivity over blood culture and comparable specificity (23,24).

Existing data pertaining to the aetiology of pneumonia and meningitis in PNG have been based on the best samples and detection

methods available at the time of study. However, it is likely that, due to the limited sensitivity of detection techniques, the relative importance of some aetiological agents has been underestimated.

Advances in bacterial identification in patient samples using molecular techniques

Molecular diagnostics, particularly the polymerase chain reaction (PCR), have revolutionized laboratory analysis due to the improved sensitivity that can be achieved, quicker time to result, potential for automation and the ability to detect non-viable microorganisms (eg, from patients undergoing antibiotic therapy).

Despite the benefits of molecular detection of pathogens, the uptake of PCR for clinical diagnosis has been surprisingly slow (25). A recent meta-analysis of studies assessing the diagnostic utility of PCR on blood for the diagnosis of IPD (1993-2009) reported an overall sensitivity of 57% (95% CI, 45.7-67.8%) and specificity of 99% (95% CI, 96.4-99.5%) (26). However, there was marked variability between methodologies in the 29 eligible studies, including blood sample type (eg, whole blood, serum, plasma), PCR method (standard, nested, real-time), the pneumococcal gene targeted by PCR (*ply*, *lytA*, PBP 2b, *psaA*, SPN9802), the study design (cohort versus case-control), age (adults versus children) and the source of infection (pneumonia only versus any IPD). Meta-regression analysis found that only PCR method, study design, age and source of infection significantly affected diagnostic accuracy (26). All analyses were highly heterogeneous with consistent estimates obtained only for recent, real-time methods.

Thus it can be argued that molecular methods for aetiological diagnosis of pneumococcal pneumonia and meningitis are not yet advanced enough to supplant traditional culture techniques; however, many studies have demonstrated the benefits of complementing the two approaches. At the time of writing, the most recent published paper using both culture and real-time *lytA* PCR on blood in children with pneumonia identified *S. pneumoniae* in 47 of 292 patients; real-time PCR (using 200 µl of whole blood) identified 45/47 cases, whereas culture (using 4-6 ml of blood) identified 11/47 cases (27). Similarly, identification of *S.*

pneumoniae and other pathogens in CSF from patients with meningitis was markedly improved by the addition of real-time PCR analysis (28,29).

Specificity is a critically important consideration for molecular methods. When the *ply* gene was used, pneumococcal DNA was detected in the blood of 17% of healthy controls (30), most likely as a result of pneumococcal carriage; however, with the more accepted *lytA* gene, reports of positive results in those lacking clinical symptoms are rare. For children in PNG who typically experience continuous and dense pneumococcal nasopharyngeal colonization from an early age (31,32), the risk of false positives is likely to be higher than for populations included in the published studies. However, a recently completed study of densely colonized Australian Indigenous children without pneumonia demonstrated that all 39 serum samples were negative for *S. pneumoniae* and *H. influenzae* using real-time PCR assays for *lytA* and *glpQ* (J.Y.R. Lai, M.J. Binks, M. Kaestli, A.J. Leach and H.C. Smith-Vaughan, unpublished data). A study specifically assessing the sensitivity and specificity of real-time PCR assays for aetiological diagnosis of bacteraemic pneumonia is necessary in a high-risk population such as children in PNG.

Early lung aspirate studies in PNG identified NTHi as an important pathogen in pneumonia, with 54% of all *H. influenzae* isolated from the lung and 23% from the blood being NTHi (4). NTHi is generally accepted to be a mucosal pathogen and rarely a cause of invasive disease, though the dogma is being challenged with increasing reports of invasive disease due to NTHi in some countries (33,34). It may be that blood culture methods underestimate the bacteraemic burden of NTHi and the introduction of real-time PCR analysis of blood will better define this burden. For PNG, an *H. influenzae* target such as *glpQ* (Protein D) (35) would be appropriate; however, in order to specifically identify *H. influenzae* type b disease, a PCR targeting the *cap* region (36) will also be necessary. These assays could supplement pneumococcal PCR assays at relatively little additional cost. Furthermore, quantitative broad-based PCR assays for multiple bacterial pathogens are continually being developed and improved upon, which may soon permit detection of bacterial load in blood samples in PNG (37).

At PNGIMR, real-time PCR is routinely conducted for other projects including HIV and enteric disease research. Therefore, an extension of the work for application to aetiological diagnosis of bacteraemic pneumonia and meningitis is feasible with appropriate funding. A significant advantage of using molecular detection methods is that samples can be collected and stored at different clinical sites and then sent to a central reference laboratory for testing, allowing collection of standardized nationwide data. A further benefit of the real-time platform is the ability to quantify product and estimate bacterial load in patient samples. Indeed, there is a positive correlation between bacterial load and disease severity; for example, Malawian children with pneumococcal meningitis were less likely to survive when higher pneumococcal DNA concentrations were detected in their blood and CSF (38). The addition of PCR detection to complement blood and CSF culture methods will certainly provide important knowledge of the aetiology of pneumonia and meningitis in PNG, which may have important implications for treatment and evaluation of interventions.

Pneumococcal serotype surveillance

There are two pneumococcal conjugate vaccines that are contenders for introduction to PNG in 2013 and a decision must be made on which will have the greater impact. One vaccine, PCV10, is composed of polysaccharide from 10 of the possible 92 different pneumococcal serotypes (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F), most of which are conjugated to an immunogenic NTHi carrier protein (Protein D) (39). The other vaccine covers more pneumococcal serotypes with polysaccharide from 13 different pneumococcal serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) (40) but it has no NTHi component. As the current vaccination strategy for pneumococcal disease is serotype-dependent, it is important to monitor which pneumococcal serotypes are causing disease (in addition to assessing the overall rates of ALRI and pneumococcal and NTHi disease). This will help to identify which of the two pneumococcal conjugate vaccines is best suited to PNG and will also be important for measuring the effectiveness of whichever vaccine is introduced. The current strategy for identifying pneumococcal serotypes is the Quellung reaction, which

requires isolation of the pneumococcus. Pools of serotype-specific serum are incubated with the bacteria and where there is a positive reaction the bacteria clump together and the capsule swells, as observed under a microscope; then the positive serum pool is further investigated on an individual basis to identify the pneumococcal serotype. This is a valuable technique though subjective, expensive and labour-intensive and requires a high skill level. As the Quellung reaction can only be conducted on pneumococcal isolates, the low culture-positive rates from patient samples (as described earlier) significantly limit serotype-specific surveillance of IPD.

Molecular serotyping by PCR has recently been developed for the pneumococcus. This technique has been shown to have good (29), or improved (41), sensitivity and high specificity on pneumococcal isolates when compared with the 'gold standard' Quellung reaction. More importantly, molecular serotyping can identify pneumococcal serotypes in culture-negative clinical samples (29,41,42), which is not possible with the Quellung reaction. Another advantage of molecular serotyping is an improved ability to identify multiple pneumococcal serotypes in one sample, which is often underestimated by culture where only a few colonies are serotyped (43). This is particularly valuable for pneumococcal carriage studies, where colonization with multiple serotypes is common, and of special relevance to PNG, where one study reported that two serotypes were obtained from 29% of nasopharyngeal aspirates (427/1449) and three serotypes from 3% of specimens (48/1449) (32). In a recent neonatal PCV study, nasopharyngeal carriage of multiple serotypes was ~10% (D. Lehmann, C. Aho, A. Michael, P. Jacoby and colleagues, unpublished data). A limiting factor of the direct pneumococcal serotyping PCR is low pneumococcal DNA levels, which is often the case for blood collected from IPD patients. However, the sensitivity of molecular serotyping of clinical samples can be enhanced by using real-time PCR (42). Direct real-time PCR serotyping of blood from 80 patients with PCR-confirmed IPD has recently been shown to identify the pneumococcal serotype in 93% of samples, whereas only 14% of the 80 samples were culture-positive (27). This study highlights the potential of real-time PCR for improving not only the detection of pneumococcal disease but also serotype surveillance through the use of culture-negative samples.

Antigen detection-based techniques have been developed where serotype-specific monoclonal antibodies are conjugated to beads for use with the Bioplex platform. This is a sensitive and specific technique that can also identify pneumococcal serotypes in culture-negative samples. The limiting factors are the number of serotypes that can be multiplexed and the ability to obtain expensive antibodies that will only be produced for the predominant 'global serotypes'; such antibodies are unlikely to be produced for rarer serotypes, for example, serotype 46, which commonly causes meningitis in PNG (4,44). In the future pneumococcal serotype surveillance for IPD and carriage studies is likely to adopt real-time PCR serotyping directly on patient samples to complement existing culture-based techniques. There is no reason that this cannot occur in PNG, and it could greatly enhance future studies on ALRI.

Conclusion

In PNG there is a need for up-to-date aetiological data for two of the most important bacterial diseases, namely pneumonia and meningitis. Blood culture will continue to be the mainstay of diagnosis in the research setting in PNG for the foreseeable future, and ideally should be extended to diagnostic services in major regional towns. Molecular detection of bacterial (and potentially viral) pathogens from blood and CSF can supplement culture in future studies, improving sensitivity. The introduction of molecular diagnostic tools is highly achievable given that we have the equipment and training support to set up PCR or quantitative real-time PCR immediately. This will provide a more accurate depiction of the aetiology of bacterial pneumonia and meningitis in PNG, which in turn will produce evidence to inform policy-makers on appropriate vaccines for PNG and permit accurate assessment of the effectiveness of pneumococcal and Hib conjugate vaccination programs.

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Nontypeable *Haemophilus influenzae* and childhood pneumonia

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SUMMARY

Nontypeable *Haemophilus influenzae* (NTHi) is a common microbe frequently isolated from the nasopharynx of children. Bacterial pneumonia is a major cause of morbidity and mortality in children less than 5 years of age, with the burden of disease being greatest in developing countries. Determination of the bacterial aetiology of pneumonia is difficult due to sampling constraints. However, with a combination of sampling approaches, trans-thoracic fine-needle aspiration, blood culture and screened sputum, the evidence strongly suggests that NTHi is a significant causative pathogen of pneumonia in young children. However, further studies are required. The development of a new pneumococcal conjugate vaccine containing *H. influenzae* protein D has the potential to be beneficial against disease caused by NTHi, including pneumonia. With the implementation of this vaccine in many regions of the world where NTHi disease is endemic, it will be critical to introduce surveillance programs wherever it is used.

Introduction

Nontypeable *Haemophilus influenzae* (NTHi) is a common microbe isolated from the upper respiratory tract of both children and adults. Despite its commensal colonization profile NTHi has been identified as the causative agent of a number of diseases with significant socioeconomic impacts. The disease spectrum of NTHi includes serious diseases such as pneumonia, otitis media and invasive diseases, particularly bacteraemia and meningitis. In addition, NTHi is renowned for causing disease primarily associated with mucosal surfaces such as sinusitis, conjunctivitis and exacerbations of chronic bronchitis.

Carriage

In developed countries, NTHi is carried in the nasopharynx of up to 40% of children (1-3). However, children in many developing countries such as Papua New Guinea (PNG) and in indigenous nations within developed countries are heavily colonized in the nasopharynx by NTHi, as well as *Streptococcus pneumoniae* and *Moraxella*

catarrhalis within weeks of birth (4,5). Whilst it has been suggested that this very early and heavy colonization of the upper airways may downregulate mucosal immune responses (6), further studies are required to confirm this observation and to assess the clinical relevance of carriage-induced hyporesponsiveness.

Burden of respiratory disease

Respiratory infections account for greater than 50% of paediatric disease globally with greater proportions being reported in Africa and Asia (7). Pneumonia represents the greatest burden of disease, accounting for more than a third of all respiratory diseases and approximately 1.5 million deaths in children less than 5 years of age annually (8).

NTHi as a causative agent of pneumonia

Determination of the bacterial aetiology of pneumonia is difficult, with trans-thoracic fine-needle aspiration being the most reliable sample to culture. However, because of the potential risks associated with needle

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aspiration it is not used as a routine source of culture material (9). Furthermore, needle aspiration is only performed in the presence of consolidation. Hence the identified pathogens represent those associated with severe disease. Blood cultures are routinely conducted in laboratories but are only of value in cases of bacteraemic pneumonia. There are also issues of poor sensitivity of blood culture as well as the possibility that bacteria which do cross from the lung into the blood stream may not be reflective of the bacteria present in the infected lung. Blood culture favours capsular organisms and is therefore likely to underestimate the presence of NTHi in the lung. A number of studies have collected sputum from patients with pneumonia. As NTHi is often found in the upper respiratory tract of healthy subjects sputum samples can be technically criticized on the basis that contamination from the upper respiratory tract could occur. If sputum samples are to be considered it is important that they be screened for upper airway contamination by assessing the number of squamous epithelial cells (10-12). Studies comparing paired sputum and trans-tracheal aspirates (13) and sputum culture for *Haemophilus* species (14) have demonstrated that if sputum samples are selected on the basis of containing less than 25 squamous epithelial cells per low-power field and 2 or more alveolar macrophages then these sputum samples are valuable in determining the aetiology of bacterial pneumonia (13), particularly that caused by *Haemophilus* species (14). Nevertheless the collection of quality sputum samples from young children is difficult. More recently, bronchoalveolar lavage has been helpful in determining the bacterial aetiology of pneumonia, particularly as this sampling method has less risk of contamination from the upper airway secretions.

Studies in PNG in the early 1980s demonstrated that bacterial pneumonia was a major cause of child mortality (15,16). Whilst *H. influenzae* type b accounted for much of the *Haemophilus* isolated from the blood of children with bacteraemic pneumonia and the cerebrospinal fluid of children with meningitis, NTHi predominated in lung tissue of children with severe pneumonia. Furthermore, NTHi was often co-cultured in the lung tissue with *S. pneumoniae* or serotypeable *H. influenzae* (17). Outside PNG, lung aspirate studies to support an aetiological role for NTHi in

childhood pneumonia have had mixed results, suggesting that there are possibly geographical as well as socioeconomic determinants of the bacterial aetiology of pneumonia. In South Africa, Nigeria and Zimbabwe lung aspirate samples were positive for NTHi on 15%-40% of occasions (18-20). In a very recent study in The Gambia 50 lung aspirate samples obtained from children under 5 years of age were analysed by both culture and molecular typing (21). As might be predicted molecular typing was found to be much more sensitive than routine culture. *S. pneumoniae* was found in 92% of the samples whilst *H. influenzae* was found in 20%. However, *H. influenzae* was always detected with *S. pneumoniae*. All the *Haemophilus* detected was non-type b with half being NTHi. In the early 1970s, 2 studies were reported from the USA (22,23). The first of these studies (23) reported the presence of *H. influenzae* in 5% of aspirates that produced positive cultures. Unfortunately, no serotyping of the cultured *Haemophilus* was conducted and the media was not ideal for the culture of *Haemophilus*. In the second study *Haemophilus* was not cultured from any of 27 lung aspirates taken from children with pneumonia aged 2 months to 15 years. *S. pneumoniae* was the predominant bacterium isolated (22). In a study from Chile NTHi was not cultured from 31 lung aspirate samples (24). Surprisingly, in the Chilean study NTHi was not present in cultures of the nasopharynx, suggesting a possible technical problem for the culture and identification of NTHi.

Despite the disadvantages of blood culture, NTHi has been detected in 2%-10% of samples from children with bacteraemic pneumonia (17,25-27).

In two studies of community-acquired pneumonia, sputum results are informative. A study conducted in Singapore of children aged 1 month to 16.3 years (median 4.2 years) demonstrated that NTHi predominated among the *Haemophilus* species detected (94%) and was present in 22% of samples in which bacteria were identified (28). The second study, conducted in Australia, isolated bacteria from approximately a third of sputum samples collected from children aged 4-15 years; in a third of these positive samples NTHi was isolated. However, NTHi was not detected in blood cultures, where *S. pneumoniae* predominated (29).

Bronchoalveolar lavage has recently been used to assess bacterial aetiology in children with community-acquired bronchopneumonia (30). In this study *H. influenzae* was the predominant bacterium isolated with most strains being nontypeable.

Overall, the published literature suggests that NTHi can be a causative agent for bacterial pneumonia without any predisposing risk factors. However, there are some clear geographical differences and further studies are required not only to determine more accurately the distribution and burden of disease due to NTHi, but also to assess the impact of pneumococcal conjugate vaccines on NTHi disease.

The need for a vaccine

Of the bacterial pneumonias in children, NTHi pneumonia is clearly the most prevalent after that caused by *S. pneumoniae*, and in some regions NTHi may predominate or at the very least be a significant co-infecting organism. Whilst there are very poor surveillance data for NTHi pneumonia, it is not unreasonable to predict that following the introduction of pneumococcal conjugate vaccines containing a greater number of serotype valencies the prevalence of NTHi pneumonia will increase. This is most likely to occur in regions where there is high carriage load of NTHi in the upper airways. Therefore, there is a need to develop a vaccine against NTHi. When the total burden of NTHi disease (otitis media, exacerbation of chronic bronchitis and invasive disease) is considered, the case for an NTHi vaccine is even more compelling (31). The development of a vaccine by GlaxoSmithKline (GSK, Belgium), Synflorix™, where the pneumococcal polysaccharides are conjugated to an active carrier from NTHi, protein D, is an innovative step. However, it will be important to implement appropriate surveillance to monitor the impact of this vaccine on both pneumococcal and NTHi disease.

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The bacteriology of lower respiratory infections in Papua New Guinean and Australian Indigenous children

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SUMMARY

Indigenous children in Australia and children in Papua New Guinea (PNG) share a high burden of respiratory disease. In PNG the focus has been on pneumonia as a major cause of mortality. While pneumonia incidence remains high in Australian Indigenous children, improved access to better health care has resulted in reduced mortality. However, severe and recurrent pneumonia are risk factors for chronic suppurative lung disease or bronchiectasis in Australian Indigenous children. Bronchiectasis is associated with significant morbidity, and early death in adulthood. This paper includes an outline of the disease manifestations of acute and chronic lower respiratory infections. The main bacterial pathogens involved in pneumonia, bronchiolitis, bronchitis and bronchiectasis have been determined. Capsular organisms such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type b are more often implicated in acute infections, while chronic infections are frequently associated with nontypeable (noncapsular) *H. influenzae*. *Moraxella catarrhalis* is more often isolated from very young children. Possible reasons for the high burden of respiratory disease in Papua New Guinean children and Australian Indigenous (primarily Aboriginal) children include early and dense colonization with multiple species and strains of respiratory pathogens. There is a role for vaccines in preventing lower respiratory infection.

Introduction

Children in Papua New Guinea (PNG) and Indigenous (mainly Aboriginal) children in Australia share a high burden of respiratory disease. In PNG the focus has been on pneumonia as a major cause of mortality (1,2). In Australia, while the incidence of pneumonia and other lower respiratory infections remains high in Indigenous children (3), mortality has been reduced (4) as a result of improved access to better health care. However, pneumonia (particularly severe and recurrent pneumonia) has been shown to be a risk factor for chronic suppurative lung disease (CSLD) or bronchiectasis in Australian Indigenous children (5). Bronchiectasis is associated with significant morbidity, and early death in adulthood (6).

Figure 1 shows the relationship between acute and chronic respiratory diseases and the bacteria associated with them. A brief description of the main acute and chronic infections is presented, followed by evidence of their bacterial aetiology. The microbiological factors contributing to the burden of disease and the role of vaccines in preventing disease are then discussed.

Acute lower respiratory infections (ALRIs)

The two most common ALRIs resulting in hospitalization are bronchiolitis and pneumonia (7). Their importance is reflected not only in direct morbidity and mortality but also in long-term consequences. Recurrent hospitalization for ALRIs and severity of illness are risk factors for bronchiectasis in Australian Indigenous children (5). Data from The Gambia also suggest a link between severe early childhood pneumonia and later chronic lung disease (8).

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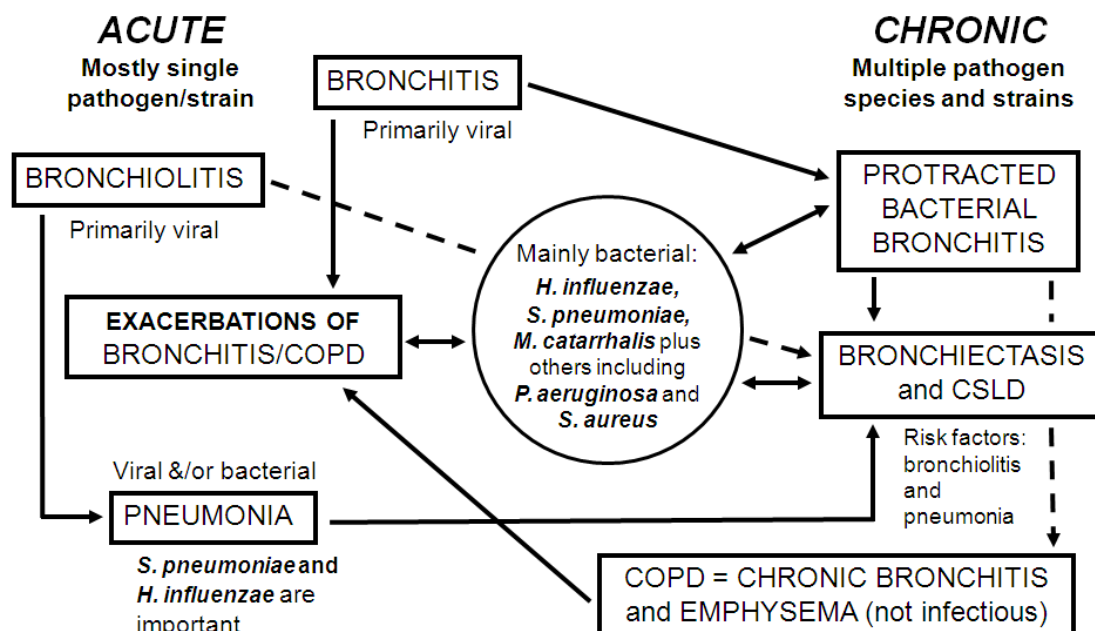
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Figure 1. Relationship between acute and chronic lower respiratory diseases and their associated pathogens: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.
COPD = chronic obstructive pulmonary disease
CSLD = chronic suppurative lung disease

Bronchiolitis is the most common ALRI in young children (usually ≤12 months of age) and is characterized by extensive inflammation of the airways and increased mucus production (7). It is a clinical diagnosis, characterized by rapid breathing and wheeze or crepitations following an upper respiratory illness, and is primarily caused by viral infection of the respiratory epithelial cells (7). There are no published data on bronchiolitis in PNG children. Indigenous infants in the Northern Territory (NT) of Australia have a higher incidence of bronchiolitis than non-Indigenous children and higher rates of coexistent clinical pneumonia (9). Bronchiolitis accounted for most of the disease burden in Indigenous infants hospitalized in the NT in the first year of life, with a rate of 227 per 1000 child-years for the years 1999-2004 (3).

Pneumonia is characterized by inflammation of the lung parenchyma (alveoli, excluding the bronchi) and congestion/abnormal alveolar filling with fluid (consolidation and exudation). It is caused by viruses and/or bacteria. As a major cause of mortality, pneumonia has a long history of research in PNG. In 1991 it was estimated that pneumonia accounted for approximately 30% of all deaths in children <5 years of age (10). From verbal autopsy reports in the Tari Basin between 1971 and 1995, pneumonia accounted for 50% and 33% of infant and toddler deaths respectively (1).

Despite a significant reduction in infant mortality in Aboriginal Australians since the 1960s, 'respiratory disease' (predominantly pneumonia) still caused 18% of all Aboriginal infant deaths in the NT between 1979 and 1983 (4,11). Morbidity rates remain high, with an ALRI incidence of 427 episodes (227 attributed to bronchiolitis) per 1000 child-years reported for Indigenous infants admitted to hospital in the NT during the years 1999-2004 (3). Compared to non-Indigenous children, Indigenous children have increased rates of pneumonia, a higher frequency of repeated hospitalizations for pneumonia and a greater propensity to develop CSLD (12).

Another lower respiratory infection, bronchitis, is defined as inflammation of the mucous membranes of the bronchi. Acute bronchitis often occurs during the course of an acute viral illness such as the common cold

or influenza. Viruses cause about 90% of cases while bacteria account for less than 10% (13). There is very little literature relating to acute bronchitis in children, presumably because it is a self-limiting disorder for which antibiotics are not usually justified (13). There are no data available specific to acute bronchitis in Papua New Guinean or Australian Indigenous children.

Chronic lower respiratory infections

Chronic wet cough may be caused by several interrelated endobronchial infections: persistent or protracted bacterial bronchitis (PBB), CSLD and bronchiectasis (14). Neutrophilic airway inflammation features in all three conditions and impaired mucociliary clearance seems to be the common risk factor, allowing bacteria to colonize the lower airway (14). Clinically these conditions overlap; whether they are different conditions or reflect severity as part of a spectrum is yet to be determined (14). Misdiagnosis of asthma is common, complicated by the fact that coexistence of asthma is not uncommon (14).

PBB is defined as the presence of chronic (>4 weeks) wet/moist cough, resolution of cough with antibiotic treatment and absence of pointers suggestive of an alternative specific cause (14). This condition has only recently been adequately characterized in children, both clinically and by bronchoscopic examination (15). As determined by bronchial lavage bacterial infection of the airways is the most common cause of chronic cough in Australian children, with few respiratory viruses detected (15). Airway neutrophilia is present in these children, and treatment is based on eradicating the bacteria with antibiotics. No data specific to Papua New Guinean or Australian Indigenous children have been found. PBB is increasingly recognized as an important diagnosis, the treatment of which may prevent progression to bronchiectasis (16).

Untreated PBB is a likely precursor to CSLD and bronchiectasis (Figure 1) (14). The term CSLD is used to describe a diagnosis where there are clinical symptoms of bronchiectasis without evidence from a chest high-resolution computed tomography (HRCT) scan (14). Bronchiectasis is defined as irreversible dilatation of peripheral airways (bronchi) which has been HRCT confirmed. At the milder end of the spectrum it appears that

radiographic changes may be reversible (17). Extra mucus tends to form and pool in the parts of the airways that are widened, rendering them prone to infection (18). Antibiotics are the mainstay of treatment: with mild bronchiectasis, a course of antibiotics is needed occasionally to clear chest infections as and when they occur; however, with more severe bronchiectasis, chest infections may return quickly following cessation of antibiotics and prophylactic antibiotics may need to be taken regularly (18).

No reference could be found for bronchiectasis in children in PNG. However, it is likely that high rates exist as with Australian Indigenous children. In Central Australia, radiologically confirmed bronchiectasis is present in 1.5% of Indigenous children aged <15 years, which is one of the highest rates recorded in the world (12). While there are management programs for cystic fibrosis (CF) (the commonest cause of bronchiectasis in non-Indigenous children) which aim to prevent disease progression, there are no parallel concerted programs or dedicated resources to manage children with non-CF bronchiectasis (who are mostly Aboriginal children in Australia). These children have significant morbidity and some succumb to premature death in adulthood (19). Non-CF bronchiectasis is now uncommon in developed countries, but persists in developing countries and other disadvantaged populations (20). These differences are partly attributed to overcrowding and poor living conditions.

While chronic obstructive pulmonary disease (COPD) is primarily a disease of adults, it is included here as a potential consequence of lower respiratory infections in childhood (Figure 1). COPD refers to chronic bronchitis and emphysema, two commonly coexisting diseases of the lungs in which the airways become narrowed. Chronic bronchitis is characterized by the presence of a productive cough and usually develops due to recurrent injury to the airways caused by inhaled irritants. While cigarette smoking is the most common cause, COPD is not simply a 'smoker's cough' but an underdiagnosed, life-threatening lung disease (21).

Acute exacerbations of chronic bronchitis (AECB) are a major contributor to morbidity and mortality in patients with COPD (22).

AECB can be caused by allergens, toxins, or acute viral or bacterial infections. However, bacterial agents are the predominant cause and the acquisition of new strains has been linked with episodes of AECB (23).

Chronic lung disease (primarily COPD) is a major problem in PNG. A survey of 510 adults in the Asaro Valley found a high prevalence of loose cough (36%) and chronic bronchitis (25%) (24). Demographic surveillance in the Tari Basin found that respiratory disease (particularly chronic lung disease in adults) accounted for 39% of all deaths (1). The role of smoking is unclear, and complicated by differences between traditional tobacco and western-style cigarettes. Grimley found that smoking was significantly related to loose cough and chronic bronchitis (24). However, Anderson and colleagues found that, unlike chronic obstructive lung disease in European populations, tobacco smoking is not an important aetiological factor in PNG (25). Although there is no direct evidence, the most likely alternative aetiologies are domestic wood smoke and acute respiratory infections in childhood (24,26).

In Australia, COPD is a serious disease that is increasing in prevalence, yet is underdiagnosed and under-recognized (27). Data on CSLD and bronchiectasis in Australian adults are lacking (28). However, bronchiectasis remains a significant cause of morbidity and mortality in Indigenous adults in Central Australia (6).

Aetiology of acute lower respiratory infections

Whilst the focus here is bacterial, recent animal and epidemiological studies indicate that respiratory viral infections increase susceptibility to bacterial superinfection, and interactions between bacteria and viruses result in greater severity of disease (29,30). Attempts to establish the aetiology of pneumonia and other ALRIs have been frustrated by the difficulty of detecting causative organisms (31). Lung aspiration is rarely done and sputum examination is difficult in young children (7). Blood cultures can only be successful when the infection has become bacteraemic, and even then their sensitivity is low, especially when antibiotics have already been administered (7). A study of the aetiology of 322 ALRI cases in 280 Central Australian Aboriginal children found only 20 blood cultures positive for bacteria

(32). Interestingly, antimicrobial substances were present in 6 of 10 blood-culture-positive cases examined, and the proportion of patients with a positive pneumolysin antibody test was not significantly different in those with or without antimicrobial substances (32). However, antibiotic resistance was not reported.

Aetiology of pneumonia

Despite problems of detection, the importance of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib) as major causes of pneumonia in children has been established by blood culture, by lung aspiration studies and, more recently, by vaccine probe studies (33). *S. pneumoniae* is the main cause of pneumonia in almost all studies around the world (34). Recent vaccine trial data indicate that in Africa it may be responsible for over 50% of severe pneumonia cases, and probably a higher proportion of fatal cases (35). This proportion may vary in different parts of the world. *H. influenzae* is also a major cause of pneumonia, with most disease caused by Hib (34). Vaccine studies from Bangladesh, Chile and The Gambia suggest that Hib causes around 20% of severe pneumonia cases (36,37). With the introduction of Hib conjugate vaccines, the incidence of pneumonia (and meningitis) caused by Hib has been greatly reduced; however, not all countries have these vaccines.

Other important pathogens causing pneumonia in children (32,34,38-41) include viruses such as respiratory syncytial virus (RSV) and influenza; other bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, *Neisseria meningitidis*, *Escherichia coli* and other enterobacteriaceae; *Chlamydia* species; and the fungus *Pneumocystis*, which is particularly important in young children with AIDS. Detailed coverage of these pathogens is beyond the scope of this review, which is primarily concerned with the three main respiratory bacteria, namely *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*.

Nontypeable *H. influenzae* (NTHi) may be an important pneumonia pathogen in children in some populations but evidence is limited (7). NTHi is rarely cultured from blood and therefore lung aspiration studies are especially valuable (42,43). The seminal

study by Shann and colleagues on the aetiology of pneumonia in 83 children in Goroka Hospital, PNG found (from lung aspirates and blood) that 40% had *H. influenzae* (19% of strains typed were Hib and 56% NTHi), 34% *S. pneumoniae* and 11% *Branhamella (Moraxella) catarrhalis* (41). Both *H. influenzae* and *S. pneumoniae* were found in 22% of children. In the same paper, the authors reported that *H. influenzae* and *S. pneumoniae* (or both) were also the most frequently isolated bacterial pathogens in 12 lung aspiration studies conducted in less developed countries (41). That *H. influenzae* pneumonia is usually caused by Hib is largely based on culture results from blood and cerebrospinal fluid, and Hib is more likely to invade the bloodstream from the lung than NTHi (41). Other serotypeable (non-Hib) *H. influenzae* and NTHi are more frequently isolated in PNG than in developed countries (44), with NTHi accounting for 23% of *H. influenzae* bacteraemia (45).

While NTHi pneumonia may also be important in Australian Indigenous children, it was not found in the Central Australian study by Torzillo and colleagues (32). Only 20 blood cultures were positive for bacteria: 11 were positive for *H. influenzae* (all typeable, 10 Hib) and 6 positive for *S. pneumoniae*. However, pneumolysin results (92 of 322 positive) suggest that at least one-third of ALRI in this population is caused by the pneumococcus. Of the 322 ALRI cases an aetiological agent was identified in 219 (68%) and in 20% of these co-infection with chlamydia and/or viral and/or other bacterial infection was found (32).

The Gambia has 20 years of experience of lung aspiration, with no deaths and few complications (46). An early lung aspiration study of 64 patients with pneumonia (51 children <10 years of age) found *S. pneumoniae* in 51% and *H. influenzae* in 24% of children: 4 of 13 typed isolates (31%) were NTHi (47); 5 (12%) of the 43 patients with a bacterial aetiology had multiple pathogens identified. Detection has improved with new molecular methods. In a more recent Gambian study, detection of *S. pneumoniae* in 50 lung aspirates from children with severe pneumonia was improved from 24% on culture to 92% using PCR, while detection of *H. influenzae* was improved from 0% on culture to 20% using PCR (48). All *H. influenzae* were found in co-infection with pneumococcus, and 5/10 were NTHi (48). Therefore NTHi would

appear to be an important pneumonia pathogen in Gambian children, though not to the same extent as in PNG (found in 8-10% versus >20% of affected children).

NTHi is a common cause of pneumonia in adults (49). In a prospective study of 170 patients with acute pneumonia in PNG, *H. influenzae* was found (in cultures of blood or lung aspirate or both) in 15 cases (9%): 7 were Hib and 3 NTHi (50). In all 15 cases *H. influenzae* was the sole organism isolated and, interestingly, chronic lung disease was significantly more common in patients with *H. influenzae* pneumonia than in patients with pneumonia due to other organisms. An earlier study in the US suggested that *H. influenzae*, both typeable and nontypeable, is a more frequent cause of pneumonia in adults than previously appreciated (51). Following widespread vaccination against Hib, NTHi was found to cause 79% of *H. influenzae* pneumonia cases in Spain (52). In a review of pneumonia studies from around the world (mainly in adults), the most common pathogens (in order of frequency) were *S. pneumoniae*, *H. influenzae*, *H. parainfluenzae*, *S. aureus*, *M. catarrhalis* and *K. pneumoniae* (53).

Aetiology of bronchiolitis

RSV bronchiolitis is the leading cause of paediatric admissions in the first year of life (54). RSV is identified in about 70% of hospitalized infants with bronchiolitis (55); other viruses implicated include adenovirus, rhinovirus, and influenza and parainfluenza viruses (7,56,57). Single viral infections are most common, but co-infection with two or more viruses is found in about a third of cases (56,58). Bacterial co-infection is generally uncommon, except in severe cases. In five studies of bronchiolitis or wheezing (presumably bronchiolitis) in infants and young children with bacterial co-infection, the bacteria detected most frequently were *H. influenzae*, *M. catarrhalis*, *S. pneumoniae* and *S. aureus* (56,57,59,60 and KMH and colleagues, unpublished) (Table 1). Only our Australian study reported whether *H. influenzae* strains were typeable or nontypeable (unpublished data).

In studies listed in Table 1 all children were hospitalized, except in the Danish study, in which children with no wheeze were compared to children with wheezy episodes or clinical pneumonia; wheezy episodes were

TABLE 1

STUDIES REPORTING UPPER OR LOWER AIRWAY BACTERIOLOGY IN CHILDREN WITH BRONCHIOLITIS (OR WHEEZING)

Study	Year	Population	Median age	Number and Condition	Specimen	Haemophilus influenzae	Streptococcus pneumoniae	Moraxella catarrhalis	Staphylococcus aureus
Thorburn (59)	2006	United Kingdom	1.6 months (interquartile range 0.5 to 4.6 months)	165 severe RSV bronchiolitis	Lower airway secretions	17% any growth 10% >10^5 CFU/ml	7% any growth 4% >10^5 CFU/ml	11% any growth 5% >10^5 CFU/ml	13% any growth 6% >10^5 CFU/ml
Franz (56)	2010	Germany	0.8 years (range 0 to 16 years)	244 (60%) wheezing 160 (40%) pneumonia	Nasopharyngeal aspirates	21% any growth	11% any growth	17% any growth	11% any growth
Bisgard (57)	2010	Denmark	12 months	279 No wheeze	Hypopharyngeal aspirates	26%	38%	29%	20%
			14 months	400 Wheeze		44%	46%	50%	15%
			15 months	144 Pneumonia		47%	59%	52%	9%
Duttweiler (60)	2004	Switzerland	1.7 months (range 0 to 5.8 years)	56 severe RSV bronchiolitis	Tracheal aspirates	30%	16%	21%	14%
Hare and colleagues, unpublished data	2010	Australia (35 Indigenous, 13 non-Indigenous)	4.9 months (range 0.5 to 11.2 months)	48 bronchiolitis	Nasopharyngeal swabs	44% (all NTHi)	23%	44%	10%

RSV = respiratory syncytial virus; CFU = colony-forming units; NTHi = nontypeable *Haemophilus influenzae*

independently associated with both bacterial and viral infection (57). In the German study of children hospitalized for acute virus-induced wheezing (60%) or pneumonia (40%), the median age was 0.8 years, with pneumonia more often found in older children (56). A positive bacterial culture was found in 198 (63%) of the nasopharyngeal aspirates collected on admission; 97 (31%) had "abundant pathogenic bacteria" and were included in viral co-infection analyses, where *H. influenzae* dominated in infections due to RSV identified as the sole virus and *M. catarrhalis* in infections due to RSV identified simultaneously with other viruses (56). The UK study found that up to 40% of children with severe RSV bronchiolitis had lower airway bacterial infection and were at increased risk of developing bacterial pneumonia (59).

Interestingly, *M. catarrhalis* was isolated more often than *S. pneumoniae* and almost as often as *H. influenzae* in all studies listed in Table 1. This probably reflects the young age of bronchiolitis patients, since *M. catarrhalis* mainly colonizes the very young or the very old (61). Data suggest that *M. catarrhalis* carriage is highest in children <18 months of age. While *M. catarrhalis* has long been known as an important pathogen in adults with COPD (62), it is also now considered an important pathogen in lower respiratory tract infections in children (63). However, even though *M. catarrhalis* was isolated from 11% of children in Shann and colleagues' pneumonia study (41), *M. catarrhalis* carriage is uncommon in Papua New Guinean children (D. Lehmann, personal communication); the reasons for this are unclear.

Aetiology of chronic lower respiratory infections

Only two studies were found reporting bacteriological findings in children with persistent bacterial bronchitis. In a prospective study of 108 (mainly non-Indigenous) Australian children with chronic wet cough (median age 2.6 years), 40% were diagnosed with PBB, with a positive bacterial culture $\geq 10^5$ colony-forming units (cfu)/ml bronchoalveolar lavage (BAL) fluid (15). Pathogens included *H. influenzae* (type unspecified, 47% of children), *S. pneumoniae* (35%) and *M. catarrhalis* (26%). More than one organism grew in significant numbers in an unspecified number of patients. A second

study of 81 UK children with PBB (median age 3.75 years) similarly found that the most commonly isolated organisms were *H. influenzae* (type unspecified) and *S. pneumoniae* (16). Pathogens were grown in over half of 51 cough swabs, with *H. influenzae* cultured in 81%, *S. pneumoniae* in 37% and both in 30% of positive swabs. Other organisms detected were *Moraxella* (unspecified) and other streptococci. Bronchoscopy culture results from 19 patients were reported to be similar to cough swab results but details were not given (16).

CSLD and bronchiectasis are associated with persistent infection with the same organisms in the airways as those found in PBB. The significance of *H. influenzae* in bronchiectasis of children was noted more than 50 years ago (64). After *H. influenzae* was found in 63% of 100 bronchoscopic aspirations from young adults, the authors detected *H. influenzae* in 84% of 32 children aged 4-15 years with purulent bronchiectasis (64). In both studies, the majority of strains were non-encapsulated (NTHi). These authors quoted from a previous study that stated "non-encapsulated *Hemophilus* (*influenzae*) is a pathogen" and "the etiology of bronchitis and that of bronchiectasis cannot possibly be understood if the part played by *Hemophilus* infection is overlooked." After observing a total of 204 relapses during 306 patient-months, mostly associated with reappearance of *H. influenzae*, the authors concluded that NTHi "is responsible for keeping the chronic inflammatory process smouldering in bronchiectatic individuals" (64).

The main bacteria associated with non-CF bronchiectasis in six more recent studies in children are listed in Table 2 (20,65-69). *H. influenzae* was the most common pathogen identified in all six studies (specified as NTHi in two studies), followed by *S. pneumoniae* and (in varying order of frequency) *M. catarrhalis*, *Pseudomonas* sp. and *S. aureus*. It is noteworthy that *M. catarrhalis* was isolated more frequently from the Australian Indigenous children, who were also younger than children in the other studies (Table 2). *H. parainfluenzae*, *Streptococcus pyogenes* and *K. pneumoniae* were listed as pathogens in one study each (20,65,67). Co-infection was reported in three studies: two or more organisms were isolated in 5% of New Zealand children (65), 15% of UK patients (66) and 18% of Australian Indigenous children with

bronchiectasis (69); if the denominator included only those with lower airway infection (defined as $>10^4$ cfu/ml BAL fluid), 33% had co-infection in the Australian study (69). The Australian study also reported a high proportion of multiple strains within pathogen species isolated from the lower airways: 67% for NTHi, 25% for *S. pneumoniae* and 22% for *M. catarrhalis* (69). The highest rates of NTHi isolation were among Australian Indigenous children and children in the New Zealand study, 80% of whom were of Pacific Islander or Maori descent (65,69). While *H. influenzae* also features in CF bronchiectasis, two other main pathogens are *P. aeruginosa* and *S. aureus* (39). In a study of non-CF bronchiectasis in 61 Central Australian adults (97% Indigenous), *H. influenzae* (type unspecified) was isolated from 38 (81%) of 47 positive sputum cultures, *P. aeruginosa* from 12 (26%) and *S. pneumoniae* from 9 (19%), and 21 (45%) had multiple pathogens isolated (6).

Common bacterial pathogens identified during acute exacerbations of COPD include *H. influenzae*, *S. pneumoniae* and *M. catarrhalis* (70). Exacerbations may also be associated with infection with *S. aureus* and *P. aeruginosa* (71). Viruses associated with exacerbations include rhinovirus, influenza virus, parainfluenza virus, coronavirus, adenovirus and RSV (71). An early study found that, while NTHi may have pathogenic potential in patients with COPD, *H. parainfluenzae* should be considered as normal flora (72). More recent studies suggest that *H. parainfluenzae* may have a pathogenic role in COPD (73), and it has been listed as a pathogen commonly associated with AECB (74).

Microbiological factors contributing to the burden of disease

It is likely that recurrent aspiration into the lower airways of pathogenic bacteria originating in the nasopharynx is an important contributor to lower respiratory infections (69,75-77). It is therefore of relevance that Papua New Guinean and Australian Aboriginal infants have high rates of nasopharyngeal colonization by potentially pathogenic respiratory bacteria from early infancy (78,79). During acute respiratory infections, repeated aspiration of bacteria-laden nasopharyngeal (NP) secretions may overwhelm local pulmonary defences, helping to initiate endobronchial infection, inflammation and airway injury, the central

tenet of Cole's 'vicious circle' hypothesis for the origins of bronchiectasis (80). High concordance between NP and lower airway (BAL) strains of respiratory bacteria in Australian Indigenous children with bronchiectasis suggests recent aspiration of NP secretions (69). Compared to the NP secretions, a higher proportion of BAL fluids harbour multiple strains, many of which are not found in the NP (69). It is plausible that recurrent aspiration over time leads to accumulation and persistence of strains in the lower airway, where immune clearance is poor. Recurrent acute respiratory infections therefore contribute to the development of chronic lung disease.

Australian Indigenous children (and most likely Papua New Guinean children) have higher bacterial loads than non-Indigenous children (81). Children from these populations also carry a greater diversity of species and strains. While carriage of multiple pneumococcal serotypes is reportedly rare (<3%) in other populations (82), 20%-30% of carriage-positive children in PNG and The Gambia as well as Australian Aboriginal children carry multiple serotypes (83-85). Carriage-positive Australian Aboriginal and Papua New Guinean children also harbour multiple *H. influenzae* strains: 44% have multiple ribotypes and 53% have multiple biotypes (83,86).

It is possible that the child's immune response to repeated acquisition of new strains may be overwhelmed, since antibody responses to pneumococci and *H. influenzae* are strain-specific (87,88). Nevertheless, while NTHi is a major cause of respiratory disease, it rarely causes systemic infection because it does elicit effective humoral immunity (89). However, chronic infection with NTHi in bronchiectasis is associated with non-clearing adaptive immunity that may be important in the pathogenesis of bronchial infection (90).

In addition, antibiotic regimens developed in low-risk populations may be inadequate for eradication of infections where there is a high bacterial load. Antimicrobial efficacy is based on pharmacodynamic and pharmacokinetic parameters including the requirement to maintain a sufficient concentration for an adequate period of time at the site of infection (91). Minimum inhibitory concentrations determined in first-world settings may not be appropriate for the

TABLE 2

STUDIES REPORTING LOWER AIRWAY BACTERIOLOGY IN CHILDREN WITH NON-CF BRONCHIECTASIS

Study	Year	Population	Age (years)	Number	Specimen	Haemophilus influenzae	Streptococcus pneumoniae	Moraxella catarrhalis	Pseudomonas sp.	Staphylococcus aureus
Edwards (65)	2003	New Zealand*	1 to 17 (median 10)	60	Sputum	55% (NTHi)	8%	5%	2%	0%
Karadag (20)	2005	Turkey	1 to 17.5 (mean 7.4)	111	Sputum	23% (type not specified)	14%	4%	6%	10%
Li (66)	2005	England	3.1 to 18.1 (median 12.1)	136	Cough swab, sputum or BAL	39% (type not specified)	17%	2%	11%	4%
Eastham (67)	2004	England	1.6 to 18.8 (median 7.2)	93	Cough swab, sputum or BAL	48% (type not specified)	22%	17%	6%	8%
Kapur (68)	2009	Australia	3 to 17 (median 5.5)	30	Sputum and BAL (85 specimens)	32% (type not specified)	15%	8%	8%	5%
Hare (69)	2010	Australia (Indigenous children)	0.7 to 10.1 (median 2.3)	45	BAL	78% any growth (NTHi) 47% >10^4 CFU/ml	33% any growth 18% >10^4 CFU/ml	27% any growth 20% >10^4 CFU/ml	4% any growth 0% >10^4 CFU/ml	9% any growth 4% >10^4 CFU/ml

*80% Pacific Islander or Maori descent
CF = cystic fibrosis; NTHi = nontypeable *Haemophilus influenzae*: BAL = bronchoalveolar lavage fluid; CFU = colony-forming units
All percentages are based on number of specimens

significantly higher bacterial loads found in disadvantaged populations (81). For example, azithromycin was highly effective (clinical success rate >80%) in treating acute otitis media (OM) in non-Indigenous children (92), whereas the same regimen achieved a success rate of only 50% in Australian Aboriginal children (93).

It has been suggested that early and dense colonization and carriage of multiple strains of potentially pathogenic respiratory bacteria may help to explain the chronicity of carriage and persistence of OM in Australian Aboriginal infants (79,86). These factors may also explain the recurrence and persistence of lower respiratory infections and the development of chronic lung disease in Australian Indigenous and Papua New Guinean children. The importance of household transmission and crowding as risk factors for carriage of respiratory pathogens has been demonstrated (94). A study in the Asaro Valley of PNG found that cohabitation with an adult complaining of chronic cough was a risk factor for ALRI in children (Rohan Grimley, 'Cohabitation with an adult complaining of chronic cough as a risk factor for acute respiratory infections (ARI) in

children', unpublished report, 1989). While respiratory infections are primarily spread by airborne droplets, indirect spread by hands is also believed to play a role. Hand contamination has been demonstrated in PNG (95), and shown to be higher in Indigenous than in non-Indigenous children in the NT (96). Poor hygiene, overcrowding and hand contamination are likely to facilitate frequent transmission of respiratory bacteria, contributing to high rates of infection. Figure 2, adapted from an OM model (97) which was in turn adapted from Cole's original model (80), shows an 'extended vicious circle' hypothesis to explain high rates of respiratory infection among Papua New Guinean and Australian Indigenous children.

Vaccines

New vaccines are needed to help reduce this heavy burden of disease. Conjugate vaccines have had a major impact on targeted diseases (including pneumonia) in countries where the vaccines have been introduced. Hib disease is now almost unknown in developed countries, and incidence of invasive disease and pneumonia caused by serotypes included in the 7-valent
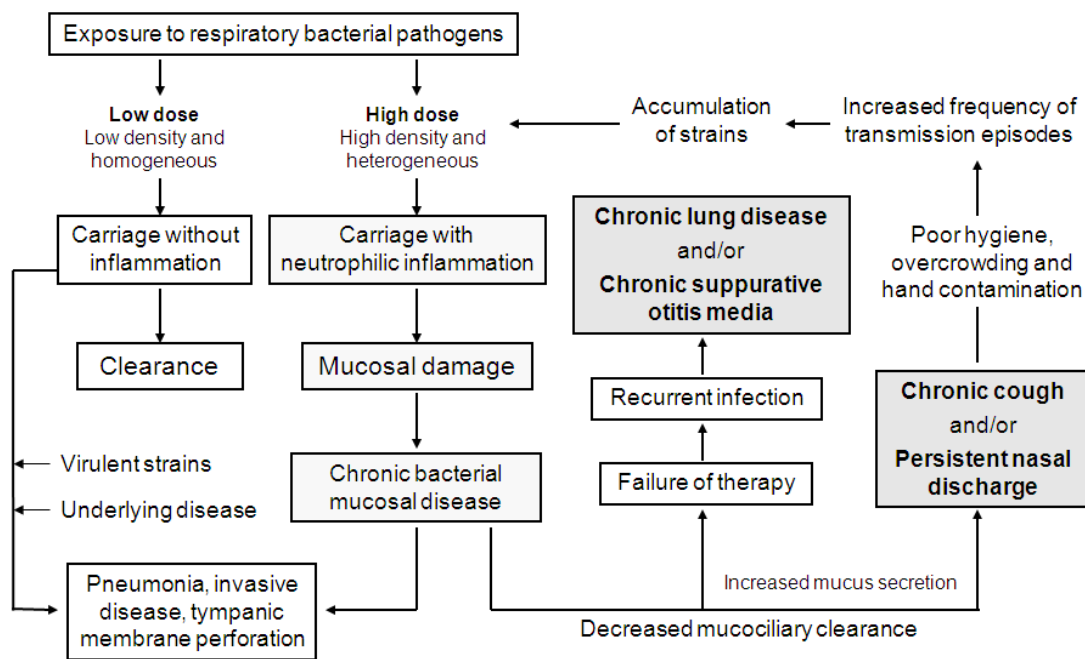

Figure 2. Extended vicious circle hypothesis explaining high rates of respiratory infection in Papua New Guinean and Australian Indigenous children.
Adapted from Wiertsema and Leach (97)

pneumococcal conjugate vaccine Prevenar® (which includes serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) have been substantially reduced (98,99). Hib vaccine was introduced to Australia in 1993 and resulted in a substantial decline in incidence of Hib disease, while Prevenar was introduced in 2001 for Indigenous and other high-risk infants (100,101). PNG only recently (in 2008) introduced a Hib vaccine, and has not yet introduced a pneumococcal conjugate vaccine into their infant vaccination schedule.

New conjugate vaccines on the market provide additional protection. The pneumococcal-nontypeable *H. influenzae* protein D conjugate vaccine, PHiD-CV (Synflorix®), provides protection against three additional serotypes (1, 5 and 7F) compared to Prevenar, and has been shown to protect against OM due to NTHi (102). At time of writing it is unknown whether Synflorix affords protection against pneumonia or other lower respiratory infections. Synflorix replaced Prevenar on the NT immunization schedule in October 2009. Prevenar13® has the same 10 serotypes as Synflorix plus an additional three (3, 6A and 19A), but does not have an NTHi component. This presents a difficult choice for vaccine policy-makers in regions with high rates of NTHi and pneumococcal infection. A trial in the NT aims to evaluate a combination schedule with both Synflorix and Prevenar13, while a study in PNG aims to assess safety and immunogenicity of each of these two vaccines in the routine accelerated 1-2-3-month schedule, followed by a booster at age 9 months with pneumococcal polysaccharide vaccine or no booster.

An oral vaccine containing killed NTHi (Broncostat®) significantly reduced the incidence of acute bronchitis in patients with chronic bronchitis in the Asaro Valley of PNG (103). This vaccine had earlier been found to afford protection in an Australian trial (104). A review of the PNG study and five Australian trials found that the vaccine reduced the number and severity of exacerbations for up to 6 months after vaccination, and the authors concluded that a large clinical trial was needed (105). NTHi was the most commonly isolated bacterium during an exacerbation, and the oral vaccine reduced carriage of NTHi in the upper respiratory tract (105). Recently a phase 2 clinical trial demonstrated that an NTHi oral immunotherapeutic vaccine (HI-1640V) reduced the number and severity of acute exacerbations of COPD (106). Results are consistent with the idea that oral NTHi enhances mucosal protection (107). It is possible that such a vaccine may reduce the incidence and/or severity of other respiratory infections caused by *H. influenzae*, namely bronchiolitis, pneumonia and bronchiectasis.

In studies of carriage, aetiology and vaccine impact, there is a need to differentiate between NTHi and non-haemolytic *H. haemolyticus*, a respiratory tract commensal (108). In a study of otitis-prone and healthy children in Western Australia, researchers found that 12% of NP isolates previously identified as NTHi were *H. haemolyticus* (109). Studies are underway to differentiate NTHi from *H. haemolyticus* in NP swabs from children who took part in a pneumococcal conjugate vaccine trial in PNG, and from NP and lower airway specimens from Indigenous Australian children with bronchiectasis.

Discussion

There are similarities and differences in the types of bacteria causing bronchiolitis, bronchitis, pneumonia and bronchiectasis in children. *S. pneumoniae* and *H. influenzae* feature in all respiratory bacterial infections, while *M. catarrhalis* is more likely to be associated with infections in infants with bronchiolitis or young children with pneumonia or bronchiectasis. The capsular organisms *S. pneumoniae* and Hib appear to be more important in acute respiratory infections and invasive disease, while NTHi features more in chronic lung disease. Acute infections are usually caused by single pathogens, although this is less true for Papua New Guinean and Australian Indigenous children, who frequently carry multiple species and strains of respiratory bacteria and suffer a high burden of respiratory disease. Chronic infections are more often associated with multiple pathogen species and strains. It also appears that NTHi may be more important in PNG children and Indigenous children in Australia and New Zealand than in more advantaged populations, though evidence is limited since in many studies *H. influenzae* serotype is not reported.

Ideally, children with pneumonia (especially severe and/or recurrent pneumonia) or persistent symptoms should be evaluated for an underlying condition such as bronchiectasis, since they are at risk of

developing chronic lung disease. There is also little doubt that improved living conditions (better nutrition and hygiene, less crowding and reduced indoor air pollution) would help reduce the high bacterial load and transmission of potential pathogens, and thus have a positive impact on disease outcomes. In the short to medium term, the rollout of new vaccines may provide more practical protection.

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Streptococcus pneumoniae serogroups and colony morphology: a look back

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SUMMARY

From 1985 to 1987, *Streptococcus pneumoniae* isolates were collected from children under 5 years of age in the Asaro Valley, Papua New Guinea as part of a study on bacterial colonization and respiratory tract infections. Data on serogroup and colony morphology were collected to survey serogroups and associated colony morphologies present in the area and to assess whether colony morphology can be indicative of serogroup. In total, 5989 colonies were examined; serogroups 6, 10, 14, 15, 19, 23, 33, 34, 35 and non-serotypeable strains were the most common and accounted for 77% of all the colonies, with serogroups 6, 19 and 23 accounting for 48%. The majority of colonies displayed the typical draughtsman morphology, though serogroup 10 and non-serotypeable isolates most often displayed a raised colony morphology. Of the 15 mucoid colonies identified 73% were serotype 3, though only 29% of serotype 3 isolates were mucoid. Thus colony morphology is of limited value in identifying the pneumococcal serogroup/serotype apart from mucoid colonies, which are likely to be serotype 3.

Introduction

Pneumonia is the leading killer of children under the age of five worldwide, and the Gram-positive bacterium *Streptococcus pneumoniae* (the pneumococcus) is the most common aetiological agent (1). *S. pneumoniae* is frequently carried in the nasopharynx of children and though colonization is generally asymptomatic, it is a prerequisite for diseases such as pneumonia and otitis media. Furthermore, carriage serves as a reservoir for maintaining strains of *S. pneumoniae* in human populations. Rates of colonization and subsequent disease are particularly high in developing countries. Studies on *S. pneumoniae* acquisition and carriage in Papua New Guinea (PNG) found that 100% of infants are colonized with *S. pneumoniae* by the age of three months (2,3). More recently, carriage rates of greater than 80% at the age of three months have been reported (4).

S. pneumoniae is a diverse species that is classified into immunologically distinct serotypes based upon capsular polysaccharides present on the bacterial surface. Over 90 different serotypes have been identified. Some are related to each other and belong to a single serogroup such as serotypes

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15B and 15C within serogroup 15, whereas some serogroups consist of a single serotype, such as serotype 3. *S. pneumoniae* is typically grown on blood agar and identified using phenotypic tests such as the presence of α-haemolysis, bile solubility and optochin sensitivity (5). The Quellung reaction, performed by incubation of bacteria with specific antisera, is considered the gold standard serotyping method (6). *S. pneumoniae* colony morphology can vary, although colonies typically display raised edges and a concave centre, referred to as a 'draughtsman' shape. Serotypes 3, 8 and 37 are generally associated with a mucoid colony morphology (7, 8). To our knowledge, a systematic examination of colony morphology of different pneumococcal serotypes identified in the nasopharynx has not been reported previously. Between 1985 and 1987 nasal swabs were collected monthly from children in highland communities located in the Asaro Valley near Goroka in the Eastern Highlands Province (3). *S. pneumoniae* isolates obtained from these samples were examined for colony morphology and serogroup in order to assess the variety of pneumococcal strains present in this population and determine if colony morphology can be used to identify particular serogroups.

Materials and Methods

A total of 1449 nasal swabs were collected from 158 study participants aged <5 years (3). Swabs were placed in Amies transport medium before plating on 5% horse blood agar and pathogens were identified as described by Montgomery and colleagues (3). In brief, *S. pneumoniae* was identified by standard methods using selective media. Four colonies were picked from each primary culture that grew and were subcultured; morphologically different colonies were selected if present. Colonies were then serogrouped with Statens Serum Institut antisera and colony morphology recorded. At the time of this study we were unable to do factor typing to establish serotypes within the same serogroup. We therefore report all results as serogroups. Each colony was recorded as having a single phenotype, either the typical or atypical draughtsman morphology or one of the common variants, such as flat, raised, irregular, mucoid or elongated. In total, 5989 colonies were examined.

Results and Discussion

A total of 37 different serogroups were identified from the samples. Table 1 lists the ten most common serogroups found in the 5989 colonies examined and, for each, the percentage of colonies that displayed the described morphology. Serogroups 6, 10, 14, 15, 19, 23, 33, 34, 35 and non-serotypeable strains were the most common and accounted for 77% of all the colonies, with serogroups 6, 19 and 23 accounting for 48% of colonies. The draughtsman morphology was the most common morphology for all of these serogroups with the exception of serogroup 10 and non-serotypeable strains, both of which most often displayed a raised colony morphology. The data suggest that colony morphology cannot be used to predict serogroup, as the draughtsman morphology dominated the majority of serogroups. The exception was serotype 3 (not included in table), which was the only serotype to frequently display a mucoid phenotype. Of the 15 mucoid colonies identified in this large dataset, 11 (73%) were serotype 3. Hence if a mucoid colony is present, it is likely to be a serotype 3. However, the utility of using the mucoid morphology to identify serogroup 3 is limited as it was only observed in 29% of the 38 colonies that were serotype 3. While serogroups 8 and 37 are known to be associated with the mucoid morphology (8), no mucoid colonies were observed for the 24 isolated serogroup 8 pneumococci, and we did not isolate serogroup 37 in this extensive carriage study. Colony morphology can be useful for identifying the presence of multiple serotypes in the same sample, although the same serogroup can display different morphologies (Table 1). Carriage of multiple serogroups is common in Papua New Guinean children: in this study, one-third of specimens contained 2 to 4 serogroups (3). Factor typing would no doubt have identified even more samples with simultaneous carriage of multiple capsular types.

In summary, these data provide a detailed evaluation of pneumococcal serogroups and associated colony morphologies carried by Papua New Guinean children living near Goroka during the mid-1980s. Although serotyping technologies have advanced since then, it is interesting to note that the most common serogroups at that time are still among the most prevalent in a period before

TABLE 1

NUMBER OF COLONIES, PERCENTAGE OF 5989 COLONIES EXAMINED AND ASSOCIATED COLONY MORPHOLOGIES OF THE TEN MOST COMMON SEROGROUPS OF *STREPTOCOCCUS PNEUMONIAE*

Serogroup*	6	10	14	15	19	23	33	34	35	NT
No of isolates	1246	158	297	251	1024	634	244	134	222	394
% of total	20.8	2.6	5.0	4.2	17.1	10.6	4.1	2.2	3.7	6.6
Draughtsman	44.4	19.0	41.4	43.4	38.7	37.1	42.6	55.2	31.1	32.0
Atypical draughtsman	12.0	17.1	19.5	17.1	11.8	16.7	20.9	15.7	9.0	13.2
Irregular	6.0	0.6	4.7	6.4	6.0	7.6	4.1	3.0	8.1	3.8
Flat	18.9	22.8	17.5	15.1	21.0	18.9	12.7	11.9	22.5	10.2
Raised	11.2	40.5	13.5	15.1	19.0	15.5	17.2	13.4	18.9	37.8
Elongated	2.1	0.0	0.0	0.8	1.1	1.4	1.6	0.0	0.9	0.3
Other	5.5	0.0	3.4	2.0	2.5	2.8	0.8	0.7	9.5	2.8

*For each serogroup, the number of colonies identified and the overall percentage of the 5989 colonies examined are shown in bold; the percentages of colonies displaying a designated colony morphology are listed in italics
NT = non-serotypeable

the introduction of pneumococcal conjugate vaccine in PNG (4). Although colony morphology is insufficient for identifying most serotypes, it can be a useful tool for identifying the presence of multiple serotypes in a single sample and can indicate the presence of mucoid serotypes. In more recent years, examination of the morphology of *S. pneumoniae* has also assessed opacity, since a transparent colony phenotype is associated with increased adherence and colonization compared to opaque phase variants of the same strain (9).

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Human immunodeficiency virus and respiratory disorders: clinical and diagnostic considerations

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SUMMARY

Respiratory infections are a major health burden for the people of Papua New Guinea (PNG) who are positive for human immunodeficiency virus (HIV). In the face of an ongoing HIV epidemic, little is known about the epidemiology and aetiology of respiratory infections in people living with HIV in PNG. In this article we provide an overview of the most important respiratory pathogens in HIV-positive people globally, focusing primarily on adults. Particular attention is given to respiratory viruses, bacterial pathogens such as *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*, and *Pneumocystis jiroveci*. In doing so we highlight the need for a better understanding of the aetiology of respiratory infections in HIV-positive people in PNG. A study is underway that aims to determine the aetiology of common infectious illnesses in HIV-positive people in PNG, focusing on respiratory infections, diarrhoeal diseases and febrile illness. The results of this study should guide future prevention, diagnostic and treatment strategies.

Introduction

Human immunodeficiency virus (HIV) was first detected in Papua New Guinea (PNG) in 1987. Since then the infection has spread, although to what extent remains unknown. It was estimated that 34,100 people were living with HIV in 2009, with an adult seroprevalence of 0.9% (1). Regardless of the exact number of people currently living with the virus, it is clear that many circumstances exist in PNG that favour the ongoing spread of HIV. Of the almost 7 million people living in PNG approximately 85% live in rural areas. The majority of the population practise subsistence-based agriculture and about one-third of the population live on less than US$1.25 per day. Access to services, health care and education is often poor for these rural people, who have low disposable incomes. In addition, previous studies have demonstrated a high rate of sexually transmitted infections

(STIs) in the community (2), suggesting that sexual practices are conducive to the spread of HIV in PNG.

A national program for antiretroviral therapy (ART) was commenced in 2004, with the rapid scale-up of treatment services throughout the country. There are now 174 treatment centres providing ART country-wide. There are over 6700 people receiving treatment, an estimated 75% of people in need of treatment (1). The success of ART to date has relieved the burden of HIV on the broader health system to some degree. However, with the ongoing spread of the disease and prolonged use of ART, HIV remains a considerable burden on the health care system. Given the nature of the human immunodeficiency virus (which itself has a pathological effect on the respiratory system by depleting local T-cell immunity, as well as causing generalized immunosuppression) and the already high burden of respiratory

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infections in PNG (3), the interaction between HIV and respiratory infections in PNG warrants further attention. To date there is a paucity of such data specific to PNG. In the absence of local data a review of the literature from other low-income settings may provide an insight to what we might expect in PNG.

HIV and respiratory viruses

Most common viral respiratory pathogens are not normally considered to be opportunistic infections. Outcomes for viral respiratory tract infections are, however, starkly different in countries with poor health services and low rates of HIV treatment from those in more affluent countries where HIV treatments are provided to all that require them. Recent data pertaining to children admitted to a hospital in rural Mozambique demonstrated that around half the children with severe pneumonia had one or more respiratory viruses detected. The most commonly detected viruses were, in order of frequency, rhinoviruses, adenoviruses, respiratory syncytial virus, human metapneumovirus, influenza viruses, parainfluenza viruses and enteroviruses. In this study, where 25% of children were HIV infected, the rate of viral infection was between 5.5 and 16 times higher in HIV-infected children than in non-HIV-infected children and the in-hospital case fatality was around 7 times higher at 18% (4).

Vaccination to prevent influenza is recommended in HIV-infected patients (5). The response to influenza vaccine may be suboptimal in those patients with lower CD4 cell counts, those who have already developed acquired immunodeficiency syndrome (AIDS) and those not receiving ART (6). However, a normal antibody response has been detected in all studies addressing this issue and, as with vaccination against important bacterial pathogens (see section on bacterial pneumonia in HIV), there remains considerable debate over the use of vaccines in HIV-positive people in resource-poor settings.

In 2009 a new strain of pandemic H1N1 influenza emerged. The clinical course in a group of 15 HIV-infected children was observed, all of whom were on ART and had CD4 cell counts >350 cells/mm^3. The clinical course and viral shedding kinetics were not different to those seen in non-HIV-infected children (7). In adults also the clinical picture is similar in those infected with HIV and those not infected. Antiviral medication effective against influenza (including pandemic H1N1) is available: oseltamivir (oral tablets) and zanamivir (inhaled agent). Neither drug interacts with antiretroviral medications; however, they are likely to be less available in low-income countries such as PNG than in high-income countries. Fortunately, to date, few cases of pandemic H1N1 have been detected in PNG.

Various other viruses are associated with respiratory infection in HIV-positive people. In profoundly immunosuppressed individuals, cytomegalovirus (CMV) can cause pneumonitis along with serious ocular and gastrointestinal infections (8). Measles can cause pneumonitis, which can be more severe in HIV-infected individuals. There is a case report of vaccine-strain pneumonitis after measles-mumps-rubella (MMR) vaccination in a severely immunodeficient person (9). The benefits of MMR vaccine far outweigh the risk and, as such, non-immune individuals should be vaccinated. Vaccine coverage and seroconversion following measles vaccination in PNG are suboptimal (10,11); inadequate overall vaccine coverage is evidenced by a high rate of subacute sclerosing panencephalitis in children reported recently in Madang Province (12). Thus HIV-positive people in PNG may be at risk of respiratory complications due to measles infection because of low vaccination coverage.

Bacterial pneumonia and HIV infection

Bacterial pneumonia is a major health concern in HIV-positive people, even in those with access to ART. In a meta-analysis of bacterial pneumonia in adults with HIV, Feikin and colleagues found the incidence of bacterial pneumonia to be up to 25 times higher in HIV-positive people than in the general population in both low-income and developed settings (13).

Globally, the most common cause of bacterial pneumonia in HIV-infected individuals is *Streptococcus pneumoniae*, commonly referred to as the pneumococcus (13). It is not normally considered an opportunistic pathogen. The pneumococcus is a major cause of pneumonia, meningitis and bacteraemia in the general population throughout the world (14) and it has long been recognized as an important pathogen

in both childhood and adult pneumonia in PNG (15,16). In HIV-infected individuals, *S. pneumoniae* accounts for approximately 40% of cases in whom a specific diagnosis is made. Other common causes of non-mycobacterial bacterial pneumonia in HIV-positive people include *Haemophilus influenzae* (10%-15% of cases), *Staphylococcus aureus* (~5%) and Gram-negative bacilli, in particular *Pseudomonas* spp. (~5%). Other organisms that are less commonly sought in diagnosis such as *Chlamydophila pneumoniae* (17) may also cause community-acquired pneumonia (CAP) in HIV-positive people, although the role of such pathogens is poorly understood, particularly in low-income countries.

Although the most recent data are over 20 years old (18), it is likely that the main cause of bacterial CAP in children (regardless of HIV status) in PNG remains *S. pneumoniae*. Given the high burden of CAP in children and adults in PNG (3) and the high carriage rates of *S. pneumoniae* (19) (which facilitates the circulation of the pathogen within the community), it is likely that *S. pneumoniae* is an extremely important, and perhaps underrated, cause of CAP in HIV-positive people in PNG.

The introduction of ART has led to a reduction in bacterial pneumonia rates in industrialized countries (20), although rates remain higher in HIV-positive people than in the general population. With the rollout of ART in many low-income countries, similar reductions to those in industrialized countries are likely to be observed though robust epidemiological data are difficult to obtain. In PNG the rollout of ART has been largely successful to date in terms of access to medication, but assessment of the effectiveness of ART by way of viral load, CD4 count or clinical indicators has not been conducted. Nonetheless, in individual cases there have been improved health outcomes for HIV-positive people accessing ART in PNG.

In addition to ART, cotrimoxazole (Septrin) prophylaxis is now commonplace in both low-income and industrialized settings, and is recommended by the World Health Organization (WHO). In PNG cotrimoxazole is routinely administered to registered HIV-positive people. Although no studies have been conducted in PNG, numerous studies have demonstrated that cotrimoxazole

prophylaxis reduces morbidity and mortality associated with HIV infection and is cost-effective in low-income settings (21). Moreover, cotrimoxazole prophylaxis has been shown to protect against pneumonia in a low-income setting (22). It is likely that cotrimoxazole prophylaxis has conferred positive health outcomes for people living with HIV in PNG.

The use of pneumococcal vaccines in HIV-positive people has been a contentious issue since French and colleagues demonstrated a detrimental effect of 23-valent pneumococcal polysaccharide vaccine (23vPPV) in a randomized controlled trial in Ugandan adults (23). However, a follow-up to that study found that vaccinated participants had better survival rates than unvaccinated controls, particularly those with a CD4 count of 200-500 cells/mm^3 at the time of vaccination (24). An immunogenicity study conducted in Spain showed that patients with a CD4 count above 200 cells/mm^3 if on highly active ART (HAART) or 350 cells/mm^3 if they had never received HAART mounted a satisfactory response to 23vPPV (25). The availability of pneumococcal conjugate vaccines (PCV) has not clarified the issue of pneumococcal vaccination in HIV-positive people. Greater increases in IgG levels in people vaccinated with PCV (as a revaccination 3-8 years after PPV) compared to those in people revaccinated with PPV were short-lived, with no significant difference in immune response after 180 days (26). A study in Malawi demonstrated a protective effect of the 7-valent PCV (7vPCV). Adults (88% of whom were HIV-positive) who had recently had an episode of invasive pneumococcal disease (IPD) were given two doses of 7vPCV. HIV-positive participants receiving the vaccine were protected from IPD caused by vaccine serotypes (and vaccine-related serotype 6A) relative to controls (placebo), with efficacy highest in the first 12 months following vaccination (27). 50% of IPD in the placebo group was caused by non-vaccine serotypes. The coverage of the serotypes that commonly cause disease in HIV-positive people in low-income countries may be limited with PCV, as is the case in the broader community, although conjugate vaccines providing a broader coverage are now available (10-valent and 13-valent). It is not possible to make sound recommendations on the suitability of pneumococcal vaccines in HIV-positive

people in PNG on the current evidence available; however, 23vPPV is currently recommended in some countries and should be considered for use in PNG if the burden of pneumococcal disease in HIV-positive people is shown to be high in this country.

Tuberculosis and HIV infection

Around one-third of the world's population is infected with *Mycobacterium tuberculosis*, and immunodeficiency associated with HIV infection greatly increases the risk of progression from latent to active tuberculosis (TB). TB can be the first presentation of HIV infection, or it may develop as an opportunistic infection. In people whose immune system is relatively normal (CD4 cell count above 350/mm^3) the presentation of TB is similar to that in the non-HIV-infected population; however, as the immunity wanes there is an increased likelihood of extrapulmonary and disseminated disease. The radiological appearances, similarly, are typical in the early stages of HIV infection with upper lobe infiltrates. With increasing immunosuppression, infiltrates may appear in other areas of the lung or have a miliary appearance (8).

The capacity to diagnose tuberculosis in PNG is limited and reliance on clinical diagnosis is all too common. Normally the appearance of acid-fast bacilli in sputum or other clinical specimens is needed for diagnosis. Ideally culture and susceptibility testing are performed in order to confirm susceptibility to commonly used antimycobacterial agents; however, culture is not routinely conducted in PNG. The WHO recommends that HIV-positive patients with any of the four following symptoms – cough, fever, weight loss or night sweats – be evaluated for tuberculosis, and that those without any of these symptoms receive 6 months of isoniazid preventive therapy. Tuberculin skin testing is no longer recommended for decision-making in relation to isoniazid prophylaxis (28). The treatment of TB should not normally be delayed; however, if the clinician considers that it is safe to do so, treatment of HIV could be postponed 2-8 weeks to avoid confusion with adverse drug reactions and to avoid immune reconstitution inflammatory syndrome (IRIS).

The recent reports of multi-drug-resistant TB (MDR-TB) in Western Province of PNG are of grave concern. Of the 15 Papua New Guinean patients with MDR-TB seen in the Torres Strait of Australia between 2001 and 2006, 10 were seen in the last 2 years. One of these patients was HIV positive and was one of the eight patients known or presumed to have died. The MDR-TB cases represented 25% of all TB seen in PNG citizens during the study period (29). A poor response to standard treatment for tuberculosis will be a clue to the presence of MDR-TB in the absence of culture and susceptibility testing. The treatment of MDR-TB is complex, and the potential for drug interactions with antiretroviral drugs used in the treatment of HIV must be considered (30).

There have been recent advances in the diagnosis of tuberculosis. An exciting example of technology that could be of relevance in PNG is the use of molecular diagnostic methods (polymerase chain reaction – PCR) for both the presence of *Mycobacterium tuberculosis* and the presence of rifampicin resistance (a marker for MDR-TB). A commercial assay has recently been introduced in the research setting in PNG. An appealing feature of molecular-based assays is the ability to generate results within hours and with a minimum of operator handling, making it safe to perform diagnostics in laboratories without the level of protective equipment required for culture and susceptibility testing. The assay is highly sensitive (92%, markedly better than microscopy) and specific (99%); the rifampicin susceptibility testing accuracy was also very high (31,32).

Pneumocystis pneumonia

The importance of *Pneumocystis jiroveci* pneumonia (commonly referred to as PCP) in low-income countries is subject to debate. PCP is known to be an important cause of respiratory infection in HIV-positive people in the industrialized world (33); however, studies have revealed differing incidences in low-income settings. In the early stages of the HIV epidemic the incidence of PCP in African nations was low (generally <15%); however, more recent data are indicative of an increasing incidence (often 30%-40%) (33,34). This apparent increase in incidence may, in part, be due to improved sensitivity of diagnostic methods used in later studies; however, improved diagnosis does not fully explain the observed increase (34). In low-

income countries outside Africa PCP appears to be common, often accounting for 15%-30% of respiratory illnesses in HIV-positive patients (34). The burden of PCP in industrialized countries has decreased following the introduction of cotrimoxazole prophylaxis and ART (33), and a similar decrease may occur in low-income countries with the widespread availability of cotrimoxazole prophylaxis and the rollout of ART.

Contributing to the uncertainty of the PCP burden in low-income countries is the difficulty of diagnosing PCP. *Pneumocystis jiroveci* is often found in the lungs of healthy individuals, in much the same way as *M. tuberculosis*. Thus, when sensitive diagnostic methods (such as PCR) are used, the detection of the causative organism is not necessarily indicative of disease. Serology is also of limited diagnostic value due to carriage in healthy individuals. Use of serum biomarkers such as α-D-glucan may be useful (35). The organism is unable to be cultured. More complex detection methods, including immunofluorescence assays and PCR, have been used, but such assays do not lend themselves to widespread use in low-income settings. Moreover, obtaining meaningful diagnostic specimens can be difficult as infected people do not always have a productive cough. In industrialized settings hypertonic nebulized saline-induced sputum or bronchoalveolar lavage is used to obtain diagnostic specimens, but these are not routinely conducted in many low-resource settings. The use of easily collected samples, such as saline gargles, may be relevant in low-resource settings (36), and is currently being evaluated in a study in PNG.

No data exist on the importance of PCP in HIV-positive people in PNG, largely on account of the complexities associated with laboratory diagnosis of PCP. On the basis of a serological study conducted in children with pneumonia in Goroka in the 1980s (37), exposure to *P. jiroveci* is likely for HIV-positive people in PNG. With the high burden of TB and bacterial pneumonia in the country, the impact of PCP as the sole cause of respiratory infection may not be as great in PNG as in other countries. However, co-infection of PCP with other respiratory pathogens has been commonly observed in low-income countries. *M. tuberculosis*, pneumococcus, *S. aureus* and respiratory viruses have all been isolated from HIV-infected patients diagnosed with PCP (38,39). It is likely that co-infection with two or more respiratory pathogens occurs in PNG also, and PCP may be important in the aetiology of respiratory disease in HIV-positive people in PNG. With the rollout of ART in PNG PCP may become less of a burden as the infection usually occurs in severely immunosuppressed patients.

Conclusion

Due to the lack of routine diagnostic capacity within the country, little is known about the true aetiology of respiratory infections in HIV-positive people in PNG. Clinical diagnosis is complicated by atypical disease presentation in immunocompromised people. While extrapolations can be made from findings in other low-income countries, marked geographical variations are observed in opportunistic infections in HIV-positive people (eg, prevalence of *Penicillium marneffei* in Southeast Asia). The high burden of respiratory infections such as bacterial pneumonia and TB in the broader population in PNG suggests that these commonly circulating pathogens may play a major role in respiratory infections in the HIV-positive population. Optimal treatment varies greatly according to the pathogen causing disease – viral, bacterial, mycobacterial or fungal – and thus a better understanding of the aetiology of respiratory infections in HIV-positive people would improve health outcomes. A study entitled 'An investigation into the causes of concurrent infections in HIV-positive people in PNG: a greater knowledge will improve diagnosis and treatment' is underway and should provide much needed data on the major causes of respiratory (and other) infections in HIV-positive people in PNG.

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Melioidosis – an uncommon but also under-recognized cause of pneumonia in Papua New Guinea

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SUMMARY

Melioidosis is being increasingly recognized as an important cause of severe, acute community-acquired pneumonia in various tropical regions. The chronic form of melioidosis can also mimic tuberculosis. Studies have established that, while uncommon in the Port Moresby region, melioidosis is an important cause of pneumonia and sepsis in the Balimo region of Western Province. Phylogenetic analyses of strains of *Burkholderia pseudomallei* from Papua New Guinea have shown them to be more closely related to strains of *B. pseudomallei* from Australia than to strains from Southeast Asia. This is consistent with the proposed origins of *B. pseudomallei* in Australia, with subsequent spread out of Australia to Southeast Asia during the last ice age. Further surveillance across Papua New Guinea is likely to unmask other locations where *B. pseudomallei* occurs in the environment and where melioidosis is currently not being diagnosed.

Background

It is 100 years since Whitmore and Krishnaswami first described the clinical disease melioidosis in Burmese patients dying from sepsis with pneumonia and multi-organ abscesses (1). Melioidosis is caused by the environmental bacterium *Burkholderia pseudomallei*, which is present in soil and surface water in endemic locations (2). It is most commonly found in northeast Thailand, Singapore, Malaysia and northern Australia but is being increasingly recognized elsewhere in the tropical and subtropical regions of Asia and Southeast Asia (3). Recent cases have also been documented from Africa, countries in the Indian Ocean, Central and South America and the Caribbean (4). Around half of patients with melioidosis present as a community-acquired pneumonia and case fatality ranges from under 15% in locations with state-of-the-art intensive care facilities to over 50% in some rural locations with limited health resources. Up to 80% of patients with melioidosis have an identified predisposing risk factor, with diabetes being the most common, present in 40-60% of cases (5). The majority of cases of melioidosis pneumonia present as an acute community-acquired pneumonia. Around half are bacteraemic and those at the most severe end of the spectrum die rapidly from fulminant septicaemic pneumonia. Around 20% of patients with melioidosis pneumonia have a more chronic illness, with cough, fever and weight loss that may be present for 2 months or longer and with chest X-ray changes that mimic tuberculosis. Indeed it is not unusual in endemic locations for cases of melioidosis to be incorrectly diagnosed and treated as 'smear-negative' tuberculosis.

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The history of melioidosis in Papua New Guinea

In 1963 *B. pseudomallei* was reported to have been isolated from a tree-climbing kangaroo in Port Moresby (6). The first suggestion that human melioidosis may occur in Papua New Guinea was when two fatal cases of chronic/reactivated melioidosis reported from Australia were attributed to World War 2 service in Papua New Guinea more than 20 years earlier (7,8). Between 1965 and 1980 at least 5 other human cases of melioidosis were documented from Port Moresby, with 3 being fatal (9-11). A further fatal case of melioidosis from Port Moresby occurred in 1987 (12).

Laboratory diagnosis of melioidosis requires facilities which are not present in much of Papua New Guinea and even in the laboratory recognition and identification of *B. pseudomallei* can be problematic (13). The difficulties diagnosing melioidosis were illustrated by the 1987 case reported from Port Moresby General Hospital (12). In that case a 28-year-old male died the day after being admitted with severe community-acquired pneumonia (Figure 1). A Gram-negative bacillus was recovered from blood cultures but was unable to be identified. The organism was sent to the Papua New Guinea Institute of Medical Research in Goroka, where it was recognized to be an unusual pathogen and referred to the New Zealand Communicable Disease Centre. Eventually it was identified in New Zealand as *B. pseudomallei*, by which time it was several months after the patient had died.

Nevertheless, despite the sporadic confirmation of melioidosis from Port Moresby, past serological studies indicated that melioidosis is indeed rare in that location within Papua New Guinea; two limited studies failed to detect antibodies to *B. pseudomallei* (9,14), in contrast to a seroprevalence of 5.7% seen in north Queensland (15).
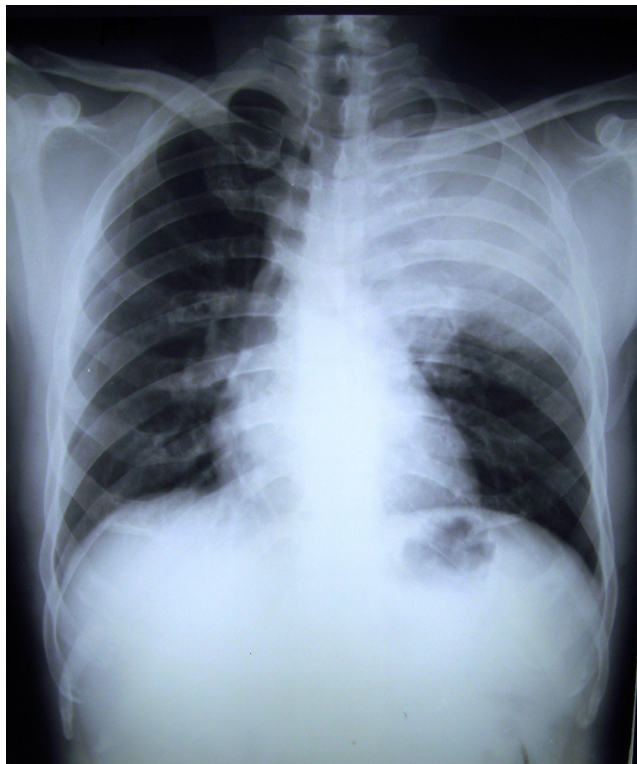
Figure 1. Chest X-ray from a rapidly fatal case of melioidosis in a 28-year-old male at Port Moresby General Hospital.

A focus of melioidosis in the Balimo region of Western Province

In contrast to the rarity of melioidosis in Port Moresby, an endemic focus of melioidosis has been found and evaluated in the Balimo region of Western Province. Investigations followed an unpublished 1983 case report of fatal melioidosis from the Balimo Health Centre. 8 cases of culture-confirmed melioidosis from the Balimo region were diagnosed during 16 months of study which spanned two periods in the 1990s (16). Notable was that 6 of the 8 cases were children under the age of 15 years. Unmasking this focus of melioidosis in a remote rural location in Papua New Guinea was only possible through the establishment of laboratory procedures specific for the culture and identification of *B. pseudomallei.* Serological surveys also undertaken as part of that project showed a seroprevalence for *B. pseudomallei* of 8.2% in local children, which is at the higher end of the seroprevalence rates seen in endemic northern Australia (3,15). Subsequent environmental studies confirmed the presence of *B. pseudomallei* in soil from the region, especially in wet locations near the lagoon where children frequently had close contact with the environment during robust play (17). Overall 2.6% of 274 soil samples were culture-positive for *B. pseudomallei.*

The Balimo studies highlighted not just the difficulties in diagnosing melioidosis in locations with limited or no laboratory facilities, but also the potentially severe nature of the disease and the especially high mortality when antibiotics for effectively treating melioidosis are not affordable or available. While chloramphenicol, cotrimoxazole and doxycycline have activity against *B. pseudomallei,* the importance of using ceftazidime or a carbapenem such as meropenem for initial therapy of melioidosis was shown in a major study from Thailand, where the use of ceftazidime halved the mortality from melioidosis (18). In the Balimo Health Centre, as elsewhere in Papua New Guinea, ceftazidime and meropenem are not available and in the Balimo study all four patients with bacteraemic melioidosis died (16). Nevertheless, where gold standard therapy for melioidosis is not available, high-dose cotrimoxazole should be considered for presumptive therapy if melioidosis is confirmed or suspected, such as in patients with tuberculosis-like pulmonary infection but not responding to standard tuberculosis therapy (3).

Results from further Port Moresby studies

Between 2000 and 2002 we undertook enhanced laboratory surveillance for *B. pseudomallei* at the Port Moresby General Hospital Pathology Department and at the Central Public Health Laboratory, Port Moresby. From 2285 blood cultures tested from patients at Port Moresby General Hospital, 2 (0.09%; 95% CI 0.01% - 0.32%) were positive for *B. pseudomallei*. At the Central Public Health Laboratory, 1309 sputum samples from 529 patients were selectively cultured for *B. pseudomallei*. These patients were being assessed for possible tuberculosis and 112/529 (21%) were confirmed as smear positive for tuberculosis on microscopy, indicative of the extremely high rates of tuberculosis seen in Papua New Guinea. When the same sputum samples were cultured for *B. pseudomallei* by placement in Ashdown's selective broth, only 1/1309 was positive for *B. pseudomallei.* Therefore in this extensive surveillance for cases of melioidosis in Port Moresby, 3 cases were identified over a 2-year period, confirming that melioidosis does occur in Port Moresby but is a very uncommon cause of community-acquired pneumonia in that region and is rarely being mistaken for tuberculosis.

The origins of *Burkholderia pseudomallei* in Papua New Guinea

Recent phylogenetic analysis of a large set of *B. pseudomallei* from many locations globally has provided strong evidence that *B. pseudomallei* most likely originated in Australia, having evolved in the local environment from an ancestral *Burkholderia* species (19). Subsequent spread is thought to have occurred across Wallace's Line to Southeast Asia, most likely during the last ice age (20,000 years ago or earlier) when sea levels were much lower. During that period a land bridge connected Papua New Guinea to Australia. Preliminary analysis of *B. pseudomallei* strains from Papua New Guinea using multilocus sequence typing (MLST) (20) has confirmed that they are more closely related to Australian *B. pseudomallei* strains than to *B. pseudomallei* from Southeast Asia.

B. pseudomallei from the Balimo endemic

region show limited genetic diversity, with multilocus sequence type (ST) ST 267 predominant. Strains from cases from Port Moresby show different STs. One strain from Papua New Guinea (ST 246) shares 5/7 MLST alleles with two different STs from the Darwin region of the Northern Territory of Australia (BJC and M. Mayo, unpublished data), where incidence rates of melioidosis are amongst the highest in the world (5).

In conclusion, melioidosis is uncommon in the Port Moresby region but is an important cause of pneumonia and sepsis in the Balimo region of Western Province. Further surveillance across the country is likely to unmask other locations where *B. pseudomallei* occurs in the environment and where melioidosis is currently not being diagnosed. Nevertheless, given the difficulties with laboratory diagnosis of melioidosis in rural settings with limited facilities and given that the best antibiotics for decreasing the mortality from melioidosis are expensive and generally not available in Papua New Guinea, mortality from this enigmatic infection will continue to occur and will mostly remain unrecognized.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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Influenza in the Pacific

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SUMMARY

Influenza A and B viruses cause significant human disease worldwide through regular outbreaks and epidemics of seasonal influenza, and occasional pandemics when a novel influenza A virus emerges. Whereas Australia and New Zealand have well-established systems for community and laboratory-based surveillance of influenza, most other countries of the Pacific are only beginning to develop such systems with the support of various global and regional agencies and networks. Here we describe the role of the World Health Organization Global Influenza Surveillance Network and other organizations in laboratory-based influenza surveillance in the region and review some of the available data on seasonal and pandemic influenza in the developed and developing countries of the Pacific. The particular features of the Pacific Island countries and territories as small dispersed island communities, together with the greater susceptibility of indigenous people to the severe effects of influenza, highlight the importance of developing local laboratory-based surveillance systems. Such systems will improve the understanding, detection and control of seasonal influenza while also providing early warning of the emergence of potential pandemic viruses.

Introduction

Human-adapted influenza A and influenza B viruses circulate continuously in the world, causing significant mortality, morbidity and economic loss. It is estimated that as many as 5%-10% of the world's population are infected with an influenza virus each year, resulting in 3-5 million severe cases and about 250,000-500,000 deaths globally per annum (1). Although infection can induce a long-lived protective antibody response, population immunity selects for variant influenza viruses whose major surface proteins, the haemagglutinin (HA) and the neuraminidase (NA), have mutated sufficiently to avoid antibody neutralization ('antigenic drift'). Such seasonal influenza viruses cause outbreaks and epidemics in many areas of the world each year.

New influenza A viruses, not previously adapted to humans, emerge at irregular intervals and cause pandemics. Molecular evolutionary analyses of the viruses from the last four pandemics, in 1918-1919 (reconstructed viruses), 1957, 1968 and 2009, have indicated that all arose by re-assortment of the genomes of avian, swine and/or human influenza viruses, introducing a new HA and, in most cases, a new NA to which the great majority of the global human population lacked protective immunity ('antigenic shift') (2). Because most people are immunologically naive to the pandemic virus, a large proportion of the world's population may be infected within the first year or two of its emergence.

Information about the impact of seasonal and pandemic influenza viruses on countries of the Pacific is highly variable in scope and depth. Most data are available for the industrialized countries of Australia and New Zealand, which have well-established infectious disease surveillance and health

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care systems. Important data have also been collected from time to time in less developed countries, often through the singular efforts of individual researchers and institutions. However, with recognition of the potential impact of an influenza pandemic, highlighted by the emergence of severe acute respiratory syndrome (SARS) in 2003 and the re-emergence of highly pathogenic avian A(H5N1) influenza in 2003-2004, recent years have seen the strengthening of surveillance networks in the Pacific as elsewhere.

Here we outline the role of the World Health Organization (WHO) and other international and regional institutions in influenza surveillance in the Pacific, then review some of the published and other available data on the impact of seasonal and pandemic influenza in this region. Finally we try to draw these threads together to identify some of the significant questions and needs for influenza surveillance, particularly in less developed countries and territories of the Pacific.

Influenza surveillance in the Pacific

Effective laboratory-based surveillance systems for influenza, as for many other infectious diseases, are limited in the Pacific outside Australia and New Zealand. The remoteness, individuality and scarce resources of some of the 22 Pacific Island countries and territories (PICTs) have impeded the development of such systems. With little access to influenza vaccines and many other challenges to the delivery of health care, it is not surprising that influenza has not been a priority. Nevertheless, laboratory-based surveillance has been established in some countries with the support of international networks, as outlined below.

The WHO Global Influenza Surveillance Network

Recognizing the potential for influenza viruses to cause devastating pandemics as in 1918-1919, the WHO Global Influenza Surveillance Network (GISN) was established in 1948 to facilitate the early detection of new influenza virus variants (3). By late 2010, the network had expanded to include 135 WHO National Influenza Centres (NICs) in 105 countries, 5 WHO Collaborating Centres for Reference and Research on

Influenza (CCs), 11 WHO H5 Reference Laboratories and 4 Essential Regulatory Laboratories. This large and growing network is coordinated by the Global Influenza Programme at the WHO's headquarters in Geneva.

WHO NICs are usually national or provincial diagnostic or reference laboratories. Their roles in GISN are to undertake influenza surveillance within their country, to report regularly on influenza prevalence to the WHO and to send a selection of recent influenza virus-containing clinical specimens or virus isolates to one of the WHO CCs in London, Atlanta, Melbourne, Tokyo or Beijing.

At the WHO CC, the viruses undergo detailed antigenic and genetic characterization, antiviral drug susceptibility testing and other analyses as required. The collective data from the many thousands of viruses analysed by the WHO CCs each year enable the major lineages of influenza A and B viruses circulating in humans to be determined and reveal the emergence and spread of, for example, antigenic drift variants and drug-resistant strains. WHO CCs also isolate some viruses directly from clinical specimens into embryonated hens' eggs in order to have a suite of potential vaccine candidate strains at hand in the event that seasonal influenza vaccines require updating because of antigenic drift in one or more of the three component viruses.

The five WHO CCs, along with representatives of the H5 Reference Laboratories and the Essential Regulatory Laboratories and other experts, meet in February and September each year to review the available data and to assist WHO in making recommendations on suitable virus strains to be included in influenza vaccines for the coming winter in the northern hemisphere and the southern hemisphere, respectively (4). If strain changes are recommended, appropriate candidate vaccine viruses isolated in the WHO CCs are distributed to the vaccine manufacturers.

The ability to update influenza vaccines with contemporary circulating viruses is one of the benefits of the virological surveillance undertaken by GISN. Of even greater global importance is the frequent and increasingly comprehensive monitoring of influenza viruses circulating in humans. This allows

the rapid detection and reporting of significant changes in seasonal influenza viruses – such as the emergence and rapid global spread of oseltamivir-resistant seasonal H1N1 viruses in 2007-2008 (5,6) – and, most importantly, increases the chance of early detection of a novel influenza A subtype with pandemic potential.

GISN and other contributing laboratories in the Pacific

In addition to the WHO CC in Melbourne, there are eight WHO NICs in the Pacific (Figure 1). Five of these are in Australia and New Zealand (at the Victorian Infectious Diseases Reference Laboratory in Melbourne, the Institute of Clinical Pathology and Medical Research in Sydney, PathWest in Perth, the Institute of Environmental Science and Research (ESR) in Wellington and Auckland Hospital in Auckland). Another three are in Fiji (at the Center for Communicable Disease Control, Mataika House, in Suva), New Caledonia (at the Pasteur Institute in Noumea) and Papua New Guinea (PNG) (at the PNG Institute of Medical Research in Goroka). All

of the WHO NICs in the Pacific are active in submitting a selection of influenza viruses, either as clinical specimens or cultured isolates, to the WHO CC in Melbourne.

Laboratory-based influenza surveillance in the Pacific is greatly enhanced by the activities of a number of other public health laboratories in the region which are not currently designated as WHO NICs but have developed with strong support from other regional institutions (Figure 1). The Secretariat of the Pacific Community (SPC) is an intergovernmental organization that provides technical and policy advice to PICTs (7). In collaboration with partner organizations, including the Pasteur Institute of New Caledonia (IPNC) and the WHO CC in Melbourne, SPC has coordinated and implemented a program funded by the United States (US) Centers for Disease Control and Prevention (CDC) aimed at improving influenza surveillance in the Pacific. This project has allowed PICTs to improve the assessment of influenza burden, their laboratory-based surveillance and their pandemic influenza preparedness (8). Other institutions, such as the Pacific Paramedical Training Centre

Figure 1. Map of the Pacific region showing the location of laboratories participating in regional influenza surveillance: WHO National Influenza Centres (closed circles); the WHO Collaborating Centre for Reference and Research on Influenza, Melbourne (open circle); PICT laboratories participating in laboratory-based influenza surveillance (closed triangles) including Kosrae, Chuuk, Pohnpei and Yap from the Federated States of Micronesia (open triangles). This map was kindly provided by SPC and modified with their approval. WHO = World Health Organization; PICT = Pacific Island Country and Territory; SPC = Secretariat of the Pacific Community.

(PPTC) and ESR in New Zealand, play an important role in supporting laboratory-based surveillance activities in the region, while the Pacific Public Health Surveillance Network (PPHSN) is a voluntary network of countries and organizations which has been dedicated to public health surveillance and response to health challenges, including influenza, since 1996 (9). As a result of this coordinated effort, many PICTs now submit samples to WHO CCs and other reference laboratories such that representative virus isolates from the Pacific enter the GISN.

Seasonal influenza in the Pacific

The health and economic burden of disease from influenza in the Pacific is likely to be significant. In Australia, for example, one analysis attributed an annual average of 310,000 general practitioner consultations and 18,400 hospitalizations to influenza at a direct cost to government of $115 million (10). Although only about 40 deaths per annum are directly attributed to influenza in Australia, the impact on mortality from other conditions is understood to be much higher. Little comparable information is available for most other countries of the Pacific. In general, however, both disease severity and mortality from influenza are likely to be higher in less developed communities due to such factors as concomitant infections, poorer nutrition, limited access to health care, the absence of community influenza vaccination programs, different approaches to health and the impact of community lifestyle on virus transmission.

Isolated populations of more remote PICTs may be at particular risk because their infrequent exposure to influenza viruses results in lower cross-reactive immunity induced by earlier virus strains. For example, in 1964, an A(H2N2) outbreak occurred on several isolated islands in the Yap district: few inhabitants had pre-epidemic antibodies to this subtype and almost the entire population was affected, with high morbidity and a case fatality rate of 1%-6.5% (11). In 1964 also, serological evidence was obtained that the population of the Caroline island of Fais had not been exposed to influenza A viruses since 1924 when the 1918 pandemic virus first reached this remote place (12), or to influenza B viruses since 1940 (11). Such prolonged lack of exposure to influenza viruses may be less common today when few communities are truly isolated from the rest of the world.

In temperate climates, influenza is highly seasonal, with the great majority of cases occurring over an 8-12 week period in the winter, usually between June and September in the southern hemisphere and between December and March in the northern hemisphere. Although the exact timing and scale of influenza activity vary from year to year, this seasonal pattern is apparent in Australia, where the great majority of people live in a temperate or subtropical climate, and in New Zealand, which is entirely temperate. Seasonality allows these countries to mitigate the community impact of influenza through government-sponsored vaccination programs in the autumn.

The available data indicate that influenza viruses circulate with variable intensity in PICTs and outbreaks can occur at any time of year (13,14). PICTs are also particularly vulnerable to importation of influenza viruses by visitors and residents returning from temperate countries of the northern or southern hemisphere during their influenza season. For example, laboratory-based surveillance in New Caledonia found discontinuous circulation of influenza viruses with a peak each year in the cool season (May-October) and other peaks, sometimes coincident with the northern hemisphere season and the return of many travellers from France (14). Similarly, return of two infected people from New Zealand led to an explosive influenza A(H3N2) outbreak in Niue in May-June 1983 that infected an estimated 41% of the population (15). The geographical, climatic and cultural diversity of PICTs can be expected to influence the annual incidence of influenza in the Pacific by affecting behaviour and travel patterns of visitors and residents.

In PNG, influenza has been detected in the wet and dry seasons and may cause sporadic outbreaks and large epidemics, despite low overall population density and the remoteness of many villages (13). A serological survey conducted amongst residents of 47 remote villages in the Western Province in 2001-2002 confirmed circulation of type A (H1N1 and H3N2) and type B viruses, with some differences noted in prevalence rates between different villages; the peak of seroprevalence was consistent with increased influenza activity during the wet season (16). The impact of isolation on immunity noted above may account for the observation in 1964 that disease caused by a

type B influenza virus became more severe as it spread from the east coast into the highlands, where it was associated with over 100 deaths (13).

Analyses of viruses submitted by various PICTs to the Melbourne WHO CC indicate that the influenza virus strains circulating throughout the Pacific are generally similar to those isolated elsewhere in the world (unpublished data). Indeed on several occasions over the last 15 years, viruses isolated by the Melbourne WHO CC from specimens provided by laboratories in the Pacific have been recommended by WHO for inclusion in seasonal influenza vaccines for the northern and southern hemispheres (Table 1).

Pandemic influenza in the Pacific

Influenza pandemics differ from seasonal epidemics in several ways. The overall burden of influenza is likely to be markedly higher in a pandemic than during inter-pandemic periods. Pandemic influenza may occur outside the normal influenza season and with two or more waves of infections within a year. Pandemics also alter the circulation of seasonal viruses: in the three pandemics of the 20th century, the previously circulating influenza A virus subtype was rapidly replaced by the new virus which then, after a period of pandemic spread, settled down into a seasonal pattern of circulation until it in turn was replaced by the next pandemic virus. Thus, H1N1 probably replaced an H3-like virus in 1918-1919, H2N2 replaced H1N1 in 1957 and H3N2 replaced H2N2 in 1968. The re-emergence of H1N1 in 1977, probably from a laboratory, did not cause a global pandemic nor did it lead to replacement of H3N2, presumably because most of the world's population had been exposed to related viruses before 1957.

The 20th century pandemics

The pandemics of the last century demonstrated that the impact of new influenza

TABLE 1

Viruses from Pacific countries recommended by the World Health Organization for inclusion in seasonal influenza vaccines

Type (subtype)	Influenza virus	WHO vaccine recommendation
A(H1N1)	A/New Caledonia/20/99	SH 2000 – 2007 NH 2000/1 – 2006/7
	A/Solomon Islands/3/2006	NH 2007/8 SH 2008
	A/Brisbane/59/2007	NH 2008/9 – 2009/10 SH 2009
A(H3N2)	A/Sydney/5/97	SH 1998 – 1999 NH 1998/9 – 1999/2000
	A/Wellington/1/2004	SH 2005
	A/Brisbane/10/2007	SH 2008 – 2009 NH 2008/9 – 2009/10
	A/Perth/16/2009	SH 2010 – 2011 NH 2010/11
B	B/Brisbane/60/2008	NH 2009/10 – 2010/11 SH 2010 – 2011

WHO = World Health Organization
SH = southern hemisphere
NH = northern hemisphere
Dates indicate the winters to which the recommendations apply

A viruses on mortality and morbidity can vary markedly between pandemics and between populations. Estimated deaths attributed to the pandemic viruses ranged from about 1 million in 1968 to 50 million in 1918-1919 (about 2.5% of the world's population), due at least in part to differences between the viruses themselves. However, the large literature on these pandemics also highlights the markedly higher mortality experienced in developing countries compared with the developed world, in isolated compared with urbanized communities and, in some countries, in indigenous compared with non-indigenous communities (17). For example, it is estimated that the 1918-1919 pandemic virus killed about 4.2% of the Maori population compared with 0.55% of the caucasian population in New Zealand (17).

The effects of the 1918 pandemic were particularly severe in some of the PICTs (reviewed in 18). Western Samoa (Samoa today) was probably the worst affected with over 7500 deaths, about 25% of the population, attributable to pandemic influenza. By contrast, neighbouring American Samoa introduced strict maritime quarantine and recorded no cases or deaths. Elsewhere in the Pacific, pandemic influenza claimed the lives of 16%, 6% and 5% of the populations of Tonga, Nauru and Fiji, respectively, while PICTs serviced exclusively by Australian vessels, including the Solomon Islands, Kiribati and Vanuatu, were spared due to maritime quarantine imposed on incoming and outgoing ships. Quarantine of arriving vessels protected Australia only until January 1919, after which the virus spread rapidly and caused approximately 12,000 deaths (0.24% of the population).

As noted above, the A(H2N2) epidemic in remote islands of the Yap district in 1964 was probably the first exposure of that population to the 1957 pandemic virus and mortality was high (11). During the 1968 pandemic, the new A(H3N2) virus quickly spread through many previously isolated areas of PNG, contributing to the deaths of more than 3000 people over a few months in 1969 (13).

The A(H1N1) 2009 pandemic

The 2009 influenza pandemic displayed both similarities with and differences from the 20th century pandemics:

- The speed and intensity of modern airline travel ensured that the virus reached most major ports within a few weeks of its first detection in Mexico and the USA in late April. Cases were confirmed in all continents within 14 weeks of the first known case in Mexico.

- The new virus quickly became the predominant influenza virus circulating in humans. Within a few months, the previous seasonal A(H1N1) viruses had almost disappeared.

- The virus preferentially affected younger people, especially children and teenagers. As a result, schools were a major avenue of spread and children were frequently the origin of household infections.

The experience of the 2009 pandemic in the Pacific mirrored that in many other parts of the world.

In Australia, there had been little seasonal influenza activity before the arrival of the pandemic virus. The first active case of A(H1N1) 2009 was identified in Melbourne in a traveller from the US on 20 May but the virus had probably been circulating there undetected and confirmed case numbers escalated rapidly from that point (19). While timing varied around the country, Australia overall experienced a single pandemic wave which peaked in the third week of July and then declined to baseline by September-October (20). The predominant seasonal influenza viruses detected in the 2009 winter were of the A(H3N2) subtype but their numbers were low compared with detections of the pandemic A(H1N1) 2009 virus. The first cases of pandemic influenza in New Zealand were detected in two high school groups who returned from North America on 25 and 28 April (week 18) (21,22). These cases were apparently contained and the pandemic wave started in earnest in weeks 24 and 25. Seasonal A(H1N1) viruses were also circulating in New Zealand at that time but were rapidly overtaken by A(H1N1) 2009 viruses.

Although rates of laboratory testing were high in Australia and New Zealand, especially early in the pandemic wave, the data suggested that many cases were mild and therefore would not have been formally diagnosed. As the pandemic progressed,

however, it also became apparent that some people suffered severe illness. About two-thirds of severe and fatal cases were associated with risk factors, including pregnancy, asthma, chronic obstructive pulmonary disease, diabetes, obesity, malignancy and immunosuppressive medication; a significant proportion of the remaining cases were in previously healthy, young people (23,24). While most health care systems coped well with the extra demands imposed by pandemic cases and policies, intensive care units in Australia and New Zealand carried an exceptional load of severely ill influenza patients, many requiring mechanical ventilation, with unprecedented extent and duration of use of extracorporeal membrane oxygenation to manage acute respiratory distress syndrome (23,25). Indigenous people were disproportionately represented among serious cases: Aboriginal and Torres Strait Islander Australians were 10 times more likely to be hospitalized than other Australians (26) and Maori and Pacific Islander people were, respectively, 5 and 7 times more likely to be hospitalized than those of European origin (27).

Several authors have collated data from PICTs as outlined below. While case numbers might be under-reported in these countries compared with industrialized nations, it is likely that hospitalizations and deaths that occurred in hospitals have been comprehensively recorded in at least some of the PICTs.

The Western Pacific Regional Office of the WHO (28) noted that, during 2009, cases of A(H1N1) 2009 were reported by all but three Pacific Island countries, Niue, Pitcairn Islands and Tokelau. Incidence was often very high in countries with small populations, including Cook Islands, the Marshall Islands, New Caledonia, Palau, Tuvalu, and Wallis and Futuna, where it ranged between about 200 and 540 per 100,000 population. Overall, PICTs recorded 21 deaths, or 0.22 per 100,000, compared with 0.08/100,000 for the whole Western Pacific Region. Most deaths, in the Pacific as elsewhere, were recorded in people aged 15-64 years.

In the French territories of the Pacific, New Caledonia, French Polynesia and Wallis and Futuna, the first pandemic wave commenced at a different time in each territory and then lasted approximately 8 weeks (29). Cases were detected among travellers from Australia, the USA and France but school exchanges with Australia and New Zealand and the return of students from holidays or study abroad appear to have contributed to community spread. Estimated attack rates ranged from 16-18% in New Caledonia to 38% in Futuna; numbers of hospitalizations and deaths were small and linked especially to diabetes, heart and lung disease, obesity, neuromuscular diseases in children and Oceanic origin.

The higher vulnerability of indigenous populations of the Pacific to pandemic influenza noted in 1918-1919 was observed again in 2009 and was apparently reflected in both clinical attack rates and the risk of severe disease and death (30). For example, in addition to the Australian and New Zealand experience noted above, the death rate among indigenous inhabitants of New Caledonia was 5.3 times higher than among non-indigenous people; the relatively high attack rates recorded in the French territories of the Pacific may therefore reflect the high proportion of indigenous people in those populations (30). Some of the contributing factors are likely to be the same as in earlier pandemics: for example, poverty, malnutrition, crowding, bacterial co-infection, high rates of pregnancy, poor access to health services and the small size of some islands. A role for genetic factors also has not been excluded. Other factors may differ from those of the past, such as obesity, diabetes, asthma and HIV-associated immunosuppression. However, the contribution of such factors may also vary between countries. For example, the incidence of diabetes, obesity and chronic respiratory diseases is higher among indigenous than non-indigenous people in Australia and New Caledonia, whereas the incidence of chronic respiratory disease is lower among Pacific peoples than Maori and others in New Zealand (27,30).

In recent years, the Melbourne WHO CC has received approximately 2500 influenza virus samples (clinical specimens and cultured virus isolates) per annum from around the Asia-Pacific region, with relatively small numbers from PICTs. Following the emergence of the pandemic virus, however, the WHO CC was requested by several countries to confirm first cases and subsequently received markedly elevated numbers of samples from a larger number of PICTs than in previous years (Table 2).

Detailed antigenic and genetic analyses of these viruses showed that they were closely related to those circulating in Australia, New Zealand and other parts of the world and would therefore be covered by pandemic vaccines containing the reference virus A/California/7/2009.

After the decline of the pandemic wave in September-October 2009, Australia and New Zealand detected only sporadic cases of influenza until the winter of 2010, when both countries experienced a moderate influenza season (31,32). In New Zealand the great majority of typed viruses were A(H1N1) 2009 while in Australia typed viruses comprised approximately 70% A(H1N1) 2009 with the remainder including both type B and, to a lesser extent, A(H3N2) viruses. Clinical reporting systems suggest that the virulence of circulating A(H1N1) 2009 viruses has not changed significantly from 2009 (32). The lower influenza prevalence reported in 2010

is likely to reflect lower testing rates and, more importantly, elevated population immunity to A(H1N1) 2009 due to exposure during 2009 or subsequent vaccination, as indicated by several recent serological surveys (eg, 33-35).

Recent information is lacking for most PICTs. However, New Caledonia has reported that influenza activity detected between July and September 2010 was mostly due to type B viruses (36). Compared with 2009, the WHO CC in Melbourne has received smaller numbers of samples from a more limited range of PICTs during 2010. Of those analysed at the time of writing, viruses from Fiji, Guam and PNG have mainly been A(H1N1) 2009 and the remainder (from the Federated States of Micronesia and New Caledonia) have been type B viruses.

These data support the idea that the 2009 pandemic virus is following a similar pattern

TABLE 2

PACIFIC ISLAND COUNTRIES AND TERRITORIES (PICTS) THAT SUBMITTED VIRUSES TO THE MELBOURNE WORLD HEALTH ORGANIZATION COLLABORATING CENTRE FOR REFERENCE AND RESEARCH ON INFLUENZA*

Countries	2006	2007	2008	2009
Number of PICTs submitting viruses	3	4	7	15
Number of samples received from:				
Fiji	0	0	5	465
French Polynesia	20	6	0	59
Guam	0	21	19	275
New Caledonia	40	16	2	38
Papua New Guinea	0	8	39	223
Solomon Islands	3	0	13	39
Total from selected PICTs	63	51	78	1099
Total from all PICTs	**63**	**51**	**99**	**2074**

*The table shows the total number of PICTs that submitted virus samples in the indicated years, the total numbers of samples received from PICTs and the numbers submitted by selected individual PICTs

of circulation to seasonal influenza viruses in the Pacific, as observed elsewhere in the world and consistent with the WHO's declaration of the end of the pandemic on 10 August 2010. A(H3N2) and type B viruses are co-circulating with A(H1N1) 2009 to varying extents in different countries while the previous seasonal A(H1N1) lineage is no longer being detected.

The WHO Consultation on the Composition of Influenza Vaccines for the Southern Hemisphere 2011, held in late September 2010, concluded, from detailed strain analyses of viruses received by the four WHO CCs and data submitted by NICs, that A(H1N1) 2009 viruses have not yet undergone significant antigenic drift and remain closely related to the vaccine strain A/California/7/2009 (36). This was also true for the A(H1N1) 2009 viruses recently submitted to the Melbourne WHO CC by countries of the Pacific. Although a new genetic subclade emerged in Australia, New Zealand and Singapore during 2010, it has remained antigenically similar to A/California/7/2009 to date (37).

Strengthening influenza surveillance in the Pacific

Although the available data indicate that both seasonal and pandemic influenza can cause significant illness in people of the Pacific, there are many gaps in our knowledge of this disease in the less developed countries of the region. These gaps include information on the contribution of influenza infection to the health and economic well-being of Pacific communities, on the seasonality of influenza activity, on major avenues of virus transmission and on clinical, environmental and cultural risk factors for severe disease and death from influenza infection. Such information would assist governments, aid agencies and other international authorities in allocating resources for effective influenza detection, prevention and treatment, particularly for the most vulnerable indigenous populations.

Laboratory-based surveillance of influenza infection is critical for obtaining this information, supported by robust communicable disease surveillance – for example, of influenza-like illness – in order to identify outbreaks and monitor disease burden (18). However, laboratories in the PICTs face significant challenges in the adoption of contemporary testing technologies: they often lack suitable facilities and equipment, laboratory technicians may have limited formal training, and they may not have access to external quality control and proficiency testing procedures. It is therefore important that laboratory techniques are appropriate to local conditions and are supported by training and external quality control. Work is also needed in other areas, including sample collection, storage and shipping to reference laboratories, and education of laboratory staff and clinicians in the use and limitations of different testing technologies for surveillance versus diagnosis and clinical management.

The work being undertaken by the WHO, SPC, PPHSN, ESR, CDC and other agencies and networks to establish laboratory and field surveillance for influenza deserves strong and continuing support. There is also an important role for the more established laboratories, not only in Australia and New Zealand, but also in, for example, Fiji, New Caledonia, PNG and Guam, in mentoring and supporting smaller PICT laboratories. The justification need not be altruistic. Strong laboratory-based surveillance is a cornerstone of pandemic preparedness, building local awareness of influenza and improving the chance of detecting and responding to an emerging outbreak in the region.

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A neonatal pneumococcal conjugate vaccine trial in Papua New Guinea: study population, methods and operational challenges

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SUMMARY

Infants in Papua New Guinea (PNG) are at a high risk of invasive pneumococcal disease, and a substantial burden of this falls on children less than six months old. PNG is planning to introduce a pneumococcal conjugate vaccine for infants in the near future, but to make the maximum impact neonatal immunization will have to be considered. To provide evidence on safety and immunogenicity for neonatal and early infant immunization, we undertook an open randomized controlled trial of 7-valent pneumococcal conjugate vaccine (7vPCV). 318 children received 7vPCV at ages 0, 1 and 2 months or at 1, 2 and 3 months or not at all. All children received 23-valent pneumococcal polysaccharide vaccine at age 9 months. This was a large and complex trial: village reporters visited participants weekly during the first year and fortnightly for a further 6 months and nurses monitored self-reported morbidity and collected many thousands of biological samples. The study team was remarkably successful in achieving the study aims, with 18-month follow-up completed on 77% of enrolled children and over 80% of scheduled samples collected. While the results of the trial will be reported elsewhere, this paper discusses the design of the study and dissects out some of the main reasons for its successful completion. Strong community engagement was an essential factor in success and the principles of equitable partnership and service provision led to a strong research partnership. A two-stage consent process, comprising primary assent followed by later informed consent, led to a high drop-out before initial enrolment, but an outstanding retention of those enrolled in the study. We conclude that factors such as strong community participation, reciprocity and a good relationship between the study team and participants are just as important as the technical elements of laboratory testing and data handling in ensuring the success of a vaccine trial in PNG.

Introduction

It is estimated that every year there are 2 million deaths from pneumonia in children under five years of age, the majority of them occurring in the third world. *Streptococcus pneumoniae* (pneumococcus) is a major cause of pneumonia and meningitis and is

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responsible for over 800,000 of these deaths (1,2). In Papua New Guinea (PNG) pneumonia is the most common cause of serious illness and mortality, with a substantial burden of disease occurring in the first 6 months of life (3,4), and infants are at high risk of invasive pneumococcal disease (IPD). In the Asaro Valley, Eastern Highlands Province (EHP), the mortality rate for acute lower respiratory tract infections (ALRIs), predominantly pneumonia, in infants has been reported to be 25/1000 live births/year; 56% of all ALRI deaths in children aged <5 years occur under age 6 months (4). We have previously found that the pneumococcus accounts for 46% of bacteraemic pneumonia (5), with 26% and 63% of cases occurring before ages 3 and 6 months, respectively. The pneumococcus also accounts for almost half of the cases of bacterial meningitis (6,7). The contribution of the pneumococcus to pneumonia and meningitis may now be even greater following introduction of *Haemophilus influenzae* type b (Hib) vaccine into PNG's expanded program of immunization (EPI) in 2008.

We have previously reported the efficacy and effectiveness of 23-valent pneumococcal polysaccharide vaccine (when first used it was 14-valent) (PPV; 23-valent includes serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F) in preventing mortality and severe morbidity due to ALRI in the highlands of PNG when given between the ages of 6 months and 5 years (8-11). However, given the high proportion of ALRI deaths occurring before the age of 6 months, earlier intervention is clearly needed. A 7-valent pneumococcal conjugate vaccine (7vPCV, which includes serotypes 4, 6B, 9V, 14, 18C, 19F & 23F conjugated to the carrier protein CRM_{197}) given as a 3-dose schedule from age 2 months onwards has led to a dramatic reduction in IPD incidence in industrialized countries (12-14) and associated benefits through herd immunity (15). In African infants, a 9-valent PCV given at 6, 10 and 14 weeks of age with no booster dose was efficacious against IPD due to the relevant PCV serotypes and in The Gambia there was a 37% reduction in the incidence of chest X-ray-proven pneumonia and a 16% reduction in overall mortality (16,17).

Pneumococcal conjugate vaccines including 10 or 13 different pneumococcal serotypes have recently been licensed and will replace 7vPCV in countries already implementing pneumococcal vaccination. The Global Alliance for Vaccines and Immunization (GAVI) and the World Health Organization (WHO) have committed to the introduction of PCVs for infants in GAVI-eligible countries (including PNG) using novel funding mechanisms. Introduction of PCV in PNG through this funding mechanism is planned for 2013. In order to obtain the earliest possible protection against invasive disease, achieve optimal coverage and reduce the burden of early pneumococcal carriage, a schedule including neonatal immunization needs to be considered.

To provide an evidence base for the PCV dosage schedule in PNG, we have investigated the safety and immunogenicity of neonatal immunization with 7vPCV in an open randomized controlled trial conducted through the PNG Institute of Medical Research (PNGIMR) in the Eastern Highlands Province of PNG between 2005 and 2009. The findings of the trial in respect of safety, immunogenicity and efficacy will be reported elsewhere, but conducting such a complex, logistically challenging study in a developing country context raises many issues that are worthy of discussion in themselves. This article describes the study population, details the study methodology, and discusses the operational challenges and lessons learned during the conduct of this vaccine trial.

Methods

Study design

We conducted an open randomized controlled trial of 7vPCV given at ages 0, 1 and 2 months (neonatal group) or at 1, 2 and 3 months (infant group) or no 7vPCV (control group). This study aimed to address the following questions:

1. Is immunization with 7vPCV safe and immunogenic in PNG infants when administered according to the standard 1-2-3-month immunization schedule in PNG?

2. Does neonatal immunization with 7vPCV provide a more favourable antibody response in early childhood than immunization starting at 1 month of age, without compromising safety?

3. Does neonatal or early infant immunization with 7vPCV induce immunological memory to the relevant pneumococcal polysaccharides?

4. Does neonatal or early infant immunization with 7vPCV affect nasopharyngeal carriage of vaccine and non-vaccine serotypes?

5. What is the impact of early colonization with vaccine serotypes on antibody responses (systemic and mucosal) to neonatal or early infant immunization with 7vPCV?

6. Is the response of PNG infants to pneumococcal antigens governed by developmental factors controlling postnatal maturation of immune competence?

7. Is there evidence that early pneumococcal carriage leads to tolerance or impaired responses to vaccine or non-vaccine pneumococcal antigens?

8. With respect to immunological safety of neonatal immunization with 7vPCV:

a. Does neonatal immunization with PCV interfere with humoral and cellular immune responses to concomitant vaccines (diphtheria toxoid, tetanus toxoid and measles)?

b. Does neonatal immunization with PCV interfere with normal maturation of the immune system, in particular Th1 functions which mature rapidly during infancy?

9. Does PCV reduce the incidence of respiratory morbidity in PNG infants in the first year of life?

Study location

The study took place in the Asaro Valley (including Goroka town) located 6° south of the equator, at an altitude of between 1500 and 1900 metres above sea level. In the 2000 National Census (http://www.spc.int/prism/country/pg/stats/Special_Products/prod.html), Goroka town (EHP's provincial capital) had a population of 19,523 while the adjacent Lowa Census Division, encompassing the rural area where we conducted the study, had a population of 39,440. In rural areas people live in hamlets and are primarily subsistence farmers but obtain cash through sales of coffee and market produce. Living standards vary widely in the town: many houses are built in a modern style with strong and long-lasting materials such as an iron roof and cement walls and floors, but some houses in the poorer settlements are more traditional in style and made of bush materials. There is also semi-permanent housing made of a combination of bush materials and strong building materials, the latter often recycled. Modern houses are connected to the town water and electricity supply, but this is less common in the settlements.

Recruitment, assent and consent process

After we had informed communities about the study through community and church gatherings, local village reporters identified pregnant women during monthly home visits conducted for demographic surveillance. A nurse explained the study to pregnant women using a flip chart, and explanatory pamphlets were distributed. A nurse also recruited women through the antenatal clinic (ANC) at Goroka General Hospital (GGH). It was important to seek assent antenatally from both pregnant women and their husbands, so they had adequate time to consider their participation in a separate environment from the actual birth. A study nurse was based on the labour ward at GGH to seek consent from women who had already given their assent and to enrol participants as soon as possible after delivery. Initially, a study nurse was available at GGH only during the day. But, as we were missing many mothers who had already assented, once additional funds had been identified, we appointed nurses to monitor the labour ward 24 hours a day. In order not to preoccupy mothers during labour, consent was sought as soon as considered appropriate after delivery when an information sheet detailing study procedures was given to mothers. However, during labour a study nurse did request permission from the mother to collect cord blood. If subsequent consent to full participation was not obtained the cord blood was discarded.

Inclusion criteria for the study were as follows:

• baby seen within 24 hours of birth

- mother intending to live within an hour's drive of Goroka for at least 2 years

- birthweight >2000 g

- no acute neonatal infection or severe asphyxia at birth

- no severe congenital abnormality.

While most babies were born in hospital, children born before arrival at the hospital were eligible for inclusion in the study if brought to the hospital within 24 hours. Parents could withdraw consent at any time without detriment.

Enrolment, immunization and follow-up

Following delivery, the baby was examined by a paediatrician. If eligible and the mother gave consent, the child was enrolled into the study. Information was collected on parity, number of antenatal attendances, laboratory investigations during pregnancy, illness and treatment given during pregnancy, and date of maternal tetanus toxoid vaccination.

The vaccine schedule is shown in Table 1. Following enrolment children were randomized to the neonatal, infant or control group using a computer-generated random number list, and this assignment was specified inside a sealed envelope with the next sequential number. Throughout the study laboratory staff were blinded to the group allocation. Since 7vPCV had not previously been evaluated in this population, the first 52 infants were randomized to the infant 7vPCV or control group. An appointed Data Safety Monitoring Board (DSMB) reviewed the safety data in these first 52 children and approved continuation of the study with inclusion of both neonatal and infant vaccine groups. The neonatal dose of 7vPCV was given by a study nurse within 72 hours of birth. Other routine vaccinations to be given at birth (BCG, oral polio vaccine, hepatitis B) were generally given within 72 hours of birth either by labour ward staff or study nurses. Mothers and their children were taken home by the research team, which helped us to locate them for subsequent visits. A nurse or driver collected study participants living in the rural areas for subsequent follow-up visits. Follow-up cards were handed to mothers, with the next appointment date noted. Study participants living in Goroka town were asked to come

directly to the PNGIMR clinic but were sought by a nurse or driver if they did not attend for follow-up. All subsequent investigational and most routine vaccinations (hepatitis B, polio vaccine and diphtheria-tetanus-whole-cell-pertussis-Hib vaccine) were given by study nurses at the PNGIMR clinic. As there were no specimens to be collected at age 6 months, children could receive the scheduled measles vaccine at PNGIMR or at other immunization clinics. At the time of the study Hib vaccine was not part of the routine immunization schedule. However, all study participants received DTP/Hib vaccine (TetrActHib™) (Table 1).

Following immunization, children were monitored at the clinic for 1 hour and seen either in the clinic or in their homes 48-96 hours post vaccination when they were examined for local or systemic reactions. While a post-vaccination visit would ideally have been conducted within 48 hours of vaccination to optimize the likelihood of detecting reactions to vaccination, we opted for a 48-96-hour follow-up visit for logistical reasons (for example, follow-up on Monday after vaccination on a Friday). Participants ceased to be in the study if they suffered an allergic reaction to vaccination, had inadvertently been given DTP (instead of DTP/Hib) at another clinic, or had received the wrong pneumococcal vaccine. Nevertheless, all such children continued to be followed and to receive medical attention as required.

Morbidity surveillance

To ensure the safety of neonatal or early 7vPCV vaccination and determine any potential benefits of 7vPCV on respiratory morbidity, village reporters conducted weekly surveillance of study participants in rural areas throughout the first year of life and then fortnightly to age 18 months. The weekly follow-up helped maintain the interest of participants' parents in the study and encourage parents to bring babies for treatment if sick. Parents were keen to attend the PNGIMR clinic since they had assistance with transport and their children were seen faster and had easier access to treatment than through the routine services. Morbidity data collected by reporters could provide information on signs and symptoms in participants who died, which was helpful when other clinical records were not available or were incomplete. Furthermore,

TABLE 1

SCHEDULE OF IMMUNIZATIONS, SPECIMEN COLLECTION AND MORBIDITY SURVEILLANCE FROM BIRTH TO AGE 18 MONTHS

	Birth	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	6 months	9 months	10 months	18 months
Neonatal PCV group	PCV				PCV	PCV				PPV		
Infant PCV group					PCV	PCV	PCV			PPV		
Control group	-				-	-	-			PPV		
Other vaccines to all children	HepB, BCG, OPV				DTP/Hib, HepB, OPV	DTP/Hib, OPV	DTP/Hib, OPV, HepB		Measles	Measles		
Pernasal swab		X	X	X	X		X			X		X
Saliva 1-2 ml		X			X	X	X	X		X	X	X
Blood for serum	X*					X	X	X		X	X	X
Blood for PBMC	X*						X			X	X	X
Morbidity surveillance												

*Cord blood sample

PCV: 7-valent pneumococcal-CRM$_{197}$ conjugate vaccine; PPV: 23-valent pneumococcal polysaccharide vaccine; HepB: hepatitis B vaccine; BCG: Bacille Calmette-Guérin anti-tuberculosis vaccine; OPV: oral polio vaccine; DTP: diphtheria-tetanus-whole-cell-pertussis vaccine; Hib: *Haemophilus influenzae* type b-tetanus toxoid conjugate vaccine (PRP-T); PBMC: peripheral blood mononuclear cells

comparisons between community-based surveillance and self-reported surveillance at the PNGIMR clinic would be possible. Hospital admissions were either known through the study nurses' referrals or detected through daily visits to the paediatric ward at GGH on weekdays. Finally, child health record books were reviewed at each follow-up visit to identify any unreported events. These books were photocopied and stored with other study records.

Specimen collection

Table 1 shows the types of specimen and ages when they were to be collected. In addition to cord blood (up to 50 ml), a further 6 blood samples were to be collected during the first 18 months of life.

Blood for *serum* (up to 2 ml) was collected to measure serotype-specific IgG antibody responses to 7vPCV and PPV, to conduct functional assays and to measure responses to pneumococcal outer membrane proteins PspA, PsaA and pneumolysin (Ply). Serum samples were aliquotted and stored at -80°C. All antibody assays were done at PNGIMR.

Heparinized blood for peripheral blood mononuclear cells (PBMC) (up to 4 ml) was collected for T-cell cytokine studies to examine the development of pneumococcal and vaccine-specific immunity as well as overall development of Th1/Th2 immunity. After immediate processing at PNGIMR, PBMC were cryopreserved and then sent in batches in a dry shipper to the Telethon Institute for Child Health Research (TICHR) in Perth, Australia. After cells were separated plasma was also stored at PNGIMR to measure pneumococcal antibodies if there was insufficient serum available. In addition, neutrophils were separated and stored at -80°C for DNA extraction for an adjunct study investigating genetic factors that may increase risk of ALRI.

Saliva samples were collected using 2-6 eye spears to determine mucosal serotype-specific IgA responses to pneumococcal vaccines and pneumococcal outer membrane proteins. All samples were stored at -80°C for investigation at PNGIMR.

Pernasal swabs (PNSs) were collected to determine the effect of early upper respiratory tract (URT) carriage on subsequent responses to 7vPCV and the development of pneumococcal immunity and also the impact of neonatal or early infant 7vPCV and a PPV booster on URT carriage. In addition, PNS samples have been used to identify respiratory viruses present in sick and healthy children. PNS samples were stored in skim milk tryptone-glucose-glycerol broth at -80°C until culture and characterization of isolates were done at PNGIMR.

We collected blood and a PNS for culture from children with fever >38°C or suspected moderate or severe pneumonia or meningitis. Chest X-rays were requested for children with suspected pneumonia and were read by a paediatrician. Malaria blood slides were collected on children with fever >38°C.

Randomization and sample size calculations

Sample size requirements assumed 80% power and a 5% significance level. Eligible infants were randomized in blocks of 26. Comparison of serious adverse events in the first 52 infants randomized to infant or control groups would allow proportions of adverse events of for example 10% in the control group versus 48% in the treated group, or 5% vs 40%, to be determined. Distributions of published pneumococcal vaccine responses (18-20) indicated a standard deviation of serotype-specific IgG concentration of around 1 on the natural logarithmic scale at all ages. Given 70% seroprotection (>0.5 µg/ml) after 1 dose of 7vPCV at age 6 weeks (18), the sample size of 100 per group (with a minimum of 90 evaluable due to loss to follow-up) would allow this proportion to be estimated with 95% CI of 60-79% and 80% power at the 5% significance level to detect a 20% difference from the neonatal (ie 90%) or control group (ie 50%).

For ALRI hospital admissions, assuming a rate of 0.0425 per child month under 1 year (21), a 50% decrease in either vaccinated group compared with controls would be detectable. With the given sample size, we would detect differences in carriage rates between the groups of between 40% and 22% or lower, from age 1 month onwards.

Study and data management

To ensure that any problems or queries were addressed as soon as possible, investigators in PNG and Australia attended monthly

teleconferences. All data collection forms were checked manually before entry into a computer file using Filemakerpro version 7.

Data Safety Monitoring Board and ethical approval

A DSMB was established, which included independent clinicians in PNG and Australia. All serious adverse events were reported to an appointed Papua New Guinean Safety Monitor for review and clinical details were sent to the Chair of the DSMB. The Chair of the DSMB was informed immediately when a death occurred. A quarterly report was prepared by the investigators and sent to the Chair of the DSMB. This included a summary of all serious adverse events and status of enrolment and follow-up.

Ethical approval to conduct the study was obtained from the Medical Research Advisory Committee of PNG and from the Princess Margaret Hospital for Children Ethics Committee in Perth, Australia. This trial is registered at ClinicalTrials.gov under registration number NCT00219401.

Results

Assent and enrolment

A total of 448 mothers gave assent, of whom 312 subsequently gave their consent for 318 of their children to take part in the study. There were 4 sets of twins and 2 sets of siblings in the study and thus the 312 mothers had 314 deliveries and 318 children. Figure 1 illustrates the flow of women who assented to have their children in the study, the number of children successfully enrolled and randomized to receive first dose of 7vPCV neonatally or at age 1 month or no 7vPCV, the number seen at each time point, and the number of children in each group who were excluded, lost to follow-up or died. Participants were randomized to the neonatal (n = 104), infant (n = 105) and control (n = 109) groups (Figure 1). Of these 318 children 241 (76%) were from the rural areas, with no difference between neonatal, infant and control groups. Of the 314 deliveries 17/239 (7%) in the rural areas and 5/75 (7%) in Goroka town took place at home.

There were 6 children excluded from the study on medical grounds, all of whom subsequently died: 2 were HIV (human immunodeficiency virus) positive (both in the neonatal group), 3 were found to have congenital heart disease (1 in the neonatal group and 2 controls) and 1 control was diagnosed as possible Hirschsprung's disease. Of the remaining 312, 30 parents (10%) withdrew consent, 16 (5%) migrated out of the study area and 10 (3%) were lost to follow-up, with no difference between neonatal, infant or control groups.

Of the 26 children who were lost to follow-up or who migrated out of the area, the majority (57%) left the study after age 9 months while a further 31% left the study between 2 and 9 months of age. In some cases, participants could not be located for some months, but then returned and re-entered the study until completion of the follow-up period. One-third of the 30 parents who withdrew their consent did so before children were aged 2 months. In PNG society it is advisable to seek consent from the father as well as the mother. However, this was not always possible if the father was not present after delivery and although the mother may have given her consent, if a father subsequently did not agree to their child being in the study, the consent was withdrawn. Some mothers did not wish to disappoint the study team and avoided the team member who came to collect them for follow-up. Later, we were informed by the village reporters that they wished to withdraw their child from the study.

There were 2 deaths during the study period: 1 child in the control group died of fire burns and gastroenteritis at age 6 months and 1 child assigned to the infant vaccination group died of pneumonia before receiving the first dose of 7vPCV. We were informed of 2 other children who died after exiting the study: 1 control who had migrated out of the area died of pneumonia at age 11 months and 1 child in the infant vaccination group died of suspected intussusception at age 20 months.

Protocol violations

There were 6 protocol violations related to 7vPCV and 4 related to PPV, and 4 children received DTP rather than DTP/Hib when they attended an immunization clinic other than the PNGIMR clinic. These make up the 14 protocol violations in Figure 1. In addition there were 5 protocol deviations that were not considered of sufficient relevance to outcome to warrant exclusion from the study – for example, 2 children received additional DTP doses at other immunization clinics during an intensive DTP immunization campaign
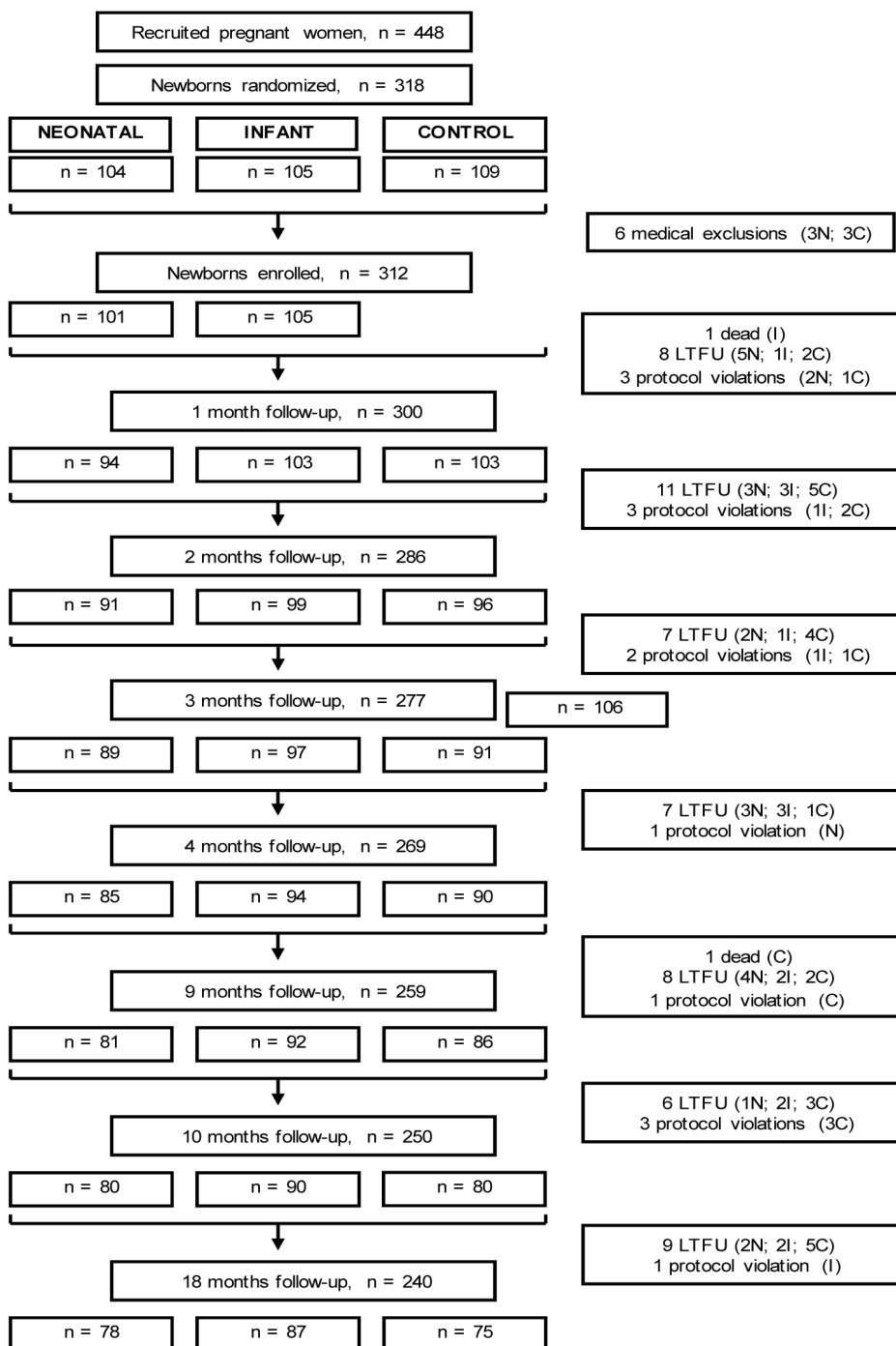
Recruited pregnant women, n = 448

Newborns randomized, n = 318

NEONATAL	INFANT	CONTROL
n = 104	n = 105	n = 109

6 medical exclusions (3N; 3C)

Newborns enrolled, n = 312

n = 101	n = 105

1 dead (I)
8 LTFU (5N; 1I; 2C)
3 protocol violations (2N; 1C)

1 month follow-up, n = 300

n = 94	n = 103	n = 103

11 LTFU (3N; 3I; 5C)
3 protocol violations (1I; 2C)

2 months follow-up, n = 286

n = 91	n = 99	n = 96

7 LTFU (2N; 1I; 4C)
2 protocol violations (1I; 1C)

3 months follow-up, n = 277		n = 106

n = 89	n = 97	n = 91

7 LTFU (3N; 3I; 1C)
1 protocol violation (N)

4 months follow-up, n = 269

n = 85	n = 94	n = 90

1 dead (C)
8 LTFU (4N; 2I; 2C)
1 protocol violation (C)

9 months follow-up, n = 259

n = 81	n = 92	n = 86

6 LTFU (1N; 2I; 3C)
3 protocol violations (3C)

10 months follow-up, n = 250

n = 80	n = 90	n = 80

9 LTFU (2N; 2I; 5C)
1 protocol violation (I)

18 months follow-up, n = 240

n = 78	n = 87	n = 75

Figure 1. Flow diagram indicating number recruited into the study, number enrolled, randomized to neonatal or infant PCV or control groups, and number excluded or lost to follow-up in the course of the study.
PCV = 7-valent pneumococcal conjugate vaccine; N = Neonatal group; I = Infant group; C = Control group; LTFU = lost to follow-up (includes not located, withdrew consent, migration).
Numbers (n) are total excluding deaths, LTFU and protocol violations.

following concern about a potential pertussis outbreak.

Follow-up of study participants

Follow-up of study participants was extremely time-consuming, particularly in the rural areas. Sometimes we had to travel out more than 3 times to locate a child, despite support from village reporters. The good relationship between the study team and the guardians of the study babies was crucial and led to the good follow-up rate and high success rate for specimen collection up to age 18 months. Of the 312 children enrolled into the study, 86% successfully completed the correct immunization schedule (ie without loss from the study or protocol violation) at age 4 months and 83% at 9 months, and 77% completed 18 months of follow-up. Most mothers from urban settlements or rural areas saw the benefit of free treatment and care given by study nurses as an important reason to decide to participate in the study.

Characteristics of mothers

The characteristics of 312 mothers who had 314 deliveries are shown in Table 2. The median age of rural mothers was 25 years and urban mothers 26 years. Of the 314 deliveries, 292 (93%) occurred in hospital; 13 (59%) of the deliveries that occurred outside hospital were among women with parity of 2 or more compared with 121 (41%) of those who delivered in hospital (Yates χ^2 = 1.93, p = 0.16). Approximately one-third of mothers were primigravida while 23% were gravida 4 or more. Data regarding laboratory tests (VDRL [Venereal Disease Research Laboratory test for syphilis] and HIV) and tetanus toxoid immunization in pregnancy were more often available for women living in Goroka town than for those living in rural areas. Among those for whom laboratory results were available there was no difference in the proportion that were positive between the rural and urban areas. HIV screening was introduced at the GGH antenatal clinic a few months before the study started and HIV results were known on 75% of mothers, all of whom were negative. However, some mothers attending the ANC chose not to have an HIV test. Later, a rapid test for HIV was introduced on the labour ward to screen the mothers with no HIV results before delivery. Nonetheless, two HIV-positive babies were detected later in the study whose mothers had been HIV negative at the time of testing

during pregnancy. Smoking in pregnancy was more common among those living in Goroka than among women from the rural areas (Yates χ^2 = 2.93, 1df, p = 0.09). There was no difference in the characteristics of mothers whose children were randomized to neonatal, infant or control groups (data not shown).

Characteristics at birth of children enrolled in the study

Among children enrolled from rural areas, 54% were male, with similar distribution between assigned groups, while in the urban area the sex distribution varied between assigned groups: 8 (28%), 11 (55%) and 18 (64%) were male in the neonatal, infant and control groups, respectively (χ^2 = 8.21, 2df, p = 0.016). Table 3 shows that estimated gestational age, birthweight, length and head circumference were similar among offspring of women from rural and urban areas. Few children were delivered by caesarean section. There was also no difference in the characteristics of children between the assigned vaccine and control groups (data not shown). Table 4 shows that children received 7vPCV and PPV in a timely manner according to their assigned group.

Specimen collection

Cord blood was collected on the labour ward at GGH from 38% of enrolled mothers (Table 5). Most PNSs at age 1-3 weeks were collected at babies' homes, but all subsequent samples were collected at the PNGIMR clinic. A high proportion of samples were successfully collected from age 1 week onwards among children who were still in the study at the various time points (Table 5). Of the 312 enrolled children, a sample for PBMC was successfully collected from 82% of children at age 3 months and 80% at ages 9, 10 and 18 months. Blood for serum was successfully collected from 90% of the 312 children at 2 months, 87% at 4 months, 72% at 9 months and 78% at 18 months. Pernasal swabs and saliva were collected from more than 90% of enrolled children during the first 3 months, 86% at 9 months and 81% at 18 months. On 16 occasions parents declined blood collection from their children because they felt the babies were too traumatized by the blood collection process. But all parents allowed blood collection if their children were sick. On 44 occasions we were unable to collect blood after two attempts and on a further 48 occasions volumes of blood

TABLE 2

CHARACTERISTICS OF MOTHERS OF STUDY PARTICIPANTS LIVING IN THE RURAL AREAS WITHIN ONE HOUR'S DRIVE FROM GOROKA AND FROM GOROKA TOWN (URBAN)

	Rural	Urban
Total deliveries*	239	75
Mother's age (median) (years)	25	26
minimum-maximum	17-45	17-48
Gravida		
1	81 (34%)	22 (29%)
2	54 (23%)	23 (31%)
3	49 (21%)	13 (17%)
4+	55 (23%)	17 (23%)
Delivered in hospital	222 (93%)	70 (93%)
Tetanus toxoid vaccine in pregnancy		
Yes	186 (78%)	67 (89%)
No	10 (4%)	3 (4%)
Unknown	43 (18%)	5 (7%)
VDRL done		
Yes	190 (79%)	69 (92%)
No	13 (5%)	1 (1%)
Unknown	36 (15%)	5 (7%)
VDRL		
Positive	8 (4%)	3 (4%)
Negative	181 (95%)	66 (96%)
Unknown	1 (1%)	-
HIV test done (none positive)		
Yes	173 (72%)	63 (84%)
No	14 (6%)	1 (1%)
Unknown	52 (22%)	11 (15%)

Mean haemoglobin (g/dl)	12.1	12.2
minimum-maximum	10-14	8-15
Number with known result	189	68
Mother smoking in pregnancy		
Yes	15 (6%)	9 (12%)
No	217 (91%)	55 (73%)
Unknown	7 (3%)	11 (15%)

*Of the 312 mothers who participated in the study 2 gave birth twice during the study period
VDRL = Venereal Disease Research Laboratory test for syphilis
HIV = human immunodeficiency virus

TABLE 3

CHARACTERISTICS OF CHILDREN AT BIRTH IN RURAL AND URBAN AREAS

	Rural	Urban
Mean birthweight (kg) (of those born in hospital)*	3.23	3.18
Range	2.2-5.5	2.01-4.4
Number	221	72
Mean length (cm)	50.24	49.92
Range	40-59	41-57
Number	222	71
Mean head circumference (cm)	33.40	33.34
Range	29-39	30-38
Number	220	73
Mean estimated gestational age (weeks)	39.57	39.33
Range	36-44	35-43
Number	236	76
Delivery by caesarean section (of those born in hospital with a known mode of delivery)	5 (2.3%)	1 (1.4%)

*Note that only children weighing >2000 g were eligible for inclusion in the study

TABLE 4

MEAN AGE (STANDARD DEVIATION) IN DAYS OF VACCINATION WITH 7-VALENT PNEUMOCOCCAL CONJUGATE VACCINE (PCV) AND 23-VALENT PNEUMOCOCCAL POLYSACCHARIDE VACCINE (PPV) IN THE NEONATAL PCV, INFANT PCV AND CONTROL GROUPS

		Neonatal	Infant	Control
PCV	dose 1	1.3 (0.9)	30.0 (2.6)	–
	dose 2	30.2 (3.1)	60.9 (5.3)	–
	dose 3	61.9 (5.5)	91.5 (6.7)	–
PPV		279.2 (16.8)	275.2 (13.8)	275.1 (13.8)

were insufficient. Blood collection was particularly hard in older infants. However, we did manage to collect blood a day or two after the initial attempt for 13 of the routine specimens. 9 blood samples for PBMC were clotted by the time they reached the laboratory.

Unfortunately, not all PNSs could be cultured for bacteria as a result of freezer failure on two occasions, and the loss of liquid nitrogen in a dry shipper resulted in loss of some PBMC samples. Details of available samples will be reported with results of the different laboratory investigations.

Discussion

In Papua New Guinea, children under the age of six months are at substantive risk of invasive pneumococcal disease. The PNG Department of Health plans to reduce the burden of pneumococcal disease in the country by introducing a conjugate pneumococcal vaccine, but current vaccination schedules would miss a portion of children in this vulnerable young group. There is an urgent need to establish the safety and immunogenicity of this vaccine in very young children, and to discount possible interference with other scheduled EPI vaccines. To our knowledge only one other neonatal pneumococcal conjugate vaccine study has been carried out, in Kenya, but in that study cell-mediated immunity was not investigated (22). In PNG we conducted an open randomized controlled trial of 7vPCV given to neonates at ages 0, 1 and 2 months and to infants at 1, 2 and 3 months, compared to non-immunized controls. This was carried

out in a cohort of over 300 children over an 18-month period. While trial results will be reported elsewhere, much can also be learned from discussion of the methodology and operational challenges of such a large complex trial in a semi-rural developing country context.

The study required follow-up of all children weekly for the first year and fortnightly for the subsequent 6 months. It also necessitated the collection of numerous biological samples, including blood, serum, saliva and pernasal swabs. The study team was outstandingly successful in achieving these ambitious goals. The full eighteen months of follow-up was successfully completed on 77% of enrolled children and over 80% of the many thousands of biological samples scheduled were collected for laboratory analysis. Attention to detail in two particular aspects of the trial design was thought to be a major influence on the success of this study in recruiting and retaining the cohort, namely the level of community engagement and the ongoing consent process.

The PNGIMR has a long experience of community engagement in semi-rural PNG to draw on and has learned many lessons from other large studies, such as the malaria vaccine trial (23); lessons learned by some of the investigators from community-based trials in Aboriginal communities in Australia also had strong relevance (24). The concepts of provision of service and establishment of true partnerships with local communities, to develop respectful, equitable research relationships, were fundamental principles in conducting the study.

TABLE 5

NUMBER (%), BLOOD VOLUMES AND MEAN NUMBER OF PBMC* OF SPECIMENS COLLECTED AT ROUTINE FOLLOW-UP VISITS FROM BIRTH TO AGE 18 MONTHS

Age	Birth†	1 week	2 weeks	3 weeks	1 month	2 months	3 months	4 months	9 months	10 months	18 months
Total number‡	**312**	**311**	**307**	**305**	**303**	**292**	**285**	**278**	**270**	**263**	**254**
Number of blood samples for serum	118 (37.8%)					281 (96.2%)		270 (97.1%)	225 (83.3%)	215 (81.7%)	243 (95.7%)
Mean volume (ml)	1.4					0.72		0.78	0.58	0.58	0.66
(Min-Max)	(0.1-17.1)					(0.01-4.8)		(0.04-7)	(0.06-1.4)	(0.04-1.4)	(0.05-1.6)
Number of blood samples for PBMC*	120 (38.5%)						257 (90.2%)		251 (93.0%)	249 (94.7%)	250 (98.4%)
Mean volume (ml)	36.3						1.85		1.92	1.27	2.04
(Min-Max)	(5-55)						(0.2-5.0)		(0.5-4)	(0.5-4)	(0.6-4)
Mean number of cells x 10^6	74.0						7.04		7.42	7.53	7.16
(Min-Max)	(3.2-527.1)						(0.05-66.11)		(0.01-25.01)	(0.3-24.9)	(0.7-25.9)
Number of saliva samples					296 (97.7%)	289 (99.0%)	282 (98.9%)	278 (100%)	267 (98.9%)	262 (99.6%)	253 (99.6%)
Number of pernasal swabs		307 (98.7%)	304 (99.0%)	298 (97.7%)	297 (98.0%)		281 (98.6%)		267 (98.9%)		253 (99.6%)

*peripheral blood mononuclear cells
†cord blood
‡numbers exclude those who did not meet inclusion criteria (eg congenital heart disease, HIV+ve) and those who were lost to follow-up or withdrew before relevant time point
Min-Max = minimum-maximum

Engaging community support, rather than concentrating specifically on trial recruitment, is absolutely essential in PNG. Ahead of any activity, inclusive information sessions were held in the community at venues such as church gatherings and positive consensus for the study was gained from community leaders. Local village-based reporters were then engaged by the project to carry out demographic surveillance and to identify eligible pregnant mothers for approach. This had the dual benefit of recruitment through familiar faces and the provision of employment into the study area, as a reciprocal benefit. The provision of a study clinic with free transport and treatment for study participants (and their relatives) was also identified by mothers as a strong motivating factor and a distinct proof of service provision. Even with this strong foundation, tracking down children for clinic visits was difficult and time consuming, and could take 2 or 3 trips out to distant villages to locate the child. Neither did it stop the embroilment of the study in village politicking, as disruptive unfounded rumours were spread from time to time, for example that the vaccine was untested and unsafe, or that blood was being sold for profit to overseas organizations. The presence of village-based staff did, however, allow the rapid recognition of these problems and the deployment of the study team into the area to directly counteract the issues raised with valid information.

The consent process used was also a carefully considered element of the trial. As the women needed to be recruited at delivery, when clearly their major focus would not be a trial investigator, a two-stage process of assent and consent was used. When pregnant women were identified as potential recruits, they were visited by a study nurse who explained the project using a standard flip chart and left explanatory pamphlets for her to discuss with her wider family. If the woman then gave assent, when she came to the labour ward for delivery a study nurse was present to seek consent. Again, information sheets were left with the mother to discuss with family and it was made clear that they could withdraw consent at any time. Using this strategy, out of 448 women that gave assent 312 gave consent (6 of them twice); the substantial number of those withholding consent was a good indication that consideration had been given to the implications of joining the study and undoubtedly contributed to the excellent retention of those who did give consent. Interestingly, a significant number of those who withdrew consent did so because the husband had not been present at the time of antenatal assent or the delivery consent and later pushed the often embarrassed woman to withdraw. There is a necessity of involving men in the consent process, because of the power dynamic of the marital relationship, and our finding is reflected in other treatment trials conducted elsewhere in PNG (25).

In summary, for a large and complex trial in a developing country context one of course needs technical expertise in the laboratory testing and data handling required, but the success of a trial is likely to hinge on factors outside the technical implementation. Strong community participation, reciprocity and a good relationship between the study team and the participants are vitally important considerations.

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List of Medical Research Projects in Papua New Guinea

Approved or Noted

By the Medical Research Advisory Committee in 2009

Malaria in Pregnancy (MiP): acceptability of Intermittent Preventive Treatment in Pregnancy (IPTp) in Madang, Papua New Guinea

Dr Suparat Phuanukoonnon (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Cytoadhesion and rosetting: implications in paediatric malaria

Dr Ivo Mueller (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Collaborative research on *P. vivax* and *P. falciparum* diagnostic blood stage antigen discovery

Dr Ivo Mueller (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

A detailed assessment of encephalitis in PNG children

Dr Laurens Manning (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

The detection of sexually transmitted infections in suspected victims of child abuse in the Eastern Highlands Province, Papua New Guinea

Dr Andrew Greenhill (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Severe anaemia in PNG children: an additional sub-study of the severe childhood illness study

Dr Laurens Manning (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

ICEE measurement barriers to eye care, utilization of services and vision-specific quality of life in PNG

Dr Jambi Garap (Eye Care PNG, Port Moresby General Hospital, Free Mail Bag, Boroko, NCD 111, Papua New Guinea)

Clinical epidemiology of sago haemolytic disease in PNG

Dr Miila Gena (Immunology and Microbiology, School of Veterinary and Biomedical Sciences, James Cook University, Townsville, Queensland, Australia)

Modelling costs and efficiency of primary health care services in PNG

Professor Brett Inder (Department of Econometrics, Monash University, Clayton, VIC 3800, Australia)

Study of the erythrocytic invasion profiles of placental isolates

Dr Ivo Mueller (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

Effect of liver and blood-stage treatment on subsequent *Plasmodium* reinfection and morbidity

Dr Inoni Betuela (Papua New Guinea Institute of Medical Research, PO Box 400, Maprik, East Sepik Province 533, Papua New Guinea)

Optimization of multiplex PCRs for the simultaneous detection of various genital ulcer causing agents

Ms Janet Gare (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Artemether-lumefantrine clinical evaluation study

Dr Manuel Hetzel, Dr Ivo Mueller and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Fc receptor mediated humoral/cellular interaction in immunity to malaria

Dr Louis Schofield, Dr Danielle Stanisic, Dr Ivo Mueller, Mr Livingstone Tavul and Mr

Jack Taraika (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

Cost-effectiveness analysis of intermittent preventive treatment with azithromycin-containing regimens in addition to long-lasting insecticide treated nets for the prevention of malaria infection, anaemia and control of sexually transmitted diseases during pregnancy in Papua New Guinea
Dr Ivo Mueller, Dr Silke Lutzelschwab, Dr Kara Hanson, Dr Suparat Phuanukoonnon, Mr Mexy Kakazo and Dr Elisa Sicuri (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

Oil Search (Ltd) – Malaria Surveillance Program
Mr Ross Hutton (Oil Search Health Services, PO Box 842, Port Moresby, NCD 121, Papua New Guinea)

The study of knowledge, attitudes and behaviour of caregivers of children <5 years, pregnant women and health service providers to the treatment and prevention of malaria in the four regions of Papua New Guinea
Mr Mexy Kakazo (Faculty of Health Sciences, Divine Word University, PO Box 483, Madang, Madang Province 511, Papua New Guinea)

Mechanisms of immune maturation in Papua New Guinean infants
Dr Anita van den Biggelaar, Prof. Peter Siba and Mr William Pomat (Institute for Child Health Research, PO Box 855, West Perth, WA 6872, Australia)

Epidemiology of tuberculosis: active case detection in sentinel sites across Papua New Guinea
Dr Suparat Phuanukoonnon and Ms Serej Ley (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Molecular parasitology of severe childhood *Plasmodium vivax* infection in children in Madang Province, Papua New Guinea
Dr Laurens Manning (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)
An investigation into the causes of concurrent infections in HIV-positive people in PNG: a greater knowledge will improve diagnosis and treatment
Dr Andrew Greenhill and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Study of *Plasmodium falciparum* parasite ligand recognition and invasion into CR1 deficiency erythrocytes
Mr Livingstone Tavul (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

Effect of fat bioavailability of artemisinin combination therapy in PNG children with malaria
Dr John Benjamin (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

Genetic diversity of novel *Plasmodium falciparum* and *P. vivax* antigens
Dr Ingrid Felger (Swiss Tropical Institute, Socinstrasse 57, 4002 Basel, Switzerland)

Women's and men's experience of PMTC in PNG: a gendered sociocultural analysis of barriers and facilitators for program engagement
Ms Martha Kupul (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

A research proposal to investigate pharmacology, genetics and sociology in relation to antimalarial drugs and their implications for malaria control in Papua New Guinea
Prof. Francis Hombhanje (Divine Word University, PO Box 483, Madang, Madang Province 511, Papua New Guinea)

Note:

These projects have been examined and cleared by the MRAC but they have not all started, nor is there any guarantee that they all will, since in many cases this still depends on funding. It should be noted that the project funds for the MRAC were deleted from the Health Budget from 1997 to 2009.

Information about these projects may be obtained from the investigators or from the

Chairperson of the Medical Research Advisory Committee (Director of Research and Monitoring, Department of Health, PO Box 807, Waigani, NCD 131)

MEDLARS BIBLIOGRAPHY

P UBLICATIONS OF RELEVANCE TO P APUA N EW G UINEA AND M ELANESIA

Bibliographic Citation List generated from MEDLARS

1 **Aguayo F, Anwar M, Koriyama C, Castillo A, Sun Q, Morewaya J, Eizuru Y, Akiba S.**
Human papillomavirus-16 presence and physical status in lung carcinomas from Asia.
Infect Agent Cancer 2010 Nov 16;5:20.
    BACKGROUND: Although human papillomavirus (HPV) genome has been detected in lung cancer, its prevalence is highly variable around the world. Higher frequencies have been reported in far-east Asian countries when compared with European countries. The present study analysed the HPV-16 presence in 60 lung carcinomas from the Asian countries China, Pakistan and Papua New Guinea. RESULTS: HPV-16 was present in 8/59 (13%) samples. According to histological type, HPV-16 was detected in 8/18 (44%) squamous cell carcinomas (SQCs), which were mainly from Pakistan; 0/38 (0%) adenocarcinomas (ACs), which were mainly from China; and in 0/4 (0%) small cell carcinomas (SCLCs). The observed histological difference was statistically significant ($p < 0.001$). HPV-16 viral load was also determined using real-time polymerase chain reaction (qRT-PCR); it ranged between 411 to 2345 copies/100 ng of genomic DNA. HPV-16 genome was found integrated into the host genome in every HPV-16 positive carcinoma. CONCLUSION: These results support the notion that HPV-16 infection is highly associated with SQCs in Pakistan. Our results show a frequent HPV-16 integration in SQCs, although the low viral load casts doubt with respect to a direct etiological role of HPV in lung carcinomas from Asia. Additional HPV-16 characterization is necessary to establish a direct or indirect etiological role of HPV in this malignancy.

2 **Alquezar DE, Hemmerter S, Cooper RD, Beebe NW.**
Incomplete concerted evolution and reproductive isolation at the rDNA locus uncovers nine cryptic species within *Anopheles longirostris* from Papua New Guinea.
BMC Evol Biol 2010 Dec 24;10:392.
    BACKGROUND: Nuclear ribosomal DNA (rDNA) genes and transcribed spacers are highly utilized as taxonomic markers in metazoans despite the lack of a cohesive understanding of their evolution. Here we follow the evolution of the rDNA second internal transcribed spacer (ITS2) and the mitochondrial DNA cytochrome oxidase I subunit in the malaria mosquito *Anopheles longirostris* from Papua New Guinea (PNG). This morphospecies inhabits a variety of ecological environments indicating that it may comprise a complex of morphologically indistinguishable species. Using collections from over 70 sites in PNG, the mtDNA was assessed via direct DNA sequencing while the ITS2 was assessed at three levels – crude sequence variation through restriction digest, intragenomic copy variant organisation (homogenisation) through heteroduplex analysis and DNA sequencing via cloning.

RESULTS: Genetic evaluation of over 300 individuals revealed that *A. longirostris* comprises eight ITS2 PCR-RFLP genotypes and nine ITS2 heteroduplex genotypes showing distinct copy variant organization profiles after PCR amplification. Seven of these nine genotypes were found to be sympatric with other genotypes. Phylogenetic analysis of cloned ITS2 PCR products and mtDNA COI confirmed all nine clades with evidence of reproductive isolation at the rDNA locus. Compensatory base changes in the ITS2 secondary structure or in pseudoknots were absent when closely related species were assessed. Individuals from each ITS2 genotype showed the same copy variant heteroduplex profile suggesting that the rDNA array is fixed within each genotype. CONCLUSION: The centromere-proximal position of the rDNA array in *Anopheles* mosquitoes has probably reduced interchromosomal recombination leaving intrachromosomal events responsible for the observed pattern of concerted evolution we see in these mosquitoes. The stability of these intragenomic ITS2 copy variants within individuals and interbreeding populations suggests that rDNA is moving as a single evolutionary unit through natural populations to fixation and has provided a complementary diagnostic tool to the restriction digest for studying genetic discontinuities and species boundaries. In this, the utility of the ITS2 as a universal taxonomic marker is probably contingent on several factors pertaining to spacer dimensions and the genomic location of the rDNA array with respect to recombination and proximity to regions potentially under selection.

3 **Ashwell HE, Barclay L.**
Problems measuring community health status at a local level: Papua New Guinea's health information system.
Rural Remote Health 2010 Oct-Dec;10(4):1539. Epub 2010 Dec 6.
    INTRODUCTION: The Papua New Guinea Department of Health monitors the performance of the health system using a computerized national health information system. This article draws on the recent evaluation of a national-wide donor-project community development initiative to highlight the problems of the lack of disaggregated village health data. This data could be used to monitor health status, health worker performance and intervention impact. METHODS: An extensive outcome evaluation conducted in 2006 used qualitative and quantitative data. The in-depth study covered 10 provinces (50%) and 19 districts (21%), obtaining data from 175 health personnel informal interviews and 77 community focus group discussions. Quantitative data from the health information system were examined for validation of the qualitative findings over a 7-year period (1998-2004). RESULTS: Healthier lifestyle and enhanced social and economic wellbeing were claimed by the

community to be the result of the project intervention. The evaluation found village claims of post-project improved physical health, increased use of health services and reduced maternal and child mortality could not be substantiated statistically. Health-centre data failed to provide a complete and accurate assessment of community health status within the national health information system. CONCLUSION: This article highlights problems in evaluating community interventions or local service performance if reliable village-level data is absent. The health information system does not allow reporting of villages separately or the tracking of changes in health status over time according to identifiable villages. Assessing changes in physical health status is not possible without village-level baseline data to measure illness trends and improvements in health in identifiable villages. There is a need for policy changes to occur at national level to prevent loss of aid-post data from the system. Future planning for community health intervention strategies needs to include disaggregated village-level baseline data against which to measure changes in community health status over time.

4   **Ballif M, Hii J, Marfurt J, Crameri A, Fafale A, Felger I, Beck HP, Genton B.**
Monitoring of malaria parasite resistance to chloroquine and sulphadoxine-pyrimethamine in the Solomon Islands by DNA microarray technology.
Malar J 2010 Oct 6;9:270.

BACKGROUND: Little information is available on resistance to anti-malarial drugs in the Solomon Islands (SI). The analysis of single nucleotide polymorphisms (SNPs) in drug resistance associated parasite genes is a potential alternative to classical time- and resource-consuming in vivo studies to monitor drug resistance. Mutations in *pfmdr1* and *pfcrt* were shown to indicate chloroquine (CQ) resistance, mutations in *pfdhfr* and *pfdhps* indicate sulphadoxine-pyrimethamine (SP) resistance, and mutations in *pfATPase6* indicate resistance to artemisinin derivatives. METHODS: The relationship between the rate of treatment failure among 25 symptomatic *Plasmodium falciparum*-infected patients presenting at the clinic and the pattern of resistance-associated SNPs in *P. falciparum* infecting 76 asymptomatic individuals from the surrounding population was investigated. The study was conducted in the SI in 2004. Patients presenting at a local clinic with microscopically confirmed *P. falciparum* malaria were recruited and treated with CQ+SP. Rates of treatment failure were estimated during a 28-day follow-up period. In parallel, a DNA microarray technology was used to analyse mutations associated with CQ, SP and artemisinin derivative resistance among samples from the asymptomatic community. Mutation and haplotype frequencies were determined, as well as the multiplicity of infection. RESULTS: The in vivo study showed an efficacy of 88% for CQ+SP to treat *P. falciparum* infections. DNA microarray analyses indicated a low diversity in the parasite population with one major haplotype present in 98.7% of the cases. It was composed of fixed mutations at position 86 in *pfmdr1*, positions 72, 75, 76, 220, 326 and 356 in *pfcrt*, and positions 59 and 108 in *pfdhfr*. No mutation was observed in *pfdhps* or in *pfATPase6*. The mean multiplicity of infection was 1.39. CONCLUSION: This work provides the first insight into drug resistance markers of *P. falciparum* in the SI. The obtained results indicated the presence of a very homogenous *P. falciparum* population circulating in the community. Although CQ+SP could still clear most infections, seven fixed mutations associated with CQ resistance and two fixed mutations related to SP resistance were observed. Whether the absence of mutations in *pfATPase6* indicates the efficacy of artemisinin derivatives remains to be proven.

5   **Baumann F, Bourrat MB, Pauillac S.**
Prevalence, symptoms and chronicity of ciguatera in New Caledonia: results from an adult population survey conducted in Noumea during 2005.
Toxicon 2010 Oct;56(5):662-667. Epub 2009 Oct 14.

Ciguatera is a widespread ichthyosarcotoxism which causes gastrointestinal, neurological and cardiovascular disturbances. Investigations conducted by ORSTOM in 1992 highlighted a prevalence of 25% in the adult population of Noumea, New Caledonia. The main objective of our study was to estimate the prevalence of ciguatera and the persistence of symptoms by sex and by ethnicity among adult patients of a nurse clinic in Noumea in 2005. Investigations were conducted from 1st January to 15th June 2005. During this period, 559 patients were included: 165 males and 394 females. Among them, 37.8% were poisoned at least once in their life. This rate was independent of gender and ethnicity, but was significantly higher in age groups above 40 years. Neurological signs were more frequent (>80%) than gastrointestinal (<50%) and cardiac signs (<15%). Symptoms presented no difference between ethnic or gender groups, even for subjective signs. Most poisonings were due to carnivorous fishes, but nearly all species living in the lagoon were implicated. Symptoms persisted more than one year for 34% of the population, in both Melanesians and Caucasians. This study shows a significant increase of ciguatera prevalence, and its chronicity for 1/5 of European cases.

6   **Bhullar N, Maikere J.**
Challenges in mass drug administration for treating lymphatic filariasis in Papua, Indonesia.
Parasit Vectors 2010 Aug 11;3:70.

BACKGROUND: The World Health Organization (WHO) Global Program to Eliminate Lymphatic Filariasis relies on mass drug administration (MDA) of two drugs annually for 4 to 6 years. The goal is to reduce the reservoir of microfilariae in the blood to a level insufficient to maintain transmission by the mosquito vector. In 2008, the international medical aid organization Médecins Sans Frontières (MSF) performed the first round of an MDA in the high-burden area of Asmat district, in Papua, Indonesia. We report the challenges faced in this MDA on a remote Indonesian island and propose solutions to overcome these hurdles in similar future contexts. RESULTS: During the MDA, we encountered difficult challenges in accessing as well as persuading the patient population to take the antifilarial drugs. Health promotion activities supporting treatment need to be adapted and repetitive, with adequate time and resources allocated for accessing and communicating with local, seminomadic populations. Distribution of bednets resulted in an increase in MDA coverage, but it was still below the 80-85% target. CONCLUSIONS: MDA for lymphatic filariasis is how the WHO has planned to eliminate the disease from endemic areas. Our programmatic experience will hopefully help inform future campaign planning

in difficult-to-access, high-burden areas of the world to achieve target MDA coverage for elimination of lymphatic filariasis.

7   **Breed AC, Field HE, Smith CS, Edmonston J, Meers J.**
Bats without borders: long-distance movements and implications for disease risk management.
Ecohealth 2010 Jun;7(2):204-212. Epub 2010 Jul 20.
    Fruit bats of the genus *Pteropus* (commonly known as flying-foxes) are the natural hosts of several recently emerged zoonotic viruses of animal and human health significance in Australia and Asia, including Hendra and Nipah viruses. Satellite telemetry was used on nine flying-foxes of three species (*Pteropus alecto* n=5, *P. vampyrus* n=2 and *P. neohibernicus* n=2) to determine the scale and pattern of their long-distance movements and their potential to transfer these viruses between countries in the region. The animals were captured and released from six different locations in Australia, Papua New Guinea, Indonesia and Timor-Leste. Their movements were recorded for a median of 120 (range, 47-342) days with a median total distance travelled of 393 (range, 76-3011) km per individual. *Pteropus alecto* individuals were observed to move between Australia and Papua New Guinea (Western Province) on four occasions, between Papua New Guinea (Western Province) and Indonesia (Papua) on ten occasions, and to traverse Torres Strait on two occasions. *Pteropus vampyrus* was observed to move between Timor-Leste and Indonesia (West Timor) on one occasion. These findings expand upon the current literature on the potential for transfer of zoonotic viruses by flying-foxes between countries and have implications for disease risk management and for the conservation management of flying-fox populations in Australia, New Guinea and the Lesser Sunda Islands.

8   **Breed AC, Yu M, Barr JA, Crameri G, Thalmann CM, Fa Wang L.**
Prevalence of henipavirus and rubulavirus antibodies in pteropid bats, Papua New Guinea.
Emerg Infect Dis 2010 Dec;16(12):1997-1999.
    To determine seroprevalence of viruses in bats in Papua New Guinea, we sampled 66 bats at 3 locations. We found a seroprevalence of 55% for henipavirus (Hendra or Nipah virus) and 56% for rubulavirus (Tioman or Menangle virus). Notably, 36% of bats surveyed contained antibodies to both types of viruses, indicating concurrent or consecutive infection.

9   **Brian G, Fischer-Harder K, Sikivou B, Qoqonokana MQ, Szetu J, Ramke J.**
Diabetic eye disease among adults in Fiji with self-reported diabetes.
Clin Experiment Ophthalmol 2010 Dec;38(9):867-874. doi: 10.1111/j.1442-9071.2010.02361.x.
    BACKGROUND: To characterize diabetic eye disease and its management among adults aged ≥40 years with self-reported diabetes in Fiji. METHODS: During a population-based cross-sectional survey using multistage cluster random sampling, participants reported health information, including whether a doctor had diagnosed diabetes. HbA1c and visual acuity were measured. Diabetic eye disease was assessed using 90-dioptre lens dilated funduscopy. RESULTS: Of those enumerated, 1381 (73.0%) participated, with 222

reporting diabetes. Twenty fundi were not examined (19 due to cataract). Of the remaining 424 eyes, 75.5% had no diabetic disease, 1.2% had proliferative retinopathy, 7.5% had active significant maculopathy and 0.7% had burnt-out/treated disease. By person, 27.2% had retinopathy and/or maculopathy in at least one eye. Mean HbA1c (9.9 ± 2.3%) for this group was significantly higher (p = 0.004) than for those without eye disease. Vision-threat occurred in at least one eye of 11.5%. Diabetes (predominantly maculopathy) caused pinhole acuity <6/18, <6/60 and <3/60 for 3.8%, 1.1% and 0.7% of eyes, respectively. No person was bilaterally blind (<6/60) due to diabetes, but 2.3% (all on oral antiglycaemics alone) were 6/60 bilaterally. Compared with recent diabetes diagnosis, diagnosis >10 years ago was predictive of any (odds ratio [OR] 8.13; 95% confidence interval [CI] 3.28-20.21; p <0.001) and vision-threatening (OR 5.25; 95% CI 1.71-16.12; p = 0.004) eye disease. Although 80.6% claimed regular general diabetes checkups, only 36.5% recalled previous dilated ocular examination. Four eyes had received laser treatment. CONCLUSION: There was evidence of failure of management of diabetes and its eye complications. Both need to be improved if increasing diabetes-related visual disability is to be avoided.

10   **Chandra G, Bhattacharjee I, Chatterjee S.**
A review on *Anopheles subpictus* Grassi – a biological vector.
Acta Trop 2010 Jul-Aug;115(1-2):142-154. Epub 2010 Feb 11.
    Anopheles subpictus is a complex of four isomorphic sibling species A, B, C and D and is recognized as a primary vector of malaria, a disease of great socio-economic importance, in the Australasian Zone, Celebes, Portuguese Timor and South East Asia and a secondary vector in Sri Lanka. This species is also a vector of some helminths and arboviruses. This species has been reported so far from nineteen countries of the Oriental and Australasian Zones. *An. subpictus* complex is the most abundant anopheline in most parts of the Indian subcontinent, with a widespread distribution eastwards and southwards to Papua New Guinea, westwards to Iran and northwards to China. Resistance to insecticide is alarming in many parts of the world. Different aspects of this important mosquito species including attempts related to its control have been discussed which will be highly useful for carrying out further research.

11   **Chandrashekaran IR, Adda CG, MacRaild CA, Anders RF, Norton RS.**
Inhibition by flavonoids of amyloid-like fibril formation by *Plasmodium falciparum* merozoite surface protein 2.
Biochemistry 2010 Jul 20;49(28):5899-5908.
    Merozoite surface protein 2 (MSP2) is a glycosylphosphatidylinositol (GPI)-anchored protein expressed abundantly on the surface of *Plasmodium falciparum* merozoites. The results of a phase 2 trial in Papua New Guinean children showed MSP2 to be a promising malaria vaccine candidate. MSP2 is intrinsically unstructured and forms amyloid-like fibrils under physiological conditions. Oligomers containing beta-strand interactions similar to those in amyloid fibrils may be a component of the fibrillar surface coat on *P. falciparum* merozoites. As the propensity of MSP2 to form fibrils in solution also

has the potential to impede its development as a vaccine candidate, finding an inhibitor that specifically inhibits fibrillogenesis may enhance vaccine development. In this study, we tested the ability of three flavonoids, EGCG, baicalein and resveratrol, to inhibit MSP2 fibrillogenesis and found marked inhibition with EGCG but not with the other two flavonoids. The inhibitory effect and the interactions of the flavonoids with MSP2 were characterized using NMR spectroscopy, thioflavin T fluorescence assays, electron microscopy, and other biophysical methods. EGCG stabilizes soluble oligomers and blocks fibrillogenesis by preventing the conformational transition of MSP2 from a random coil to an amyloidogenic beta-sheet structure. Structural comparison of the three flavonoids indicates an association between their propensity for autoxidation and their fibril inhibitory activity; the activity of EGCG can be attributed to the vicinal hydroxyl groups present in this flavonoid and their ability to form quinones. The molecular mechanism of fibril inhibition by EGCG appears to be complex and involves noncovalent binding followed by covalent modification of the protein. Although the addition of EGCG appears to be an effective means of stabilizing MSP2 in solution, the covalent modification of MSP2 would most likely not be acceptable in a vaccine formulation. However, these small molecule inhibitors of MSP2 fibril formation will be useful as mechanistic probes in studying oligomerization and fibril assembly of MSP2.

12 **Cléro E, Leux C, Brindel P, Truong T, Anger A, Teinturier C, Diallo I, Doyon F, Guénel P, de Vathaire F.**
Pooled analysis of two case-control studies in New Caledonia and French Polynesia of body mass index and differentiated thyroid cancer: the importance of body surface area.
Thyroid 2010 Nov;20(11):1285-1293. Epub 2010 Oct 9.
    BACKGROUND: New Caledonia and French Polynesia have among the world's highest thyroid cancer incidence rates. Studies have demonstrated a relationship between anthropometric parameters and the prevalence of cancer. In this study we evaluated further the relationship between body mass index (BMI) and other anthropometric parameters and the incidence of thyroid cancer in the New Caledonia and French Polynesia populations. METHODS: We performed a pooled analysis of two case-control studies in New Caledonia and French Polynesia. We included a total of 554 cases (65 men and 489 women) of differentiated thyroid cancers and 776 population control subjects matched on sex, age and study. Anthropometric factors (height, weight, BMI, body fat percentage [BF%] and body surface area [BSA]), at age 18 and before diagnosis, were analyzed by conditional logistic regression, adjusting for other independent risk factors. RESULTS: A high proportion of cases (73%) were overweight (25-29.9 kg/m^2) or obese ($\geq$30 kg/m^2) before diagnosis of thyroid cancer (against 57% of control subjects). An increased risk of thyroid cancer was observed with greater height, weight, BMI, BF% and BSA. The association of thyroid cancer risk with height, weight, BMI and BF% did not remain when adjustment was made for BSA. By comparison, the odds ratios for the highest versus the lowest quartile of BSA at age 18 were 3.97 (95% confidence interval, 2.57-6.15; p <0.001)

for women and 4.06 (95% confidence interval, 1.03-16.06; p = 0.04) for men. The association between thyroid cancer risk and each of the anthropometric factors did not depend on tumor size or menopausal status before diagnosis. CONCLUSION: Among anthropometric factors, BSA plays a dominant role in thyroid cancer risk and explains the apparent role of BMI.

13 **Cummings LJ.**
One midwife's journey; stories from Papua New Guinea and beyond.
Midwifery Today Int Midwife 2010 Autumn;(95):55-56, 69.

14 **Davy CP, Sicuri E, Ome M, Lawrence-Wood E, Siba P, Warvi G, Mueller I, Conteh L.**
Seeking treatment for symptomatic malaria in Papua New Guinea.
Malar J 2010 Oct 6;9:268.
    BACKGROUND: Malaria places a significant burden on the limited resources of many low income countries. Knowing more about why and where people seek treatment will enable policy makers to better allocate the limited resources. This study aims to better understand what influences treatment-seeking behaviour for malaria in one such low-income country context, Papua New Guinea (PNG). METHODS: Two culturally, linguistically and demographically different regions in PNG were selected as study sites. A cross sectional household survey was undertaken in both sites resulting in the collection of data on 928 individuals who reported suffering from malaria in the previous four weeks. A probit model was then used to identify the factors determining whether or not people sought treatment for presumptive malaria. Multinomial logit models also assisted in identifying the factors that determined where people sought treatment. RESULTS: Results in this study built upon findings from other studies. For example, while distance in PNG has previously been seen as the primary factor in influencing whether any sort of treatment will be sought, in this study cultural influences and whether it was the first, second or even third treatment for a particular episode of malaria were also important. In addition, although formal health care facilities were the most popular treatment sources, it was also found that traditional healers were a common choice. In turn, the reasons why participants chose a particular type of treatment differed according to whether they were seeking an initial or subsequent treatment. CONCLUSIONS: Simply bringing health services closer to where people live may not always result in a greater use of formal health care facilities. Policy makers in PNG need to consider within-country variation in treatment-seeking behavior and the important role of traditional healers and also ensure that the community fully understands the potential implications of not seeking treatment for illnesses such as malaria at a formal health care facility.

15 **Decaro JA, Decaro E, Worthman CM.**
Sex differences in child nutritional and immunological status 5-9 years post contact in fringe highland Papua New Guinea.
Am J Hum Biol 2010 Sep-Oct;22(5):657-666.
    OBJECTIVES: This study examines sex differences in vulnerability among children experiencing rapid culture change that may reflect distinct microecologies driven by differential

parental investment and/or sex-specific life history strategies. Apparent female growth canalization may be a life history strategy favouring growth over maintenance but also may reflect sex-differentiated selection for resilience based on unequal treatment during early life. METHODS: Stature, weight, and serum measures of C-reactive protein (CRP, an inflammation marker) and Epstein-Barr virus antibodies (EBV, a humoral immune response marker) were collected longitudinally among children/adolescents aged 5-20 years (N = 65), 5-9 years after sustained contact in a fringe highland hunter-horticulturalist group from the Schrader Range in Papua New Guinea exhibiting male preference and sex-biased survival. It was hypothesized that girls would exhibit canalization, with better nutritional status than boys; lower maintenance investment would yield lower female immune activation; and because of differential survivorship, females would appear increasingly canalized as early conditions for girls worsened relative to boys. RESULTS: Girls had greater arm circumference z-scores than boys, less frequent stunting, and lower CRP despite high pathogen load. Average nutritional status for girls improved over time as the sex ratio became increasingly male biased and the condition of female infants reportedly worsened. CONCLUSIONS: Both canalization and survivorship effects were found. Although a life history perspective on female canalization can help explain developmental outcomes in populations undergoing rapid culture change amid adversity, possible sex differences in the strength of survivorship effects that select for resiliency should not be ignored.

16 **du Toit R, Palagyi A, Brian G.**
The development of competency-based education for mid-level eye care professionals: a process to foster an appropriate, widely accepted and socially accountable initiative.
Educ Health (Abingdon) 2010 Aug;23(2):368. Epub 2010 Jul 23.

INTRODUCTION: The Western Pacific region has a dearth of appropriately educated eye care providers and training programs and large and increasing eye health needs. METHOD: To ensure regional eye health needs would be met, an iterative process sought triangulations between the literature and consultations with local stakeholders from various fields. This information was used to develop competencies to meet quality standards for educational outcomes. A framework for social accountability was used to evaluate the proposed educational initiative and the subsequent eye care service the graduates could provide. RESULTS: Current human resource development and deployment is inadequate to protect and restore ocular and visual health in the region. Some of these service needs could be met by task-shifting from conventional health professionals to appropriately trained mid-level personnel. A competency-based curriculum was developed to meet eye care needs and define this new cadre of mid-level professionals in relation to other professionals. This initiative met the relevance, equity, cost-effectiveness and quality criteria for social accountability. DISCUSSION: The consultative process resulted in broad acceptance of the need for an appropriately educated mid-level cadre that could be recruited, educated, deployed, supported and retained in the Western Pacific region to supplement and substitute for established eye

care professionals. This process also provided validation of the initiative prior to implementation, as being appropriate to the region and meeting educational standards and social accountability criteria for outcomes. It could be replicated in other regions that wish to develop such an education for new cadres of health care professionals.

17 **Fernandez LS, Sykes ML, Andrews KT, Avery VM.**
Antiparasitic activity of alkaloids from plant species of Papua New Guinea and Australia.
Int J Antimicrob Agents 2010 Sep;36(3):275-279. Epub 2010 Jun 26.

New drugs are needed to help overcome the increasing problem of drug resistance in parasites that cause diseases such as malaria and trypanosomiasis. In this study, alkaloid compounds isolated from extracts of the plants *Flindersia amboinensis*, *Stephania zippeliana* and *Voacanga papuana* from Papua New Guinea and *Flindersia acuminata* from Australia were examined for their antiparasitic activity against *Plasmodium falciparum* strains and *Trypanosoma brucei brucei* as well as their cytotoxicity against the mammalian cell lines HEK 293 and HeLa. The most active compound, dimethylisoborreverine (DMIB), showed submicromolar activity, with 50% inhibitory concentration (IC_{50}) values between 20 nM and 810 nM both against drug-sensitive and drug-resistant *P. falciparum* strains, along with moderate selectivity against *T. b. brucei* and mammalian cells. Stage specificity studies revealed that *P. falciparum* trophozoite-stage parasites were more susceptible to DMIB than ring- or schizont-stage parasites. DMIB-treated trophozoites showed changes in food vacuole morphology, with an apparent reduction in haemozoin formation that does not appear to be inhibited via the direct binding of haem. These findings suggest a potential for indole alkaloids from *Flindersia* spp. as new antiparasitic agents.

18 **Fotinatos N, Warmington A, Walker T, Pilbeam M.**
Knowledge and perceptions of cervical cancer and healthcare in Vanuatu.
Health Promot J Austr 2010 Aug;21(2):127-129.

ISSUE ADDRESSED: There is increasing evidence of unacceptably high levels of cervical cancer abnormalities in Vanuatu. The purpose of this research was to determine cervical health awareness in local women from rural and urban environments. METHODS: Women from hospitals, health clinics and small local villages were invited to participate in a health survey. This investigated health knowledge, current information sources and perceived limitations in accessing health information. RESULTS: A total of 422 surveys were undertaken, a response rate of 93% in urban centres and 95% in rural areas. There was a direct relationship between the number of school years completed and awareness of cancer. Nurses, doctors and village health workers all played a vital role in providing women's health care information. General embarrassment and a lack of knowledge were the greatest limitations reported to affect the ability and confidence for women to investigate health concerns. CONCLUSIONS: Vanuatu women are poorly educated regarding health issues, particularly cervical cancer. Strategies to improve cervical cancer awareness may include travelling workshops, an active media campaign and the introduction of culturally sensitive education programs tailored to

formal and non-formal environments. Programs should inform whole communities and health care professionals.

19 **Frances SP, Bugoro H, Butafa C, Cooper RD.**
Field evaluation of deet against *Anopheles farauti* at Ndendo (Santa Cruz) Island, Solomon Islands.
J Med Entomol 2010 Sep;47(5):851-854.

Field efficacy studies comparing two formulations of deet (N,N-diethyl-3-methylbenzamide) against mosquitoes were conducted on Ndendo Island, Solomon Islands. The repellent study was conducted at Pala village in November 2008, and the only mosquito species collected was *Anopheles farauti* Laveran. A formulation containing 35% deet in a gel provided >95% protection for 2 hours, whereas a formulation containing 40% deet in ethanol in a spray applicator provided >95% for only 1 hour. This field study demonstrated again that repellents containing deet provide a relatively short period of complete protection against *Anopheles* spp.

20 **Gonçalves DU, Proietti FA, Ribas JG, Araújo MG, Pinheiro SR, Guedes AC, Carneiro-Proietti AB.**
Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases.
Clin Microbiol Rev 2010 Jul;23(3):577-589.

Human T-cell leukemia virus type 1 (HTLV-1), the first human retrovirus to be discovered, is present in diverse regions of the world, where its infection is usually neglected in health care settings and by public health authorities. Since it is usually asymptomatic in the beginning of the infection and disease typically manifests later in life, silent transmission occurs, which is associated with sexual relations, breastfeeding and blood transfusions. There are no prospects of vaccines and screening of blood banks and in prenatal care settings is not universal. Therefore, its transmission is active in many areas such as parts of Africa, South and Central America, the Caribbean region, Asia and Melanesia. It causes serious diseases in humans, including adult T-cell leukemia/lymphoma (ATL) and an incapacitating neurological disease (HTLV-associated myelopathy/tropical spastic paraparesis [HAM/TSP]) besides other afflictions such as uveitis, rheumatic syndromes and predisposition to helminthic and bacterial infections, among others. These diseases are not curable as yet, and current treatments as well as new perspectives are discussed in the present review.

21 **Gosden C.**
Archaeology. When humans arrived in the New Guinea Highlands.
Science 2010 Oct 1;330(6000):41-42.

22 **Gutiérrez JM, Williams D, Fan HW, Warrell DA.**
Snakebite envenoming from a global perspective: towards an integrated approach.
Toxicon 2010 Dec 15;56(7):1223-1235. Epub 2009 Nov 29.

Snakebite envenoming is a neglected public health challenge of compelling importance in many regions of the world, particularly sub-Saharan Africa, Asia, Latin America and Papua New Guinea. Addressing the problem of snakebite effectively demands an integrated multifocal approach, targeting complex problems and involving many participants. It must comprise: (a) Acquisition of reliable information on the incidence and mortality attributable to snakebite envenoming, and the number of people left with permanent sequelae; (b) Improvements in production of effective and safe antivenoms, through strategies aimed at strengthening the technological capacity of antivenom manufacturing laboratories; (c) Increasing the capacity of low-income countries to produce specific immunogens (snake venoms) locally, and to perform their own quality control of antivenoms; (d) Commitments from regional producers to manufacture antivenoms for countries where antivenom production is not currently feasible; (e) Implementation of financial initiatives guaranteeing the acquisition of adequate volumes of antivenom at affordable prices in low-income countries; (f) Performance of collaborative studies on the safety and effectiveness of antivenoms assessed preclinically and by properly designed clinical trials; (g) Development of antivenom distribution programmes tailored to the real needs and epidemiological situations of rural areas in each country; (h) Permanent training programmes for health staff, particularly in rural areas where snakebites are frequent; (i) Implementation of programmes to support those people whose snakebites resulted in chronic disabilities; (j) Preventive and educational programmes at the community level, with the active involvement of local organizations and employing modern methods of health promotion. Such an integrated approach, currently being fostered by the Global Snake Bite Initiative of the International Society on Toxinology and by the World Health Organization, will help to alleviate the enormous burden of human suffering inflicted by snakebite envenoming.

23 **Hamelin C, Salomon C, Cyr D, Gueguen A, Lert F.**
Childhood sexual abuse and adult sexual health among indigenous Kanak women and non-Kanak women of New Caledonia.
Child Abuse Negl 2010 Sep;34(9):677-688.

OBJECTIVES: Few studies have addressed the long-term consequences of adverse childhood experiences among women in Oceania, in particular among indigenous women. This paper aims to report prevalences of childhood sexual abuse (CSA) and to assess the negative sexual health consequences in adulthood by comparing indigenous Kanak to non-Kanak women in New Caledonia. METHODS: Data come from a population survey on violence against women and health. Face-to-face interviews were conducted in 2002-2003 with adult women randomly selected from the electoral list. Separate models for Kanak (n=329) and non-Kanak women (n=426) were performed. Regression models adjusted for relevant socio-demographic factors were conducted to estimate the odds ratios for the associations between childhood sexual abuse and adult sexual health outcomes. RESULTS: A non-significant difference between Kanak (11.8%) and non-Kanak women (14.4%) was found for the prevalence of CSA. Among Kanak women, CSA increases the risk of sexually transmitted infections, of non-desired sexual intercourse with an intimate partner and of experience of adult sexual violence. However, use of modern contraception as an adult was more frequent among CSA Kanak victims, as compared to other Kanak women. Among non-Kanak women, only abortion appeared significantly associated with CSA. CONCLUSIONS AND

PRACTICE IMPLICATIONS: The findings show that in all ethnic communities of New Caledonia, a history of child sexual abuse is not rare among women. They also shed light on the long-term consequences of CSA, suggesting that the effect of CSA may differ according to ethnic membership and subsequent social stratification and gender norms. Efforts to break the silence around violence against girls and establish a stronger foundation are required in New Caledonia. Prevention programs on violence against women and sexual health that take into account the cultural and social heterogeneity are needed.

24 **Harris I, Sharrock WW, Bain LM, Gray KA, Bobogare A, Boaz L, Lilley K, Krause D, Vallely A, Johnson ML, Gatton ML, Shanks GD, Cheng Q.**
A large proportion of asymptomatic *Plasmodium* infections with low and sub-microscopic parasite densities in the low transmission setting of Temotu Province, Solomon Islands: challenges for malaria diagnostics in an elimination setting.
Malar J 2010 Sep 7;9:254.
      BACKGROUND: Many countries are scaling up malaria interventions towards elimination. This transition changes demands on malaria diagnostics from diagnosing ill patients to detecting parasites in all carriers including asymptomatic infections and infections with low parasite densities. Detection methods suitable to local malaria epidemiology must be selected prior to transitioning a malaria control programme to elimination. A baseline malaria survey conducted in Temotu Province, Solomon Islands in late 2008, as the first step in a provincial malaria elimination programme, provided malaria epidemiology data and an opportunity to assess how well different diagnostic methods performed in this setting. METHODS: During the survey, 9,491 blood samples were collected and examined by microscopy for *Plasmodium* species and density, with a subset also examined by polymerase chain reaction (PCR) and rapid diagnostic tests (RDTs). The performances of these diagnostic methods were compared. RESULTS: A total of 256 samples were positive by microscopy, giving a point prevalence of 2.7%. The species distribution was 17.5% *Plasmodium falciparum* and 82.4% *Plasmodium vivax*. In this low transmission setting, only 17.8% of the *P. falciparum* and 2.9% of *P. vivax* infected subjects were febrile ($\geq$38°C) at the time of the survey. A significant proportion of infections detected by microscopy, 40% and 65.6% for *P. falciparum* and *P. vivax* respectively, had parasite density below 100/µL. There was an age correlation for the proportion of parasite density below 100/µL for *P. vivax* infections, but not for *P. falciparum* infections. PCR detected substantially more infections than microscopy (point prevalence of 8.71%), indicating a large number of subjects had sub-microscopic parasitemia. The concordance between PCR and microscopy in detecting single species was greater for *P. vivax* (135/162) than for *P. falciparum* (36/118). The malaria RDT detected the 12 microscopy and PCR-positive *P. falciparum*, but failed to detect 12/13 microscopy and PCR-positive *P. vivax* infections. CONCLUSION: Asymptomatic malaria infections and infections with low and sub-microscopic parasite densities are highly prevalent in Temotu province where malaria transmission is low. This presents a challenge for elimination since the large proportion of the parasite

reservoir will not be detected by standard active and passive case detection. Therefore effective mass screening and treatment campaigns will most likely need more sensitive assays such as a field deployable molecular based assay.

25 **Henrich J, Henrich N.**
The evolution of cultural adaptations: Fijian food taboos protect against dangerous marine toxins.
Proc Biol Sci 2010 Dec 22;277(1701):3715-3724. Epub 2010 Jul 28.
      The application of evolutionary theory to understanding the origins of our species' capacities for social learning has generated key insights into cultural evolution. By focusing on how our psychology has evolved to adaptively extract beliefs and practices by observing others, theorists have hypothesized how social learning can, over generations, give rise to culturally evolved adaptations. While much field research documents the subtle ways in which culturally transmitted beliefs and practices adapt people to their local environments, and much experimental work reveals the predicted patterns of social learning, little research connects real-world adaptive cultural traits to the patterns of transmission predicted by these theories. Addressing this gap, we show how food taboos for pregnant and lactating women in Fiji selectively target the most toxic marine species, effectively reducing a woman's chances of fish poisoning by 30 per cent during pregnancy and 60 per cent during breastfeeding. We further analyse how these taboos are transmitted, showing support for cultural evolutionary models that combine familial transmission with selective learning from locally prestigious individuals. In addition, we explore how particular aspects of human cognitive processes increase the frequency of some non-adaptive taboos. This case demonstrates how evolutionary theory can be deployed to explain both adaptive and non-adaptive behavioural patterns.

26 **Herbert B.**
Nurses the life support of impoverished but welcoming Solomon Islands.
Nurs NZ 2010 Aug;16(7):24-25.

27 **Hodgdon HE, Yoon KS, Previte DJ, Kim HJ, Aboelghar GE, Lee SH, Clark JM.**
Determination of knockdown resistance allele frequencies in global human head louse populations using the serial invasive signal amplification reaction.
Pest Manag Sci 2010 Sep;66(9):1031-1040.
      BACKGROUND: Pediculosis is the most prevalent parasitic infestation of humans. Resistance to pyrethrin- and pyrethroid-based pediculicides is due to knockdown resistance (*kdr*)-type point mutations in the voltage-sensitive sodium channel alpha-subunit gene. Early detection of resistance is crucial for the selection of effective management strategies. RESULTS: *Kdr* allele frequencies of lice from 14 countries were determined using the serial invasive signal amplification reaction. Lice collected from Uruguay, the United Kingdom and Australia had *kdr* allele frequencies of 100%, while lice from Ecuador, Papua New Guinea, South Korea and Thailand had *kdr* allele frequencies of 0%. The remaining seven countries investigated, including seven US populations, two Argentinian populations and populations from Brazil, Denmark, Czech Republic, Egypt and Israel, displayed variable *kdr* allele

frequencies, ranging from 11 to 97%. CONCLUSION: The newly developed and validated SISAR method is suitable for accurate monitoring of *kdr* allele frequencies in head lice. Proactive management is needed where *kdr*-type resistance is not yet saturated. Based on sodium channel insensitivity and its occurrence in louse populations resistant to pyrethrin- and pyrethroid-based pediculicides, the T917I mutation appears to be a key marker for resistance. Results from the Egyptian population, however, indicate that phenotypic resistance of lice with single or double mutations (M815I and/or L920F) should also be determined.

28 **Horii T, Shirai H, Jie L, Ishii KJ, Palacpac NQ, Tougan T, Hato M, Ohta N, Bobogare A, Arakaki N, Matsumoto Y, Namazue J, Ishikawa T, Ueda S, Takahashi M.**
Evidences of protection against blood-stage infection of *Plasmodium falciparum* by the novel protein vaccine SE36.
Parasitol Int 2010 Sep;59(3):380-386. Epub 2010 May 20.

An effective malaria vaccine is a public health priority. Proteins expressed during the blood-stage of the parasite life cycle have been proposed as good vaccine candidates. No such blood-stage vaccine, however, is available against *Plasmodium falciparum*, the deadliest *Plasmodium* species. We show here that *P. falciparum* serine repeat antigen 5 (SERA5) is a potential vaccine immunogen. We have constructed a new recombinant molecule of SERA5, namely SE36, based on the previously reported SE47' molecule, by removing the serine repeats. Epidemiological study in the holo-endemic population of Solomon Islands shows highly significant correlation of sero-conversion and malaria protective immunity against this antigen. Animal experiments using non-human primates, and a human phase 1a clinical trial, assessed SE36 vaccine immunogenicity. Vaccination of squirrel monkeys with SE36 protein and aluminum hydroxyl gel (SE36/AHG) conferred protection against high parasitemia and boosted serum anti-SE36 IgG after *P. falciparum* parasite challenge. SE36/AHG was highly immunogenic in chimpanzees, where serum anti-SE36 IgG titers last more than one year. Phase 1a clinical trial (current controlled trials, ISRCTN78679862) demonstrated the safety and immunogenicity of SE36/AHG with 30 healthy adults and 10 placebo controls. Three subcutaneous administrations of 50 and 100 microg dose of SE36/AHG were well tolerated, with no severe adverse events, and resulted in 100% sero-conversion in both dose arms. The current research results for SE36/AHG provide initial clinical validation for future trials and suggest clues/strategies for further vaccine development.

29 **Kelly A, Worth H, Man N, Nosi S, Emori R, Mek A, Akuani F, Kupul M, Kepa B, Walizopa L, Pirpir L, Cangah B, Siba P, Frankland A, Rawstorne P.**
Barriers and facilitators for adherence to antiretroviral therapy in Papua New Guinea.
Curr HIV Res 2010 Dec;8(8):630-637.

Papua New Guinea (PNG) is in a phase of scaling up access to antiretroviral therapy (ART), and adherence to the newly available drug therapy is becoming an important issue. This paper examines adherence to ART in a sample of 374 HIV-positive people in six provinces in PNG. Participants were recruited to the study using non-probability sampling. 62% of participants reported complete adherence (no missed or late doses in the past week) and 79% reported not missing any doses in the last week. Revival church members were significantly more likely to report having missing a treatment dose(s) (66%). Those living in the Highlands and those attending Catholic health clinics were significantly more likely to be adherent to their treatment. Age, gender, marital status, education level and employment type did not show significant association with treatment adherence. Adherence rates in PNG are not alarming, indicating that people with HIV can adhere to treatment despite the challenges of living in PNG.

30 **Kelly GC, Hii J, Batarii W, Donald W, Hale E, Nausien J, Pontifex S, Vallely A, Tanner M, Clements A.**
Modern geographical reconnaissance of target populations in malaria elimination zones.
Malar J 2010 Oct 20;9:289.

BACKGROUND: Geographical Reconnaissance (GR) operations using Personal Digital Assistants (PDAs) and Global Positioning Systems (GPS) have been conducted in the elimination provinces of Temotu, Solomon Islands and Tafea, Republic of Vanuatu. These operations aimed to examine modern approaches to GR to define the spatial distribution of target populations to support contemporary malaria elimination interventions. METHODS: Three GR surveys were carried out covering the outer islands of Temotu Province (October-November, 2008); Santa Cruz Island, Temotu Province (February 2009) and Tanna Island, Tafea Province (July-September 2009). Integrated PDA/GPS handheld units were used in the field to rapidly map and enumerate households, and collect associated population and household structure data to support priority elimination interventions, including bed net distribution, indoor residual spraying (IRS) and malaria case surveillance. Data were uploaded and analysed in customized Geographic Information System (GIS) databases to produce household distribution maps and generate relevant summary information pertaining to the GR operations. Following completion of field operations, group discussions were also conducted to review GR approaches and technology implemented. RESULTS: 10,459 households were geo-referenced and mapped. A population of 43,497 and 30,663 household structures were recorded during the three GR surveys. The spatial distribution of the population was concentrated in coastal village clusters. Survey operations were completed over a combined total of 77 field days covering a total land mass area of approximately 1103.2 km^2. An average of 45 households, 118 structures and a population of 184 people were recorded per handheld device per day. Geo-spatial household distribution maps were also produced immediately following the completion of GR fieldwork. An overall high acceptability of modern GR techniques and technology was observed by both field operations staff and communities. CONCLUSION: GR implemented using modern techniques has provided an effective and efficient operational tool for rapidly defining the spatial distribution of target populations in designated malaria elimination zones in Solomon Islands and Vanuatu. The data generated are being used for the strategic implementation and scaling-up of priority interventions, and will be essential for establishing future surveillance using spatial decision

support systems.

31 **Kerbrat AS, Darius HT, Pauillac S, Chinain M, Laurent D.**
Detection of ciguatoxin-like and paralysing toxins in *Trichodesmium* spp. from New Caledonia lagoon.
Mar Pollut Bull 2010;61(7-12):360-366. Epub 2010 Jul 16.

Marine pelagic cyanobacteria *Trichodesmium* are widespread in the New Caledonia lagoon. Blooms of these Oscillatoriales are suspected to be a potential source of toxins in the ciguatera food chain and were previously reported to contain certain types of paralysing toxins. In the present study, toxicity experiments were conducted on lipid- and water-soluble extracts of freeze-dried samples of these cyanobacteria. Lipid-soluble fractions revealed a ciguatoxin-like activity in both in vivo (mouse bioassay) and in vitro (mouse neuroblastoma cells assay and receptor binding assay using tritiated brevetoxin-3) assays. The water-soluble fractions tested on mice exhibited neurotoxicity with paralytic symptoms. These toxicities have also been observed with benthic filamentous cyanobacteria within the Oscillatoriales order, also collected in New Caledonia. This study provides unprecedented evidence of the toxicity of *Trichodesmium* species from the New Caledonia lagoon. This survey also demonstrates the possible role of these cyanobacteria in ciguatera fish poisoning.

32 **Keven JB, Henry-Halldin CN, Thomsen EK, Mueller I, Siba PM, Zimmerman PA, Reimer LJ.**
Pyrethroid susceptibility in natural populations of the *Anopheles punctulatus* group (Diptera: Culicidae) in Papua New Guinea.
Am J Trop Med Hyg 2010 Dec;83(6):1259-1261.

The development of insecticide resistance has compromised mosquito control efforts in many parts of the world. Papua New Guinea (PNG) has a long history of dichlorodiphenyltrichloroethane (DDT) use and currently distributes pyrethroid-treated nets for malaria control. This study is the first to investigate the status of pyrethroid resistance in the *Anopheles punctulatus* group, the major malaria and filariasis vectors of PNG. The study used World Health Organization standard susceptibility bioassays to detect knockdown phenotypes and a novel nested polymerase chain reaction to detect the knockdown resistant (*kdr*) allele in these vectors. Our results show 100% susceptibility to pyrethroids in all populations surveyed and an absence of the *kdr* allele.

33 **Kuwahata M, Wijesinghe R, Ho MF, Pelecanos A, Bobogare A, Landry L, Bugora H, Vallely A, McCarthy J.**
Population screening for glucose-6-phosphate dehydrogenase deficiencies in Isabel Province, Solomon Islands, using a modified enzyme assay on filter paper dried bloodspots.
Malar J 2010 Aug 5;9:223.

BACKGROUND: Glucose-6-phosphate dehydrogenase deficiency poses a significant impediment to primaquine use for the elimination of liver stage infection with *Plasmodium vivax* and for gametocyte clearance, because of the risk of life-threatening haemolytic anaemia that can occur in G6PD deficient patients. Although a range of methods for screening G6PD deficiency have been described, almost all require skilled personnel, expensive laboratory equipment and freshly collected blood, and are time consuming, factors that render them unsuitable for mass-screening purposes. METHODS: A published WST8/1-methoxy PMS method was adapted to assay G6PD activity in a 96-well format using dried blood spots, and we used it to undertake population screening within a malaria survey undertaken in Isabel Province, Solomon Islands. The assay results were compared to a biochemical test and a recently marketed rapid diagnostic test. RESULTS: Comparative testing with the biochemical and rapid diagnostic tests indicated that results obtained by filter paper assay were accurate providing that blood spots were assayed within 5 days when stored at ambient temperature and 10 days when stored at 4 degrees. Screening of 8541 people from 41 villages in Isabel Province, Solomon Islands revealed the prevalence of G6PD deficiency as defined by enzyme activity <30% of normal control was 20.3% and a prevalence of severe deficiency that would predispose to primaquine-induced hemolysis (WHO Class I-II) of 6.9%. CONCLUSIONS: The assay enabled simple and quick semi-quantitative population screening in a malaria-endemic region. The study indicated a high prevalence of G6PD deficiency in Isabel Province and highlights the critical need to consider G6PD deficiency in the context of *P. vivax* malaria elimination strategies in Solomon Islands, particularly in light of the potential role of primaquine mass drug administration.

34 **Laman M, Manning L, Hwaiwhange I, Vince J, Aipit S, Mare T, Warrel J, Karunajeewa H, Siba P, Mueller I, Davis TM.**
Lumbar puncture in children from an area of malaria endemicity who present with a febrile seizure.
Clin Infect Dis 2010 Sep 1;51(5):534-540.

BACKGROUND: Although routine lumbar puncture (LP) is often recommended as part of the assessment of fever-associated seizures in children, accumulating evidence questions its value and reveals a decrease in its frequency. Our primary hypothesis was that children who present with a single seizure but with no clinical signs of meningism or coma do not require LP as part of initial diagnostic assessment. METHODS: We prospectively followed up 377 children aged 2 months through 10 years who presented with at least 1 fever-associated seizure to Modilon Hospital, Madang, Papua New Guinea, from November 2007 through July 2009. Clinical management was performed by hospital staff according to national pediatric guidelines. RESULTS: Of 188 children with a single seizure and 189 children with multiple seizures, 139 (73.9%) and 154 (81.5%), respectively, underwent a LP as part of their initial assessment. Of the 130 children with a single seizure but no evidence of meningism (ie, neck stiffness, positive Kernig's or Brudzinski's sign, and bulging fontanele) or coma (Blantyre Coma Score 2), none (95% confidence interval, 0%-3.6%) had proven or probable acute bacterial meningitis, and only 1 patient had viral encephalitis (subacute sclerosing panencephalitis). Eighty-one of these children (62.3%) had a final diagnosis of a simple febrile seizure. Proven or probable acute bacterial meningitis was more common in children with a single seizure and meningism or coma (10; 17.2%) and in those with multiple seizures without or with meningism or coma (2 [2.0%] and 30 [33.7%], respectively). CONCLUSIONS: Initial LP is unnecessary when careful clinical assessment

indicates features of a simple febrile seizure.

35 **Le Hello S, Falcot V, Lacassin F, Mikulski M, Baumann F.**
Risk factors for carbapenem-resistant *Acinetobacter baumannii* infections at a tertiary care hospital in New Caledonia, South Pacific.
Scand J Infect Dis 2010 Dec;42(11-12):821-826. Epub 2010 Jun 21.

In New Caledonia, South Pacific, *Acinetobacter baumannii* is a nosocomial pathogen. OXA-23 carbapenem-resistant *A. baumannii* (CRAB) has been ranked third among all multidrug-resistant (MDR) bacteria at the main hospital of Nouméa in New Caledonia (24.8%, 50/202 isolates). In the present study, risk factors and outcomes for 50 patients with CRAB infection were compared with those of 152 patients infected with other MDR bacteria. Independent risk factors for infection with CRAB were respiratory ward admission (odds ratio 2.8, 95% confidence interval 1.1-7.1) and previous treatment with quinolones, β-lactams and anti-MRSA antibiotics. The 30-day mortality was higher for CRAB infections than for other MDR infections (14% vs 3.3%, p = 0.006). These findings highlight the importance of knowing specific local characteristics relating to the ecology and patterns of resistance of MDR bacteria so as to avoid the emergence of unexpected pan-resistant bacteria.

36 **Le Hello S, Watson M, Levy M, Marcon S, Brown M, Yvon JF, Missotte I, Garin B.**
Invasive serotype 1 *Streptococcus pneumoniae* outbreaks in the South Pacific from 2000 to 2007.
J Clin Microbiol 2010 Aug;48(8):2968-2971. Epub 2010 Jun 9.

In New Caledonia, Wallis and Futuna, and French Polynesia, an active surveillance system was established to monitor pneumococcal serotype prevalence between 2000 and 2007. The most prevalent serotype was serotype 1, which belonged to the major clonal complex sequence type 306 (ST306) and was responsible for invasive pneumococcal disease outbreaks.

37 **Lee EJ, Koki G, Merriwether DA.**
Characterization of population structure from the mitochondrial DNA vis-à-vis language and geography in Papua New Guinea.
Am J Phys Anthropol 2010 Aug;142(4):613-624.

Situated along a corridor linking the Asian continent with the outer islands of the Pacific, Papua New Guinea has long played a key role in understanding the initial peopling of Oceania. The vast diversity in languages and unique geographical environments in the region have been central to the debates on human migration and the degree of interaction between the Pleistocene settlers and newer migrants. To better understand the role of Papua New Guinea in shaping the region's prehistory, we sequenced the mitochondrial DNA (mtDNA) control region of three populations, a total of 94 individuals, located in the East Sepik Province of Papua New Guinea. We analyzed these samples with a large data set of Oceania populations to examine the role of geography and language in shaping population structure within New Guinea and between the region and Island Melanesia. Our results from median-joining networks, star-cluster age estimates and population genetic analyses show that while highland New Guinea populations seem to be the oldest settlers, there has been significant

gene flow within New Guinea with little influence from geography or language. The highest genetic division is between Papuan speakers of New Guinea versus East Papuan speakers located outside of mainland New Guinea. Our study supports the weak language barriers to genetic structuring among populations in close contact and highlights the complexity of understanding the genetic histories of Papua New Guinea in association with language and geography.

38 **Lin E, Tavul L, Michon P, Richards JS, Dabod E, Beeson JG, King CL, Zimmerman PA, Mueller I.**
Minimal association of common red blood cell polymorphisms with *Plasmodium falciparum* infection and uncomplicated malaria in Papua New Guinean school children.
Am J Trop Med Hyg 2010 Oct;83(4):828-833.

Southeast Asian ovalocytosis (SAO), α(+)-thalassemia, and low expression of complement receptor 1 (CR1) have been associated with protection against severe *Plasmodium falciparum* malaria. In a cohort of children 5-14 years of age the effect of α(+)-thalassemia, SAO (*SLC4A1Δ27*), CR1 polymorphisms, and Gerbich negativity (*GYPCΔex3*) on risk of *P. falciparum* infections and uncomplicated illness were evaluated. The risk of acquiring polymerase chain reaction (PCR)-diagnosed *P. falciparum* infections was significantly lower for α(+)-thalassemia heterozygotes (hazard ratio [HR]: 0.56) and homozygotes (HR: 0.51) than wild-type children. No such differences were seen in light microscopy diagnosed infections (p = 0.71) nor were α(+)-thalassemia genotypes associated with a reduced risk of uncomplicated *P. falciparum* malaria. No significant associations between the risk of *P. falciparum* infection or illness were observed for any of the other red blood cell polymorphisms (p >0.2). This suggests that these polymorphisms are not associated with significant protection against *P. falciparum* blood-stage infection or uncomplicated malaria in school-aged children.

39 **Maharaj JC, Venketasubramanian N.**
Burden of stroke in Fiji.
Int J Stroke 2010 Aug;5(4):321-322.

40 **Marshall K, Shakya S, Greenhill AR, Padill G, Baker A, Warner JM.**
Antibiosis of *Burkholderia ubonensis* against *Burkholderia pseudomallei*, the causative agent for melioidosis.
Southeast Asian J Trop Med Public Health 2010 Jul;41(4):904-912.

Melioidosis, caused by *Burkholderia pseudomallei*, is an enigmatic infectious disease that afflicts individuals in many tropical and developing regions. Treatment is hampered by the organism's innate antibiotic resistance and the disease's non-pathognomic presentation. Recently, added attention has been given to this organism due to its classification as a potential biowarfare agent. Therefore, methods of preventing acquisition of infection are needed. We investigated antagonism between *Burkholderia* spp and *B. pseudomallei* derived from the same ecological niche in a melioidosis endemic region in Papua New Guinea. Isolates of environmentally derived non-pseudomallei *Burkholderia* spp (n=16) were screened for antibiosis against 27 *B. pseudomallei* isolates. Three isolates subsequently identified as

B. ubonensis produced specific antagonistic activity against all *B. pseudomallei* isolates tested. The antagonistic compound in a cell-free state was obtained from a representative producing strain, with subsequent biological characterization revealing a pepsin sensitive peptide moiety consistent with a bacteriocin-like compound. To our knowledge, this is the first report of antagonistic activity demonstrated by near-neighbor *Burkholderia* against *B. pseudomallei*. This antagonism may be important in the micro-ecology of *B. pseudomallei*, and could also have application in the biocontrol of this pathogen.

41 **McAdam M, Sakita J, Tarivonda L, Pang J, Frazer IH.**
Evaluation of a cervical cancer screening program based on HPV testing and LLETZ excision in a low resource setting.
PLoS One 2010 Oct 7;5(10):e13266.
    We conducted studies in Vanuatu to evaluate potential screening and treatment strategies to assist with control of cervical cancer. In a pilot study of 496 women, visual inspection and cytology were evaluated as screening tests for detection of CIN 2 or worse (CIN 2+), observed in 21 of 206 subjects biopsied on the basis of abnormal visual inspection or cytology. Sensitivity of visual inspection with Lugol's Iodine for detection of CIN 2+ on biopsy was 0.63, specificity was 0.32, and the positive predictive value was 0.09. For HSIL cytology, sensitivity was 0.99, specificity was 0.77, and the positive predictive value was 0.88. HSIL cytology was significantly more sensitive and had a significantly higher PPV for CIN 2+ than visual inspection (p <0.01). In a further study of 514 women, we compared testing for HR HPV and cytology as predictors of biopsy proven CIN 2+. Sensitivity of HSIL cytology for CIN 2+ as established by loop excision of the cervix was 0.81, specificity was 0.94, and positive predictive value was 0.48. Sensitivity of a positive test for HR HPV for detection of CIN 2+ was non-significantly different from cytology at 0.81, specificity was 0.94, and positive predictive value was 0.42. Combining the two tests gave a significantly lower sensitivity of 0.63, a specificity of 0.98, and a positive predictive value of 0.68. For women over 30 in a low resource setting without access to cytology, a single locally conducted test for high risk HPV with effective intervention could reduce cervical cancer risk as effectively as intervention based on cytology conducted in an accredited laboratory.

42 **McEvoy BP, Lind JM, Wang ET, Moyzis RK, Visscher PM, van Holst Pellekaan SM, Wilton AN.**
Whole-genome genetic diversity in a sample of Australians with deep Aboriginal ancestry.
Am J Hum Genet 2010 Aug 13;87(2):297-305.
    Australia was probably settled soon after modern humans left Africa, but details of this ancient migration are not well understood. Debate centers on whether the Pleistocene Sahul continent (composed of New Guinea, Australia and Tasmania) was first settled by a single wave followed by regional divergence into Aboriginal Australian and New Guinean populations (common origin) or whether different parts of the continent were initially populated independently. Australia has been the subject of relatively few DNA studies even though understanding regional variation in genomic structure and diversity will be important if disease-association mapping methods are to be successfully evaluated and applied across populations. We report on a genome-wide investigation of Australian Aboriginal SNP diversity in a sample of participants from the Riverine region. The phylogenetic relationship of these Aboriginal Australians to a range of other global populations demonstrates a deep common origin with Papuan New Guineans and Melanesians, with little evidence of substantial later migration until the very recent arrival of European colonists. The study provides valuable and robust insights into an early and important phase of human colonization of the globe. A broader survey of Australia, including diverse geographic sample populations, will be required to fully appreciate the continent's unique population history and consequent genetic heritage, as well as the importance of both to the understanding of health issues.

43 **McIver LJ, Kippin AN, Parish ST, Whitehead OG.**
HIV, malaria and pneumonia in a Torres Strait Islander male – a case report.
Commun Dis Intell 2010 Dec;34(4):448-449.
    This report presents the case of a middle-aged Torres Strait Islander male with HIV who contracted *Plasmodium vivax* malaria in Papua New Guinea. His presentation included clinical and radiological features of pneumonia and he required inpatient treatment for 13 days. This study reviews the literature concerning co-infection with HIV and malaria, which is an uncommon combination in Australia, discusses the public health risks posed by patients with malaria in the Torres Strait, given the presence of a known vector, and suggests strategies to reduce the disease burden posed by malaria in this patient and other Torres Strait Islanders travelling to Papua New Guinea under the terms of the Torres Strait Treaty.

44 **Méjean A, Peyraud-Thomas C, Kerbrat AS, Golubic S, Pauillac S, Chinain M, Laurent D.**
First identification of the neurotoxin homoanatoxin-a from mats *of Hydrocoleum lyngbyaceum* (marine cyanobacterium) possibly linked to giant clam poisoning in New Caledonia.
Toxicon 2010 Oct;56(5):829-835. Epub 2009 Nov 4.
    We report the first identification of homoanatoxin-a from benthic marine cyanobacteria (*Hydrocoleum lyngbyaceum*) samples collected in Lifou (Loyalty Islands, New Caledonia), where cases of giant clam (*Tridacna maxima*) intoxications were recorded during a severe ciguatera fish poisoning outbreak. Homoanatoxin-a was also detected in extracts of giant clams harvested in the surroundings of the contaminated area suggesting the possible link between these poisoning events and the occurrence of potentially neurotoxic *Hydrocoleum*.

45 **Mermond S, Berlioz-Arthaud A, Estivals M, Baumann F, Levenes H, Martin PM.**
Aetiology of community-acquired pneumonia in hospitalized adult patients in New Caledonia.
Trop Med Int Health 2010 Dec;15(12):1517-1524. doi: 10.1111/j.1365-3156.2010.02653.x. Epub 2010 Oct 18.
    OBJECTIVE: To describe the aetiology of community-acquired pneumonia (CAP) in hospitalized adult patients in New Caledonia, a French archipelago in the South Pacific. METHODS: Confirmed CAP patients (n=137) were enrolled

prospectively. Pathogens were detected by culture, molecular methods, serology on paired sera, immunofluorescence on nasopharyngeal swabs and antigen detection in urine. RESULTS: The aetiology of CAP was determined in 82 of 137 cases (59.8%), of which 31 exhibited two or more pathogens (37.8%). 117 pathogens were detected: *Streptococcus pneumoniae* was the most common one (41.0%), followed by influenza virus A (22.1%) and *Haemophilus influenzae* (10.2%). The frequency of atypical bacteria was low (6.0%). The most frequent and significant coinfection was *S. pneumoniae* with influenza A virus (p=0.004). Influenza virus was detected from nasopharyngeal swabs in four patients (15.4% of patients tested for influenza) and by PCR from pulmonary specimens in 15 patients (57.7%). CONCLUSIONS: *S. pneumoniae* is the leading cause of CAP in New Caledonian adults. Viral-bacterial co-infections involving *S. pneumoniae* and influenza virus are very common during the winter. Such adult patients hospitalized with CAP are a clear sentinel group for surveillance of influenza. Vaccination against influenza and *S. pneumoniae* should be strengthened when risk factors are identified.

46 **Moodie M, Richardson J, Rankin B, Iezzi A, Sinha K.**
Predicting time trade-off health state valuations of adolescents in four Pacific countries using the Assessment of Quality-of-Life (AQoL-6D) instrument.
Value Health 2010 Dec;13(8):1014-1027. doi: 10.1111/j.1524-4733.2010.00780.x. Epub 2010 Sep 3.

OBJECTIVES: Pacific Obesity Prevention in Communities (OPIC) is a community-based intervention project targeting adolescent obesity in Australia, New Zealand, Fiji and Tonga. The Assessment of Quality of Life Mark 2 (AQoL-6D) instrument was completed by 15,481 adolescents to obtain a description of the quality of life associated with adolescent overweight and obesity, and a corresponding utility score for use in a cost-utility analysis of the interventions. This article describes the recalibration of this utility instrument for adolescents in each country. METHODS: The recalibration was based on country-specific time trade-off (TTO) data for 30 multiattribute health states constructed from the AQoL-6D descriptive system. Senior secondary students, in a classroom setting, responded to 10 health state scenarios each. These TTO interviews were conducted for 24 groups, comprising 279 students in the four countries, resulting in 2790 completed TTO scores. The TTO scores were econometrically transformed by regressing the TTO scores upon predicted scores from the AQoL-6D to produce country-specific algorithms. The latter incorporated country-specific 'corrections' to the Australian adult utility weights in the original AQoL. RESULTS: This article reports two methodological elements not previously reported. The first is the econometric modification of an extant multiattribute utility instrument to accommodate cultural and other group-specific differences in preferences. The second is the use of the TTO technique with adolescents in a classroom group setting. Significant differences in utility scores were found between the four countries. CONCLUSION: Statistical results indicate that the AQoL-6D can be validly used in the economic evaluation of both the OPIC interventions and other adolescent programs.

47 **Nursing New Zealand.**
Nursing in Fiji under pressure.
Nurs NZ 2010 Dec-2011 Jan;16(11):13.

48 **O'Connor PM, Scarr BC, Lamoureux EL, Le Mesurier RT, Keeffe JE.**
Validation of a quality of life questionnaire in the Pacific Islands.
Ophthalmic Epidemiol 2010 Dec;17(6):378-386. Epub 2010 Nov 16.

PURPOSE: To adapt an existing validated quality of life instrument, the Impact of Vision Impairment (IVI) questionnaire, for Pacific Island countries. METHODS: Following in-depth interviews (n=24) and a pilot study (n=67), the original 32-item IVI questionnaire was translated and adapted in Vanuatu. The Melanesian IVI (IVI_M) was administered to participants not previously involved in the pilot study (n=189). RESULTS: Participants included 117 (62%) with mild, moderate or severe vision impairment, 39 with unilateral loss and 33 with normal vision. Eighty-six percent of the original 32 items were deemed relevant by 90% of participants. Items displaying floor effects were removed (4), 2 were combined and 3 items rephrased to reflect Melanesian-specific activities, resulting in a 23-item IVI_M. Nineteen items were relevant to both the Melanesian and Australian contexts including all 8 items related to the emotional reaction to vision loss. IVI_M demonstrated content and construct validity and reliability and discriminated visually healthy populations from those with vision impairment. Vision impairment of <6/18 negatively affected quality of life. CONCLUSION: While the adaptation process demonstrated the need for culturally relevant instruments, it also highlighted the value of adapting an existing validated instrument for use in cross-cultural research rather than developing a new instrument from first principles.

49 **O'Farrell N, Moi H; IUSTI/WHO European STD guidelines Editorial Board. Collaborators: Radcliffe K, Babayan K, Barton S, Janier M, Jensen JS, Khotenashvili L, van de Laar M, van der Meijden W, Moi H, Neumann M, Patel R, Robinson A, Ross J, Sherrard J, Unemo M.**
European guideline for the management of donovanosis, 2010.
Int J STD AIDS 2010 Sep;21(9):609-610.

Donovanosis is a rare sexually transmitted infection now mainly seen in sporadic cases in Papua New Guinea, South Africa, India, Brazil and Australia. The causative organism is *Calymmatobacterium granulomatis* though a proposal has been put forward that the organism be reclassified as *Klebsiella granulomatis* comb. nov. The incubation period is approximately 50 days with genital papules developing into ulcers that increase in size. Four types of lesion are described – ulcerogranulomatous, hypertrophic, necrotic and sclerotic. The diagnosis is usually confirmed by microscopic identification of characteristic Donovan bodies on stained tissue smears. More recently, polymerase chain reaction (PCR) methods have been developed. The recommended treatment is azithromycin 1 g weekly until complete healing is achieved.

50 **Pacific Malaria Initiative. Collaborators: Henri I, Iamaher S, Iavro J, Kalomuana MJ, Mera K, Taleo G, Alungo B, Bobogare A, Boaz L, Dagi E, Hou B, Leo B, Memua N, Moses S, Newa A, Osiwane L, Raoga R, Rose N, Salopuka L,**

Tanen A, Young J, Atkins C, Auliff A, Bain LM, Cheng Q, Cooper R, Harris IE, Ebringer A, Edstein MD, Elmes N, Gray KA, Humphries J, Johnson ML, Krause D, Lilley K, MacKenzie D, McPherson B, Perrin R, Shanks GD, Sharrock WW, Staley J, Waters NC, Atkinson JA, Clements A, Dove G, Forsyth S, Kelly G, Reid LM, Riley I, Vallely A, Whittaker M.
Malaria on isolated Melanesian islands prior to the initiation of malaria elimination activities.
Malar J 2010 Jul 26;9:218.

BACKGROUND: The Australian Government's Pacific Malaria Initiative (PacMI) is supporting the National Malaria Program in both Solomon Islands and Vanuatu, complementing assistance from the Global Fund for AIDS, Tuberculosis and Malaria (GFATM). Two remote island groups – Tafea Province, Vanuatu and Temotu Province, Solomon Islands – have been selected by the governments of both countries as possible malaria elimination areas. To provide information on the prevalence and distribution of the disease within these island groups, malariometric surveys were conducted during the wet seasons of 2008. METHODS: In Tafea Province, a school-based survey was conducted which included the 2-12 y age group, while in Temotu a village-based all-ages survey was conducted. An effort was made to sample villages or schools from as wide an area as possible on all islands. Diagnosis was initially based on Giemsa-stained blood slides followed by molecular analysis using polymerase chain reaction (PCR). RESULTS: In Tafea Province, 73% (5238/7150) of children (2-12 y) were surveyed and in Temotu Province, in the all-ages survey, 50.2% (8742/17410) of the provincial population participated in the survey. In both Vanuatu and Solomon Islands malariometric surveys of their southern-most islands in 2008 showed relatively low over-all malaria parasite prevalence (2 to 3%). Other features of malaria in these island groups were low parasitaemia, low gametocyte carriage rates, low spleen rates, low malaria-associated morbidity, a high incidence of asymptomatic infections, and a predominance of *Plasmodium vivax* over *Plasmodium falciparum*. CONCLUSION: For various reasons malaria rates are declining in these provinces providing a favourable situation for local malaria elimination. This will be advanced using mass distribution of bed nets and selective indoor residual spraying, the introduction of rapid diagnostic tests and artemisinin combination therapy, and intensive case detection and surveillance. It is as yet uncertain whether malaria parasites can themselves be sustainably eliminated from entire Melanesian islands, where they have previously been endemic. Key issues on the road to malaria elimination will be continued community involvement, improved field diagnostic methods and elimination of residual *P. vivax* parasites from the liver of asymptomatic persons.

51 **Pell C, Straus L, Phuanukoonnon S, Lupiwa S, Mueller I, Senn N, Siba P, Gysels M, Pool R.**
Community response to intermittent preventive treatment of malaria in infants (IPTi) in Papua New Guinea.
Malar J 2010 Dec 22;9:369.

BACKGROUND: Building on previous acceptability research undertaken in sub-Saharan Africa this article aims to investigate the acceptability of intermittent preventive treatment of malaria in infants (IPTi) in Papua New Guinea (PNG). METHODS: A questionnaire was administered to mothers whose infants participated in the randomised placebo controlled trial of IPTi. Mothers whose infants participated and who refused to participate in the trial, health workers, community reporters and opinion leaders were interviewed. Men and women from the local community also participated in focus group discussions. RESULTS: Respondents viewed IPTi as acceptable in light of wider concern for infant health and the advantages of trial participation. Mothers reported complying with at-home administration of IPTi due to perceived benefits of IPTi and pressure from health workers. In spite of patchy knowledge, respondents also demonstrated a demand for infant vaccinations and considered non-vaccination to be neglect. There is little evidence that IPTi has negative impacts on attitudes to EPI, EPI adherence or existing malaria prevention practices. CONCLUSION: The degree of similarity between findings from the acceptability studies undertaken in sub-Saharan Africa and PNG allows some generalization relating to the implementation of IPTi outside of Africa: IPTi fits well with local health cultures, appears to be accepted easily and has little impact on attitudes towards EPI or malaria prevention. The study adds to the evidence indicating that IPTi could be rolled out in a range of social and cultural contexts.

52 **Perez J, Goarant C.**
Rapid *Leptospira* identification by direct sequencing of the diagnostic PCR products in New Caledonia.
BMC Microbiol 2010 Dec 22;10:325.

BACKGROUND: Most of the current knowledge of leptospirosis epidemiology originates from serological results obtained with the reference Microscopic Agglutination Test (MAT). However, inconsistencies and weaknesses of this diagnostic technique are evident. A growing use of PCR has improved the early diagnosis of leptospirosis but a drawback is that it cannot provide information on the infecting *Leptospira* strain which provides important epidemiologic data. Our work is aimed at evaluating if the sequence polymorphism of diagnostic PCR products could be used to identify the infecting *Leptospira* strains in the New Caledonian environment. RESULTS: Both the *lfb1* and *secY* diagnostic PCR products displayed a sequence polymorphism that could prove useful in presumptively identifying the infecting leptospire. Using both this polymorphism and MLST results with New Caledonian isolates and clinical samples, we confirmed the epidemiological relevance of the sequence-based identification of *Leptospira* strains. Additionally, we identified one cluster of *L. interrogans* that contained no reference strain and one cluster of *L. borgpetersenii* found only in the introduced Rusa deer *Cervus timorensis russa* that is its probable reservoir. CONCLUSIONS: The sequence polymorphism of diagnostic PCR products proved useful in presumptively identifying the infecting *Leptospira* strains. This could contribute to a better understanding of leptospirosis epidemiology by providing epidemiological information that cannot be directly attained from the use of PCR as an early diagnostic test for leptospirosis.

53 **Price RN, Marfurt J, Chalfein F, Kenangalem E, Piera KA, Tjitra E, Anstey NM, Russell B.**
In vitro activity of pyronaridine against multidrug-

resistant *Plasmodium falciparum* and *Plasmodium vivax*.
Antimicrob Agents Chemother 2010 Dec;54(12):5146-5150. Epub 2010 Sep 27.

Pyronaridine, a Mannich base antimalarial, has demonstrated high in vivo and in vitro efficacy against chloroquine-resistant *Plasmodium falciparum*. Although this drug has the potential to become a prominent artemisinin combination therapy, little is known about its efficacy against drug-resistant *Plasmodium vivax*. The in vitro antimalarial susceptibility of pyronaridine was assessed in multidrug-resistant *P. vivax* (n = 99) and *P. falciparum* (n = 90) isolates from Papua, Indonesia, using a schizont maturation assay. The median 50% inhibitory concentration (IC_{50}) of pyronaridine was 1.92 nM (range, 0.24 to 13.8 nM) against *P. falciparum* and 2.58 nM (range, 0.13 to 43.6 nM) against *P. vivax*, with in vitro susceptibility correlating significantly with chloroquine, amodiaquine and piperaquine (r(s) [Spearman's rank correlation coefficient] = 0.45 to 0.62; p <0.001). *P. falciparum* parasites initially at trophozoite stage had higher IC_{50}s of pyronaridine than those exposed at the ring stage (8.9 nM [range, 0.6 to 8.9 nM] versus 1.6 nM [range, 0.6 to 8.9 nM], respectively; p = 0.015), although this did not reach significance for *P. vivax* (4.7 nM [range, 1.4 to 18.7 nM] versus 2.5 nM [range, 1.4 to 15.6 nM], respectively; p = 0.085). The excellent in vitro efficacy of pyronaridine against both chloroquine-resistant *P. vivax* and *P. falciparum* highlights the suitability of the drug as a novel partner for artemisinin-based combination therapy in regions where the two species are coendemic.

54 **Qoqonokana MQ, Brian G, Ramke J, Garcia J, Szetu J.**
Diabetic retinopathy in a hospital eye clinic population in Honiara, Solomon Islands.
Clin Experiment Ophthalmol 2010 Dec;38(9):862-866. doi:10.1111/j.1442-9071.2010.02356.x.

BACKGROUND: To determine the presence, severity and context of diabetic retinopathy among diabetic adults using hospital eye clinic services in Honiara, Solomon Islands. METHODS: Fifty consecutive known diabetic patients aged ≥20 years were interviewed and underwent Snellen distance vision testing and complete ocular examination, including dilated 90 D funduscopy. Diabetic retinopathy was graded according to the International Clinical Diabetic Retinopathy and Macular Oedema Disease Severity Scales. RESULTS: Participants were predominantly Melanesian (94%). Mean age was 53.6 ± 10.7 years; 42% were female; and 34% were rural dwellers. Diabetes had been diagnosed <5, 5-10 and >10 years ago for 42%, 34% and 24% of participants. However, 54% denied ever having had a dilated fundus examination. Proliferative retinopathy, severe non-proliferative changes and maculopathy were present in 5, 12 and 26 eyes, respectively. For the 32 eyes with pinhole acuity ≤6/12, diabetic eye disease was the cause for 20, with all but one due to maculopathy. Diabetes reduced the pinhole vision of five eyes to ≤6/60. Twenty-four per cent of participants had diabetes-related pinhole vision ≤6/12 in at least one eye. Those diagnosed with diabetes 5-10 and >10 years were 17.5 and 58.8 times more likely to have such an eye compared with those recently (<5 years) diagnosed (extended Mantel-Haenszel χ^2 = 11.570, p <0.001). Participants were not well informed

about diabetic eye disease. CONCLUSIONS: Diabetic eye disease is now a significant contributor to this clinic's workload. A population-based survey is needed to quantify the problem and inform design and delivery of eye services for this chronic disease.

55 **Raghukumar R, Vali L, Watson D, Fearnley J, Seidel V.**
Antimethicillin-resistant *Staphylococcus aureus* (MRSA) activity of 'Pacific propolis' and isolated prenylflavanones.
Phytother Res 2010 Aug;24(8):1181-1187.

The need to discover and develop alternative therapies to treat methicillin-resistant *Staphylococcus aureus* (MRSA) infections is timely. This study was undertaken to purify and identify some anti-MRSA constituents from propolis, a natural product from the beehive traditionally used in folk medicine for its antimicrobial properties. A crude extract of propolis originating from the Solomon Islands ('Pacific propolis') was screened, using an agar dilution assay, in vitro against 15 MRSA clinical isolates. Results revealed activity worthy of further investigation, and subsequent purification work on this crude extract afforded 23 fractions. Further purification of active fractions led to the isolation of compounds 1-4, characterized upon analysis of their spectroscopic data (1D- and 2D-NMR, MS) and by comparison with the literature, as the prenylflavanones propolin H (1), propolin G (2), propolin D (3) and propolin C (4). This study is the first to report the anti-MRSA activity of 'Pacific propolis' and the presence of prenylflavanones in the propolis sample selected. The anti-MRSA activity of propolin D (3) (MIC 8-16 mg/L) and propolin C (4) (MIC 8-32 mg/L) is reported for the first time.

56 **Ralph AP, Ardian M, Wiguna A, Maguire GP, Becker NG, Drogumuller G, Wilks MJ, Waramori G, Tjitra E, Sandjaja, Kenagalem E, Pontororing GJ, Anstey NM, Kelly PM.**
A simple, valid, numerical score for grading chest X-ray severity in adult smear-positive pulmonary tuberculosis.
Thorax 2010 Oct;65(10):863-869.

BACKGROUND: The grading of radiological severity in clinical trials in tuberculosis (TB) remains unstandardised. The aim of this study was to generate and validate a numerical score for grading chest X-ray (CXR) severity and predicting response to treatment in adults with smear-positive pulmonary TB. METHODS: At a TB clinic in Papua, Indonesia, serial CXRs were performed at diagnosis, 2 and 6 months in 115 adults with smear-positive pulmonary TB. Radiographic findings predictive of 2-month sputum microscopy status were used to generate a score. The validity of the score was then assessed in a second data set of 139 comparable adults with TB, recruited 4 years later at the same site. Relationships between the CXR score and other measures of TB severity were examined. RESULTS: The estimated proportion of lung affected and presence of cavitation, but not cavity size or other radiological findings, significantly predicted outcome and were combined to derive a score given by percentage of lung affected plus 40 if cavitation was present. As well as predicting 2-month outcome, scores were significantly associated with sputum smear grade at diagnosis (p <0.001), body mass index, lung function, haemoglobin, exercise tolerance and quality of life (p <0.02 for each). In

the validation data set, baseline CXR score predicted 2-month smear status significantly more accurately than did the proportion of lung affected alone. In both data sets, CXR scores decreased over time (p <0.001). CONCLUSION: This simple, validated method for grading CXR severity in adults with smear-positive pulmonary TB correlates with baseline clinical and microbiological severity and response to treatment, and is suitable for use in clinical trials.

57 **Randall LM, Kenangalem E, Lampah DA, Tjitra E, Mwaikambo ED, Handojo T, Piera KA, Zhao ZZ, de Labastida Rivera F, Zhou Y, McSweeney KM, Le L, Amante FH, Haque A, Stanley AC, Woodberry T, Salwati E, Granger DL, Hobbs MR, Price RN, Weinberg JB, Montgomery GW, Anstey NM, Engwerda CR.**
Age-related susceptibility to severe malaria associated with galectin-2 in highland Papuans.
J Infect Dis 2010 Jul 1;202(1):117-124.

   BACKGROUND: Age and host genetics are important determinants of malaria severity. Lymphotoxin-alpha (LTalpha) has been associated with the development of cerebral malaria (CM) and other severe malaria (SM) syndromes. Mutations in genes regulating LTalpha production contribute to other acute vascular diseases and may contribute to malaria pathogenesis. METHODS: We tested the association between rs7291467, a single-nucleotide polymorphism (SNP) in the LTalpha-related gene encoding galectin-2 (*LGALS2*), disease severity and function in a case-control study of ethnic highland Papuan adults and children with SM (n = 380) and asymptomatic malaria-exposed controls (n = 356) originating from a non-malaria-endemic region but residing in a lowland malaria-endemic area of Papua, Indonesia. RESULTS: The *LGALS2* SNP showed a significant association with susceptibility to SM (including CM) in children (odds ratio, 2.02 [95% confidence interval, 1.14-3.57]) but not in adults. In SM, the C allele at rs7291467 was associated with enhanced galectin-2 transcript levels. In a separate group of Tanzanian children originating from a malaria-endemic region, we found preservation of the major ancestral *LGALS2* allele and no association with susceptibility to CM. CONCLUSIONS: Results suggest differences in the inflammatory contribution to the development of SM between children and adults in the same population and potential differences between individuals originating from malaria-endemic and non-malaria-endemic areas.

58 **Randall LM, Kenangalem E, Lampah DA, Tjitra E, Mwaikambo ED, Handojo T, Piera KA, Zhao ZZ, de Labastida Rivera F, Zhou Y, McSweeney KM, Le L, Amante FH, Haque A, Stanley AC, Woodberry T, Salwati E, Granger DL, Hobbs MR, Price RN, Weinberg JB, Montgomery GW, Anstey NM, Engwerda CR.**
A study of the TNF/LTA/LTB locus and susceptibility to severe malaria in highland Papuan children and adults.
Malar J 2010 Oct 29;9:302.

   BACKGROUND: Severe malaria (SM) syndromes caused by *Plasmodium falciparum* infection result in major morbidity and mortality each year. However, only a fraction of *P. falciparum* infections develop into SM, implicating host genetic factors as important determinants of disease outcome. Previous studies indicate that tumour

necrosis factor (TNF) and lymphotoxin alpha (LTα) may be important for the development of cerebral malaria (CM) and other SM syndromes. METHODS: An extensive analysis was conducted of single nucleotide polymorphisms (SNPs) in the TNF, LTA and LTB genes in highland Papuan children and adults, a population historically unexposed to malaria that has migrated to a malaria-endemic region. Generated p-values for SNPs spanning the LTA/TNF/LTB locus were corrected for multiple testing of all the SNPs and haplotype blocks within the region tested through 10,000 permutations. A global p-value of <0.05 was considered statistically significant. RESULTS: No associations between SNPs in the TNF/LTA/LTB locus and susceptibility to SM in highland Papuan children and adults were found. CONCLUSIONS: These results support the notion that unique selective pressure on the TNF/LTA/LTB locus in different populations has influenced the contribution of the gene products from this region to SM susceptibility.

59 **Redman-Maclaren ML, Maclaren DJ, Asugeni R, Fa'anuabae CE, Harrington H, Muse A, Speare R, Clough AR.**
"We can move forward": challenging historical inequity in public health research in Solomon Islands.
Int J Equity Health 2010 Nov 5;9:25.

   BACKGROUND: In resource-poor countries, such as Solomon Islands, the research agenda on health is often dominated by researchers from resource-rich countries. New strategies are needed to empower local researchers to set directions for health research. This paper presents a process which seeks to enable a local and potentially more equitable research agenda at a remote hospital in Solomon Islands. METHODS: In preparation for a health research capacity-building workshop at Atoifi Adventist Hospital, Malaita, Solomon Islands, a computer-based search was conducted of Solomon Islands public health literature. Using a levels-of-agreement approach publications were categorised as: a) original research, b) reviews, c) program descriptions and d) commentaries or discussion. Original research publications were further sub-categorised as: i) measurement, ii) descriptive research and iii) intervention studies. Results were reviewed with Solomon Islander health professionals in a focus group discussion during the health research workshop. Focus group participants were invited to discuss reactions to literature search results and how results might assist current or future local researchers to identify gaps in the published research literature and possible research opportunities at the hospital and surrounding communities. Focus group data were analysed using a grounded theory approach. RESULTS: Of the 218 publications meeting inclusion criteria, 144 (66%) were categorised as 'original research', 42 (19%) as 'commentaries/discussion', 28 (13%) as 'descriptions of programs' and 4 (2%) as 'reviews'. Agreement between three authors' (MRM, DM, AC) independent categorisation was 'excellent' (0.8 <κ). The 144 'original research' publications included 115 (80%) 'descriptive studies' (κ = 0.82); 19 (13%) 'intervention studies' (κ = 0.77); and 10 (7%) 'measurement studies' (κ = 0.80). Key themes identified in the focus group discussion challenged historical inequities evident from the literature review. These included: i) who has done/is doing research in Solomon Islands (largely non-Solomon Islanders); ii) when the research was done (research needs to

keep up to date); iii) amount of published research (there should be more); iv) types of research (lack of intervention and operational research); v) value of published research (important); vi) gaps in published literature (need more research about nursing); vii) opportunities for research action (start small); viii) support required to undertake research at the hospital and in surrounding communities (mentoring and partnering with experienced researchers). CONCLUSIONS: A search and collaborative review of public health literature for Solomon Islands at a health research capacity building workshop has uncovered and challenged historical inequity in the conduct and access to public health research. Emerging Solomon Islander researchers at a remote hospital are now working to set priorities and strengthen local research efforts. These efforts have highlighted the importance of collaboration and mentoring for Solomon Islanders to instigate and implement public health research to improve the health of individuals and communities served by this remote hospital.

60 **Redman-Maclaren ML, Maclaren DJ, Solomon J, Muse A, Asugeni R, Harrington H, Kekuabata E, Speare R, Clough AR.**
Research workshop to research work: initial steps in establishing health research systems on Malaita, Solomon Islands.
Health Res Policy Syst 2010 Oct 31;8:33.

INTRODUCTION: Atoifi Adventist Hospital is a 90 bed general hospital in East Kwaio, Malaita, Solomon Islands providing services to the population of subsistence villagers of the region. Health professionals at the hospital and attached College of Nursing have considerable human capacity and willingness to undertake health research. However, they are constrained by limited research experience, training opportunities, research systems, physical infrastructure and access to resources. This brief commentary describes an 'Introduction to Health Research' workshop delivered at Atoifi Adventist Hospital in September 2009 and efforts to move from 'research workshop' to 'research work'. THE APPROACH: Using a participatory-action research approach underpinned by decolonising methodologies, staff from Atoifi Adventist Hospital and James Cook University (Queensland, Australia) collaboratively designed, implemented and evaluated a health research workshop. Basic health research principles and methods were presented using active learning methodologies. Following the workshop, Atoifi Adventist Hospital and Atoifi College of Nursing staff, other professionals and community members reported an increased awareness and understanding of health research. The formation of a local Research Committee, improved ethics review procedures and the identification of local research mentors followed the week-long workshop. The workshop has acted as a catalyst for research activity, increasing structural and human resource capacity for local health professionals and community leaders to engage in research. DISCUSSION AND CONCLUSIONS: Participants from a variety of educational backgrounds participated in, and received benefit from, a responsive, culturally and linguistically accessible health research workshop. Improving health research systems at a remote hospital and aligning these with local and national research agendas is establishing a base to strengthen public health research and practice on Malaita, Solomon Islands.

61 **Reich D, Green RE, Kircher M, Krause J, Patterson N, Durand EY, Viola B, Briggs AW, Stenzel U, Johnson PL, Maricic T, Good JM, Marques-Bonet T, Alkan C, Fu Q, Mallick S, Li H, Meyer M, Eichler EE, Stoneking M, Richards M, Talamo S, Shunkov MV, Derevianko AP, Hublin JJ, Kelso J, Slatkin M, Pääbo S.**
Genetic history of an archaic hominin group from Denisova Cave in Siberia.
Nature 2010 Dec 23;468(7327):1053-1060.

Using DNA extracted from a finger bone found in Denisova Cave in southern Siberia, we have sequenced the genome of an archaic hominin to about 1.9-fold coverage. This individual is from a group that shares a common origin with Neanderthals. This population was not involved in the putative gene flow from Neanderthals into Eurasians; however, the data suggest that it contributed 4-6% of its genetic material to the genomes of present-day Melanesians. We designate this hominin population 'Denisovans' and suggest that it may have been widespread in Asia during the Late Pleistocene epoch. A tooth found in Denisova Cave carries a mitochondrial genome highly similar to that of the finger bone. This tooth shares no derived morphological features with Neanderthals or modern humans, further indicating that Denisovans have an evolutionary history distinct from Neanderthals and modern humans.

62 **Reiling L, Richards JS, Fowkes FJ, Barry AE, Triglia T, Chokejindachai W, Michon P, Tavul L, Siba PM, Cowman AF, Mueller I, Beeson JG.**
Evidence that the erythrocyte invasion ligand PfRh2 is a target of protective immunity against *Plasmodium falciparum* malaria.
J Immunol 2010 Nov 15;185(10):6157-6167. Epub 2010 Oct 20.

Abs targeting blood-stage Ags of *Plasmodium falciparum* are important in acquired immunity to malaria, but major targets remain unclear. The *P. falciparum* reticulocyte-binding homologs (PfRh) are key ligands used by merozoites during invasion of erythrocytes. PfRh2a and PfRh2b are functionally important members of this family and may be targets of protective immunity, but their potential role in human immunity has not been examined. We expressed eight recombinant proteins covering the entire PfRh2 common region, as well as PfRh2a- and PfRh2b-specific regions. Abs were measured among a cohort of 206 Papua New Guinean children who were followed prospectively for 6 mo for reinfection and malaria. At baseline, Abs were associated with increasing age and active infection. High levels of IgG to all PfRh2 protein constructs were strongly associated with protection from symptomatic malaria and high-density parasitemia. The predominant IgG subclasses were IgG1 and IgG3, with little IgG2 and IgG4 detected. To further understand the significance of PfRh2 as an immune target, we analyzed PfRh2 sequences and found that polymorphisms are concentrated in an N-terminal region of the protein and seem to be under diversifying selection, suggesting immune pressure. Cluster analysis arranged the sequences into two main groups, suggesting that many of the haplotypes identified may be antigenically similar. These findings provide evidence suggesting that PfRh2 is an important target of protective immunity in humans and that Abs act by controlling blood-stage parasitemia and support its potential for vaccine development.

63 **Richards JS, Stanisic DI, Fowkes FJ, Tavul L, Dabod E, Thompson JK, Kumar S, Chitnis CE, Narum DL, Michon P, Siba PM, Cowman AF, Mueller I, Beeson JG.**

Association between naturally acquired antibodies to erythrocyte-binding antigens of *Plasmodium falciparum* and protection from malaria and high-density parasitemia.

Clin Infect Dis 2010 Oct 15;51(8):e50-e60.

BACKGROUND: Antibodies targeting blood stage antigens are important in protection against malaria, but the principle targets remain unclear. Erythrocyte-binding antigens (EBAs) are important erythrocyte invasion ligands used by merozoites and may be targets of protective immunity, but there are limited data examining their potential importance. METHODS: We examined antibodies among 206 Papua New Guinean children who were treated with antimalarials at enrollment and observed prospectively for 6 months for reinfection and malaria. Immunoglobulin (Ig) G, IgG subclasses, and IgM to different regions of EBA175, EBA140 and EBA181 expressed as recombinant proteins were assessed in comparison with several other merozoite antigens. RESULTS: High levels of IgG to each of the EBAs were strongly associated with protection from symptomatic malaria and high density parasitemia, but not with risk of reinfection per se. The predominant IgG subclasses were either IgG1 or IgG3, depending on the antigen. The predominance of IgG1 versus IgG3 reflected structural features of specific regions of the proteins. IgG3 was most strongly associated with protection, even for those antigens that had an IgG1 predominant response. CONCLUSIONS: The EBAs appear important targets of acquired protective immunity. These findings support their further development as vaccine candidates.

64 **Rosanas-Urgell A, Mueller D, Betuela I, Barnadas C, Iga J, Zimmerman PA, del Portillo HA, Siba P, Mueller I, Felger I.**

Comparison of diagnostic methods for the detection and quantification of the four sympatric *Plasmodium* species in field samples from Papua New Guinea.

Malar J 2010 Dec 14;9:361.

BACKGROUND: Accurate diagnosis of *Plasmodium* infections is essential for malaria morbidity and mortality reduction in tropical areas. Despite great advantages of light microscopy (LM) for malaria diagnosis, its limited sensitivity is a critical shortfall for epidemiological studies. Robust molecular diagnostic tools are thus needed. METHODS: The present study describes the development of a duplex quantitative real time PCR (qPCR) assay, which specifically detects and quantifies the four human *Plasmodium* species. Performance of this method was compared to PCR-ligase detection reaction-fluorescent microsphere assay (PCR_LDR_FMA), nested PCR (nPCR) and LM, using field samples collected from 452 children one to five years of age from the Sepik area in Papua New Guinea. Agreement between diagnostic methods was calculated using kappa statistics. RESULTS: The agreement of qPCR with other molecular diagnostic methods was substantial for the detection of *P. falciparum*, but was moderate for the detection of *P. vivax*, *P. malariae* and *P. ovale*. *P. falciparum* and *P. vivax* prevalence by qPCR was 40.9% and 65.7% respectively. This compares to 43.8% and 73.2% by nPCR and 47.1% and 67.5% by PCR_LDR_FMA. *P. malariae* and *P. ovale*

prevalence was 4.7% and 7.3% by qPCR, 3.3% and 3.8% by nPCR, and 7.7% and 4.4% by PCR_LDR_FMA. Prevalence by LM was lower for all four species, being 25.4% for *P. falciparum*, 54.9% for *P. vivax*, 2.4% for *P. malariae* and 0.0% for *P. ovale*. The quantification by qPCR closely correlated with microscopic quantification for *P. falciparum* and *P. vivax* samples ($r^2 = 0.825$ and $r^2 = 0.505$, respectively). The low prevalence of *P. malariae* and *P. ovale* did not permit a solid comparative analysis of quantification for these species. CONCLUSIONS: The qPCR assay developed proved optimal for detection of all four *Plasmodium* species. Densities by LM were well reflected in quantification results by qPCR, whereby congruence was better for *P. falciparum* than for *P. vivax*. This likely is a consequence of the generally lower *P. vivax* densities. Easy performance of the qPCR assay, a less laborious workflow and reduced risk of contamination, together with reduced costs per sample through reduced reaction volume, opens the possibility to implement qPCR in endemic settings as a suitable diagnostic tool for large epidemiological studies.

65 **Rosewell A, Ropa B, Posanai E, Dutta SR, Mola G, Zwi A, MacIntyre CR.**

Shigella spp. antimicrobial drug resistance, Papua New Guinea, 2000-2009.

Emerg Infect Dis 2010 Nov;16(11):1797-1799.

66 **Rudge JW, Phuanukoonnon S, Nema KH, Mounier-Jack S, Coker R.**

Critical interactions between Global Fund-supported programmes and health systems: a case study in Papua New Guinea.

Health Policy Plan 2010 Nov;25 Suppl 1:i48-i52.

In Papua New Guinea, investment by the Global Fund to Fight AIDS, Tuberculosis and Malaria (the Global Fund) has played an important role in scaling up the response to HIV and tuberculosis (TB). As part of a series of case studies on how Global Fund-supported programmes interact with national health systems, we assessed the nature and extent of integration of the Global Fund portfolios within the national HIV and TB programmes, the integration of the HIV and TB programmes within the general health system, and system-wide effects of Global Fund support in Papua New Guinea. The study relied on a literature review and 30 interviews with key stakeholders using the Systemic Rapid Assessment Toolkit and thematic analysis. Global Fund-supported activities were found to be largely integrated, or at least coordinated, with the national HIV and TB programmes. However, this has reinforced the vertical nature of these programmes with respect to the general health system, with parallel systems established to meet the demands of programme scale-up and the performance-based nature of Global Fund investment in the weak health system context of Papua New Guinea. The more parallel functions include monitoring and evaluation, and procurement and supply chain systems, while human resources and infrastructure for service delivery are increasingly integrated at more local levels. Positive synergies of Global Fund support include engagement of civil-society partners, and a reliable supply of high-quality drugs which may have increased patient confidence in the health system. However, the severely limited and overburdened pool of human resources has been skewed towards the three diseases, both at management

and service delivery levels. There is also concern surrounding the sustainability of the disease programmes, given their dependence on donors. Increasing Global Fund attention towards health system strengthening was viewed positively, but should acknowledge that system changes are slow, difficult to measure and require long-term support.

67 **Russell FM, Carapetis JR, Satzke C, Tikoduadua L, Waqatakirewa L, Chandra R, Seduadua A, Oftadeh S, Cheung YB, Gilbert GL, Mulholland EK**.
Pneumococcal nasopharyngeal carriage following reduced doses of a 7-valent pneumococcal conjugate vaccine and a 23-valent pneumococcal polysaccharide vaccine booster.
Clin Vaccine Immunol 2010 Dec;17(12):1970-1976. Epub 2010 Oct 13.

This study was conducted to evaluate the effect of a reduced-dose 7-valent pneumococcal conjugate vaccine (PCV) primary series followed by a 23-valent pneumococcal polysaccharide vaccine (23vPPS) booster on nasopharyngeal (NP) pneumococcal carriage. For this purpose, Fijian infants aged 6 weeks were randomized to receive 0, 1, 2 or 3 PCV doses. Within each group, half received 23vPPS at 12 months. NP swabs were taken at 6, 9, 12 and 17 months and were cultured for *Streptococcus pneumoniae*. Isolates were serotyped by multiplex PCR and a reverse line blot assay. There were no significant differences in PCV vaccine type (VT) carriage between the 3- and 2-dose groups at 12 months. NP VT carriage was significantly higher (p <0.01) in the unvaccinated group than in the 3-dose group at the age of 9 months. There appeared to be a PCV dose effect in the cumulative proportion of infants carrying the VT, with less VT carriage occurring with more doses of PCV. Non-PCV serotype (NVT) carriage rates were similar for all PCV groups. When groups were pooled by receipt or nonreceipt of 23vPPS at 12 months, there were no differences in pneumococcal, VT or NVT carriage rates between the 2 groups at the age of 17 months. In conclusion, there appeared to be a PCV dose effect on VT carriage, with less VT carriage occurring with more doses of PCV. By the age of 17 months, NVT carriage rates were similar for all groups. 23vPPS had no impact on carriage, despite the substantial boosts in antibody levels.

68 **Russell FM, Carapetis JR, Tikoduadua L, Paeds D, Chandra R, Seduadua A, Satzke C, Pryor J, Buadromo E, Waqatakirewa L, Mulholland EK.**
Invasive pneumococcal disease in Fiji: clinical syndromes, epidemiology, and the potential impact of pneumococcal conjugate vaccine.
Pediatr Infect Dis J 2010 Sep;29(9):870-872.

Invasive pneumococcal disease (IPD) epidemiology and the potential impact of the pneumococcal conjugate vaccine in Fiji are documented. The annual incidence was 26.5 and 10.9 in those aged <5 and ≥55 years per 100,000, respectively. The case fatality rate was 9.4% and 67% in <5 and >65 year olds, respectively. One pneumococcal death and case would be prevented in <5 year olds for every 1930 and 128 infants vaccinated with 7vPCV, respectively.

69 **Satzke C, Ortika BD, Oftadeh S, Russell FM, Robins-Browne RM, Mulholland EK, Gilbert GL.**
Molecular epidemiology of *Streptococcus pneumoniae* serogroup 6 isolates from Fijian children, including newly identified serotypes 6C and 6D.
J Clin Microbiol 2010 Nov;48(11):4298-4300. Epub 2010 Sep 1.

Multilocus sequence typing (MLST) was applied to all unique serotype 6C and 6D isolates and a random selection of serotype 6B and 6A isolates from nasopharyngeal swabs from Fijian children enrolled in a recent vaccine trial. The results suggest that Fijian serotype 6D has arisen independently from both serotypes 6A/C and 6B.

70 **Schultz L, Wapling J, Mueller I, Ntsuke PO, Senn N, Nale J, Kiniboro B, Buckee CO, Tavul L, Siba PM, Reeder JC, Barry AE.**
Multilocus haplotypes reveal variable levels of diversity and population structure of *Plasmodium falciparum* in Papua New Guinea, a region of intense perennial transmission.
Malar J 2010 Nov 23;9:336.

BACKGROUND: The South West Pacific nation of Papua New Guinea has intense year round transmission of *Plasmodium falciparum* on the coast and in the low-lying inland areas. Local heterogeneity in the epidemiology of malaria suggests that parasites from multiple locations will need to be surveyed to define the population biology of *P. falciparum* in the region. This study describes the population genetics of *P. falciparum* in thirteen villages spread over four distinct catchment areas of Papua New Guinea. METHODS: Ten microsatellite loci were genotyped in 318 *P. falciparum* isolates from the parasite populations of two inland catchment areas, namely Wosera (number of villages (n) = 7) and Utu (n = 1), and two coastal catchments, Malala (n = 3) and Mugil (n = 3). Analysis of the resultant multilocus haplotypes was done at different spatial scales (2-336 km) to define the genetic diversity (allelic richness and expected heterozygosity), linkage disequilibrium and population structure throughout the study area. RESULTS: Although genetic diversity was high in all parasite populations, it was also variable with a lower allelic richness and expected heterozygosity for inland populations compared to those from the more accessible coast. This variability was not correlated with two proxy measures of transmission intensity, the infection prevalence and the proportion of multiple infections. Random associations among the microsatellite loci were observed in all four catchments showing that a substantial degree of out-crossing occurs in the region. Moderate to very high levels of population structure were found but the amount of genetic differentiation (FST) did not correlate with geographic distance suggesting that parasite populations are fragmented. Population structure was also identified between villages within the Malala area, with the haplotypes of one parasite population clustering with the neighbouring catchment of Mugil. CONCLUSION: The observed population genetics of *P. falciparum* in this region is likely to be a consequence of the high transmission intensity combined with the isolation of human and vector populations, especially those located inland, and migration of parasites via human movement into coastal populations. The variable genetic diversity and population structure of *P. falciparum* has important implications for malaria control strategies and warrants further fine scale sampling throughout Papua New Guinea.

71 **Scott RM, Buckley HR.**
Biocultural interpretations of trauma in two prehistoric Pacific Island populations from Papua New Guinea and the Solomon Islands.
Am J Phys Anthropol 2010 Aug;142(4):509-518.

Two Pacific Island skeletal samples originating from the inland site of Nebira, Papua New Guinea (1230-1650) and a coastal site on the small island of Taumako, Solomon Islands (1530-1698) were examined for evidence of skeletal trauma using a biocultural approach. The types of trauma identified were cranial trauma, postcranial fractures, and piercing and sharp force trauma. Both samples exhibit trauma (Nebira, n = 9/28, 32.1%; Taumako, n = 17/133, 12.8%). Postcranial fractures are significantly higher in males from Nebira (Fisher Exact p value = 0.025). The prevalence of cranial trauma (n = 6/28, 21.4%) is significantly higher in Nebira individuals (Fisher Exact p value = 0.007). There is no conclusive evidence of piercing trauma at Nebira unlike Taumako, which has four individuals with evidence of piercing or sharp force trauma. Both samples show evidence of interpersonal violence and warfare. The results suggest the environment may have contributed to the pattern of trauma at these sites. These patterns are discussed within their cultural and environmental contexts.

72 **Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH.**
Virology, epidemiology, pathogenesis, and control of enterovirus 71.
Lancet Infect Dis 2010 Nov;10(11):778-790. Epub 2010 Oct 18.

First isolated in California, USA, in 1969, enterovirus 71 (EV71) is a major public health issue across the Asia-Pacific region and beyond. The virus, which is closely related to polioviruses, mostly affects children and causes hand, foot and mouth disease with neurological and systemic complications. Specific receptors for this virus are found on white blood cells, cells in the respiratory and gastrointestinal tract, and dendritic cells. Being an RNA virus, EV71 lacks a proofreading mechanism and is evolving rapidly, with new outbreaks occurring across Asia in regular cycles, and virus gene subgroups seem to differ in clinical epidemiological properties. The pathogenesis of the severe cardiopulmonary manifestations and the relative contributions of neurogenic pulmonary oedema, cardiac dysfunction, increased vascular permeability and cytokine storm are controversial. Public health interventions to control outbreaks involve social distancing measures, but their effectiveness has not been fully assessed. Vaccines being developed include inactivated whole-virus, live attenuated, subviral particle and DNA vaccines.

73 **Srikantiah P, Ghidinelli M, Bachani D, Chasombat S, Daoni E, Mustikawati DE, Nhan do T, Pathak LR, San KO, Vun MC, Zhang F, Lo YR, Narain JP.**
Scale-up of national antiretroviral therapy programs: progress and challenges in the Asia Pacific region.
AIDS 2010 Sep;24 Suppl 3:S62-S71.

BACKGROUND: There has been tremendous scale-up of antiretroviral therapy (ART) services in the Asia Pacific region, which is home to an estimated 4.7 million persons living with HIV/AIDS. We examined treatment scale-up, ART program practices and clinical outcome data in the nine low-and-middle-income countries that share over 95% of the HIV burden in the region. METHODS: Standardized indicators for ART scale-up and treatment outcomes were examined for Cambodia, China, India, Indonesia, Myanmar, Nepal, Papua New Guinea, Thailand and Vietnam using data submitted by each country to the WHO/The Joint United Nations Programme on HIV/AIDS (UNAIDS)/UNICEF joint framework tool for monitoring the health sector response to HIV/AIDS. Data on ART program practices were abstracted from National HIV Treatment Guidelines for each country. RESULTS: At the end of 2009, over 700,000 HIV-infected persons were receiving ART in the nine focus countries. Treatment coverage varies widely in the region, ranging from 16 to 93%. All nine countries employ a public health approach to ART services and provide a standardized first-line nonnucleoside reverse transcriptase inhibitor-based regimen. Among patients initiated on first-line ART in these countries, 65-88% remain alive and on treatment 12 months later. Over 50% of mortality occurs in the first 6 months of therapy, and losses to follow-up range from 8 to 16% at 2 years. CONCLUSION: Impressive ART scale-up efforts in the region have resulted in significant improvements in survival among persons receiving therapy. Continued funding support and political commitment will be essential for further expansion of public sector ART services to those in need. To improve treatment outcomes, national programs should focus on earlier identification of persons requiring ART, decentralization of ART services, and the development of stronger healthcare systems to support the provision of a continuum of HIV care.

74 **Stanisic DI, Mueller I, Betuela I, Siba P, Schofield L.**
Robert Koch redux: malaria immunology in Papua New Guinea.
Parasite Immunol 2010 Aug;32(8):623-632.

Over a century ago, the malaria expedition of the brilliant microbiologist Robert Koch to the Dutch East Indies (Indonesia) and German New Guinea (now Papua New Guinea, or PNG), resulted in profound observations that are still central to our current understanding of the epidemiology and acquisition of immunity to the malaria parasite *Plasmodium*. The tradition of malaria research in PNG pioneered by Koch continues to this day, with a number of recent studies still continuing to elucidate his original concepts and hypotheses. These include age and exposure-related acquisition of immunity, species-specific and cross-species immunity, correlates of protective immunity and determining the prospects for anti-malaria vaccines.

75 **Steel A, Gubler DJ, Bennett SN.**
Natural attenuation of dengue virus type-2 after a series of island outbreaks: a retrospective phylogenetic study of events in the South Pacific three decades ago.
Virology 2010 Sep 30;405(2):505-512.

Dengue is an expanding arboviral disease of variable severity characterized by the emergence of virus strains with greater fitness, epidemic potential and possibly virulence. To investigate the role of dengue virus (DENV) strain variation on epidemic activity we studied DENV-2 viruses from a series of South Pacific islands experiencing outbreaks of varying intensity and clinical severity. Initially appearing in 1971 in Tahiti and Fiji, the virus was responsible for subsequent epidemics in

American Samoa, New Caledonia and Niue Island in 1972, reaching Tonga in 1973, where there was near-silent transmission for over a year. Based on whole-genome sequencing and phylogenetic analysis on 20 virus isolates, Tonga viruses were genetically unique, clustering in a single clade. Substitutions in the pre-membrane (prM) and nonstructural genes NS2A and NS4A correlated with the attenuation of the Tongan viruses and suggest that genetic change may play a significant role in dengue epidemic severity.

76 **Stewart L, Usher K.**
The impact of nursing leadership on patient safety in a developing country.
J Clin Nurs 2010 Nov;19(21-22):3152-3160. doi: 10.1111/j.1365-2702.2010.03285.x.
AIM: This article is a report of a study to identify the ways nursing leaders and managers in a developing country have an impact on patient safety. BACKGROUND: The attempt to address the problem of patient safety in health care is a global issue. Literature addressing the significant impact that nursing leadership has on patient safety is extensive and focuses almost exclusively on the developed world. DESIGN: A critical ethnography was conducted with senior registered nursing leaders and managers throughout the Fiji Islands, specifically those in the Head Office of the Fiji Ministry of Health and the most senior nurse in a hospital or community health service. METHOD: Semi-structured interviews were conducted with senior nursing leaders and managers in Fiji. Thematic analysis of the interviews was undertaken from a critical theory perspective, with reference to the macro socio-political system of the Fiji Ministry of Health. RESULTS: Four interrelated issues regarding the nursing leaders and managers' impact on patient safety emerged from the study. Empowerment of nursing leaders and managers, an increased focus on the patient, the necessity to explore conditions for front-line nurses and the direct relationship between improved nursing conditions and increased patient safety mirrored literature from developed countries. CONCLUSION: The findings have significant implications for developing countries and it is crucial that support for patient safety in developing countries become a focus for the international nursing community. RELEVANCE TO CLINICAL PRACTICE: Nursing leaders and managers' increased focus on their own place in the hierarchy of the health care system and on nursing conditions as these affect patient safety could decrease adverse patient outcomes. The findings could assist the global nursing community to better support developing countries in pursuing a patient safety agenda.

77 **Summerhayes GR, Leavesley M, Fairbairn A, Mandui H, Field J, Ford A, Fullagar R.**
Human adaptation and plant use in highland New Guinea 49,000 to 44,000 years ago.
Science 2010 Oct 1;330(6000):78-81.
After their emergence by 200,000 years before the present in Africa, modern humans colonized the globe, reaching Australia and New Guinea by 40,000 to 50,000 years ago. Understanding how humans lived and adapted to the range of environments in these areas has been difficult because well-preserved settlements are scarce. Data from the New Guinea highlands (at an elevation of ~2000 meters) demonstrate the exploitation of the endemic

nut *Pandanus* and yams in archaeological sites dated to 49,000 to 36,000 years ago, which are among the oldest human sites in this region. The sites also contain stone tools thought to be used to remove trees, which suggests that the early inhabitants cleared forest patches to promote the growth of useful plants.

78 **Truong T, Baron-Dubourdieu D, Rougier Y, Guénel P.**
Role of dietary iodine and cruciferous vegetables in thyroid cancer: a countrywide case-control study in New Caledonia.
Cancer Causes Control 2010 Aug;21(8):1183-1192. Epub 2010 Apr 2.
Exceptionally high incidence rates of thyroid cancer have been reported in New Caledonia, particularly in Melanesian women. To clarify the reasons for this elevated incidence, we conducted a countrywide population-based case-control study in the multiethnic population of Caledonian women. The study included 293 cases of thyroid cancer and 354 population controls. Based on a food frequency questionnaire, we investigated the role in thyroid cancer of food items rich in iodine – such as seafood – and of vegetables containing goitrogens – such as cruciferous vegetables. A measure of total daily iodine intake based on a food composition table was also used. Our findings provided little support for an association between thyroid cancer and consumption of fish and seafood. We found that high consumption of cruciferous vegetables was associated with thyroid cancer among women with low iodine intake (OR = 1.86; 95% CI: 1.01-3.43 for iodine intake <96 microg/day). The high consumption of cruciferous vegetables among Melanesian women, a group with mild iodine deficiency, may contribute to explain the exceptionally high incidence of thyroid cancer in this group.

79 **Vallely A, Page A, Dias S, Siba P, Lupiwa T, Law G, Millan J, Wilson DP, Murray JM, Toole M, Kaldor JM.**
The prevalence of sexually transmitted infections in Papua New Guinea: a systematic review and meta-analysis.
PLoS One 2010 Dec 23;5(12):e15586.
BACKGROUND: The potential for an expanded HIV epidemic in Papua New Guinea (PNG) demands an effective, evidence-based and locally appropriate national response. As sexually transmitted infections (STIs) may be important co-factors in HIV transmission nationally, it is timely to conduct a systematic review of STI prevalences to inform national policy on sexual health and HIV/STI prevention. METHODOLOGY/PRINCIPAL FINDINGS: We undertook a systematic review and meta-analysis of HIV and STI prevalences in PNG, reported in peer-reviewed and non-peer-reviewed publications for the period 1950-2010. Prevalence estimates were stratified by study site (community or clinic-based), geographic area and socio-demographic characteristics. The search strategy identified 105 reports, of which 25 studies (10 community-based; 10 clinic-based; and 5 among self-identified female sex workers) reported STI prevalences and were included in the systematic review. High prevalences of chlamydia, gonorrhoea, syphilis and trichomonas were reported in all settings, particularly among female sex workers, where pooled estimates of 26.1%, 33.6%, 33.1%

and 39.3% respectively were observed. Pooled HIV prevalence in community-based studies was 1.8% (95% CI:1.2-2.4) in men; 2.6% (95% CI:1.7-3.5) in women; and 11.8% (95% CI:5.8-17.7) among female sex workers. CONCLUSIONS/ SIGNIFICANCE: The epidemiology of STIs and HIV in PNG shows considerable heterogeneity by geographical setting and sexual risk group. Prevalences from community-based studies in PNG were higher than in many other countries in the Asia-Pacific. A renewed focus on national STI/HIV surveillance priorities and systems for routine and periodic data collection will be essential to building effective culturally-relevant behavioural and biomedical STI/HIV prevention programs in PNG.

80 **Wollstein A, Lao O, Becker C, Brauer S, Trent RJ, Nürnberg P, Stoneking M, Kayser M.**
Demographic history of Oceania inferred from genome-wide data.
Curr Biol 2010 Nov 23;20(22):1983-1992. Epub 2010 Nov 11.

BACKGROUND: The human history of Oceania comprises two extremes: the initial colonizations of Near Oceania, one of the oldest out-of-Africa migrations, and of Remote Oceania, the most recent expansion into unoccupied territories. Genetic studies, mostly using uniparentally inherited DNA, have shed some light on human origins in Oceania, particularly indicating that Polynesians are of mixed East Asian and Near Oceanian ancestry. Here, we use ~1 million single nucleotide polymorphisms (SNPs) to investigate the demographic history of Oceania in a more detailed manner. RESULTS: We developed a new approach to account for SNP ascertainment bias, used approximate Bayesian computation simulations to choose the best-fitting model of population history, and estimated demographic parameters. We find that the ancestors of Near Oceanians diverged from ancestral Eurasians ~27 thousand years ago (kya), suggesting separate initial occupations of both territories. The genetic admixture in Polynesian

history between East Asians (~87%) and Near Oceanians (~13%) occurred ~3 kya, prior to the colonization of Polynesia. Fijians are of Polynesian (~65%) and additional Near Oceanian (~35%) ancestry not found in Polynesians, with this admixture occurring considerably after the initial settlement of Remote Oceania. Our data support a greater contribution of East Asian women than men in the admixture history of Remote Oceania and highlight population substructure in Polynesia and New Guinea. CONCLUSIONS: Despite the inherent ascertainment bias, genome-wide SNP data provide new insights into the genetic history of Oceania. Our approach to correct for ascertainment bias and obtain reliable inferences concerning demographic history should prove useful in other such studies.

81 **Yeo TW, Lampah DA, Tjitra E, Piera K, Gitawati R, Kenangalem E, Price RN, Anstey NM.**
Greater endothelial activation, Weibel-Palade body release and host inflammatory response to *Plasmodium vivax*, compared with *Plasmodium falciparum*: a prospective study in Papua, Indonesia.
J Infect Dis 2010 Jul 1;202(1):109-112.

Pathogenic mechanisms underlying vivax malaria are poorly understood, with few studies comparing endothelial and inflammatory responses with falciparum malaria. In adults with uncomplicated vivax or falciparum malaria, we compared plasma measurements of endothelial Weibel-Palade body release (angiopoietin-2) and activation (ICAM-1, E-selectin), as well as selected cytokines. Despite a lower median parasite count, angiopoietin-2 concentrations were higher in patients with vivax malaria than in those with falciparum malaria. Per peripheral parasite, median plasma angiopoietin-2, ICAM-1, E-selectin, interleukin-6 and interleukin-10 concentrations were higher in patients with malaria due to *Plasmodium vivax*. *P. vivax* induces greater endothelial Weibel-Palade body release and activation and greater host inflammatory responses than *Plasmodium falciparum*.

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REFEREES 2006-2009

To maintain its standards the Journal is dependent on the willingness and dedication of its referees, colleagues who review all papers submitted to the Editor for publication and make the Journal's peer review system a reality.

We on the editorial staff recognize that we could not function without our referees (or reviewers). Some papers submitted to the Journal are accepted with little change and some are rejected (but only after 2 referees have independently concurred in this opinion). However, the majority of papers are made acceptable only after considerable revision, following the reports of the referees. In most cases this entails a great deal of work on the part of each referee. On occasions, other colleagues are consulted about specific matters related to a submitted paper, to improve or correct it before publication.

We acknowledge the help of the following colleagues who have contributed reports on papers which were published in the Journal - or rejected - during the period 2006-2009. Many have undertaken this task more than once, some many times. We thank them all for their essential contribution to the Journal. We apologize for any omissions - since each year we have a focus issue with a guest editor, the work of some referees may not have come to our attention.

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4 **Cochrane RG.** A critical appraisal of the present position of leprosy. In: Lincicome DP, ed. International Review of Tropical Medicine. New York: Academic Press, 1961:1-42.

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