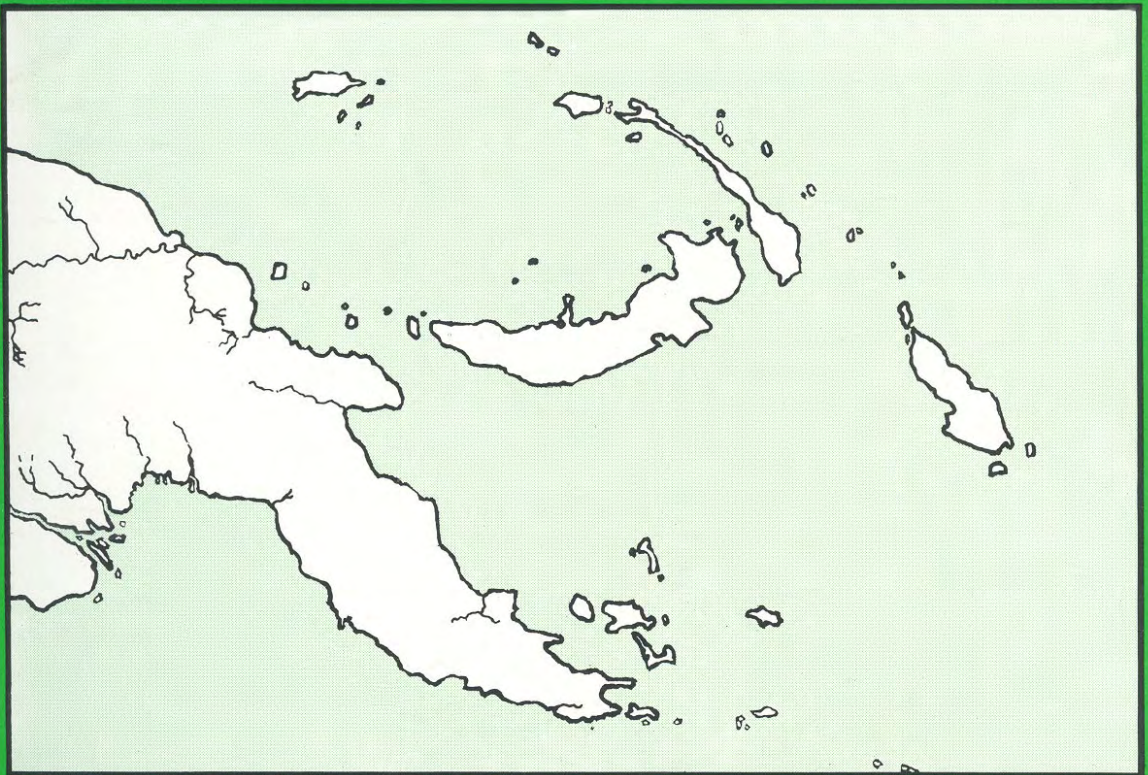


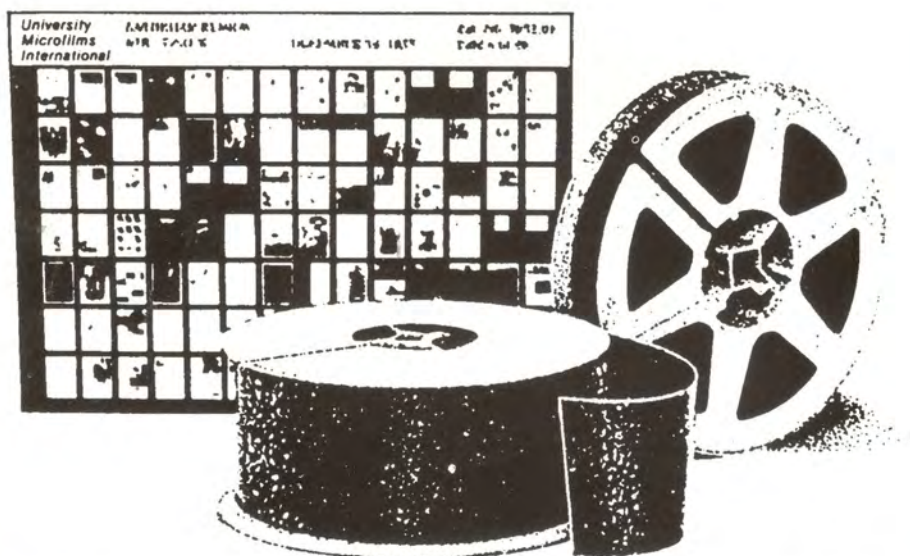
ISSN 0031-1480

PAPUA NEW GUINEA MEDICAL JOURNAL



VOL. 51, NO 3-4, SEPTEMBER-DECEMBER 2008

this publication is available in microform



Please send me additional information

Name _____

Institution _____

Street _____

City _____

State _____ Zip _____

University Microfilms International

300 North Zeeb Road
Dept. P.R.
Ann Arbor, MI 48106
U.S.A.

18 Bedford Row
Dept. P.R.
London, WC1R 4EJ
England

Medical Society of Papua New Guinea

Executive 2008

President:	Mathias Sapuri
Vice-President:	Nicholas Mann
Secretary:	Sylvester Lahe
Treasurer:	Harry Aigeeleng
Executive Member:	Osborne Liko

ACKNOWLEDGEMENT

We are grateful to the Government of Australia through AusAID for providing funding for the publication of this issue of the Journal.

The Editors



Australian Government
Aid Program

Supported by the Australian Government, AusAID

Disclaimer

The views expressed in this publication are those of the authors and not necessarily those of the Australian Agency for International Development (AusAID).

Papua New Guinea Medical Journal

ISSN 0031-1480

September-December 2008, Volume 51, Number 3-4

EDITORS: PETER M. SIBA, NAKAPI TEFUARANI, FRANCIS HOMBHANJE

Editorial Committee

B. Amoa	V. Golpak
G. Hiawalyer	J. Millan
G. Mola	A. Saweri
J. Vince	

Assistant Editor: Cynthea Leahy

Emeritus Editor: Michael Alpers

Email: pngmedj@pngimr.org.pg
Web page: <http://www.pngimr.org.pg>

- ★ Registered at GPO, Port Moresby for transmission by Post as a Qualified Publication.
- ★ Printed by Moore Printing for the Medical Society of Papua New Guinea.
- ★ Authors preparing manuscripts for publication in the *Journal* should consult 'Information for Authors' inside back cover.

CONTENTS

MEMORIAL TO DR HELENA VRBOVA

EDITORIAL

- A memorial tribute to Helena Vrbova *M.P. Alpers* 73

TRIBUTES

- Helena Vrbova: a very special person *G. Vrbova* 80
- Helena Vrbova – a personal tribute *D.J. Jolley* 86
- Helena Vrbova, malaria epidemiologist *B.A. Darlow* 90
- Helena Vrbova: a reminiscence *S.J. Oppenheimer* 92
- Dr Helena Vrbova – a pioneer in malaria research *J. Stace* 94
- Helena Vrbova – an appreciation *A.O. Lucas* 95
- Personal observations on the characteristics of scientists *J. Taime* 96
- Tribute from Gonoa village, sent to her family at the time of Helena's death
Gonoa Village and J.S. Moir 98
- Bibliography of Helena Vrbova 99

ORIGINAL ARTICLES

- Women's groups and the marketing of health interventions – a Tanzanian experience *D. Charlwood* 102
- Effective diagnostic tests and anthelmintic treatment for *Strongyloides stercoralis* make community control feasible *J.M. Shield and W. Page* 105
- The relationship between undernutrition and humoral immune status in children with pneumonia in Papua New Guinea *A.W. Cripps, D.C. Otczyk, J. Barker, D. Lehmann and M.P. Alpers* 120
- Alpha⁺-thalassaemia and malaria in Melanesia: epidemiological perspectives *F.J.I. Fowkes and K.P. Day* 131

ORIGINAL ARTICLES

- Does Integrated Management of Childhood Illness (IMCI) make a difference to the assessment of sick children in Papua New Guinea? *M. Moti and J.D. Vince* 138
- Glycophorin C* Δ^{exon3} is not associated with protection against severe anaemia in Papua New Guinea *L. Tavul, I. Mueller, L. Rare, E. Lin, P.A. Zimmerman, J. Reeder, P. Siba and P. Michon* 149
- Is a 'convenience' sample useful for estimating immunization coverage in a small population? *J.E. Weir and C. Jones* 155

MEDICAL RESEARCH PROJECTS IN PAPUA NEW GUINEA 160

MEDLARS BIBLIOGRAPHY 163

EDITORIAL

A memorial tribute to Helena Vrbova

Dr Helena Vrbova came to the Papua New Guinea Institute of Medical Research (PNGIMR) on 20 July 1979. Her time in Papua New Guinea (PNG) came to an abrupt end when she died in Madang on 9 May 1982, less than 3 weeks from her 30th birthday. She had planned originally to celebrate her birthday with her family in London, where she was going on recreational leave after fulfilling her contract at the Institute. In addition, she had been granted extended unpaid leave to undertake a Masters degree at the London School of Hygiene and Tropical Medicine, for which she had been awarded a scholarship from the Medical Research Council of the UK. Her tragic and untimely death was an enormous loss to tropical epidemiology and medicine: in part because of her achievements, which were already considerable, but principally because of her potential for a brilliant career in medical research, her commitment to health and well-being in the tropical world, her ability to engage with people in every walk of life, and her determination to make a difference to the world.

On 9 May 2007 it was 25 years since Helena died. Her friends and colleagues then began a commitment to honour her memory and celebrate her vitality, her compassion, her outstanding research abilities and her achievements. We agreed upon the idea of a memorial in the *Papua New Guinea Medical Journal*. We are grateful to the Chief Editor of the Journal, Professor Peter Siba, for the ready support which he gave to this idea. In the end, for some, the task of writing about Heli, even in a professional way, proved to be too hard. Even after this passage of time, we all find that she readily comes to life as a vivid memory. This common experience makes it abundantly clear that the pain of her death and the regret of losing her are still powerfully present among her friends and colleagues. I sympathize therefore with those who felt unable to contribute to this written memorial. Several of Heli's friends submitted photographs and I include a selection of them without attribution in this brief editorial overview.

However, some have succeeded in recording their memories of Heli and of the pleasures of working with her, and others have paid tribute to her by submitting scientific papers dedicated to her memory. The most remarkable contribution comes from her mother (1). It is a beautiful and frank account of Helena's birth and growing-up and the dramatic events in her early life. Gerta Vrbova has also written a more extensive account of her own early life (2), which is dedicated to Helena. As Gerta says in her reminiscences here, Helena's father escaped from Auschwitz and alerted the Hungarian authorities as to the real extent of the horror in the Nazi concentration camps. He wrote a book about his extraordinary exploits (3), which have also been described by George Klein (4). George is a friend and colleague with a keen interest in Papua New Guinea because of his lifetime passion for studying Burkitt lymphoma; although he never knew Helena he was much affected by her death. When he sent me a copy of his book *Pietà* he specifically drew my attention to its second part (5) since it was deeply relevant to the discussions we had had after Helena's death.

Helena's interest in Papua New Guinea arose through her determination, as a young doctor, to work on tropical diseases, which are the diseases of poverty and of the third world, and she was introduced to the burgeoning malaria research program of the PNGIMR by Graham Brown. She was immediately interested in coming to PNG. I was impressed by her credentials and her personality and offered her a position in Madang, which she quickly accepted. She arrived in Madang soon afterwards. It is clear from the testimonies of her colleagues, Damien Jolley (6), Brian Darlow (7), Stephen Oppenheimer (8) and John Stace (9), that she was a great success in this position. She was intelligent and hard-working, with a passion for detail (Figure 1), she was open and warm to everyone she worked with (Figure 2), and she was physically very fit (Figure 3): a perfect combination for a field epidemiologist. She was widely loved and respected, not least by the Papua New Guinean staff of the Institute, as witnessed by John Taime (10), and by the



Figure 1. Helena getting the record straight.



Figure 2. Helena collecting fingerprick blood from children for malaria blood smears in Karimui.



Figure 3. Field team at Karimui, 1981. From the left – Phil Harvey, Helena Vrbova, Robin Hide, Jane Barker.

village people of Gonoa (11), where she set up the Institute's first longitudinal study of malaria. She was a beautiful person, who was much admired in her lifetime.

Helena's research career began in England. Characteristically, it involved research into a possible new intervention for an important disease (1,12). However, though the technique of transplanting islets of Langerhans proved to be successful, it was remote from a possible application in diabetes mellitus and Helena wanted to work on a disease where she would be closer to the action. Malaria fitted the bill perfectly. She achieved much in her time in PNG, as her bibliography shows (12). She took part in all the Institute's work on malaria. As well as conducting epidemiological and clinical intervention studies on malaria (Figures 4 and 5), the IMR established diagnostic, culture and entomology laboratories in Yagaum, which was a major achievement; falciparum malaria strains from Madang established in culture in those early days have been used throughout the world ever since – for a recent example of the use of MAD20 see Dent et al. (13). I came to Madang from Goroka to work

as often as I could. On these occasions I interacted with Heli mostly in the field, where we enjoyed engaging with the people of our study communities. However, occasionally, we attended to cultures in the lab, usually at night; there I learned also to appreciate her manual dexterity. She was certainly an accomplished all-rounder as a scientist.

Helena understood implicitly the inherent need for community members involved in research studies to be participants in the research, an important part of the Institute's policy of conduct and an underpinning principle of our fieldwork, which had to be established in practice whenever we moved into a new area to work (14). The staff of the PNGIMR got used to talking about community 'participants', not 'subjects'. It is interesting that some people – like Heli – 'get it' and understand without being told; others never 'get it' even after they have correctly carried out the process according to rule and conducted good work in the field. Heli was also a good manager – and one might make similar comments about that skill.

Helena was a committed feminist, though



Figure 4. Helena at work in the field in the Madang area. Photograph courtesy of Gerta Vrbova.



Figure 5. Helena at work in the field in Madang with Sam Pariva. Sam Pariva was a member of the chemoprophylaxis study team that included other PNGIMR staff and John Stace. After the project finished, Sam became a national Member of Parliament. Not long after his retirement he died. We take this opportunity of honouring his memory as another pioneer in the malaria research program of the PNGIMR. Photograph courtesy of Gerta Vrbova.

not in any way an ideologue. She was fiercely independent, and could ignore the rules and cause no offence, because of the force of her personality: she always dressed in shorts when she worked in the field (just like the men – Figure 6). She would have given strong support to our women's health program, which was established, initially in Madang (15), after her time. She would also have enjoyed Derek Charlwood's account of engaging women in health projects in Tanzania (16), and would have been understanding – though never complacent or cynical – about the outcome.

Though based in Yagaum Helena visited the highlands on several occasions, following the first grand escapade described by Damien Jolley (6) and alluded to by Stephen Oppenheimer (8). She visited the various epidemiological studies ongoing in highland populations, including the kuru project. When she visited the kuru-affected region the bridge over the river between the villages of Miarasa and Yasubi was out and we had to spend time negotiating a steep ford in order to make our way; that place has in my mind been

associated with Helena ever since. She worked in Karimui (Figures 2, 3 and 7), with colleagues Jim Tulloch, Robin Hide, Jane Barker, Phil Harvey and Jenny Shield, in a multidisciplinary study that included the investigation of infection with intestinal parasites. It is a pleasure to have the dedicated paper by Jenny Shield (written with her colleague Wendy Page) in this memorial issue (17): Jenny was the parasitologist at the IMR when the studies in Karimui were conducted (18,19).

Our malaria research program was established in collaboration with colleagues in Australia, in Melbourne, Sydney, Canberra, Brisbane and Newcastle. Allan Cripps was part of the Newcastle team and he has contributed a memorial paper, with colleagues who include Jane Barker (seen in Figure 3), on a study carried out in children with pneumonia in Goroka (20). In Madang many of our later studies on malaria examined the extraordinary malaria-protective genetic polymorphisms established over millennia in human populations through evolutionary selection by malaria, especially ovalocytosis



Figure 6. Field staff in Madang. From the left – Maria Passingan, Peter Heywood, Helena Vrbova, Sean Gibney. Photograph, from the permanent collection on the noticeboard at Yagaum, courtesy of John Taime.



Figure 7. Helena collecting fingerprick blood during the malaria survey in Karimui.

(21,22) and alpha-thalassaemia (23,24). We are privileged to have a comprehensive up-to-date review of alpha-thalassaemia and malaria by Freya Fowkes and Karen Day (25) in this memorial issue.

Helena's plans after she left PNG were to get further training in epidemiology and then to continue her work on malaria. I suspect that she would have spent some time in Africa but that the malaria research program in PNG that she helped to initiate would eventually have drawn her back. Whichever way it might have gone, I am sure that we would all have benefited from her contribution to easing the global burden of malaria. Sadly, it was not to be. We regret that loss deeply as we honour her memory. Nevertheless, I believe that her vitality and commitment live on in a significant measure in the lives, work and achievements of those who knew her, including those who have contributed here and others who have remembered her but preferred not to write.

Helena was passionate about human rights and especially committed to the advancement of women. She was not afraid of expressing love for her fellow human beings – and anger at all forms of injustice, prejudice and hate. Certainly we can say that her influence spread widely and will never be forgotten by all who knew and loved her – and also by those who

met her only briefly, as witnessed by Adetokunbo Lucas (26). The legacy that Helena left, as a person and as a scientist, is no mean achievement for one who died so young. The *Papua New Guinea Medical Journal* is proud to create the opportunity for this legacy to be acknowledged and her memory honoured.

Michael P. Alpers

Centre for International Health
ABCRC, Shenton Park Campus
Curtin University of Technology
GPO Box U1987
Perth, WA 6845
Australia
m.alpers@curtin.edu.au
Formerly Director of the Papua New Guinea
Institute of Medical Research, 1977-2000

REFERENCES

- 1 **Vrbova G.** Helena Vrbova: a very special person. *PNG Med J* 2008;51:80-85.
- 2 **Vrbová G.** Trust and Deceit: A Tale of Survival in Slovakia and Hungary, 1939-1945. London: Vallentine Mitchell, 2006.
- 3 **Vrba R, Bestic A.** 44070 – The Conspiracy of the Twentieth Century. New expanded edition of I Cannot Forgive (1963). Bellingham, Washington

- State: Star & Cross Publishing House, 1989.
- 4 **Klein G.** The ultimate fear of the traveler returning from hell. Chapter 5 of *Pietà*. Cambridge, Massachusetts: MIT Press, 1992:125-159.
 - 5 **Klein G.** *Suicides*. Part Two of *Pietà*. Cambridge, Massachusetts: MIT Press, 1992:17-95.
 - 6 **Jolley DJ.** Helena Vrbova – a personal tribute. *PNG Med J* 2008;51:86-89.
 - 7 **Darlow BA.** Helena Vrbova, malaria epidemiologist. *PNG Med J* 2008;51:90-91.
 - 8 **Oppenheimer SJ.** Helena Vrbova: a reminiscence. *PNG Med J* 2008;51:92-93.
 - 9 **Stace J.** Dr Helena Vrbova – a pioneer in malaria research. *PNG Med J* 2008;51:94.
 - 10 **Taime J.** Personal observations on the characteristics of scientists. *PNG Med J* 2008;51:96-97.
 - 11 **Gonoa Village, Moir JS.** Tribute from Gonoa village, sent to her family at the time of Helena's death. *PNG Med J* 2008;51:98.
 - 12 **Vrbova H.** Bibliography. *PNG Med J* 2008;51:99-101.
 - 13 **Dent AE, Yohn CT, Zimmerman PA, Vulule J, Kazura JW, Moormann AM.** A polymerase chain reaction/ligase detection reaction-fluorescent microsphere assay to determine *Plasmodium falciparum* MSP-119 haplotypes. *Am J Trop Med Hyg* 2007;77:250-255.
 - 14 **Reeder JC, Taime J.** Engaging the community in research: lessons learned from the malaria vaccine trial. *Trends Parasitol* 2003;19:281-282.
 - 15 **Gillett JE.** The Health of Women in Papua New Guinea. Papua New Guinea Institute of Medical Research Monograph No 9. Goroka: Papua New Guinea Institute of Medical Research, 1990.
 - 16 **Charlwood D.** Women's groups and the marketing of health interventions – a Tanzanian experience. *PNG Med J* 2008;51:102-104.
 - 17 **Shield JM, Page W.** Effective diagnostic tests and anthelmintic treatment for *Strongyloides stercoralis* make community control feasible. *PNG Med J* 2008;51:105-119.
 - 18 **Barker J, Harvey PWJ, Hide RL, Shield JM, Tulloch JL, Vrbova H.** Nutrition, malaria, intestinal parasitosis and morbidity in Karimui. Report of the Karimui Epidemiological Survey carried out by SLUP (Simbu Land Use Project) and IMR (Institute of Medical Research), 24 Aug-4 Sep 1981:122p.
 - 19 **Shield JM, Hide RL, Harvey PWJ, Vrbova H, Tulloch JL.** Hookworm (*Necator americanus*) and *Strongyloides fuelleborni*-like prevalence and egg count with age in highlands fringe people of Papua New Guinea. *PNG Med J* 1987;30:21-26.
 - 20 **Cripps AW, Otczyk DC, Barker J, Lehmann D, Alpers MP.** The relationship between undernutrition and humoral immune status in children with pneumonia in Papua New Guinea. *PNG Med J* 2008;51:120-130.
 - 21 **Genton B, Al-Yaman F, Mgone CS, Alexander NDE, Paniu MM, Alpers MP, Mokela D.** Ovalocytosis and cerebral malaria. *Nature* 1995;378:564-565.
 - 22 **Mgone CS, Koki G, Paniu MM, Kono J, Bhatia KK, Genton B, Alexander NDE, Alpers MP.** Occurrence of the erythrocyte band 3 (AE1) gene deletion in relation to malaria endemicity in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1996;90:228-231.
 - 23 **Allen SJ, O'Donnell A, Alexander NDE, Alpers MP, Peto TEA, Clegg JG, Weatherall DJ.** α -thalassaemia protects children against disease caused by other infections as well as malaria. *Proc Natl Acad Sci USA* 1997;94:14736-14741.
 - 24 **Fowkes FJI, Allen SJ, Allen A, Alpers MP, Weatherall DJ, Day KP.** Increased microerythrocyte count in homozygous α -thalassaemia contributes to protection against severe malarial anaemia. *PLoS Med* 2008;5(3):e56(0494-0501). doi:10.1371/journal.pmed.0050056.
 - 25 **Fowkes FJI, Day KP.** Alpha+-thalassaemia and malaria in Melanesia: epidemiological perspectives. *PNG Med J* 2008;51:131-137.
 - 26 **Lucas AO.** Helena Vrbova – an appreciation. *PNG Med J* 2008;51:95.

Helena Vrbova: a very special person

GERTA VRBOVA¹

Autonomic Neuroscience Institute, University College Medical School, University College London, England

In 1982, on the 9th of May, a dark cloud carried the sun away, for on that day you, my firstborn daughter Helena, died and left us for ever. I could visualize the beach house near Madang in Papua New Guinea, where you went to die. The unreal beauty of the spot could have been paradise, and yet when I heard that it was there that you died, it turned into the most sinister dark place on earth.

Taking a job in faraway Papua New Guinea was but one of the manifestations of your intense desire to help people. You went there to fight malaria, and stayed for 3 years. You coped with your loneliness and had the strength to offer medical care to villagers that had never been cared for, and worked hard for the people there. You achieved so much and I cannot understand the despair that drove you to opt out from life. I remember I felt a sense of unreality when I visited you in Madang. The beauty of the place, and all the alien plants, animals and smells made me feel that I was already on a different plane between being alive and dead; maybe this was already paradise and death not so far away. The loneliness, and isolation from everything familiar, must have engulfed you and in a moment of despair perhaps you forgot how much we loved you, and instead of coming back home to us, as you were supposed to, you departed, just before your 30th birthday, on that dreadful journey from where there is no return.

I keep wondering what I could have done to prevent your death. Maybe when you were old enough to understand, I should have explained to you how important you were for me and your father as well as the family. It was your birth and later your person that gave me the strength to live after the war. I was the only Holocaust survivor of our close family and your father Rudi one of the few people who escaped from Auschwitz to tell the

outside world about the horrors of the camp. Although Rudi and I were married and lived together in Prague, Czechoslovakia we were terribly lonely, and bruised by our experiences during the Holocaust. Perhaps at the time we did not even comprehend how 'damaged' we both were, and it is only with time that I can see how it must have affected us all. It was you and your birth that helped me to live and do something useful with my life.

Helena, your first few years of life

I remember feeling you growing inside me and the exquisite pleasure of knowing that a new life was being created. Just a few years before, Rudi and I were both destined to die in the Holocaust, and now together we had created a new life, that was you Helena. I have never felt as content, or at ease with myself and the world, as when we were together and shared the same body.

When it was time for you to leave me you did it so gently. One morning at the end of May 1952 you were complete and ready to leave me. I went to the maternity ward in Prague, on my own. Rudi had to go and pick up a fridge for our small flat, and I took the tram to go to hospital. I arrived at the hospital and announced to the porter that I had started labour. The labour pains were by then every five minutes and they were quite intense. The porter looked at me and said: "You don't look as though you are in labour. You are too slim to be nine months pregnant. In addition, when women come to deliver a baby they don't come alone, and they bring a suitcase and a cushion and not just a briefcase like you. I think you had better go home." I asked him to call Dr Brodski, who was my obstetrician and friend. Dr Brodski came to see me immediately, and admitted me to the labour ward. He examined me and said: "You were right, you are in labour, your cervix is dilated

¹ Autonomic Neuroscience Institute, Royal Free and University College Medical School, University College London, Royal Free Campus, Rowland Hill Street, London NW3 2PF, England, United Kingdom
ucgavev@ucl.ac.uk

and the baby should be delivered within the next 2 hours." Soon after this, Helena, you were born and it hardly hurt at all. A cheerful midwife wrapped you in a shawl and brought you to me. She smiled and said: "You have a beautiful baby." You had a lot of dark hair, a smooth dark face, a small nose and, when you opened your huge eyes and looked around, the whole world seemed different. To me you seemed to be the most exquisite human being on earth.

Rudi came to see us later. He gave me a hug and kiss, and then took you into his arms and said to me, "Gerti, if someone had told me in Auschwitz that in 10 years' time I will be a father to such an exquisite baby I would not have believed it. Yet here she is, our daughter." I asked Rudi: "What should we call her?" "Let's call her Helena after my mother, and because she certainly will be every bit as beautiful as Helen of Troy." Rudi was always concerned with beauty. He was much too interested in appearances. While I saw the inner beauty of your person in those huge baby eyes of yours, Rudi saw the physical beauty you would acquire when you grew into a woman. Still, you were named Helena, and the name suited you. A few days later we took you to our small apartment in Dejvice. At the time it was quite a luxury to have a two-room apartment, with a bathroom and a kitchenette. One room was used as a sitting-dining room, the other room was our bedroom. We had already bought a baby-cot and that was in the bedroom, squeezed under the window. We had a baby-bath for you, and we managed to obtain a plank of wood to put on the bath where we could change your nappies, and place the baby-bath when you had a bath. We were so proud of all these preparations we had made for your arrival. We had a big party and all our friends gathered to admire you.

You were a model baby, very considerate, allowing us to sleep during the night. Breastfeeding you was a special pleasure, for this act still connected our bodies so intimately. When you were about 6 weeks old we decided to take you into the country, and rented a small apartment in the Jizera mountains in a village called Albrechtice. A friend of Rudi's had a large car and agreed to drive us there. We had a roof-rack and were able to load all the things we thought you might need into the car. This took a very long time, and when we finished packing we started on our journey. Then after we turned

the corner of our street I suddenly shouted: "Stop, and turn back home." Rudi and his friend looked at me in amazement. "Whatever for?", Rudi said. My eyes were full of tears as I explained: "Can't you see, we left the baby in the flat." Indeed, Helena was not in the car. We turned round and dashed back home and there you were, on our bed quite content, sucking your thumb. After this our journey to Albrechtice was quite uneventful.

The little flat we rented was cozy, and there was a large garden we could use. There were lush meadows around us and hills covered with trees. Not far from our flat there was a dam and a large lake, where we could swim, while you Helena slept in your pram, or on a blanket we spread out for you in the shade of a tree. The air was clear and unpolluted, and the sun was so strong that when we washed your soiled nappies and spread them on the grass still wet they turned a brilliant white by the time they dried. I spent six wonderful weeks in Albrechtice with you, and was determined to return each summer, for I loved it there.

But we had to return home to Prague. I was convinced that you needed a lot of fresh air, so in addition to taking you for long walks in the parks of Prague, I put your cot under the open window in our flat. A lady from the flat opposite to ours spotted you, lying there contentedly under the window, and one day when I was walking home with you she stopped me and asked whether she could pick you up and give you a cuddle. The lady was in her early thirties, had blond hair and a gentle face. She picked you up with loving care and tenderness, and from that moment on, I saw that a love affair had developed between the two of you. After this first encounter she came often to our flat to play with you and we called her tetinka (auntie). Thus, Helena, you found yourself a nanny, for when the time came for me to go back to work tetinka was more than pleased to take care of you, and you too were happy to be looked after by her.

Helena, you were so delightful and gave us so much pleasure that we thought we would like another child. In spite of the fact that Rudi and I did not get on well at all, or may be because of it, I badly wanted a second child. Naively, I hoped that this would make us into a more united complete family, and that the difficulty between Rudi and me

would disappear with the arrival of a new baby. And so in May 1954, just before your second birthday, Helena, your little baby sister Zuzka was born in the same maternity ward as you. She too was a lovely little girl, but looked much more fragile than you. She had delicate white skin and fluffy blond hair. We got a small bed for you and Zuzka had your cot. You were so delighted and pleased to have a little sister, but then just before we were due to come home from hospital with the baby, you got ill and had whooping cough. You were not allowed to have contact with the baby and had to stay away from our flat in tetinka's house. We were very anxious and worried about our new baby, for whooping cough was a dangerous disease in the newborn, so the pleasure and happiness was marred by this. But you recovered well from your illness, and finally could spend time with your baby sister. You helped me to change her nappies, and to bath her in your old baby-bath.

In June we went again to Albrechtice; we took a friend of yours with us, little Olga. By this time, Heli, you were very talkative, and were making up stories that you were telling us, and Zuzka. You also knew a lot of songs,

and sang lullabies to Zuzka. This year you were old enough to come with us picking wild raspberries that grew in the forest. It was a very happy summer, but it was over too fast, and even before I could get used to having now two lovely daughters I had to go back to work. Tetinka now had two little girls to look after, but I think, Helena, that you were always closer to her heart than Zuzka. You loved your little sister and took good care of her (Figure 1). Also, I was very lucky that you could talk well, for you were always able to tell me how you spent the time while I was working.

Rudi had very strange ideas about parenting. He did love you, Helena, but somehow we all had to fit into his life. My relationship with him deteriorated, and we found it more and more difficult to live together. Divorcing Rudi was a hard decision: I knew that you needed both of us but our home environment had become so unpleasant that we could not be happy. When you were 4 years old we divorced, and you and Zuza saw your father every second weekend. I think he cared about you very much.



Figure 1. The family photograph shows Helena and Zuzka in Albrechtice, Czechoslovakia, in 1956.

One day, when you were 6 years old, Rudi came and brought all your toys you had in his house. He said that he was going to work in the Soviet Union for one year, and that he would not be seeing you for a while. This alarmed me, for I knew that it was not true and that he planned to emigrate to the West.

Leaving Czechoslovakia

Life was very hard in communist Czechoslovakia, and my opportunities would have been much better in a western country, so I too wanted to leave Czechoslovakia and move to England. I had an English boyfriend, Sidney Hilton, and we wanted to get married. At that time it was impossible for a Czechoslovak national to marry a western subject and leave. I tried hard to leave Czechoslovakia, but all my efforts failed. I could have left alone but not with you and Zuzka, and that was not an option. I knew that when Rudi was abroad, we would never be able to leave, and would be trapped in Czechoslovakia.

And then suddenly I thought there was a way out. I was to attend a meeting in Poland at the beginning of September 1958. I got my passport for my trip to Poland and the return train tickets from Prague to Warsaw. When I examined my passport I discovered that apart from the usual details about my date of birth, colour of eyes etc my passport had on the third page an exit visa the wording of which caught my eye. It stated that I could go to Poland and return back through any country in Europe. This meant that I could have a stop-over wherever I wanted and not return at all. But the passport was still in my name only and was not valid for my two daughters. Thus in spite of this promising wording of the exit visa the problem as to how to get my children out of Czechoslovakia was still unsolved. Still, some very exciting ideas about possible ways of leaving Prague and moving to England with my children were constantly occupying my mind, and in fact were preventing me from thinking my scientific thoughts about work. I kept very quiet about it all and did not talk to anyone about my schemes.

I went to the meeting in Poland on my own, and got Sidney to send me an air ticket for myself, you and Zuzka to travel from Warsaw via Copenhagen to Prague. Then I returned to Prague to fetch you and Zuzka and I walked with you and Zuzka across the mountains to

Poland. There I entered your names into my passport, got a Danish transit visa and took a flight to Copenhagen. Neither you nor Zuzka had travelled on a plane and hearing about flying to Copenhagen was wonderful news for both of you.

Denmark and early days in England

We landed in Copenhagen, and Sidney was waiting for us together with a Danish friend called Morten. There was an apartment prepared for us, and we stayed there until we recovered from our border crossing and all the stress. I got a job, and you Helena went to nursery school. You were longing for your elephant with the torn ear, but I could not find a substitute for your old elephant in any of the toyshops in Copenhagen.

However, in time your Danish became perfect, you made many friends, and had a very special boy friend called Toben. The two of you were inseparable. We could not get visas to go to England and so we stayed in Denmark for a year. During the summer holiday the nursery school took all the children, including you and Zuzka, to a summer camp by the Baltic Sea for 3 weeks. You always said it was the best holiday you ever had. In the autumn, just before you were due to start proper school, we finally got permission to go to England. Shortly after we arrived I married Sidney.

We lived in a tiny apartment in Golders Green and you started at an English school. You had to learn yet another language and adjust to a different environment. This was not easy, for while Denmark had brilliant childcare, England did not, and you had a difficult time at school. However, another move into a large house and starting a new school helped, for the new school had a very active swimming club. You were an excellent swimmer and therefore a real asset for the school. The head teacher soon spotted that apart from the swimming you were also an intelligent child with exceptional academic abilities, and he helped you to settle well in this new school. It was during this time that your sister Caroline and brother Peter were born. You were delighted with each baby and became the most caring and loving older sister, a great support for me. I had a full-time job, and having you, Helena, around when I was out at work was invaluable help. I had to have nannies who looked after the children when I was at work, and you were a

responsible person who could always tell me what was happening in my absence.

It was while we lived in London that your father Rudi also moved to London. He left Czechoslovakia almost the same week as we did, and stayed in Vienna after having attended a scientific meeting. Then he went to Israel and when he heard that we were in London he moved to London to be close to you and Zuza. It was, I think, important for you to get to know him. Still it was not easy for you and Zuza, for he had not changed, and again his interaction with you was irregular and depended entirely on what suited him and not what was best for you.

Your stepfather Sidney had very British ideas about children and their role in the family, and was becoming very disciplinarian. It was hard for you to accommodate these two fathers in your life, but you dealt with it diplomatically and very effectively, in contrast to Zuza, who fought both of them all the time. Sunday lunches were a nightmare, for we used to have some roast, and Zuza did not like meat and particularly hated fat, which she refused to eat. Sidney tried to insist that she should eat these bits, and there was much screaming, shouting and upheaval. You Helena tried to bring peace, but it was distressing for everyone.

Birmingham and growing up

Just as you were settled in London, in your early teens (Figure 2), my husband Sidney was offered and accepted a Chair of Physiology in Birmingham, and that meant that we had to move again, and you and Zuza had to make yet another adjustment. Somehow you managed this new move with remarkable ease. You passed the exam to attend a very good girls' school, and settled in Birmingham quickly. You had an active social life, and enjoyed your studies. You were very creative and artistic, and it was a difficult decision for you whether to opt for science or arts subjects, but gradually you decided that you wanted to study medicine, so as to be able to help people. Little changed in our home life, and you were getting independent, and tried to organize your own life. It was while we lived in Birmingham that we acquired a small summer house by the sea in Wales, and I think it was there that you felt happiest and developed a fascination and love of the sea. You were a young person in the swinging 60s and that must have influenced you, for you were trying to be a liberated person. Liberated from what was difficult for me to understand, but you were a determined feminist, pacifist and all those things that were typical of that generation. Although you thought you were strong, you



Figure 2. Helena aged 14 and Zuza 12 in Birmingham in 1966.

were vulnerable and deep down a romantic who needed more love and care than you admitted to yourself. You passed your exams (then O-levels) and A-levels with no difficulties, and decided to go and study medicine in Edinburgh. You probably chose Edinburgh because it was far away and you could distance yourself from our turbulent family life.

Medical studies and career

It was in Edinburgh that you started your proper 'growing up' process. You had your first serious boyfriend, with whom you built a boat. But I think you missed the family, especially your sisters and brother, and felt quite lonely during those 5 years of medical studies. After you qualified you spent some time in Poole, Dorset, where you were a house officer. Your interests started to gravitate towards research and you took up a research position in the Biochemistry Department at Sussex University, where you studied diabetes and completed your MD thesis. As a result of this interest in diabetes you got involved in research on the transplantation of islets of Langerhans to use as a replacement therapy for diabetes. This was one of the first attempts to introduce such methods into medicine and although you were there for only a short time you had some publications (1,2).

Some of this work was carried out at the Charing Cross Hospital Medical School, and to our great delight you moved to London close to our family home. However, your stay here was short-lived for you became interested in tropical diseases and particularly malaria so that when you had an opportunity to do work in this field you decided to travel very far away from us, to Papua New Guinea (Figure 3). After 3 years of hard work and completion of several successful research projects you were supposed to return to London to take up a Medical Research Council scholarship awarded to you to further your studies in tropical medicine. Just a few days before you were due to leave you died, and your premature death is a tremendous loss not only to all of us who loved you, but also to humankind, for you had the potential to make further outstanding contributions to help to improve life.

REFERENCES

- 1 **Vrbova H, Theodorou NA, Tyhurst M, Howell SL.** Transplantation of islets of Langerhans from pilocarpine-pretreated rats: a method of enhancing islet yield. *Transplantation* 1979;28:433-435.
- 2 **Theodorou NA, Vrbova H, Tyhurst M, Howell SL.** Problems in the use of polycarbonate diffusion chambers for syngeneic pancreatic islet transplantation in rats. *Diabetologia* 1980;18:313-317.



Figure 3. Helena in Papua New Guinea aged 28.

Helena Vrbova – a personal tribute

DAMIEN J. JOLLEY¹

School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia

A performance of *Dimboola* at the Madang Club was the incongruous setting of my last conversation with Heli. She was a rare visitor to that venue, which she saw as a bastion of colonial racism; but MATS, the local Madang Amateur Theatrical Society, exploited the Club's atmosphere of decaying Australian culture, and its country-pub style kitchen, for its 1982 production of Hibberd's classic play.

Many of Heli's colleagues from the Papua New Guinea Institute of Medical Research (PNGIMR) were involved: Stephen Oppenheimer played the paedophile priest to perfection, James Moir was a most believable bridegroom, Graham Wood and Vicky Hoogland were parents of the bride, and I was nominally the 'director', although I use the term loosely. Heli sat politely through the three acts and two courses, and offered some kind words of encouragement to the cast and crew at the after-party. Within twenty-four hours, we heard the tragic news of Heli's death.

In the intervening 25 years, I have often wondered how she could have endured such trivia at that time, given the torment which we now know she was suffering.

I arrived in Madang on a Sunday in January 1980, and met Helena Vrbova for the first time at a planning meeting for a four-day patrol through the mountains to the south – the foothills of the Bismarck Range. The following day we set off with our census forms, pencils and clipboards, rice and tinned fish in backpacks and stout walking boots. By the fourth day I was near-catatonic with fatigue, culturally overwhelmed and pathetically relieved finally to take off my boots at my new home with Tim Spencer at Yagaum. Heli bounced past, laughing, with her raven curls gleaming in the sun, as though she had just returned from a Sunday stroll. I knew then that my time in PNG with the IMR would be

very special.

Soon after my gruelling initiation to Yagaum, I realized that its isolation some 10 km from the town of Madang would necessitate some cheap means of transport. Tony Ades was leaving PNG and kindly sold me (for next to nothing) his Honda CT125 motorbike, which was identical to Helena's. I eagerly took delivery one Saturday lunchtime and set off to the beach. I made it as far as the hill just outside the compound gates (see the dirt road at the top right of Figure 1), where I managed to fall off and break my wrist: it was the scaphoid! – twelve weeks in a cast was a prospect I dreaded, but particularly so given that several of us, including Heli, were booked for a scuba (self-contained underwater breathing apparatus) diving course in the next couple of weeks.

One advantage of working in an isolated environment with dedicated young physicians is that their skills and enthusiasm can overcome misfortune. Within hours of my X-ray, Heli and Andrew Climie between them constructed a framework of aluminium rods, old inner tubes and fibreglass around my left forearm, holding it firm ('like you're grasping a tinnie' – the spirit of Australian functional, creative orthopaedics) and, most importantly, waterproof. Thenceforth, the locals knew me as 'han bruk', a name which I secretly enjoyed.

Heli and I, with Steve, James, Tim, Graham Knowles and Derek Charwood, went on to complete our scuba course that month at the dive centre north of the township. Bill, the ex-US Marine instructor, was dubious about my safety with one arm in a cast, but happily he acquiesced and I gained my certificate.

We spent many happy days diving around the glorious coral reefs which bless Madang and its neighbouring islands and inlets. Heli

¹ Monash Institute of Health Services Research, School of Public Health and Preventive Medicine, Monash University, 43-51 Kanooka Grove, Clayton, Victoria 3168, Australia
damien.jolley@med.monash.edu.au



Figure 1. Satellite view of the Yagaum hospital compound which houses PNGIMR Madang. The 'haus meri', where Helena lived with Ali and Naomi, is circled. The main office building is the first above the road in the complex of buildings on the right.

© Google – Imagery © DigitalGlobe, GeoEye, Map data ©2009

often expressed her enthusiasm for this most relaxing of sports, and how fortunate we were to be living literally at the world's epicentre of tropical coral.

My motorcycle provided another opportunity for me to enjoy Helena's company. Together, in 1981, we rode our twin trail bikes from Madang on the north coast to Goroka in the highlands, for the inaugural PNGIMR colloquium. We travelled first southwards through the Bismarck foothills until we met the mighty Ramu River, and tracked its vast floodplain southeast to the Highlands Highway. This led us initially south, and sharply upwards through the Kassam Pass into the Eastern Highlands Province, and westwards through Kainantu, Henganofi and finally to Goroka. Many have done this trip, but few were as unprepared as Heli and I, wearing our skimpy coastal clothing, which was no protection from the cold and rain which welcomed us to the highlands. Our teeth chattered, our fingers froze, but eventually we rolled into Goroka and a warm IMR welcome from our highlands hosts. As cold as it was, I valued the experience of riding a road that had been surveyed by my brother two decades earlier, and also Helena's company, which was, as ever, uplifting.

Working with Helena was just as much fun as diving or biking with her. She was fanatically dedicated to her research work as

a tropical epidemiologist, and her enthusiasm was as infectious as the diseases we tracked. She made every effort to support the local research staff, women especially, and managed to draw out exceptional efforts from us all.

It was with Heli's encouragement, Peter Heywood's advice and Michael Alpers' patience and bankrolling, that we installed one of PNG's earliest minicomputers at the Yagaum outpost in 1981. Helena led the epidemiology group, which in turn depended on the demographers, to document the population denominators for the malaria surveys conducted by the Institute. Bravely, we decided to computerize the record-keeping and census data. A DEC PDP-11/05 computer – the digital equivalent of a Ford Model T, and about the size of a large filing cabinet – arrived by sea freight from Boston, and, miraculously, worked from the start. Since we could not trust the variable PNG power supply, a noisy power generator was installed some 10 metres from the office (you can still see the generator shed in Figure 1). We poured diesel in one end, and obtained BMDP statistical output from the other!

In today's world of laptops and the internet, such antediluvian machinery seems ludicrous and quaint. But, at the time, we were quite chuffed to have a real computer working in the jungles of PNG. A little research reveals

the puny scale of this machine by today's standards: there was only 64 kb of memory, and the 5 Mb removable hard drive discs were 40 cm in diameter, encased in heavy plastic cartridges. At first, only one operator at a time could use the computer, but later software enhancements saw us expand to four or five terminals simultaneously. David Kotale and Marcus Apo were keen learners, and I trust they continue to use their skills.

A lasting memory of Helena in Madang is our climb of Karkar volcano, in late 1980. The island of Karkar is visible as a perfect cone about 30 km directly north from Madang in the Bismarck Sea. At that time the PNGIMR maintained a house on the island, and a group of us, including visitors from Australia, ventured the four-hour ferryride from Madang. The climb from coast to caldera was arduous, with eventually only Helena and I completing the task, to be rewarded with a sulphurous but spectacular view from the crater's rim. Only 18 months had passed since two vulcanologists had died in an eruption on Karkar, so we were cautious, to say the least.

Our fears were not unfounded. During a later Institute expedition to the volcano, a sudden torrential downpour turned the ash beds into treacherous rapids and several walkers were swept down the mountain. Geraldine White, a promising young occupational therapist, an Australian volunteer from Geelong, was killed. Dave Skelton, a local forester, was lucky to escape with his life.

The spirit of Karkar had smiled on Heli and me. I was, and remain, fortunate to have known this remarkable woman and to have shared with her some exciting adventures. While the physical cause of her death may be known (though it was not formally established at inquest), the underlying pathology remains a mystery. I know that we all regret any squandered opportunities for helping her that we may have had; and, in retrospect, I am embarrassed by the banal circumstances of our last encounter.

But I owe my professional life to Heli and the Institute, and I am proud to record this public recognition of my debt.

BIBLIOGRAPHY

My joint publications with Helena Vrbova

- 1 **Vrbova H, Stace J, Climie A, Gibson FD, Heywood PF, Jolley DJ, Pariva S, Alpers MP.** The effect of chloroquine-resistant *Plasmodium falciparum* on chemoprophylaxis with amodiaquine. Abstract No 425 in Program and Abstracts of the Tenth International Congress on Tropical Medicine and Malaria, Manila, 9-15 Nov 1980.
- 2 **Darlow BA, Gibney S, Vrbova H, Jolley DJ, Stace J, Alpers MP.** Treatment of malaria with Fansidar. Abstract in Program and Abstracts of the Seventeenth Annual Symposium of the Medical Society of Papua New Guinea, Rabaul, 25-26 Sep 1981:43.
- 3 **Vrbova H, Gibney S, Gibson FD, Jolley DJ, Heywood PF, Stace J.** A trial of amodiaquine (10mg/kg) and Maloprim as chemoprophylactic agents in the Madang District. Abstract in Program and Abstracts of the Seventeenth Annual Symposium of the Medical Society of Papua New Guinea, Rabaul, 25-26 Sep 1981:45.
- 4 **Darlow BA, Vrbova H, Gibney S, Jolley DJ, Stace J, Alpers MP.** Sulphadoxine-pyrimethamine for the treatment of acute malaria in children in Papua New Guinea. I. *Plasmodium falciparum*. *Am J Trop Med Hyg* 1982;31:1-9.
- 5 **Darlow BA, Vrbova H, Gibney S, Jolley DJ, Stace J, Alpers MP.** Sulphadoxine-pyrimethamine for the treatment of acute malaria in children in Papua New Guinea. II. *Plasmodium vivax*. *Am J Trop Med Hyg* 1982;31:10-13.
- 6 **Vrbova H, Gibney S, Tiromry K, Jolley DJ, Cattani JA, Stace J, Alpers MP.** A longitudinal study of malaria in Madang Province. Abstract in Abstracts and Proceedings of the Eighteenth Annual Symposium of the Medical Society of Papua New Guinea, Port Moresby, 10-11 Sep 1982:55.
- 7 **Tulloch JL, Vrbova H, Jolley DJ, Cattani JA.** Malaria in a coastal area of Madang Province: preliminary results of an epidemiological study. Abstract in Abstracts and Proceedings of the Eighteenth Annual Symposium of the Medical Society of Papua New Guinea, Port Moresby, 10-11 Sep 1982:59,145.
- 8 **Cattani JA, Vrbova H, Tulloch JL, Jolley DJ.** Inter-cluster variation in malariometric rates in a coastal area of Papua New Guinea. In: Bryan JH, Moodie PM, eds. Malaria. Proceedings of a Conference to Honour Robert H. Black, Sydney, 10-12 Feb 1983. Canberra: Australian Government Publishing Service, 1984:112-118.
- 9 **Alpers MP, Jolley DJ, Vrbova H, Stace J, Gibney S, Tiromry K, Cattani JA.** A longitudinal study of malaria in a village population near Madang, Papua New Guinea. In: Bryan JH, Moodie PM, eds. Malaria. Proceedings of a Conference to Honour Robert H. Black, Sydney, 10-12 Feb 1983. Canberra: Australian Government Publishing Service, 1984:119-126.
- 10 **Moir JS, Tulloch JL, Vrbova H, Jolley DJ, Heywood PF, Alpers MP.** The role of voluntary

- village aides in the control of malaria by presumptive treatment of fever. 1. Selection, training and practice. *PNG Med J* 1985;28:257–266.
- 11 **Moir JS, Tulloch JL, Vrbova H, Jolley DJ, Heywood PF, Alpers MP.** The role of voluntary village aides in the control of malaria by presumptive treatment of fever. 2. Impact on village health. *PNG Med J* 1985;28:267–278.
- 12 **Cattani JA, Tulloch JL, Vrbova H, Jolley DJ, Gibson FD, Moir JS, Heywood PF, Alpers MP, Stevenson A, Clancy RL.** The epidemiology of malaria in a population surrounding Madang, Papua New Guinea. *Am J Trop Med Hyg* 1986;35:3–15.
- 13 **Vrbova H, Gibney S, Gibson FD, Jolley DJ, Heywood PF, Stace J, Trenholme KR, Alpers MP.** Chemoprophylaxis against malaria in Papua New Guinea: a trial of amodiaquine and a combination of dapsone and pyrimethamine. *PNG Med J* 1992;35:275–284.

Helena Vrbova, malaria epidemiologist

BRIAN A. DARLOW¹

Christchurch School of Medicine, University of Otago, Christchurch, New Zealand

Prue Stringer and I arrived in Papua New Guinea (PNG), intending to holiday, sometime in March 1980. I had been a paediatric registrar in both the United Kingdom and New Zealand and this fact may have helped in the offer of a job at the Papua New Guinea Institute of Medical Research (PNGIMR) in Madang to undertake a research project on the place of Fansidar (sulphadoxine-pyrimethamine) in acute malaria in children, which was funded by the World Health Organization and the World Bank. The next 9 months of the project (April 1980 to February 1981) would be one of the most interesting and fulfilling times of our lives. Heli helped me quickly get up to speed on all aspects of malaria. I worked quite closely with her on the project, recruiting children with acute malaria in the outpatient clinic at Madang Hospital, treating them either with Fansidar alone or in combination with chloroquine, in hospital at first and then following them up for a month. Because of logistical problems such follow-up had rarely been done before in PNG. Heli helped us organize the transport of the children and their families home so that we could find them again. We soon learnt that 'longwe liklik' could be anything from one to fifty miles away!

The work was completed on time and Heli's drive was a major factor in this. We showed that whilst Fansidar did treat acute falciparum malaria in childhood in most cases, there was already some resistance to the drug. In addition, Fansidar was less satisfactory than chloroquine as treatment for vivax malaria in children because fever resolution and clearance of parasitaemia were significantly slower with the former. Heli and I were co-authors on 6 publications as a result of this work (1-6), which helped to determine future treatment policies and led to further studies in vitro (7).

Prue was busy teaching at the International

School for part of the time and acted as the chauffeur to transport children in my study to their villages or settlements. As well as a full schedule in the hospital, doing general outpatient paediatrics, preparing and reading blood films to diagnose malaria and following the families at home, we were able to experience many of the natural and social delights of PNG, often in company with Heli (Figure 1), with whom we particularly enjoyed celebrations out in the villages (Figure 2). Snorkeling and scuba diving were second to none. Prue undertook a trip up to the Karkar volcano rim with Heli and Damien Jolley and we climbed Mt Wilhelm. Through the IMR wantok system we were able to visit and stay in several highland communities, the highlight probably being Tari and Lake Kutubu in the Southern Highlands.

It was always a privilege and enjoyable to work with Heli, who became a good friend and one we kept in touch with. Prue and I have been grateful for the chance to contribute to this special memorial to Heli in the *Papua New Guinea Medical Journal*, which has brought back many wonderful memories but also sadness for a life cut so tragically short.

REFERENCES

- 1 **Darlow BA, Vrbova H, Stace J, Heywood PF, Alpers MP.** Fansidar-resistant falciparum malaria in Papua New Guinea. *Lancet* 1980;2:1243.
- 2 **Darlow BA, Vrbova H, Stace J, Heywood PF, Alpers MP.** Drug-resistant strains of *Plasmodium falciparum* in Papua New Guinea. *Lancet* 1981;1:386.
- 3 **Darlow BA, Vrbova H, Stace J.** Acute malaria in children in Madang: endemicity, clinical presentation and treatment. *PNG Med J* 1981;24:85-95.
- 4 **Darlow BA, Vrbova H.** Chloroquine-resistant *Plasmodium falciparum* malaria in Madang children. *PNG Med J* 1981;24:96-98.
- 5 **Darlow BA, Vrbova H, Gibney S, Jolley DJ, Stace J, Alpers MP.** Sulfadoxine-pyrimethamine for the treatment of acute malaria in children in Papua New

¹ Department of Paediatrics, Christchurch School of Medicine, University of Otago, PO Box 4345, Christchurch, New Zealand
brian.darlow@chmeds.ac.nz

- Guinea. I. *Plasmodium falciparum*. *Am J Trop Med Hyg* 1982;31:1-9.
- 6 **Darlow BA, Vrbova H, Gibney S, Jolley DJ, Stace J, Alpers MP.** Sulfadoxine-pyrimethamine for the treatment of acute malaria in children in Papua New Guinea. II. *Plasmodium vivax*. *Am J Trop Med Hyg* 1982;31:10-13.
- 7 **Lamont G, Darlow BA.** Comparison of in vitro pyrimethamine assays and in vivo response to sulfadoxine-pyrimethamine in *Plasmodium falciparum* from Papua New Guinea. *Trans R Soc Trop Med Hyg* 1982;76:797-799.



Figure 1. Helena Vrbova in a relaxed mood in rural Madang in 1980.



Figure 2. Helena and I enjoyed the celebrations held in the village communities where we were working. Food distribution after a 'mumu' (food cooked over hot stones in an earth oven) in Gonoa, 1980.

Helena Vrbova: a reminiscence

STEPHEN J. OPPENHEIMER¹

School of Anthropology, Oxford University, United Kingdom

I worked as a paediatrician and researcher overall for five years in Papua New Guinea, the first two as a government paediatric specialist in Goroka and Wewak and the last three on secondment to the Papua New Guinea Institute of Medical Research (PNGIMR) from the Department of Tropical Paediatrics of the Liverpool School of Tropical Medicine. I was associated with the PNGIMR through Michael Alpers as Director in Goroka and Peter Heywood's tolerant support as head of the new Madang branch of the Institute. Two and a half of those last three years overlapped with Heli's time in Madang.

I had already heard of Heli before I returned to Papua New Guinea to work in Madang in 1979. Dick Ashford at the Liverpool School of Tropical Medicine, who also worked at the University of Papua New Guinea, told me about Peter Heywood and Helena Vrbova who, with others such as Health Department paediatric collaborator John Stace, formed the scientific nucleus of a new embryo unit that had been created by the PNGIMR in Madang. Dick, an experienced parasitologist himself and sometimes critical in his views, was deeply impressed with Heli's resourcefulness, intelligence and energy of application in, for her, a new discipline and challenging environment. He described her in his staccato way as "...cheerfully dashing about doing great things and setting up malaria cultures in such a short time...a great girl". He also, incidentally, had a high opinion of Peter as the head of the unit.

There was a lot of preparatory work to do before large-scale laboratory studies and fieldwork could begin. There had been a research presence in Madang long before, still represented in part by a small air-conditioned lab next to the Provincial Health Office, now visibly being eaten from inside by termites. I remember, while working there, a continuous low scratching, grinding sound punctuated occasionally by a door falling off its hinges. Although this lab continued to be

used intermittently by the IMR throughout my time there, and the established and running Camoquin study had a building in the centre of Madang, an early decision had been made to shift the centre of gravity of IMR activities to a set of empty buildings in the inland mission health centre of Yagaum (previously a well-known Lutheran hospital), including a large two-storey building that became the offices and lab.

When I arrived in November 1979, two of the available Yagaum living quarters had been cleaned and occupied: Peter Heywood's house, with its grand verandah and stunning view towards the coast, and the one next door, which was occupied by Heli and Tony Ades. They kindly invited me to stay for a short time while the house just above was cleaned and equipped as a single-male quarters. Later, Naomi Yupae and Alison Orr-Ewing joined Heli in her house, which subsequently became the Yagaum 'haus meri', with Heli, Naomi and Ali as the long-term residents.

Those first few days getting over jet-lag, cleaning rooms and settling in, imprinted in my mind the image of Heli as a cheerful, extremely intelligent, self-reliant supergirl. I cannot remember who cooked, probably all of us, and we usually dined al fresco. We all talked a lot. Heli's taste in music I remember, such as Joan Armatrading records; and books – an eclectic but very left-wing choice, such as Wilhelm Reich's dialectical materialist take on Trobriand Islands culture as described by Malinowski. The weather was very wet and the bright sun made the overgrown banks behind the house luminous green. The second day after my arrival, a snake made its way into a toilet cistern causing great commotion and indecision. At night fruit bats dropped mango stones loudly on the tin roof. We regularly drove up the north coast late afternoons to nearby lagoons to snorkel.

Heli and Tony both bought Honda 125CT four-stroke motorbikes, which they used for

¹ School of Anthropology, Oxford University, Oxford, United Kingdom
stephen.oppenheimer@ntlworld.com

preference to go into Madang town. Damien Jolley, who arrived next in January 1980, purchased Tony's from him when he left to return home. The motorbike was typical of Heli's tomboyish, independent behaviour; she was always in shorts and never seemed to wear a skirt; yet this strong persona failed to deter a string of fascinated admirers. I soon came to know Heli more as a sister, sometimes critical, but never socially exclusive.

Heli was always up for adventures and expeditions. I remember in 1980 we both attended a tropical conference in Manila; and afterwards we took planes, buses and boats to the islands down south, where we found great scuba diving. Back in Madang, dive and snorkeling trips up the north coast were a regular feature. Several times we went as far as Hansa Bay, where we were once invited to an evening village 'singsing' near the beach. Damien will, I am sure, describe his epic journey with Heli on the two bikes from Madang up the Ramu Valley across the forty fords, eventually to arrive in Goroka for a PNGIMR colloquium (1). For that meeting I drove my Toyota short wheel base (SWB) and had to overnight in Lae due to some road-blocking natural disaster, and thus managed to arrive late.

Heli and I never formally collaborated on any study, which is indeed one reason I have written a personal reminiscence, rather than a scientific appreciation. However, she was always a source of wisdom, insight and constructive critique. In my first couple of months I rewrote the protocol for my own study. I remember Heli and Peter Heywood as very helpful and supportive in this process (as they were for all things), over several meetings (2). They also generously loaned me the use of a vehicle (and a range of other facilities) for my pilot surveys, pending the purchase of my project Suzuki and my own ex-forestry SWB 4x4 wreck. Likewise, Heli, Peter and Michael Alpers did me the honour

of inviting me to their research planning meetings.

As more staff arrived and filled up the Yagaum quarters in 1980, I took advantage of the relative independence of my grant to rent my own accommodation. I moved first to the beautiful Nagada Lagoon and later to Madang town. My work necessitated a hospital base, and space was kindly allocated for me there by the Provincial Health Officer and hospital superintendent. After the move, my daily contact with the Yagaum 'campus', where Heli was based, ceased, and indeed my acquaintances and interests moved away from expatriates and more towards Papua New Guineans.

So, while the expeditions and dives continued, I saw rather less of Heli over 1981-1982. I have one clear last memory of her, prior to that last fateful weekend. I bumped into her at a large Yagaum party in early 1982. Talking to Heli then, I was surprised to find a different person, one who was in love and emotionally dependent. Gone was much of the independence of opinion and spirit, to be replaced by a personality perhaps more vulnerable and less sure, even deferring to another's opinion. However, I believe she experienced her greatest happiness in that time, although at unacceptable cost to herself, her family and friends and the scientific world.

REFERENCES

- 1 **Jolley DJ.** Helena Vrbova – a personal tribute. *PNG Med J* 2008;51:86-89.
- 2 **Oppenheimer SJ, Hendrickse RG, MacFarlane SBJ, Moody JB, Harrison C, Alpers MP, Heywood PF, Vrbova H.** Iron and infection in infancy – report on field studies in Papua New Guinea. II. Protocol and description of study cohort. *Ann Trop Paediatr* 1984;4:145-153.

Dr Helena Vrbova – a pioneer in malaria research

JOHN STACE¹

Shenton Park, Western Australia

Dr Helena Vrbova was the pioneer staff member of the Papua New Guinea Institute of Medical Research (PNGIMR) malaria research team based in the old Yagaum Lutheran Hospital, 15 km outside Madang.

I first met Helena as she was being driven on a muddy road from Madang Airport to Yagaum. She was self-assured, had a lovely smile and was stylishly dressed. She had just flown in from the UK, where she had been working in a medical research laboratory. I imagine that she had some culture shock, like most expatriates on arrival in PNG.

In the months ahead, Helena demonstrated her versatility in adapting to the hot humid climate, the relatively primitive conditions and the challenges of starting new projects. She had a sharp mind, a clarity of expression and a willingness to have fun. She worked closely with her PNGIMR colleagues and co-authored several significant papers on malaria.

All the staff at PNGIMR and all her other friends and associates were shocked to learn that Helena had chosen to end her life prematurely. Her death was felt very keenly, in particular by her friends, who would have done anything to help her if they had known of her anguish.

Helena's death was a reminder that people separated from their usual family and friends can experience profound mood swings, which may end in tragedy.

Malaria research continues, in PNG and elsewhere, and we hope that one day it will result in an effective vaccine that will bring health to millions who at present bear the full brunt of the disease. Many researchers will have contributed to that success – and one of them is Dr Helena Vrbova. As we mourn her death, even after the passage of more than 25 years, we remember her vitality and celebrate her life and work.

¹ 159 Derby Road, Shenton Park, Western Australia 6008, Australia
Formerly Specialist in Child Health, Madang General Hospital, Madang, Papua New Guinea
john.stace@westnet.com.au

Helena Vrbova – an appreciation

ADETOKUNBO O. LUCAS¹

Special Programme of Research and Training in Tropical Diseases, World Health Organization, Geneva, Switzerland

It is difficult to imagine that a quarter of a century has passed since Helena Vrbova died. It is even more remarkable that someone whom I met briefly on two occasions has left such an indelible memory. On the first occasion, Helena presented a paper at a scientific meeting in Papua New Guinea. I clearly remember the glowing terms in which I commented on her presentation of a study on malaria. As the Director of the WHO Tropical Diseases Research Programme, I was pleased that the Programme gave some support to the research team that Helena represented and I looked forward to many more research results following up on the results that she presented at the conference. The next

occasion that I met her was at the International Congress of Tropical Medicine and Malaria in the Philippines. There was a brief conversation. I obtained follow-up information about her work; it reassured me that she was on course to making many more contributions to our knowledge of the epidemiology of malaria. Alas, it was the last occasion that we met. I continue to treasure her memory as someone who used her talent in helping to relieve the suffering of poor people in developing countries. She will remain a credible role model for other researchers who plan to use science as a tool for health development in the developing world.

¹ Former Director, World Health Organization Special Programme of Research and Training in Tropical Diseases, Geneva, Switzerland
Adjunct Professor, Harvard School of Public Health, 677 Huntington Avenue, Boston, MA 02115, USA
Present address: 25 Adebajo Street, Kongi, PO Box 30917 Sec. BO, Ibadan, Nigeria
adelucas@aol.com

Personal observations on the characteristics of scientists

JOHN TAIME¹

Papua New Guinea Institute of Medical Research, Madang

From my non-science background I feel honoured to contribute a brief paper on the characteristics of scientists I have known in Papua New Guinea (PNG). From the studies they have undertaken in my country these medical scientists have developed new information and knowledge that has been useful for improving health in PNG and throughout the tropical world. As a representative of the staff of the Papua New Guinea Institute of Medical Research (PNGIMR) in Yagaum, I am pleased to add my tribute to the memorial for Dr Helena Vrbova in the *Papua New Guinea Medical Journal* and thank Professor Michael Alpers, the guest editor, and Professor Peter Siba, the Director of the Institute and Chief Editor of the Journal, for giving me this opportunity.

Medical scientific researchers conduct research under significant pressure to find new and better ways of combating major global public health problems. It is the poorer developing countries that face the most severe consequences of poor health and widespread, multiple diseases. To study diseases in these settings young doctors and scientists leave their own society, comfortable homes, families and friends to travel out to faraway countries where the culture and the political, economic and social lives of the people are totally different.

It requires courage and determination to face these difficulties and the uncertainty about whether the outcome of the proposed research will be successful or not. In carrying out their research scientists expend great effort, both of physical work and hard concentration. In addition, visiting research workers have to put aside their background and the society they come from and adapt to a new community within the nation that has sponsored their studies. Though they face difficulties and challenges in this adaptation because of cultural differences, the rough

terrain and the remoteness of the area where they are working, they confront these challenges and do not give up until their research aims and scientific mission have been accomplished. I have been impressed by their commitment and dedication. To complete a research study requires long hours working around the clock, both the sweat and hard work getting out to communities and the tireless effort of collecting and checking the data. Only then can the data be analyzed and the results provided to the national Department of Health and published in the scientific literature.

Scientists conduct their complex research activities knowing that others are doing similar studies in other parts of the world. If they have successfully discovered something new with their research they are naturally excited and their findings are made known quickly to other researchers around the world. However, if they fail, this becomes a stepping-stone for them to learn from their mistakes and plan improved research studies, with renewed drive and determination – to try out new methodologies, or switch to other more soluble research problems, until success is achieved.

Often the outcome of a research study is determined by cultural, social, political or environmental factors. This is particularly relevant in Papua New Guinea, which is a small island country with more than 800 languages and cultures. It took years for PNGIMR researchers to convince their communities about the need for and the importance of medical research. This was achieved through different forms of awareness, at community meetings, during church services, through radio programs, with the help of other sectors operating in the area, and by engaging different community groups such as schools and youth groups. In recent years, because of thorough awareness

¹ Vector-borne Disease Research Unit – Administration Manager, Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea
general@pngimr.org.pg

programs in different communities, the people have better knowledge of the importance of the research being conducted in PNG, of the various aspects of the work of PNGIMR and of the essential role that the communities themselves play when supporting or participating in these studies.

It is pleasing to report that many of the research projects of the PNGIMR have achieved their desired goals and objectives and have been very successful. This has been good for the people of PNG and has also given a good impression of PNGIMR to stakeholders such as the PNG Government, the Australian Government and funding organizations like the Bill and Melinda Gates Foundation. If such organizations acknowledge the good work that PNGIMR is doing the chances are increased of continued financial support for PNGIMR's research programs on the major disease problems affecting Papua New Guineans. Since PNG, like many other developing countries, faces a lot of health problems it has a strong need for continued research on these diseases. This funding support, dependent on the good reputation of PNGIMR, is therefore important in the long term for the health and well-being of the people of Papua New Guinea.

One recent example of the benefit of medical research is the antimalarial drug change which was approved by the national Department of Health after receiving a recommendation from researchers and clinicians. For first-line treatment of malaria chloroquine alone was changed to a combination of chloroquine and Fansidar. This was based on the work of PNGIMR showing increasing levels of chloroquine resistance in the malaria parasites circulating in PNG. Now that there is a greater demand world-wide for evidence to inform public health and clinical medicine, more attention is being paid to research workers and their findings. In the medical field now and in the future we can expect that the results of research work will be taken into consideration

and implemented through National Health Strategic Plans.

Scientists' minds are strong and sharp but their hearts are soft and humble. In researching means and ways of discovering and developing new health information, their primary motive is to serve and to give, rather than receive any personal benefit from their research, beyond the satisfaction of using their skills, doing their job well and passing on these skills and attitudes to others. At least, that is the overwhelming impression I have of the scientists I have worked with in Papua New Guinea. It is reassuring that the knowledge, drive, commitment and attention to detail of our overseas colleagues have been passed on, and continue to be passed on, to many young Papua New Guinean scientists at the PNGIMR and, in consequence, a bright group of national researchers and scientific mentors is appearing.

In conclusion, I am conscious of how much we owe to the many overseas scientists who have worked here with us. Everything I have learned about Dr Helena Vrbova makes it clear that she was an early and prime example of commitment, in the most selfless manner, to scientific research and to the people who might benefit from it. On behalf of the Papua New Guinea Institute of Medical Research, the Health Department, the staff of PNGIMR in Yagaum, and the communities of Gonoa and other villages in Madang Province, I share with Helena Vrbova's family, scientific colleagues, friends and loved ones our continued heartfelt sorrow for her untimely death. We honour her memory and pay tribute to Helena as a young, dedicated and hardworking scientist.

Helena, you and the good work you started with IMR at Yagaum in Madang are celebrated here and will continue to be remembered in the years to come. May God bless you and keep your soul at peace in your resting place.

Tribute from Gonoa village, sent to her family at the time of Helena's death

GONOA VILLAGE¹ AND JAMES S. MOIR^{1,2}

Papua New Guinea Institute of Medical Research, Madang

Editorial note:

An important part of Helena's work on malaria was a longitudinal study in Gonoa village. After she died a Memorial Clinic was established in Gonoa by her family and friends. Her work was continued by Dr James Moir and other colleagues. This condolence message from the people of Gonoa was transmitted soon after her death by James Moir to Helena's mother.

I write to you to give you and the family a message from the people of Gonoa. I went to Gonoa yesterday with two other members of the staff from Yagaum, and before we started work we had a meeting with the village people, at their request, to talk about the future of the project at Gonoa, which Heli had supervised since shortly after her arrival here in Papua New Guinea. When we arrived yesterday morning we gathered together in an open house, or 'haus win', in the centre of the village. One of the village leaders spoke to us, saying that the people at Gonoa were very upset when they heard about Heli's death, and they wanted to know whether we would continue to work there now that Heli was gone. I talked to them for a few minutes, as spokesman for the Institute of Medical Research, and told them that Heli's work at Gonoa was an essential part of our work in researching malaria, and that our involvement with the village would continue as before. After some discussion, one of the men stood up and asked me to convey a message from the people to you and the family. We were also given a small 'bilum' (netbag) with a two kina note inside, to send to you as a token in remembrance of Helena. The message they gave went as follows.

Mipela sori tru long Helena, na sori na wari tru long famili bilong em. Em i kam planti taim long mipela, na em i hat wok long lukautim mipela na bosim dispela wok.

Pastaim tru mipela stap nating, na mipela hat wok long painim marasin na lukautim pikinini bilong mipela, na mipela bagarap. Tasol dispela misis ikam insait, na kirapim gutpela wok long aid post, na ol pikinini bilong mipela i kamap gutpela. Olsem mipela hamamas tru long dispela wok bilong em, na yumi no ken lusim. Nau em i dai pinis na mipela laik givim dispela liklik bilum bilong salim igo long famili bilong em, na ol iken lukim, na tingim em, na tingim mipela. Sori tru.

This may be translated as follows.

We are very sad about Helena, and we are sorry for and feel for her family. She came many times to Gonoa, and worked hard to look after us, and supervise the work here. Before, we didn't have anything, and we had a hard time getting medicine and taking care of our children, and we were in a bad way. But this lady came here and made the aid post work well, and our children grew up strong and healthy. And so we were very happy with the work she was doing, and we mustn't allow it to stop. Now, she is dead, and we would like to give this little 'bilum' to send to her family, so that when they look at it they can remember her and remember us. We are very sorry indeed.

It was a moving and touching occasion.

In 1975, just before we graduated from medical school in Aberdeen, a classmate of ours died after a long illness, and after the funeral service at his home village, a group of us were about to leave, when our friend's mother spoke to us, and I'll never forget what she said. She told us, "Go out and do for him what he might have done for himself." I think we all feel the same about Helena, and for her as much as for anybody we are going to do our best to ensure that the work we continue to do here is a success.

¹ c/o Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea

² Present address: Sunshine Coast Private Hospital Medical Centre, 12 Elsa Wilson Drive, Buderim Queensland 4556, Australia

Bibliography of Helena Vrbova

Vrbova H, Theodorou NA, Tyhurst M, Howell SL. Transplantation of islets of Langerhans from pilocarpine-pretreated rats: a method of enhancing islet yield. *Transplantation* 1979 Nov;28(5):433-435.

Alpers MP, Heywood PF, Nurse G, Stace J, Vrbova H. Collaborative malaria research program of the Papua New Guinea Institute of Medical Research. Abstract in Proceedings of the First Meeting of Australian Research Workers on Malaria, Ingleburn, 22-24 Feb 1980:31-32.

Theodorou NA, Vrbova H, Tyhurst M, Howell SL. Problems in the use of polycarbonate diffusion chambers for syngeneic pancreatic islet transplantation in rats. *Diabetologia* 1980 Apr;18(4):313-317.

Vrbova H. Assessment of the sensitivity of *P. falciparum* to chloroquine using the micro-in-vitro technique. Presented at the Eighth South-West Pacific Malaria Conference in Port Moresby, 4-8 Aug 1980:7p.

Vrbova H, Gibson FD. Chloroquine-resistant malaria in the Madang District. Abstracts and Proceedings of the Sixteenth Annual Symposium of the Medical Society of Papua New Guinea, Madang, 19-21 Sep 1980:34-36.

Vrbova H, Stace J, Climie A, Gibson FD, Heywood PF, Jolley DJ, Pariva S, Alpers MP. The effect of chloroquine-resistant *Plasmodium falciparum* on chemoprophylaxis with amodiaquine. Abstract No 425 in Program and Abstracts of the Tenth International Congress on Tropical Medicine and Malaria, Manila, 9-15 Nov 1980.

Darlow BA, Vrbova H, Stace J, Heywood PF, Alpers MP. Fansidar-resistant falciparum malaria in Papua New Guinea. *Lancet* 1980 Dec 6;2(8206):1243.

Darlow BA, Vrbova H, Stace J, Heywood PF, Alpers MP. Drug-resistant strains of *Plasmodium falciparum* in Papua New Guinea. *Lancet* 1981 Feb 14;1(8216):386.

Darlow BA, Vrbova H, Stace J. Acute malaria in children in Madang: endemicity, clinical presentation and treatment. *PNG Med*

J 1981 Jun;24(2):85-95.

Darlow BA, Vrbova H. Chloroquine-resistant *Plasmodium falciparum* malaria in Madang children. *PNG Med J* 1981 Jun;24(2):96-98.

Barker J, Harvey PWJ, Hide RL, Shield JM, Tulloch JL, Vrbova H. Nutrition, malaria, intestinal parasitosis and morbidity in Karimui. Report of the Karimui Epidemiological Survey carried out by SLUP (Simbu Land Use Project) and IMR (Institute of Medical Research), 24 Aug-4 Sep 1981:122p.

Darlow BA, Gibney S, Vrbova H, Jolley DJ, Stace J, Alpers MP. Treatment of malaria with Fansidar. Abstract in Program and Abstracts of the Seventeenth Annual Symposium of the Medical Society of Papua New Guinea, Rabaul, 25-26 Sep 1981:43.

Vrbova H, Gibney S, Gibson FD, Jolley DJ, Heywood PF, Stace J. A trial of amodiaquine (10mg/kg) and Maloprim as chemoprophylactic agents in the Madang District. Abstract in Program and Abstracts of the Seventeenth Annual Symposium of the Medical Society of Papua New Guinea, Rabaul, 25-26 Sep 1981:45.

Darlow BA, Vrbova H, Gibney S, Jolley DJ, Stace J, Alpers MP. Sulfadoxine-pyrimethamine for the treatment of acute malaria in children in Papua New Guinea. I. *Plasmodium falciparum*. *Am J Trop Med Hyg* 1982 Jan;31(1):1-9.

Darlow BA, Vrbova H, Gibney S, Jolley DJ, Stace J, Alpers MP. Sulfadoxine-pyrimethamine for the treatment of acute malaria in children in Papua New Guinea. II. *Plasmodium vivax*. *Am J Trop Med Hyg* 1982 Jan;31(1):10-13.

Vrbova H, Heywood PF. Malaria: prevention or cure? *Medicine in Society* 1982;8(1):40-42.

Brown GV, Coppel RL, Vrbova H, Anders RF, Mitchell GF. *Plasmodium falciparum*: comparative analysis of stage-dependent protein antigens. *Exp Parasitol* 1982 Apr;53(2):279-284.

Vrbova H, Gibney S, Tiromry K, Jolley DJ,

Cattani JA, Stace J, Alpers MP. A longitudinal study of malaria in Madang Province. Abstract in Abstracts and Proceedings of the Eighteenth Annual Symposium of the Medical Society of Papua New Guinea, Port Moresby, 10-11 Sep 1982:55.

Tulloch JL, Vrbova H, Jolley DJ, Cattani JA. Malaria in a coastal area of Madang Province: preliminary results of an epidemiological study. Abstract in Abstracts and Proceedings of the Eighteenth Annual Symposium of the Medical Society of Papua New Guinea, Port Moresby, 10-11 Sep 1982:59,145.

Oppenheimer SJ, Hendrickse RG, MacFarlane SB, Moody JB, Harrison C, Alpers MP, Heywood PF, Vrbova H. Iron and infection in infancy – report on field studies in Papua New Guinea. II. Protocol and description of study cohort. *Ann Trop Paediatr* 1984 Sep;4(3):145-153.

Gibson FD, Vrbova H, Heywood PF. A comparison of results obtained for *Plasmodium falciparum* in Papua New Guinea by macro-in-vitro, micro-in-vitro and in-vivo tests for chloroquine resistance. In: Bryan JH, Moodie PM, eds. Malaria. Proceedings of a Conference to Honour Robert H Black, Sydney, 10-12 Feb 1983. Canberra: Australian Government Publishing Service, 1984:63-71.

Alpers MP, Heywood PF, Vrbova H. The collaborative integrated malaria research program of the Papua New Guinea Institute of Medical Research. In: Bryan JH, Moodie PM, eds. Malaria. Proceedings of a Conference to Honour Robert H Black, Sydney, 10-12 Feb 1983. Canberra: Australian Government Publishing Service, 1984:108-111.

Cattani JA, Vrbova H, Tulloch JL, Jolley DJ. Inter-cluster variation in malarionometric rates in a coastal area of Papua New Guinea. In: Bryan JH, Moodie PM, eds. Malaria. Proceedings of a Conference to Honour Robert H Black, Sydney, 10-12 Feb 1983. Canberra: Australian Government Publishing Service, 1984:112-118.

Alpers MP, Jolley DJ, Vrbova H, Stace J, Gibney S, Tiromry K, Cattani JA. A longitudinal study of malaria in a village population near Madang, Papua New Guinea.

In: Bryan JH, Moodie PM, eds. Malaria. Proceedings of a Conference to Honour Robert H Black, Sydney, 10-12 Feb 1983. Canberra: Australian Government Publishing Service, 1984:119-126.

Moir JS, Tulloch JL, Vrbova H, Jolley DJ, Heywood PF, Alpers MP. The role of voluntary village aides in the control of malaria by presumptive treatment of fever. 1. Selection, training and practice. *PNG Med J* 1985 Dec;28(4):257-266.

Moir JS, Tulloch JL, Vrbova H, Jolley DJ, Heywood PF, Alpers MP. The role of voluntary village aides in the control of malaria by presumptive treatment of fever. 2. Impact on village health. *PNG Med J* 1985 Dec;28(4):267-278.

Cattani JA, Tulloch JL, Vrbova H, Jolley DJ, Gibson FD, Moir JS, Heywood PF, Alpers MP, Stevenson A, Clancy RL. The epidemiology of malaria in a population surrounding Madang, Papua New Guinea. *Am J Trop Med Hyg* 1986 Jan;35(1):3-15.

Shield JM, Hide RL, Harvey PWJ, Vrbova H, Tulloch JL. Hookworm (*Necator americanus*) and *Strongyloides fuelleborni*-like prevalence and egg count with age in highlands fringe people of Papua New Guinea. *PNG Med J* 1987 Mar;30(1):21-26.

Vrbova H, Gibney S, Gibson FD, Jolley DJ, Heywood PF, Stace J, Trenholme KR, Alpers MP. Chemoprophylaxis against malaria in Papua New Guinea: a trial of amodiaquine and a combination of dapsone and pyrimethamine. *PNG Med J* 1992 Dec;35(4):275-284.

These completed papers appear in the Publication List (PL) of the Papua New Guinea Institute of Medical Research, with the exception of the 2 papers from Helena's work before she came to the Institute and 2 reports in the Unpublished Reports (UR) of the Institute. In addition, as a matter of record, there are 3 archival draft papers that have never been published, despite several attempts by various authors to get them into a form suitable for publication during the years following Helena's death.

Tulloch JL, Vrbova H, Stevenson A, Jolley DJ, Anders RF, Heywood PF, Cripps AW,

Alpers MP, Clancy RL. Antimalarial antibody levels, as determined by ELISA, in relation to other malariometric indices in an epidemiological study of malaria in coastal Papua New Guinea. [1981]

Vrbova H, Gibson FD, Darlow BA, Jolley DJ, Alpers MP. Chloroquine-resistant *Plasmodium falciparum* in Papua New

Guinea: micro-in-vitro, macro-in-vitro and in-vivo tests. [1982]

Tulloch JL, Vrbova H, Jolley DJ, Collins WE, Harvey PWJ, Hide RL, Alpers MP. The relationship of serological and malariometric indices in individuals in a cross-sectional survey of a rural population in the Karimui region of Papua New Guinea. [1982]

Women's groups and the marketing of health interventions – a Tanzanian experience

DEREK CHARLWOOD¹

Danish Bilharziasis Laboratory, Charlottenlund, Denmark

The formation of women's groups to improve their life is a good idea everywhere but is especially good in Africa and Papua New Guinea, where women have to 'hold up more than half of the sky'. It is something that Helena would most certainly have appreciated, she being the most truly liberated person one could ever meet. Here I would like to recount some of my experiences with the formation of 'Tupendane' and 'Tupendane Too' in the early 1990s in Tanzania, when the country was in transition from one-party rule to multi-party democracy.

One of the best ways of controlling *Culex* mosquitoes (those nasty ones that buzz around your head in towns and cities throughout the tropics) is to use expanded polystyrene beads as a layer over the water in septic tanks and pit latrines. A blanket of beads a few centimetres thick prevents gravid females from laying their eggs and drowns larvae whilst at the same time allowing solid matter to pass through. They refloat in latrines that have temporarily gone dry and so do not need to be applied more than once. They also reduce the smell, so they are generally a good thing all round. I had just done an experiment which showed that they worked even when applied to the large open septic tanks in the grounds of the St Francis Hospital. Going to hospital meant getting bitten – the paper I wrote on this study described thousands of larvae per cupful of water.

The beads come unexpanded in drums. The polystyrene looks a little bit like white sand. It contains a gas which, when heated to 100 degrees centigrade, expands and in so doing expands the bead. Adding the material to a pot of boiling water creates a

little bit of magic. However, unless it is expanded the gas gradually leaks out and the product loses its value.

I had a couple of drums left over from the experiment. The local pit latrines were an obvious target for the beads. We ourselves did not have the time, manpower or the funds to do this. That was our problem.

Just outside of the institute where I worked there lived a bevy of young, vivacious, single mothers making a hand-to-mouth living. Although I thought I knew them well I really had no idea how they survived. What better way to solve our problem than to become an honorary woman and establish a women's group to sell the beads. I showed them the process and explained the idea of a 'women's only' enterprise (men could be hired but could not be part of the group – myself of course excepted – well, I thought I deserved some perks!). The idea of earning some money, a rare commodity as far as these young women were concerned, was delightful (money's important when you haven't any). And so Tupendane, which means 'We like each other', was born.

I suggested that the money that they might earn from the first barrel be spent in any way that they liked, but that they save enough from the sale of the second drum so that they could buy another one and continue. They took off like wildfire. One or two went round collecting orders, another couple were responsible for boiling the water and expanding the beads (which required buying and carrying the wood etc) and another pair were responsible for the application. I as usual did not do much more than make friends with them. They had orders from all over town. People flocked to pay what I

¹ Danish Bilharziasis Laboratory, Jaegersborg Alle 1D, DK-290, Charlottenlund, Denmark
dcharlwood@dblnet.dk; jdcharlwood@gmail.com

thought was an exorbitant – but was actually considered quite reasonable – price for the beads to be put into septic tanks (with the occasional pit latrine). I nominated myself as the group's ombudsman (which meant of course that I had to leave the group – still it was fun while it lasted). Then it was my job to follow up on complaints and to make spot checks to determine that the layer of beads applied was sufficiently thick to kill larvae and to last long enough for the investment to have been worthwhile. The most accurate way of measuring bead depth is to be held upside down and extend a finger through the beads until it touches the water – or at least something that is not bead. We opted instead to develop a little gadget out of wire for the job.

These women had never been so happy. The first barrel was used in literally a few days. This was in May, the rainy season, when mosquito densities were very high. Now this was Tanzania in a time of transition: from state-run to private enterprise. So who should come along but someone from the District Health Committee to tell the women that health provision in Tanzania was free and that they should therefore deliver it for free or face proceedings. So of course they stopped. I learnt that this committee was to meet in a couple of weeks and managed to get the group on to the agenda. I attended and described the process and what diseases would be prevented if a large number of households used it. The man who had stopped them in the first place slept through it all. He was just obviously jealous of the fact that the women were earning more than he was. Anyway the upshot was that everyone agreed that the women were doing a good job and should carry on. The committee did suggest that since money was involved the issue should perhaps also be brought to the attention of the District Development Committee. I of course volunteered to go and tell them too but was told, "No, no, no. No need to bother: the committee has seven members, three of which are here now so your case will be well represented and this will really be just a formality." I felt reassured and sat through lots of other less interesting business. It was only as we were leaving that I happened to ask when the next DDC meeting would be. My informant rubbed his chin for a few seconds: "Mmm. April, I think." We were in the second week of May! The women did

eventually get permission to continue but by this time they had all started doing other things and anyway their heart was no longer in it.

Also, the beads had lost much of their gas – as I found out to my cost when I tried to determine how much was left in the remaining barrel using a kerosene lamp at night to see by. I had to stick my head in the barrel and lower the lamp to eye level. I forgot to mention that the gas is inflammable and heavier than air. The gas that had escaped was still in the barrel. When the lamp reached almost eye level there was a 'woof' and the lamp was blown out. The fact that I had to walk around with Groucho Marx style eyebrows caused much merriment at the scientific meeting I had to attend a few weeks later!

So Tupendane died. Of course, not long after, people were coming from Dar-es-Salaam to see this already famous women's group!

'Tupendane too' was only a little more successful. This consisted of my two house girls, Rukia and Consolata (and eventually the gardener – which led to their downfall). They impregnated and sold mosquito nets out of our kitchen. The nets were ones that I had bought from UNICEF in Morogoro, a town some 150 km from Ifakara. They sold the nets either individually or 'wholesale' and eventually had a cadre of salespeople putting the money up-front for lots of 10 or 20, which they then took into the wide world. We set up a system in which the receipt acted as a voucher for a free retreatment of the net, and again things were going fine (indeed there was no problem with the DHC or the DDC or the PDC or anyone else).

The problem in this case was the nets themselves (and the gardener). This was a time when people – usually men – in glass offices in Europe bought all the nets that ended up in Africa. The available information about nets in Africa that these bureaucrats already had was that they were generally in bad shape and full of holes. The information about impregnated nets was that the condition of the net did not matter once they were treated with insecticide. Since they were going to get holes anyway, someone somewhere did their maths and decided that cheap nets were the most cost-effective

option and so the nets that were being sold were of a very low denier (a measure of net strength). As a result, in a few days the nets started tearing, even with very careful use. Now a big deal had been made to customers about the treatment, what it was for and when to come for retreatment; for example, it was emphasized that it would work against malaria mosquitoes but not necessarily against *Culex* – for that people should consult

‘Tupendane’ – so everyone knew that their nets had insecticide on them. The nets gave way much sooner and more dramatically than other nets that the people had had in the past. Quite reasonably it was the insecticide that was considered responsible. Not one person came back.

Then the gardener stole the money. The enterprise was ruined. Men have a lot to answer for.

Effective diagnostic tests and anthelmintic treatment for *Strongyloides stercoralis* make community control feasible

JENNIFER M. SHIELD¹ AND WENDY PAGE²

Aboriginal Resource and Development Services and Miwatj Health Aboriginal Corporation, Nhulunbuy, Northern Territory, Australia

SUMMARY

Strongyloides stercoralis is endemic in tropical and subtropical countries, and is prevalent particularly in economically impoverished people. Although an estimated 30 to 100 million people world-wide suffer from *S. stercoralis* infection and it is a life-long disease, it remains a neglected tropical disease. Faecal testing for *S. stercoralis* is very insensitive. The prevalence of *S. stercoralis* in Indigenous Australians (up to 60%) is much higher than previously thought, and its prevalence in Papua New Guinea is likely to be much higher than currently believed. When *S. stercoralis* and the HTLV-1 virus coexist in the one person, both diseases progress more quickly than when either infection is on its own. When people become infected with *S. stercoralis*, they develop acute strongyloidiasis which may be life threatening. At any time during the course of the disease, if the immune system is suppressed, most often by corticosteroid drugs, infected people may develop hyperinfective strongyloidiasis and they will die unless the underlying *S. stercoralis* infection is effectively treated. The use of serology for diagnosis, together with ivermectin treatment, has revealed that it is possible to eradicate *S. stercoralis* from the patient, and serology can also define the effectiveness of treatment. The reservoir of infection is humans; the free-living stages are short-lived. Mass treatment may be effective at eliminating *S. stercoralis* from a community. Safe water and effective sanitation alone do not lead to elimination of *S. stercoralis*. Up-to-date knowledge of *S. stercoralis* has been revealed through the workshops of the National Strongyloides Working Group in Australia and is summarized here. Much of this information is now available on the world wide web, and the addresses of relevant web sites are given.

Introduction

Strongyloides stercoralis (Bavay 1876) Stiles and Hassal 1902 was first described in France by Bavay in 1876 as *Anguillula stercoralis*. The genus *Anguillula* was preoccupied by an eel genus, so in 1879 Grassi erected a new genus *Strongyloides* for roundworms previously known as *Anguillula*. In 1902, Stiles and Hassall showed that the correct name is *Strongyloides stercoralis* (1). This species is widespread particularly throughout tropical and subtropical areas of the world. Estimates of the number of people affected vary from

30 to 100 million (2). *S. stercoralis* occurs in Papua New Guinea (PNG) (3) but its extent is unknown. Surveys by Alan Kelly in the early 1970s did not reveal *S. stercoralis* (4). Its presence in Morobe Province was revealed in the early 1980s by using a filter paper culture technique (JMS, unpublished data).

S. stercoralis is a nematode tissue parasite that causes a life-long disease unless eradicated by treatment (5). It can cause an overwhelming infection called hyperinfection, or disseminated strongyloidiasis, when an infected person's immune system is suppressed (6). Corticosteroids play an

¹ Aboriginal Resource and Development Services, PO Box 1671, Nhulunbuy, Northern Territory 0881, Australia and La Trobe University, PO Box 199, Bendigo, Victoria 3552, Australia
jenny.shield@ards.com.au

² Miwatj Health Aboriginal Corporation, PO Box 519, Nhulunbuy, Northern Territory 0881, Australia

important role in triggering hyperinfection (7). Other drugs and conditions that suppress the immune system also lead to hyperinfection, and in about 10% of the cases the cause is not clear (8). Human T-cell leukaemia virus type 1 (HTLV-1) occurs in some parts of PNG and West Papua (9). Coinfection with *S. stercoralis* and HTLV-1 causes accelerated progression of both diseases (10) and resistance to anthelmintic treatment for *S. stercoralis* (11,12).

Although disease caused by *S. stercoralis* is now diagnosable and treatable (5), it has been neglected. It is now classified by the World Health Organization as a neglected tropical disease.

There are a number of reasons why *S. stercoralis* has been neglected. These are elaborated later. Briefly they are:

- *The disease has been difficult to diagnose.* The symptoms are non-specific and secondary infection often masks the underlying cause of the disease. Faecal testing is insensitive. Although the sensitivity of the specific IgG test is high, patients in the acute or hyperinfective phase of the disease may not have the antibodies. Consequently there has been gross underdiagnosis of the disease, as well as gross underestimation of its prevalence and its contribution to morbidity and mortality. In a retrospective study in Queensland from 1998 to 2002, of 120 hospital admission records of patients who tested positive for *Strongyloides*, only 6 gave strongyloidiasis as the primary diagnosis. The primary diagnosis of the remaining 114 was one of the following: gastrointestinal disorder, respiratory disorder, failure to thrive, genitourinary disorder, skin disorder or sepsis (13). All these conditions are features of *S. stercoralis* infection, and it is likely that the true primary cause of disease in these cases was in fact *S. stercoralis* infection.
- *The sensitivity of faecal testing is low.* Until recently, practitioners have relied on faecal testing for diagnosis. This means that there has been an underestimation of prevalence and an overestimation of the efficacy of anthelmintic drugs (14,15).

- *The disease has been difficult to cure.* There was a lack of effective drugs (until ivermectin became available). In addition, the ability of the worms to multiply in the host means that all the worms must be eliminated in order to effect a cure. So it is important to be able to determine whether a treated person has been effectively treated. The IgG diagnostic test has made this possible (16).
- *A mistaken belief that free-living stages of *S. stercoralis* persist in the environment.* In fact, the free-living stages are short-lived (17,18).

As a result, health authorities have not seen the need to carry out mass treatment to control *S. stercoralis*. Australia, in spite of having an excellent health system, has not come to grips with *S. stercoralis* in its midst. Although *S. stercoralis* is rare in mainstream Australia, it is hyperendemic throughout tropical and subtropical Indigenous Australia. Estimates of prevalence in various Indigenous settlements are between 4.5% and 60% seropositive (19-25) and between 2% and 41% positive by stool testing (19,26,27). In central Australia, it coexists with HTLV-1 (28).

In 2001, a group of concerned professionals came together at a workshop in Nhulunbuy in the Northern Territory and formed the National Strongyloides Working Group (NSWG) of the Australasian College of Tropical Medicine to investigate *S. stercoralis*. Since then, the NSWG has held biennial National Workshops on Strongyloidiasis in order to share information about *S. stercoralis* and to make recommendations on how to tackle the disease. This has resulted in bringing together information about *S. stercoralis*, some of which would not otherwise have entered the public arena.

What we now know gives us the tools to enable the disease to be controlled. This paper will summarize this information and refer to more detailed material, much of which is available on the world wide web on the Aboriginal Resource and Development Services web site at http://www.ards.com.au/health_strong.htm or the James Cook University web site at <http://www.jcu.edu.au/school/phtm/PHTM/ss/>.

***Strongyloides stercoralis* life cycle**

The life cycle is represented schematically in Figure 1. It is described in detail by Speare (5) and is available at <http://www.jcu.edu.au/school/phtm/PHTM/ss/acrrm-cd/CD-Index.pdf>. A patient education flip chart about *S. stercoralis*, the life cycle, the disease, and how to prevent and treat it, is available in plain English at <http://www.ards.com.au/StrongFront.pdf>. Infective filariform larvae are illustrated in Figure 2. There are a

number of features in the life cycle that have implications for the treatment of patients and control of the disease.

The parasitic females actively burrow through the intestinal absorptive epithelium counteracting the movement of epithelial cells as they migrate towards the tips of the villi (30), and filariform larvae can migrate anywhere in the body (31). Thus *S. stercoralis* is a tissue parasite in intimate association with the host.

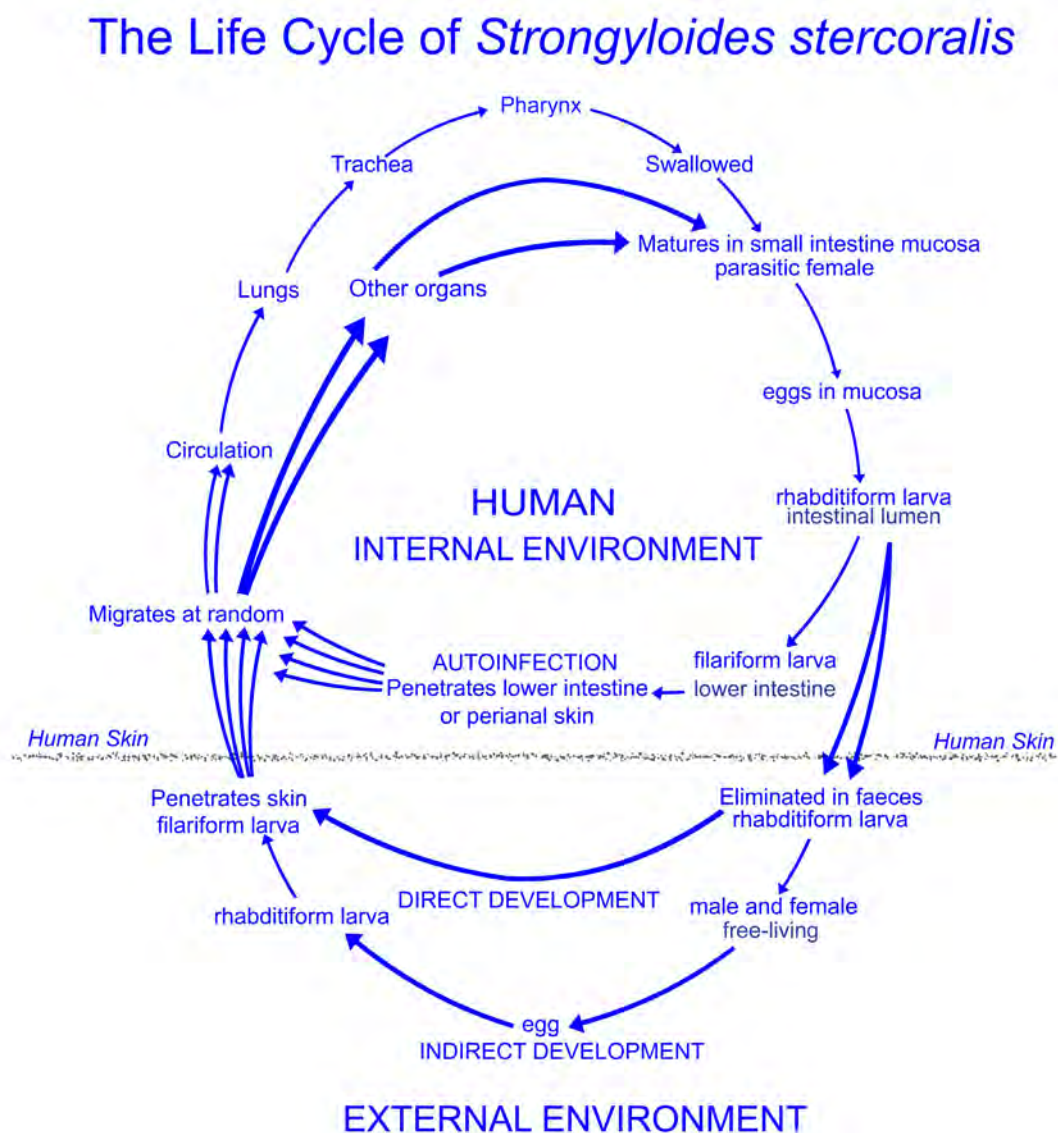


Figure 1. Schematic representation of the life cycle of *Strongyloides stercoralis*. Updated and modified from Zaman (29).



Figure 2. Infective filariform larvae of *Strongyloides stercoralis* in faeces. Photo: P. Caramello. URL <http://www.cdfound.to.it>

S. stercoralis has the ability to multiply in the body by the autoinfective cycle. Some rhabditiform larvae develop into infective filariform larvae in the lower part of the gut, and enter the body proper through the side of the lower gut or the skin around the anus (5). As a consequence, *S. stercoralis* causes a life-long disease, and hyperinfection occurs particularly when type 2 immune responses are suppressed (32). In addition, a person is cured only when treatment has eliminated every worm in the body. A single worm may reestablish a patent infection by the autoinfective cycle.

The infective larvae can migrate anywhere in the body (31). So larvae can cause symptoms anywhere in the body, and bacteria carried by the larvae from the gut may seed secondary infection anywhere in the body.

S. stercoralis does not persist in the environment. There is a maximum of one generation of free-living *S. stercoralis* (33) and the infective larvae survive for only two weeks even in ideal conditions (34). They are all dead within 3 weeks of faecal contamination. The reservoir of infection is infected people.

Infective filariform larvae have narrow tolerance limits for survival. Their optimum temperature range is 20 to 28 degrees

Celsius and they die in the refrigerator and in the heat (5). They die within a few hours on dry soil in the sun and within 3 days on dry soil in the shade (17). So it is essential to store faecal samples correctly and for as short a time as possible, particularly when using diagnostic tests that depend on the presence of viable larvae in the faeces.

Strongyloidiasis: progression of the disease

The three phases of strongyloidiasis

The progression of the disease is summarized in Table 1. There are three phases of strongyloidiasis: acute, chronic and hyperinfective (disseminated).

Acute strongyloidiasis

In the 1950s, Tanaka infected himself with infective filariform larvae through the skin and, 27 days later, rhabditiform larvae first appeared in the faeces (5). The worms multiply in the body, and as the numbers increase, the symptoms become more intense. This is acute strongyloidiasis. In children, the infection sometimes causes wasting and hypokalaemia (36). In adults, it sometimes causes dysentery (35). Some mortality may occur at this stage. Eventually

TABLE 1

PROGRESSION OF DISEASE DUE TO *STRONGYLOIDES STERCORALIS*: CHANGES IN BEHAVIOUR OF THE WORMS, LARVAL OUTPUT, EFFECTIVENESS OF THE DIAGNOSTIC IgG TEST, IMMUNE STATUS, AND SYMPTOMS AS THE DISEASE PROGRESSES*

Phase	Worms	Larvae/ml stool	Specific IgG test	Immunity	Symptoms
Acute	Number of females in gut increases via the autoinfective cycle, then larval output slows as immunity increases.	0 to >1000. Stool test negative or positive (35)	Negative, equivocal or positive	'Window period' before specific IgG levels become raised. Immune response increases, antibodies IgE, IgA, IgM and IgG increase, number of eosinophils increases.	Marked skin, lung and digestive system symptoms, severe diarrhoea, wasting and hypokalaemia in children; can be fatal (36).
Chronic	Low larval output by stunted females reduces migration of larvae in tissues. Immunity keeps the number of worms low, but worms persist.	0 to 400. Stool test usually negative	Positive or equivocal	Immune response is strong, antibodies IgE, IgA, IgM and IgG are raised, eosinophilia is present in about 70%.	Skin, lung and digestive symptoms mild to moderate and may be intermittent; at least 70% have symptoms (37).
Hyperinfective (Disseminated)	Females recover (38); numbers of females, migrating larvae and larvae in stools increase. The person will die unless they get effective treatment.	400 to >1000. Stool test usually positive	Positive, equivocal or negative	Immune suppression causes the antibodies IgE, IgA, IgM and IgG and the number of eosinophils to decrease (39).	Severe skin, lung and digestive system symptoms; other organs may be affected. Secondary infection with gut bacteria in 50%, which may present as pneumonia, meningitis or septicaemia. Case fatality rate 70% (8).

*Information from R. Speare unless otherwise indicated

the immune system responds with type 2 immunity (40) that attacks the females in the gut and the larvae migrating through the tissues. The effect on the females is marked reduction in the rate of reproduction (38,41). The immune response slows the migration of larvae through the tissues, and probably kills some of the migrating larvae (42). The overall effect is a reduction of the worm load, but the immune system is not able to eliminate the worms.

Chronic strongyloidiasis

The infection persists. This has been shown by studies of World War 2 ex-prisoners of war who acquired *S. stercoralis* in prison camps during the war and had the infection for up to 57 years (37,43,44). This phase is called chronic strongyloidiasis. The person has fewer worms, and intermittent symptoms. Infected people remain infected for the rest of their life (5).

Hyperinfective strongyloidiasis

In some people, the number of worms continues to increase slowly, and hyperinfective strongyloidiasis develops with no obvious cause. Most cases of hyperinfective strongyloidiasis are associated with suppression of the immune system, particularly type 2 immunity. This topic has been reviewed by Keiser and Nutman (32). A study of fatal strongyloidiasis in the literature showed that the most common precipitating factor was the administration of corticosteroid drugs (60%) (7,8). Some deaths were due to other conditions and other drugs that suppress type 2 immunity (30%). There was no obvious cause of hyperinfection in a few cases (10%) (8). Unfortunately, corticosteroids are sometimes given to patients for respiratory symptoms that are actually due to *S. stercoralis* larvae migrating through the lungs (45,46). An endoscopic and histopathological study of the duodenum in hyperinfection was published recently (47).

Symptoms

A summary of the symptoms is given in Table 1. The symptoms of *S. stercoralis* infection have been described in detail by Grove (37) and summarized by Speare (5). Briefly, the majority of symptoms are non-specific. This is a major impediment to diagnosis of the disease. The severity of the symptoms varies according to the number of

worms in the body, and which organs are affected depends on where the larvae have migrated to. The most frequent symptoms are associated with the skin, digestive system and lungs, but any other organ may be affected, including the joints and central nervous system. Fatigue is common. Secondary infection may make its presence felt as pneumonia, septicaemia or meningitis, or abscesses in any organ or in the muscles. The only symptom that is pathognomonic for *S. stercoralis* infection is larva currens. It consists of itchy linear urticarial rashes (Figure 3) that move at 2 to 10 cm per hour (37).

Immune regulation of *S. stercoralis* infection

In chronic strongyloidiasis, the level of infection with *S. stercoralis* is regulated by the type 2 component of the immune system. IgA, IgM, IgG and eosinophils are lower in severe strongyloidiasis than in mild or moderate strongyloidiasis (39).

The effects of antibodies on filariform larvae in the body have been investigated by experimental work in mice. Both IgG and IgM are important in killing filariform larvae in the body, but the role of eosinophils is not clear. Brigandi et al. (42) showed that eosinophils are associated with IgG-mediated killing of filariform larvae in mice. IgM together with complement and neutrophils was protective against filariform larvae (48). Similarly, IgG together with complement and neutrophils was protective against filariform larvae (40).

With respect to the stages in the gut, IgA and IgE play a role in modulating larval output (43), probably by reducing parasitic female fecundity by impairing their ability to feed and excrete. When stunted females that were not producing larvae were taken from immune dogs and transplanted into naive dogs, they recovered and reproduced (38).

The level of IgG4 rises in people who have been infected with *S. stercoralis* for a long time (49). Experimental evidence is consistent with the hypothesis that IgG4 blocks IgE-mediated immune responses (43).

Diagnostic tests

There are two main possibilities for diagnosing *S. stercoralis* available in Australia: examination of faeces for rhabditiform larvae or filariform larvae, and



Figure 3. Larva currens is pathognomonic for *Strongyloides stercoralis* infection and consists of itchy linear urticarial rashes that move at 2 to 10 cm per hour. Photo: W. Page.

examination of the blood for specific antibodies to *S. stercoralis*. Sputum and duodenal fluid are sometimes examined for *S. stercoralis* larvae.

Tests are currently being developed in the USA based on recognizing antigenic material from *S. stercoralis* in the blood or faeces (50). This may overcome the problem of changing immune status during the progression of the disease.

Faecal testing

Faecal testing has high specificity but poor sensitivity. The most sensitive faecal test is the agar plate culture.

The most common test used is the direct smear. This is a particularly insensitive test for the early stage of acute strongyloidiasis, for chronic strongyloidiasis and for the early stages of hyperinfective strongyloidiasis due to the very low larval output in the faeces. Conway et al. (51) summarized the relative sensitivity of various faecal tests. In these studies, the estimated number of people infected was the number positive by any one of a number of faecal tests. The estimated

sensitivity of the direct smear (3 studies) varied between 0% and 52%, formalin-ether concentration (3 studies) between 13% and 55%, nutrient agar plate culture (5 studies) between 78% and 100%, Harada-Mori filter paper culture (3 studies) between 7% and 58%, and Baermann concentration (1 study) 60%. The latter three tests require viable larvae, and therefore correct handling of specimens.

However, the true sensitivity is probably about half these estimates, judging by sensitivity estimates in two studies on patients who had a previous positive faecal test. In a study using the Baermann technique, 35% were positive by the first examination (14). In a study using the agar plate test, 58% were positive after one test (15).

People coinfectd with HTLV-1 are more likely to return a positive direct faecal smear. In a study in Japan, 61% of people with both *S. stercoralis* and HTLV-1 had a positive direct faecal smear whereas only 18% of those with *S. stercoralis* and no HTLV-1 had a positive direct faecal smear (52).

It is important to note that an estimate of

sensitivity depends on the mix of patients being tested. If they are mostly in the acute or hyperinfective phase, then the sensitivity of faecal testing will be higher than if they are mostly in the chronic phase, because the larval output is higher in severe strongyloidiasis (39). Also, if there are several larvae per field in a direct smear, it indicates that the patient has, or is heading for, severe disease (5).

Implications of the insensitivity of faecal testing

- The diagnosis of *S. stercoralis* infection has frequently been missed.
- People who were considered 'cured' still had the infection.
- The efficacy of drugs for *S. stercoralis* infection has been overestimated. The efficacy of thiabendazole has been thought to be high, but in fact the main effect of the drug is to inhibit reproduction in parasitic females (53), resulting in negative faecal testing after treatment.
- Grove's morbidity study (37) underestimated the extent of symptoms due to *S. stercoralis* infection because some of the 'control' group were later found to have *S. stercoralis* (D. Grove, personal communication).
- Estimations of the specificity of serology have been underestimated because false positives were defined as positive serology and negative faecal testing.

Serum-specific IgG ELISA

The first application of the use of the enzyme-linked immunosorbent assay (ELISA) to detect antibodies to *S. stercoralis* was carried out in France in 1978 (54). This test was further developed in Australia to detect IgG antibodies to *S. stercoralis* in 1981 (55).

In Australia today, blood testing consists of the determination of IgG antibodies to *Strongyloides ratti* antigen in the serum by ELISA. It is now routinely available in four major laboratories using a single source of antigen for routine testing. The sensitivity of

the test is estimated to be 93% and the specificity 95%. The specificity approaches 100% in the mid to high positive range (20).

A commercial kit tests for specific IgG using a preparation of *S. stercoralis* filariform larvae as the antigen. It gives comparable results (56). Some private laboratories offer this test.

The sensitivity of the IgG test is probably much higher for people with chronic strongyloidiasis (see Table 1). In a study that compared returned travellers with immigrants from areas where *S. stercoralis* is endemic, the sensitivity of the IgG test was lower in travellers (73%) than in immigrants (98%) (57). The authors did not offer an explanation for their results. It is likely that the travellers had contracted *S. stercoralis* recently and some were still in the 'window period' before developing immunity and producing the specific IgG antibodies, whereas most of the immigrants probably had chronic strongyloidiasis. This would account for the difference in the two groups.

People with hyperinfection may be negative with the IgG test, depending on how severely their type 2 immune responses are depressed. In addition some elderly World War 2 ex-prisoners of war with *S. stercoralis* infection are negative by the IgG test (WP, unpublished observations).

There have been efforts to improve the specificity of the test by using monoclonal antibodies. It appears that there is no single antigen from *S. stercoralis* larvae that is universally recognized by immune sera (58). So perhaps the strength of the current test is the number of antigenic components in the preparation used for testing.

Studies in Japan suggest that *Strongyloides* serology may be less sensitive in patients infected with HTLV-1. This implies that patients with borderline serology should be treated, as is done in central Australia (L. Einsiedel, personal communication).

Strongyloides kellyi, a *S. fuelleborni*-like species of *Strongyloides*, is endemic only in parts of Papua New Guinea. *S. kellyi* does not have an autoinfective cycle. It is likely that *S. kellyi* antibodies would cross-react in this test (I. Sampson, personal communication), but *S. kellyi* infections are readily identified by faecal testing. Past work

on the IgG test indicated that *Wuchereria bancrofti*, *Ascaris lumbricoides*, *Necator americanus* and *Toxocara canis* are unlikely to cross-react (55).

A major advantage of the IgG test is the opportunity to identify people with chronic strongyloidiasis so that they can be treated and relieved of their non-specific symptoms, and not become victims of hyperinfective strongyloidiasis at a later date.

Eosinophilia

Eosinophilia defined as $>400/\mu\text{l}$ varies in different series between 60% and 90% (59). 57% of patients with *S. stercoralis* at Royal Darwin Hospital from mid-1991 to mid-1992 had eosinophilia (60). Eosinophilia is also present in people infected with other helminths, so it is not a reliable test for *S. stercoralis*.

Value of the IgG test in monitoring the effectiveness of treatment

The value of the IgG test has been the subject of debate among medical practitioners in Australia. It was mistakenly believed that a positive IgG test does not distinguish between past and current infection. Though this is true for viral infections, it is not true for parasitic infections. People treated with anthelmintic drugs often returned a negative faecal test, and the practitioner then erroneously assumed that the person was cured, and a positive IgG test was assumed to be past infection rather than failure of treatment.

The advent of the drug ivermectin enabled the clarification of the value of the IgG test (16). An Australian Indigenous medical clinic instituted a monitoring program based on the identification of strongyloidiasis using the IgG test followed by treatment with albendazole for three days, and then retesting several months later. They found that although the test results showed lower IgG levels after treatment, most did not become negative (22). This result was disappointingly similar to earlier studies using thiabendazole (61-63) or albendazole (63,64) as the anthelmintic. Then they used ivermectin (200 μg per kg body weight) as the anthelmintic treatment. On retesting after six months, most of the patients treated with ivermectin were negative by the IgG test. Thus the value of the IgG test for diagnosis and the efficacy of

treatment with ivermectin were clarified at the same time. In addition, the value of the IgG test in monitoring the effectiveness of treatment was established (16). Since that time, these conclusions have been confirmed during a community control program in another Indigenous community (23). However, there are some patients who test negative by the IgG test who may still have a few worms and later the symptoms recur (WP, unpublished observations).

Anthelmintic treatment

Effects of anthelmintics on *S. stercoralis*

David Grove and others conducted a number of studies examining the effect of anthelmintic drugs on migrating larvae and parasitic adults of *S. ratti* (53). The effects of the drugs on *S. stercoralis* in people are likely to be similar. The results are summarized as follows:

- Ivermectin: there is dose-dependent eradication of adults in the gut and larvae in the tissues, and surviving larvae do not mature.
- Albendazole: there is also dose-dependent eradication of adults in the gut and larvae in the tissues.
- Thiabendazole does not kill adult worms but reduces larval output; it has no effect on larvae in the tissues.
- Cambendazole eliminates adults and larvae.
- Mebendazole kills adults but not larvae, and is between 100 and 1000 times less effective than cambendazole.

Of these drugs, thiabendazole is no longer commonly used because of the side-effects of nausea and neuropsychiatric symptoms. There were a number of trials of cambendazole during the early 1980s, but it was withdrawn from the market by the manufacturer because of rare severe reactions in cattle (61). Mebendazole is completely unreliable (61). Albendazole 400 mg for 3 days is commonly prescribed. The estimated efficacy of this regimen was 38% compared with 83% for ivermectin (65). This

is probably an overestimate for both drugs because it relied on faecal testing for defining cure. The estimate of Archibald et al. of the efficacy of albendazole at 400 mg twice daily for 3 days was 75% (66). Their definition of cure was all of three criteria: negative faecal test, negative IgG test and no symptoms. In another study, the efficacy of one dose of ivermectin at 200 µg per kg body weight was 68% and of ivermectin followed by a second course of either ivermectin or albendazole was 83%, using a negative IgG test alone as the criterion for cure (16).

One of the problems of albendazole therapy is that it must be taken on three consecutive days. This makes it difficult to ensure that all the doses have been taken. An advantage of ivermectin is that a course is one dose, so the health professional can directly observe that the drug has been swallowed.

Absorption of anthelmintics

Both albendazole and ivermectin are lipophilic and are best taken with a fatty food such as full-cream milk (67). Ivermectin may be poorly absorbed in the fasted state in a critically ill patient (68).

Resistance to anthelmintic treatment

Two studies of patients who had been treated by two courses of ivermectin and then followed up indicated that 17% and 16% respectively were still IgG positive six months after treatment (16,23). If still positive after retreatment, they may be resistant to treatment and should be treated on a regular basis (41,69).

Resistance of *S. stercoralis* to treatment with albendazole is associated with elevation of the *S. stercoralis*-specific IgG4 antibody titre at the expense of IgG1 (70). IgG4 is thought to block IgE-mediated responses in human strongyloidiasis (43).

Patients who are immunosuppressed (71,72) as well as HTLV-1 patients (11,12,73,74) are frequently resistant to treatment.

Safety of ivermectin

Ivermectin affects gamma-aminobutyric acid (GABA)-mediated nerves. In many invertebrates including roundworms, muscle

contraction is controlled by GABA-mediated nerves, and ivermectin causes paralysis. In mammals, such nerves occur only in the central nervous system, and the blood-brain barrier prevents ivermectin from entering the brain. People with conditions that may compromise the blood-brain barrier may be at risk of an adverse reaction when treated with ivermectin (5).

In general, ivermectin is a very safe drug (75). Its safety for very young children and pregnant women has not been established conclusively, so it should not be used routinely in these groups. If there is a clinical need to use ivermectin, it should be considered on a case-by-case basis. Data collected so far suggest that it is safe. During the oncocerciasis trials in Africa, some pregnant women were treated inadvertently. When the outcomes of these pregnancies were compared with the outcomes in those who were not treated, there was no significant difference between the two groups (76).

Ivermectin should be used with caution in people who may be coinfecting with the eye worm *Loa loa*. This species is endemic in parts of Africa. Individuals with high densities of *L. loa* microfilariae have developed serious adverse effects when treated with ivermectin, and some have died (77).

Coinfection with HTLV-1

The rate of *S. stercoralis* infection is significantly higher in patients with HTLV-1 infection than in patients without HTLV-1 infection (74,78).

HTLV-1 is associated with an exacerbated type 1 immune response. Coinfection with *S. stercoralis* or *Schistosoma mansoni* decreases the activation of type 1 cells, which may influence the outcome of HTLV-1 infection (79). Helminths including *S. stercoralis* induce a type 2 response. HTLV-1 decreases the type 2 immune response that is effective against *S. stercoralis* (10), leading to hyperinfective strongyloidiasis.

HTLV-1 causes decreased levels of IgE and eosinophils, both of which are important in the immune response to *S. stercoralis* (74). The high production of IFN-γ observed in patients coinfecting with HTLV-1 and *S. stercoralis* (73,80) decreases the production of IL-4, IL-5, IL-13 and IgE, molecules that participate in the host defence mechanism

against helminths (80). Although coinfection with HTLV-1 was associated with a decrease in levels of IgE and skin sensitivity, it did not affect the levels of IgG (10).

HTLV-1 patients are frequently refractory to anthelmintic treatment. Resistance to treatment with albendazole has been associated with high levels of serum IFN- γ and TGF- β -1 (73). Similarly, resistance to treatment with ivermectin by HTLV-1-positive patients has been demonstrated (11). This is probably due to impairment of immunity to *S. stercoralis* in people coinfecting with HTLV-1.

Transmission

Aspects of the life cycle and transmission of *S. stercoralis* and also effective control measures are well understood, and have been summarized by Feachem et al. (81), except that it is now known that there is a maximum of one free-living generation (33), and the stages in the soil are short-lived, about 2 weeks (34). Galliard showed in the 1950s that the worms die within a few hours in the open, or 2 to 3 days in the shade if the soil is dry (17).

Transmission may be indirect, by contact with infective filariform larvae on damp soil or damp vegetation, or direct, by faecal contamination of the skin. With rare exceptions, larvae in direct faecal smears are rhabditiform. Infective filariform larvae are observed rarely (82,83), and these can be passed on to others by faecal contamination.

There is evidence that transmission of infection occurs indoors. There have been reports of high rates of infection with *S. stercoralis* in mental institutions in USA, Canada, USSR and Chile (18) and of clustering of people infected with *S. stercoralis* in households in Jamaica (84) and Bangladesh (85). Transmission in these circumstances could be either direct or indirect.

Community control

The reservoir of infection with *S. stercoralis* is infected people. Its free-living stages are short-lived and sensitive to heat, cold and drying. This means that treatment of people has the potential to eliminate this species from the community. Prociv (26) found that, in children of an Australian Indigenous

community, prevalence of *S. stercoralis* was reduced from 26% to below 6% six months after thiabendazole treatment. This level was sustained for at least the ensuing three years. He concluded that sustained chemotherapy would have eliminated *S. stercoralis* from the community without any change in living conditions.

In situations where there is safe water, effective sanitation and good personal hygiene, an infected person does not pass on the infection. This has been shown to be true in a study of ex-prisoners of war infected with *S. stercoralis* during World War 2, none of whom gave the infection to their wives (86).

Specific IgG testing is valuable in identifying people with chronic strongyloidiasis in the communities where it is endemic, because in this situation most infected people have the chronic form of the disease.

Community control should take place in the overall context of other diseases that are present in the community. In Papua New Guinea, other parasitic diseases include *Plasmodium* spp, *Wuchereria bancrofti*, *Strongyloides kellyi*, *Necator americanus*, *Ascaris lumbricoides*, *Trichuris trichiura*, *Sarcoptes scabiei* and enteric protozoa. For example, ivermectin treatment should be effective against *Strongyloides* spp, *W. bancrofti* and *Sarcoptes scabiei*, and albendazole for three days is effective against *A. lumbricoides*, *N. americanus* and *T. trichiura*. Because *A. lumbricoides* and *T. trichiura* are persistent in the environment, ongoing treatment would be required to eliminate these from the community.

Conclusion

It is now possible to diagnose and treat *Strongyloides stercoralis* infection. This means that it is possible to identify people with chronic strongyloidiasis and treat them so that they can be relieved of their non-specific symptoms and not become victims of hyperinfective strongyloidiasis at a later date.

Recent studies have shown the value of IgG ELISA serology for identifying people with *Strongyloides* infection and monitoring the effectiveness of anthelmintic treatment. They have also shown that past treatments have been ineffective and that ivermectin is the most effective and expedient treatment

available, particularly when two courses are given.

Failed treatment can usually be identified by retesting with the serum IgG test six months after treatment. Those who return a test that is not low or negative should be retreated. This process can be used to identify people who are resistant to treatment.

Longer-term retesting perhaps at two years, or self-referral when the symptoms return, may be able to identify the few who are negative six months after treatment but are still infected. For this reason, anyone who has ever tested positive for *S. stercoralis* could still have the disease. They should receive prophylactic anthelmintic treatment before being given corticosteroids or any other medication that depresses the immune system.

HTLV-1 patients, those on immunosuppressant drugs and others who are resistant to treatment should receive anthelmintic treatment on a regular basis.

Because the free-living stages are short-lived, it is possible to achieve community control by mass treatment with ivermectin. Safe water and good sanitation alone are not sufficient to eradicate *S. stercoralis*.

ACKNOWLEDGEMENTS

The National Strongyloides Working Group and Professor Rick Speare have made possible the collection of information that comprises this paper. We thank Dr Pietro Caramello, editor of the CD Found Parasitology Atlas 2000 web site, for permission to use his photograph of *S. stercoralis* filariform larvae and Greg Stehle and Ella Fairbairn for preparing the life cycle diagram.

This paper is dedicated to the memory of Dr Helena Vrbova.

REFERENCES

- 1 **Grove DI.** Historical introduction. In: Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. London: Taylor & Francis, 1989.
- 2 **Genta RM.** Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Rev Infect Dis* 1989;11:755-767.
- 3 **Ewers WH, Jeffrey WT.** *Parasites of Man in Niugini*. Brisbane: Jacaranda Press, 1971.
- 4 **Kelly A.** *Alimentary Parasites of Man in Papua New Guinea*. Goroka: Papua New Guinea Institute of Medical Research, 1974.
- 5 **Speare R.** *Strongyloides stercoralis*: the parasite. Second National Strongyloidiasis Workshop, Brisbane, 25-26 Jul 2003. URL, www.jcu.edu.au/school/phtm/PHTM/ss/acrrm-cd/01_S.stercoralis_parasite_RS.pps
- 6 **Scowden EB, Schaffner W, Stone WJ.** Overwhelming strongyloidiasis: an unappreciated opportunistic infection. *Medicine* 1978;57:527-544. http://www.ards.com.au/Strongy/09_Scowden.pdf
- 7 **Genta RM.** Disregulation of strongyloidiasis: a new hypothesis. *Clin Microbiol Rev* 1992;5:345-355.
- 8 **Speare R, Durrheim D, White S.** Fatal strongyloidiasis: lessons from the literature. Second National Strongyloidiasis Workshop, Brisbane, 2003. URL, www.jcu.edu.au/school/phtm/PHTM/ss/acrrm-cd/06_Fatal_strongyloidiasis_RS.pps
- 9 **Takao S, Ishida T, Bhatia KK, Saha N, Soemantri A, Kayame OW.** Seroprevalence of human T-lymphotropic virus type 1 in Papua New Guinea and Irian Jaya measured using different Western blot criteria. *J Clin Virol* 2000;16:129-133.
- 10 **Porto AF, Neva FA, Bittencourt H, Lisboa W, Thompson R, Alcântara L, Carvalho EM.** HTLV-1 decreases Th2 type of immune response in patients with strongyloidiasis. *Parasite Immunol* 2001;23:503-507.
- 11 **Shikiya K, Zaha O, Niimura S, Uehara T, Ohshiro J, Kinjo F, Saito A, Asato R.** Clinical study on ivermectin against 125 strongyloidiasis patients. [Jp] *Kansenshogaku Zasshi* 1994;68:13-20.
- 12 **Terashima A, Alvarez H, Tello R, Infante R, Freedman DO, Gotuzzo E.** Treatment failure in intestinal strongyloidiasis: an indicator of HTLV-I infection. *Int J Infect Dis* 2002;6:28-30.
- 13 **Hutchinson P.** Strongyloidiasis: an investigation into prevalence. Second National Strongyloidiasis Workshop, Brisbane, 2003. URL, www.jcu.edu.au/school/phtm/PHTM/ss/acrrm-cd/07_Prevalence_PH.pps
- 14 **Dreyer G, Fernandez-Silva E, Alves S, Rocha A, Albuquerque R, Addiss D.** Patterns of detection of *Strongyloides stercoralis* in stool specimens: implications for diagnosis and clinical trials. *J Clin Microbiol* 1996;34:2569-2571. <http://jcm.asm.org/cgi/reprint/34/10/2569>
- 15 **Sato Y, Kobayashi J, Toma H, Shiroma Y.** Efficacy of stool examination for detection of *Strongyloides* infection. *Am J Trop Med Hyg* 1995;53:248-250.
- 16 **Page WA, Dempsey K, McCarthy JS.** Utility of serological follow-up of chronic strongyloidiasis after anthelmintic chemotherapy. *Trans R Soc Trop Med Hyg* 2006;100:1056-1062. http://www.ards.com.au/Strongy/08_Page_Dempsey_McCarthy.pdf
- 17 **Galliard H.** Recherches sur l'infestation expérimentale à *Strongyloides stercoralis* au Tonkin (XII). *Ann Parasitol Hum Comp* 1951;26:201-227. http://www.ards.com.au/Strongy/23_Galliard_1950.pdf
- 18 **Pawlowski ZS.** Epidemiology, prevention and control. In: Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. London: Taylor & Francis, 1989.
- 19 **Flannery G, White N.** Immunological parameters in northeast Arnhem Land Aborigines: consequences of changing settlement and lifestyles. In: Schell LM, Smith MT, Bilsborough AB, eds. *Urban Ecology and Health in the Third World*.

- Cambridge: Cambridge University Press, 1993:202-220.
http://www.ards.com.au/Strongy/01_FlanneryG_WhiteN.pdf
- 20 **Sampson I, Smith DW, MacKenzie B.** Serological diagnosis of *Strongyloides stercoralis* infection. Second National Strongyloidiasis Workshop, Brisbane, 25-26 Jul 2003.
www.jcu.edu.au/school/phtm/PHTM/ss/acrrm-cd/02_Serological-Dx-Ss-IS.pps
 - 21 **Van Ingen L.** Strongyloidiasis in an island community. Progress of a treatment programme. Second National Strongyloidiasis Workshop, Brisbane, 2003.
www.jcu.edu.au/school/phtm/PHTM/ss/acrrm-cd/10_Island_Community_control_LVI.pps
 - 22 **Page W, Dempsey K.** Report on Miwatj Strongyloidiasis Study. Implementing best practice in the eradication of chronic strongyloidiasis for clients of Miwatj Health Aboriginal Corporation. Report to Centre for Remote Health, Alice Springs, 2004.
http://www.ards.com.au/Strongy/04_Miwatj_Strongyloidiasis_Study_Report.pdf
 - 23 **Lord R.** *Strongyloides* serology from Woorabinda. Third National Strongyloidiasis Workshop, Yeppoon, 10-11 Jun 2005.
http://www.ards.com.au/Strongy/39_LordR.pdf
 - 24 **Cooper J.** Strongyloidiasis in Northern New South Wales. Fourth National Workshop on Strongyloidiasis, Adelaide, 11-12 Jul 2007.
<http://www.jcu.edu.au/school/phtm/PHTM/ss/4natwork/4-Cooper.pps>
 - 25 **Woods R, Page W.** *Strongyloides* near Kuranda: elements of a strategic plan to control strongyloides. Fourth National Workshop on Strongyloidiasis, Adelaide, 11-12 Jul 2007.
<http://www.jcu.edu.au/school/phtm/PHTM/ss/4natwork/8-Woods.pps>
 - 26 **Prociv P, Luke R.** Observations on strongyloidiasis in Queensland Aboriginal communities. *Med J Aust* 1993;158:160-163. http://www.ards.com.au/Strongy/05_Prociv_Luke.pdf
 - 27 **Aland K.** Worm project at Galiwin'ku. *Working Together* 1996;6(6):10. http://www.ards.com.au/Strongy/02_Aland_1996.pdf
 - 28 **Einsiedel L.** Strongyloidiasis and HTLV-1 infection: a dangerous liaison in Central Australia. Fourth National Workshop on Strongyloidiasis, Adelaide, 11-12 Jul 2007.
<http://www.jcu.edu.au/school/phtm/PHTM/ss/4natwork/5-Einsiedel.pps>
 - 29 **Zaman V.** Atlas of Medical Parasitology. Sydney: Adis Press, 1978.
 - 30 **Grove DI, Warton A, Yu LL, Northern C, Papadimitriou JM.** Light and electron microscopical studies of the location of *Strongyloides stercoralis* in the jejunum of the immunosuppressed dog. *Int J Parasitol* 1987;17:1257-1265.
 - 31 **Schad GA, Aikens LM, Smith G.** *Strongyloides stercoralis*: is there a canonical migratory route through the host? *J Parasitol* 1989;75:740-749.
 - 32 **Keiser PB, Nutman TB.** *Strongyloides stercoralis* in the immunocompromised population. *Clin Microbiol Rev* 2004;17:208-217.
<http://cmr.asm.org/cgi/content/full/17/1/208>
 - 33 **Yamada M, Matsuda S, Nakazawa M, Arizono N.** Species-specific differences in heterogonic development of serially transferred free-living generations of *Strongyloides planiceps* and *Strongyloides stercoralis*. *J Parasitol* 1991;77:592-594.
 - 34 **Schad GA.** Morphology and life history of *Strongyloides stercoralis*. In: Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. London: Taylor & Francis, 1989:85-104.
 - 35 **Pattison D, Speare R.** Strongyloidiasis in military and police contingents of the Regional Assistance Mission to Solomon Islands (RAMSI). Fourth National Strongyloidiasis Workshop, Adelaide, 11-12 Jul 2007.
<http://www.jcu.edu.au/school/phtm/PHTM/ss/4natwork/7-Pattison.pps>
 - 36 **Kukuruzovic R, Robins-Browne RM, Anstey NM, Brewster DR.** Enteric pathogens, intestinal permeability and nitric oxide production in acute gastroenteritis. *Pediatr Infect Dis J* 2002;21:730-739.
 - 37 **Grove DI.** Clinical manifestations. In: Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. London: Taylor & Francis, 1989:155-174.
http://www.ards.com.au/Strongy/13_Grove_Strongyloidiasis_Clinical.pdf
 - 38 **Schad GA, Thompson F, Talham G, Holt D, Nolan TJ, Ashton FT, Lange AM, Bhopale VM.** Barren female *Strongyloides stercoralis* from occult chronic infections are rejuvenated by transfer to parasite-naïve recipient hosts and give rise to an autoinfective burst. *J Parasitol* 1997;83:785-791.
http://www.ards.com.au/Strongy/32_Schad.pdf
 - 39 **Carvalho EM, Andrade TM, Andrade JA, Rocha H.** Immunological features in different clinical forms of strongyloidiasis. *Trans R Soc Trop Med Hyg* 1983;77:346-349.
 - 40 **Kerepesi LA, Nolan TJ, Schad GA, Lustigman S, De'Broski RH, Keiser PB, Nutman TB, Krolewiecki AJ, Abraham D.** Human immunoglobulin G mediates protective immunity and identifies protective antigens against larval *Strongyloides stercoralis* in mice. *J Infect Dis* 2004;189:1282-1290.
 - 41 **Grove DI.** Human strongyloidiasis. *Adv Parasitol* 1996;38:251-309.
 - 42 **Brigandi RA, Rotman HL, Leon O, Nolan TJ, Schad GA, Abraham D.** *Strongyloides stercoralis* host-adapted third-stage larvae are the target of eosinophil-associated immune-mediated killing in mice. *J Parasitol* 1998;84:440-445.
 - 43 **Atkins NS, Conway DJ, Lindo JF, Bailey JW, Bundy DAP.** L3 antigen-specific antibody isotype responses in human strongyloidiasis: correlations with larval output. *Parasite Immunol* 1999;21:517-526.
 - 44 **Gill GV, Beeching NJ, Khoo S, Bailey JW, Partridge S, Blundel JW, Luksza AR.** A British Second World War veteran with disseminated strongyloidiasis. *Trans R Soc Trop Med Hyg* 2004;98:382-386.
 - 45 **Lim L, Biggs BA.** Fatal disseminated strongyloidiasis in a previously treated patient. *Med J Aust* 2001;174:355-356.
http://www.ards.com.au/Strongy/10_lim&biggs_MJA_174_2001.pdf
 - 46 **Einsiedel L, Spelman D.** *Strongyloides stercoralis*: risks posed to immigrant patients in an Australian tertiary referral centre. *Intern Med J* 2006;36:632-637.
 - 47 **Kishimoto K, Hokama A, Hirata T, Ihama Y, Nakamoto M, Kinjo N, Kinjo F, Fujita J.** Endoscopic and histopathological study on the duodenum of *Strongyloides stercoralis* hyperinfection. *World J Gastroenterol* 2008;14:1768-1773.

- <http://www.wjgnet.com/1007-9327/14/1768.asp>
- 48 **Ligas JA, Kerepesi LA, Galioto AM, Lustigman S, Nolan TJ, Schad GA, Abraham D.** Specificity and mechanism of immunoglobulin M (IgM)- and IgG-dependent protective immunity to larval *Strongyloides stercoralis* in mice. *Infect Immun* 2003;71:6835-6843.
 - 49 **Atkins NS, Lindo JF, Lee MG, Conway DJ, Bailey JW, Robinson RD, Bundy DAP.** Humoral responses in human strongyloidiasis: correlations with infection chronicity. *Trans R Soc Trop Med Hyg* 1997;91:609-613.
 - 50 **Ravi V, Ramachandran S, Thompson RW, Andersen JF, Neva FA.** Characterization of a recombinant immunodiagnostic antigen (NIE) from *Strongyloides stercoralis* L3-stage larvae. *Mol Biochem Parasitol* 2002;125:73-81.
 - 51 **Conway DJ, Lindo JF, Robinson RD, Bundy DAP.** Towards effective control of *Strongyloides stercoralis*. *Parasitol Today* 1995;11:420-424. http://www.ards.com.au/Strongy/27_Conway_Parasitology_1995.pdf
 - 52 **Satoh M, Kiyuna S, Shiroma Y, Toma H, Kokaze A, Sato Y.** Predictive markers for development of strongyloidiasis in patients infected with both *Strongyloides stercoralis* and HTLV-1. *Clin Exp Immunol* 2003;133:391-396.
 - 53 **Grove DI.** Fifteen years research into strongyloidiasis. Fourth National Strongyloidiasis Workshop, Adelaide, 11-12 Jul 2007. <http://www.jcu.edu.au/school/phtm/PHTM/ss/4nawork/1-Grove.pps>
 - 54 **Tribouley-Duret J, Tribouley J, Appriou M, Megraud RN.** Diagnosis of strongyloidiasis using E.L.I.S.A. test. (Fr) *Ann Parasitol Hum Comp* 1978;53:641-648.
 - 55 **Carroll SM, Karthigas KT, Grove DI.** Serodiagnosis of human strongyloidiasis by an enzyme-linked immunosorbent assay. *Trans R Soc Trop Med Hyg* 1981;75:706-709.
 - 56 **Leydon J.** Comparison of strongyloides antibody levels in South-East Asian refugees before and after treatment for presumed strongyloidiasis using an in-house and a commercial enzyme immunoassay. Fourth National Strongyloidiasis Workshop, Adelaide, 11-12 Jul 2007. <http://www.jcu.edu.au/school/phtm/PHTM/ss/4nawork/4-Leydon.pps>
 - 57 **Sudarshi S, Stümpfle R, Armstrong M, Ellman T, Parton S, Krishnan P, Chiodini PL, Whitty CJM.** Clinical presentation and diagnostic sensitivity of laboratory tests for *Strongyloides stercoralis* in travellers compared with immigrants in a non-endemic country. *Trop Med Int Health* 2003;8:728-732.
 - 58 **Sato Y, Inoue F, Matsuyama R, Shiroma Y.** Immunoblot analysis of antibodies in human strongyloidiasis. *Trans R Soc Trop Med Hyg* 1990;84:403-416.
 - 59 **Genta RM.** Immunology. In: Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. London: Taylor & Francis, 1989:133-153.
 - 60 **Fisher D, McCarry F, Currie B.** Strongyloidiasis in the Northern Territory. Under-recognised and under-treated? *Med J Aust* 1993;159:88-90.
 - 61 **Grove DI.** Treatment. In: Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. London: Taylor & Francis, 1989:199-231.
 - 62 **Kobayashi J, Sato Y, Toma H, Takara M, Shiroma Y.** Application of enzyme immunoassay for postchemotherapy evaluation of human strongyloidiasis. *Diagn Microbiol Infect Dis* 1994;18:19-23.
 - 63 **Loufty MR, Wilson M, Keystone JS, Kain KC.** Serology and eosinophil count in the diagnosis and management of strongyloidiasis in a non-endemic area. *Am J Trop Med Hyg* 2002;66:749-752.
 - 64 **Karunajeewa H, Kelly H, Leslie D, Leydon J, Saykao P, Biggs BA.** Parasite-specific IgG response and peripheral blood eosinophil count following albendazole treatment for presumed chronic strongyloidiasis. *J Travel Med* 2006;13:84-91.
 - 65 **Datry A, Hilmarsdottir I, Mayorga-Safastume R, Lyagoubi M, Gaxotte P, Biligui S, Chodakewitz J, Neu D, Danis M, Gentilini M.** Treatment of *Strongyloides stercoralis* infection with ivermectin compared with albendazole: results on an open study of 60 cases. *Trans R Soc Trop Med Hyg* 1994;88:344-345. http://www.ards.com.au/Strongy/31_Datry.pdf
 - 66 **Archibald LK, Beeching NJ, Gill GV, Bailey JW, Bell DR.** Albendazole is effective treatment for chronic strongyloidiasis. *Q J Med* 1993;86:191-195.
 - 67 **Mukerjee CM, Carrick J, Walker JC, Woods RL.** Pulmonary strongyloidiasis presenting as chronic bronchitis leading to interlobular septal fibrosis and cured by treatment. *Respirology* 2003;8:536-540.
 - 68 **Looke D.** A case of *Strongyloides stercoralis* hyperinfection syndrome: the use of parenteral ivermectin and the development of an assay for serum ivermectin determination. Third National Strongyloidiasis Workshop, Yeppoon, 10-11 Jun 2005.
 - 69 **Davis JS, Currie BJ, Fisher DA, Huffarn SE, Anstey NM, Price RN, Krause VL, Zweck N, Lawton PD, Snelling PL, Selvanayagam S.** Prevention of opportunistic infections in immunosuppressed patients in the tropical Top End of the Northern Territory. *NT Dis Control Bull* 2004;11:7-13. <http://www.healthconnect.gov.au/internet/main/publishing.nsf/Content/cda-pubs-cdi-2003-cdi2704-htm-cdi2704s.htm>
 - 70 **Satoh M, Toma H, Kiyuna S, Shiroma Y, Kokaze A, Sato Y.** Association of a sex-related difference of *Strongyloides stercoralis*-specific IgG4 antibody titer with the efficacy of treatment of strongyloidiasis. *Am J Trop Med Hyg* 2004;71:107-111.
 - 71 **Shelhamer JH, Neva FA, Finn DR.** Persistent strongyloidiasis in an immunodeficient patient. *Am J Trop Med Hyg* 1982;31:746-751.
 - 72 **Fowler CG, Lindsay I, Levin J, Sweny P, Fernando ON, Moorhead JF.** Recurrent hyperinfestation with *Strongyloides stercoralis* in a renal allograft recipient. *Br Med J (Clin Res)* 1982;285:1394.
 - 73 **Satoh M, Toma H, Sato Y, Takara M, Shiroma Y, Kiyuna S, Hirayama K.** Reduced efficacy of treatment of strongyloidiasis in HTLV-1 carriers related to enhanced expression of IFN-gamma and TGF-beta1. *Clin Exp Immunol* 2002;127:354-359. <http://www.ingentaconnect.com/content/bsc/cei/2002/00000127/00000002/art00024>
 - 74 **Hirata T, Uchima N, Kishimoto K, Zaha O, Kinjo N, Hokama A, Sagugawa H, Kinjo F, Fujita J.** Impairment of host immune response against *Strongyloides stercoralis* by human T cell lymphotropic virus type 1 infection. *Am J Trop Med Hyg* 2006;74:246-249.
 - 75 **Taylor HR.** Stemming the tide of river blindness: the early years of ivermectin. *Med J Aust* 2003;179:617-619.
 - 76 **Pacqué M, Muñoz B, Poetschke G, Foese J,**

- Greene BM, Taylor HR.** Pregnancy outcome after inadvertent ivermectin treatment during community-based distribution. *Lancet* 1990;336:1486-1489.
- 77 **Pion DS, Gardon J, Kamgno J, Gardon-Wendel N, Chippaux JP, Boussinesq M.** Structure of the microfilarial reservoir of *Loa loa* in the human host and its implications for monitoring the programmes of community-directed treatment with ivermectin carried out in Africa. *Parasitology* 2004;129:613-626.
- 78 **Gotuzzo E, Terashima A, Alvarez H, Tello R, Infante R, Watts DM, Freedman DO.** *Strongyloides stercoralis* hyperinfection associated with human T cell lymphotropic virus type-1 infection in Peru. *Am J Trop Med Hyg* 1999;60:146-149.
- 79 **Porto AF, Santos SB, Muniz AL, Basilio V, Rodrigues W Jr, Neva FA, Dutra WO, Gollob KJ, Jacobson S, Carvalho EM.** Helminthic infection down-regulates type 1 immune responses in human T cell lymphotropic virus type 1 (HTLV-1) carriers and is more prevalent in HTLV-1 carriers than in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis. *J Infect Dis* 2005;191:612-618.
- 80 **Carvalho EM, Da Fonseca Porto A.** Epidemiological and clinical interaction between HTLV-1 and *Strongyloides stercoralis*. *Parasite Immunol* 2004;26:487-497.
- 81 **Feachem RG, Bradley DJ, Garelick H, Mara DD.** *Strongyloides* and strongyloidiasis. In: Sanitation and Disease: Health Aspects of Excreta and Waste Water Management. New York: Wiley, 1983:457-461.
<http://www.personal.leeds.ac.uk/~cen6ddm/SanitationDisease/sandis33.pdf>
- 82 **Eveland LK, Kenney M, Yermakov V.** Laboratory diagnosis of autoinfection in strongyloidiasis. *Am J Clin Pathol* 1975;63:421-425.
- 83 **Sing A, Leitritz L, Bogner JR, Heesemann J.** First-glance diagnosis of *Strongyloides stercoralis* autoinfection by stool microscopy. *J Clin Microbiol* 1999;37:1610-1611.
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=84850>
- 84 **Lindo JF, Robinson RD, Terry SI, Vogel P, Gam AA, Neva FA, Bundy DAP.** Age-prevalence and household clustering of *Strongyloides stercoralis* infection in Jamaica. *Parasitology* 1995;110:97-102.
http://www.ards.com.au/Strongy/24_Lindo_1995.pdf
- 85 **Conway DJ, Hall A, Anwar KS, Rahman ML, Bundy DAP.** Household aggregation of *Strongyloides stercoralis* infection in Bangladesh. *Trans R Soc Trop Med Hyg* 1995;89:258-261.
- 86 **Grove DI.** Strongyloidiasis: is it transmitted from husband to wife? *Br J Vener Dis* 1982;58:271-272.

The relationship between undernutrition and humoral immune status in children with pneumonia in Papua New Guinea

ALLAN W. CRIPPS¹, DIANA C. OTCZYK¹, JANE BARKER^{2,3}, DEBORAH LEHMANN^{2,4} AND MICHAEL P. ALPERS^{2,5}

School of Medicine, Griffith University, Gold Coast, Queensland, Australia, Papua New Guinea Institute of Medical Research, Goroka, Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, Perth and Curtin University of Technology, Perth, Western Australia

SUMMARY

Malnutrition is a significant risk factor for childhood infectious diseases in developing countries, including Papua New Guinea (PNG). Whilst the mechanisms are not fully understood there is little doubt that impairment of immune function is a major contributing factor in enhancing disease susceptibility in malnourished children. This susceptibility has been clearly shown for pneumonia in PNG. The aim of this study was to examine the effect of undernutrition on the humoral immune profile in children less than 60 months of age with pneumonia. The study was cross-sectional with measurements of nutritional status and parameters of the immune response being assessed simultaneously. The children were grouped according to age for the purpose of comparative analysis. The children were from the Goroka region of the Eastern Highlands Province of PNG and had been admitted to hospital with moderate-severe pneumonia. They were classified as undernourished (less than 80% weight for age) or nourished (greater than or equal to 80% weight for age). Serum albumin, IgG, IgA and IgM and salivary albumin and IgA were measured. Antibodies to nontypeable *Haemophilus influenzae* outer membrane protein and *Escherichia coli* O antigen were also determined in serum and saliva. Undernourished children aged less than 49 months had lower levels of serum albumin than nourished children throughout this age range. Lower values of salivary IgA were observed in infants (less than 13 months of age) than in older children, with a larger proportion of younger children having no detectable IgA. The age-related immunological profile was similar in undernourished and nourished children. At different age intervals the concentration of immunoglobulins in serum and saliva from undernourished children was generally found to be less than or the same as that from nourished children. In most cases undernourished children had lower levels of specific antibodies than nourished children but for some antibodies in some age groups the levels in the undernourished were higher. In conclusion, undernutrition was associated with hypoalbuminaemia and reduced humoral immune responses in children with pneumonia but its immunological effects varied with age in an unpredictable way.

¹ School of Medicine, Griffith Health, Gold Coast Campus, Griffith University, Queensland 4222, Australia
allan.cripps@griffith.edu.au

² Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, Eastern Highlands Province 441, Papua New Guinea

³ Present address: Taylors Road, Eureka, New South Wales 2480, Australia

⁴ Present address: Division of Population Sciences, Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, PO Box 855, West Perth, Western Australia 6872, Australia

⁵ Centre for International Health, ABCRC, Shenton Park Campus, Curtin University of Technology, GPO Box U1987, Western Australia 6845, Australia

Introduction

Maternal and child undernutrition accounts for 35% of deaths in children younger than 5 years and 11% of the total global disease burden (1). Most of this burden is borne by developing countries. Intrauterine growth restriction and child malnutrition together lead to 2.2 million deaths and 21% of disability-adjusted life-years (DALYs) in children under 5 years (1). Malnutrition impacts on the body's ability to function properly, with particularly deleterious effects in young children. For many malnourished children, inadequate food intake is secondary to endemic chronic infectious diseases. Diseases such as pneumonia, malaria, tuberculosis, diarrhoea and parasitic infections constitute the main burden of ill health among under-five children in developing countries (2). Significantly, nearly half of the deaths due to these diseases each year are associated with malnutrition (3). Indeed, the relationship between malnutrition and a higher risk of respiratory and enteric infections has been well established (4). What is becoming most evident is that the profound interactions between the pathogenicity of malnutrition, disturbances in the ontogeny of the immune system and chronic antigenic exposure from infections may have a more significant impact on child death than the direct sequelae of the nutritional deficiencies themselves.

Malnutrition is an important cause of impaired immune competence. The effect of undernutrition on the ontogeny of the immune system of the fetus during gestation and neonatal maturation may lead to deficits in immunity in infancy and early childhood. The impairments have been reported in several aspects of immunity such as cell-mediated immune responses (5-10), the complement system (5,6,10-12) and phagocytic function (13). When the immature and compromised immune system of an undernourished child is challenged by chronic and repeated infections, there is a further weakening of the immune response as evidenced by altered immune cell populations (14-18) and a generalized increase in pro-inflammatory cytokines (19). The overall effects might include altered mucosal immune defences and compromised barrier function against invasion by pathogens (20). The results concerning immunoglobulin levels and antibody synthesis to common pathogens have been variable, with depressed, normal or elevated levels being reported

(9,10,12,18,21-27).

Most of the previously published studies have involved severely malnourished children. Whilst the highest risk of mortality is associated with the most severely underweight children, in developing countries children with mild to moderate underweight status constitute the greater burden of disease because of their high prevalence (3). Whilst there is little doubt that severe nutritional stress impairs immune function, the evidence that mild to moderate malnutrition does so remains controversial. Papua New Guinea (PNG) has a significant number of children below 80% weight for age, who are classified as undernourished (28). In PNG, malnutrition accounts for 34% of deaths in children under 5 years of age whilst very low birthweight from intrauterine growth restriction is responsible for more than half of neonatal mortality (29). Pneumonia is the leading cause of death (29), with low birthweight (30), undernutrition (31-33), poor infant feeding practices (34) and depressed cell-mediated immunity (35) as significant contributing factors. Previous studies have shown that, as elsewhere in the world, *Streptococcus pneumoniae* and *Haemophilus influenzae* are the most significant bacterial pathogens in pneumonia (29,33,36,37). In PNG, children acquire *S. pneumoniae* and *H. influenzae* at an extremely early age, with all children being colonized by 3 months of age (38). A previous study suggested that early colonization may result in a 'high zone' tolerance to infection by *H. influenzae* (39). The present study was conducted to examine age-related profiles of the humoral immune responses in children with pneumonia from the Goroka area in the Eastern Highlands Province (EHP) of PNG, where heavy bacterial colonization is the norm (38). All children included in the study had moderate-severe pneumonia. The relationship between undernutrition and humoral immune status was examined in these children.

Methods

Subjects

238 children aged 6 weeks to 60 months who were admitted to the Goroka Base Hospital, EHP, PNG with a clinical diagnosis of moderate or severe pneumonia were enrolled in the study. The study children were grouped into 6 age intervals: 6 weeks-6 months, 7-12 months, 13-24 months, 25-36

months, 37-48 months and 49-60 months.

Nutritional assessment

On admission to hospital the children were weighed with minimal clothing. The children were classified as undernourished (less than 80% weight for age) or nourished (greater than or equal to 80% weight for age) (28). This is the standard method of assessing and following the nutritional status of children in PNG, though in research studies the Waterlow classification is now more commonly used (40,41).

Sample collection

Saliva (1 ml) was collected by gentle suction and blood (3 ml) by venepuncture. The samples were transported to the laboratory and serum was prepared from the blood sample. The saliva and serum were stored frozen until assayed.

Quantitation of serum albumin, IgG, IgA and IgM and salivary albumin and IgA

Serum albumin, IgG, IgA and IgM were measured by rate nephelometry. Salivary albumin and IgA were measured by electroimmunodiffusion (42). Contamination of saliva samples with breastmilk was identified by immunoelectrophoresis. Any sample containing breastmilk was excluded from the analysis.

Measurement of specific antibodies in serum and saliva

Isotype-specific antibodies to nontypeable *H. influenzae* (NTHi) outer membrane protein (OMP) and *Escherichia coli* O antigen were determined by enzyme-linked immunosorbent assay (ELISA) (39,43).

Statistical analysis

For the purpose of analysis the children were grouped into 6 age groups: ≤ 6 months, 7-12 months, 13-24 months, 25-36 months, 37-48 months and 49-60 months. The saliva data contained numerous values that were below the detectable limit of the assays. Hence, for saliva, the medians and interquartile ranges of positive values in each age group are presented, as well as the percentage of 'zero' IgA values. Statistical differences between the values in serum and saliva for the two groups were assessed by

the Mann-Whitney test or the Kruskal-Wallis test for more than two groups.

Ethical approval

The study was approved by the Medical Research Advisory Committee of Papua New Guinea through the pneumonia research program of the PNG Institute of Medical Research.

Results

Serum albumin, immunoglobulins and specific antibodies

The median levels of albumin, immunoglobulins, NTHi OMP antibodies and *E. coli* antibodies are presented in Table 1.

Serum albumin

Undernourished children less than 49 months of age had lower levels of serum albumin than nourished children (≤ 6 months, 7-12 months and 13-24 months, $p < 0.001$; 25-36 months and 37-48 months, $p < 0.05$). The serum albumin levels were the same in undernourished and nourished children in the 49-60 month age group.

Serum immunoglobulins

IgA: The IgA level in serum was significantly lower in undernourished children aged 7-12 months than in nourished children of the same age ($p < 0.001$). In the other age groups there was no difference between the levels in the nourished and the undernourished children.

IgG: At 25-36 months of age the level of serum IgG in undernourished children was lower than that observed in nourished children ($p < 0.05$). The differences between the levels in the other age groups were not considered to be significant.

IgM: The level of IgM in the serum of undernourished children was lower than in nourished children at the ages of ≤ 6 months ($p < 0.05$), 25-36 months ($p < 0.05$) and 37-48 months ($p < 0.001$).

Serum NTHi OMP and E. coli antibodies

IgA: The level of serum NTHi OMP-specific IgA antibody was higher in undernourished children 49-60 months of age than in nourished children ($p < 0.05$). The differences

between the levels in the other age groups were not considered to be significant. At 7-12 months of age the level of serum *E. coli*-specific IgA antibody was lower in undernourished than in nourished children ($p < 0.05$).

IgG: At 7-12 months and 37-48 months of age the levels of serum NTHi OMP-specific IgG antibody were lower in undernourished than in nourished children ($p < 0.05$ and $p < 0.002$, respectively) and in the other age groups the differences were not considered to be significant. Similar results were found with *E. coli*-specific IgG antibody: at 7-12 months and 37-48 months of age the levels of antibody were lower in undernourished than in nourished children ($p < 0.05$).

IgM: The level of NTHi OMP-specific IgM antibody was higher in undernourished than in nourished children at 7-12 months of age ($p < 0.01$). Serum *E. coli*-specific IgM antibody was lower in undernourished than in nourished children aged 13-24 months ($p < 0.05$), 37-48 months ($p < 0.05$) and 49-60 months ($p < 0.01$). However, at 25-36 months of age the level of *E. coli*-specific IgM antibody was higher in the undernourished children ($p < 0.05$).

Salivary albumin, IgA and IgA-specific antibodies

The median levels of positive values of albumin, IgA, NTHi OMP-specific IgA antibody and *E. coli*-specific IgA antibody and the proportion with no detectable amounts of IgA in saliva are presented in Table 2.

No significant differences were observed between undernourished and nourished children in the levels of albumin in saliva. However, at ≤ 6 months of age the level of albumin in saliva in both undernourished and nourished children was lower than in older age groups.

The level of IgA in positive samples of saliva was lower in undernourished than in nourished children at ≤ 6 months ($p < 0.05$) and 49-60 months ($p < 0.02$) of age. The level of salivary IgA in both nourished and undernourished children increased from ≤ 6 months of age to 13-24 months ($p < 0.05$) and then tended to increase with age in the nourished children and decrease in the undernourished. At ≤ 6 months of age approximately 80% of the children irrespective

of nutritional status had no detectable salivary IgA. This proportion decreased with age in both groups of children. However, after 6 months of age, there was always a greater proportion of undernourished children with no detectable salivary IgA.

The level of *E. coli*-specific IgA antibody in saliva was significantly lower in undernourished than in nourished children at 7-12 months of age ($p < 0.005$). There was an increase in *E. coli*-specific IgA antibody in the nourished children between the ages of ≤ 6 months and 7-12 months ($p < 0.05$) followed by a decline at 13-24 months ($p = 0.002$).

Discussion

It is generally accepted that undernourished children are more susceptible to infections than nourished children; this is mediated in large by an impaired immune function. The results concerning immunoglobulin and antibody synthesis, however, have been highly variable and nonspecific (9,10,12,18,21-27), with the majority of these investigations dealing with the severe forms of malnutrition, ie, marasmus and kwashiorkor (9,12,18,21,25-27). The few studies that have looked at mild malnutrition and its impact on humoral immune function have been less than convincing (10,22-24). These inconsistencies may be associated with inadequately characterized population groups. There has been some suggestion that various aspects of the immune response are affected in different ways depending on the severity and type of malnutrition (7,8), the presence of micronutrient deficiencies (44,45), the age of the children studied (27) and the existence and type of infection (9,10,15,16,17,46).

The mucosal immune system begins to develop very shortly after conception; however, human beings are born with a structurally complete but functionally immature and inexperienced mucosal immune system (47). Environmental influences such as nutrition (48,49), feeding practices (50) and intestinal colonization with microflora (51,52) play an important role in influencing the developing human immune system. The immunological abnormalities resulting from intrauterine malnutrition may persist for several months after birth (48) and may compromise postnatal immunity and host resistance to infection. The risk of

TABLE 1

MEDIAN CONCENTRATION OF SERUM ALBUMIN, IgA, IgG, IgM, NTHi OMP-SPECIFIC ANTIBODY (IgA, IgG, IgM) AND E. COLI-SPECIFIC ANTIBODY (IgA, IgG, IgM) IN NOURISHED AND UNDERNOURISHED CHILDREN AT DIFFERENT AGES

Serum	≤6 months	7-12 months	13-24 months	25-36 months	37-48 months	49-60 months							
	n	n	n	n	n	n							
Albumin (mg/l)	Nourished	34.5 ^a (30.0-41.5)	36	39.0 ^a (29.0-46.0)	20	28.5 ^a (20.8-37.3)	20	39.0 ^d (34.5-46.0)	17	42.0 ^d (22.5-42.5)	9	34.0 (28.5-42.5)	14
	Undernourished	25.0 ^a (17.0-34.3)	16	27.0 ^a (22.5-34.5)	19	16.5 ^a (15.0-19.8)	20	30.0 ^d (23.5-38.0)	17	20.0 ^d (17.0-26.5)	9	34.0 (30.3-39.5)	6
IgA (mg/l)	Nourished	1.0 (0.6-1.6)	36	1.2 ^a (0.8-1.8)	20	0.6 (0.4-1.0)	20	1.0 (0.6-1.2)	17	1.3 (0.7-2.0)	7	1.8 (1.4-2.0)	9
	Undernourished	1.1 (0.9-1.2)	16	0.7 ^a (0.4-0.9)	20	0.8 (0.5-1.1)	20	1.1 (0.8-1.6)	17	1.6 (1.0-2.3)	9	1.2 (1.0-2.1)	6
IgG (mg/l)	Nourished	11.1 (8.6-15.8)	36	13.9 (8.0-14.8)	20	15.1 (9.5-21.1)	20	16.8 ^d (12.6-20.1)	18	9.3 (7.2-11.0)	9	17.4 (12.6-25.5)	11
	Undernourished	11.1 (7.2-15.2)	16	12.1 (8.5-14.5)	20	10.8 (7.2-19.0)	20	12.6 ^d (11.1-15.0)	17	13.5 (9.5-17.4)	9	12.5 (9.0-15.9)	6
IgM (mg/l)	Nourished	1.4 ^d (0.8-1.7)	32	1.9 (1.4-2.4)	20	0.7 (0.6-0.8)	20	1.4 ^d (1.1-1.9)	17	0.5 ^a (0.4-0.9)	9	1.1 (0.8-1.5)	10
	Undernourished	0.8 ^d (0.6-1.1)	16	1.4 (1.0-3.0)	19	0.8 (0.5-2.1)	19	0.8 ^d (0.7-1.7)	17	0.2 ^a (0.2-0.3)	9	0.8 (0.6-0.8)	6
NTHi OMP-specific antibody (EU/ml)													
IgA	Nourished	66.8 (30.0-128.6)	40	90.0 (50.6-240.4)	20	53.3 (24.0-210.8)	20	127.5 (51.8-211.5)	20	684.0 (63.0-911.3)	8	87.0 ^d (54.0-111.0)	19
	Undernourished	75.0 (49.5-155.8)	25	73.2 (48.8-106.5)	20	87.0 (62.3-301.5)	20	90.0 (39.0-204.0)	19	60.0 (40.5-199.5)	9	195.0 ^d (126.0-483.0)	5

IgG	Nourished	19,291 (11,386-34,696)	30	21,997 ^d (13,182-48,903)	19	14,420 (10,746-31,444)	20	7131 (6255-11,733)	18	12,383 ^b (6220-20,768)	10	20,737 (10,085-61,782)	18
	Undernourished	17,018 (14,365-23,736)	14	12,454 ^d (7669-18,934)	19	21,287 (15,672-26,148)	20	10,385 (7055-18,405)	20	5775 ^b (5118-6182)	9	14,022 (11,560-27,861)	6
IgM	Nourished	2224 (1439-4948)	31	1438 ^c (843-2234)	20	2843 (1732-5590)	19	2425 (1237-4883)	10	nd		8460 (2030-12,861)	18
	Undernourished	1946 (1455-2484)	15	2910 ^c (1791-5494)	19	1604 (852-3432)	19	3072 (1319-6264)	20	nd		nd	
E. coli-specific antibody (EU/ml)													
IgA	Nourished	34.8 (10.2-60.0)	30	64.5 ^d (29.1-116.4)	20	40.2 (26.3-69.6)	20	61.5 (17.7-120.0)	20	nd		43.2 (13.2-118.2)	18
	Undernourished	17.0 (8.9-56.1)	24	20.4 ^d (10.8-49.4)	20	43.4 (24.2-63.0)	20	37.2 (18.0-120.0)	19	20.4 (18.9-39.3)	9	nd	
IgG	Nourished	199.0 (106.0-346.3)	24	635.5 ^d (232.4-1310.0)	16	245.6 (117.3-689.5)	15	208.5 (156.1-405.6)	16	562.6 ^d (375.4-845.9)	8	396.7 (177.1-977.0)	13
	Undernourished	144.5 (57.5-235.3)	12	283.7 ^d (130.4-472.4)	17	538.0 (178.3-817.0)	14	564.6 (147.3-918.2)	17	117.1 ^d (82.0-428.1)	9	492.2 (227.2-1402.5)	4
IgM	Nourished	204.0 (105.6-297.3)	30	265.3 (188.6-382.2)	20	330.3 ^d (254.6-455.7)	20	303.4 ^d (157.1-495.0)	19	466.8 ^d (182.5-710.7)	10	1141.2 ^c (691.8-1494.0)	17
	Undernourished	129.0 (97.0-192.3)	14	320.9 (167.8-414.5)	19	165.5 ^d (77.1-407.6)	20	657.6 ^d (238.7-1080.8)	19	160.6 ^d (114.3-280.0)	9	398.1 ^c (100.7-685.1)	6

Median values are reported with the interquartile range in parenthesis

nd = no assays were conducted or insufficient data for analysis

NTHi = nontypeable *Haemophilus influenzae*

OMP = outer membrane protein

EU = ELISA units

Difference between nourished and undernourished children for respective values within an age group:

^a p <0.001; ^b p <0.002; ^c p <0.01; ^d p <0.05

TABLE 2

MEDIAN POSITIVE VALUES OF SALIVARY ALBUMIN, IgA, NTHi OMP-SPECIFIC IgA ANTIBODY, *E. coli*-SPECIFIC IgA ANTIBODY AND THE PERCENTAGE OF ZERO IgA VALUES IN NOURISHED AND UNDERNOURISHED CHILDREN AT DIFFERENT AGES

Saliva	≤6 months	7-12 months	13-24 months	25-36 months	37-48 months	49-60 months						
	n	n	n	n	n	n						
Albumin (mg/l)												
Nourished	3.0* (2.0-15.0)	27	25.0* (21.0-36.5)	13	16.0 (6.8-24.8)	10	18.0 (12.5-42.0)	13	17.0 (15.0-20.5)	9	21.0 (17.3-31.3)	16
Undernourished	2.0* (1.0-12.0)	11	21.0* (6.0-25.0)	7	27.0 (10.0-36.0)	10	18.0 (15.0-23.0)	9	18.0 (14.8-26.0)	10	29.0 (12.0-36.0)	5
IgA (mg/l)												
Nourished	12.0* (8.8-20.8)	6	11.0† (5.0-23.0)	9	27.0*‡ (20.0-31.0)	9	27.0 (12.0-38.3)	8	40.0 (24.5-64.0)	9	40.0 ^b (21.5-52.0)	13
Undernourished	4.0*‡ (3.3-7.5)	5	16.0* (6.0-21.0)	3	40.0† (21.5-45.0)	5	29.5 (16.0-37.5)	8	25.5 (11.0-54.0)	8	7.0 ^b (6.5-25.0)	5
% zero IgA value												
Nourished	81.3	26/32	55.0	11/20	55.0	11/20	27.3	3/11	10.0	1/10	23.5	4/17
Undernourished	78.3	18/23	84.2	16/19	73.7	14/19	57.9	11/19	33.3	4/12	28.6	2/7
NTHi OMP IgA antibody (EU/ml)												
Nourished	50.6 (13.0-163.9)	17	59.9 (42.8-206.2)	14	27.6 (12.9-71.3)	7	16.8 (14.6-222.3)	5	14.6 (9.3-31.3)	4	14.9 (11.6-30.1)	13
Undernourished	35.3 (7.8-56.8)	6	54.6 (43.7-70.4)	7	nd	nd	31.9 (14.1-84.8)	12	13.0 (9.2-33.1)	6	14.7 (10.9-32.4)	3
E. coli IgA antibody (EU/ml)												
Nourished	116.1* (44.7-157.7)	11	268.6 ^{a,*#} (94.7-471.7)	10	32.6 [#] (12.9-54.6)	8	31.2 (17.0-56.6)	5	13.4 (12.5-52.8)	7	30.9 (27.7-50.1)	8
Undernourished	nd	nd	44.4 ^a (18.3-63.1)	5	42.7 (20.8-153.9)	4	40.7 (10.9-64.2)	9	31.7 (13.6-89.3)	5	17.0 (10.6-93.9)	3

Median values are reported with the interquartile range in parenthesis

nd = no assays were conducted or insufficient data for analysis

NTHi = nontypeable *Haemophilus influenzae*

OMP = outer membrane protein

EU = ELISA units

Difference between nourished and undernourished children for respective values within an age group:

^a p < 0.005; ^b p < 0.02; ^c p < 0.05

Difference between age groups for respective values within nourished or undernourished children:

* p < 0.05; [†] p < 0.005; [‡] p = 0.002

undernourishment is heightened in children where breastfeeding practices are suboptimal (53), with an associated increased risk of infections, particularly diarrhoea (54) and pneumonia (34). However, in the population under study exclusive and prolonged breastfeeding was the norm.

Our results provide some insight into the effects of infection and undernutrition on the humoral immune responses in a population of undernourished children with acute lower respiratory infection compared to similarly infected children who maintained normal nutritional status before contracting pneumonia and during their disease. Albumin, immunoglobulin and specific antibody levels in serum and saliva were contrasted between nourished infected children and undernourished infected children. Any differences observed were therefore related, directly or indirectly, to their nutritional condition.

This was a cross-sectional study with measurements of nutritional status and parameters of the immune response being assessed simultaneously. The children were divided according to their weight for age into two nutritional groups, nourished and undernourished. The children within each nutritional group were stratified into age groups for the purpose of comparative analysis. The results of this study showed that undernourished children less than 49 months of age have significantly lower levels of albumin in serum than nourished children. These findings are consistent with previous studies which have shown an association of hypoalbuminaemia with protein malnutrition (9,10,24,25,55) and more specifically with pleural effusion in children with pneumonia (56,57). Previous studies investigating the more severe forms of malnutrition have demonstrated IgG and IgM levels in serum to be decreased (7) or increased (9,12,22) or show no change (10,24,26), while the level of IgA has been more consistently demonstrated to be elevated (7,8,12,22,24,27). Serum immunoglobulin levels in mild to moderately undernourished children have generally been shown to be not significantly affected (10,23,24). This study demonstrated that in different age groups the concentration of immunoglobulins in serum and saliva in children who were undernourished was in most cases lower than or no different from that in nourished children. Undernourished children at 7-12 months had

significantly decreased levels of serum IgA in comparison with nourished children. Decreases in the level of IgM in the undernourished were observed at the age of ≤ 6 months and from 25 to 48 months. In children aged 25-36 months the IgG levels in serum were lower in the undernourished. What was most apparent in our study population was that the level of immunoglobulin present at any given time was highly dependent on the age of the child. This observation may offer some insight into the inconsistencies between previous studies, where the age range of children was anywhere from birth to 7 years of age.

In saliva, it was demonstrated that albumin does not appear to be affected by nutritional status, which is consistent with previous observations (22). The immune system at mucosal surfaces has been shown to be suppressed in severe to moderate malnutrition, with decreased levels of IgA observed in nasopharyngeal fluid, tears, saliva and duodenal fluid (21,22,25,27); in the case of mild malnutrition there was no change in salivary IgA (23). Our results support these observations, with total IgA levels in saliva being lower in undernourished children aged ≤ 6 months and 49-60 months. The age profiles in both groups up to 24 months of age show a development pattern similar to that previously described by Gleeson et al. (50) with lower values of salivary IgA observed in infants and young children and a larger proportion of younger than older children having no detectable IgA. The proportion of children with no detectable salivary IgA decreased with age from 80% at ≤ 6 months to approximately 25% at 49-60 months. However, after 6 months of age, the proportion of children with no detectable IgA in saliva was always greater in the undernourished group. Also, in the undernourished children, after the age of 24 months, and in contrast to the nourished children, the levels of salivary IgA decreased with age.

Antigenic exposure is important to the ontogeny of IgA responses to common enteric and respiratory organisms. Because of the heavy early exposure to *E. coli* and NTHi, antibodies to *E. coli* O antigen and NTHi OMP were investigated in this study as markers of the immune response to bacterial exposure. PNG children have an early bacterial colonization (29,38) characterized by a rapid development of serum and salivary IgA and

specific IgA antibody, which was nevertheless lower than that in Australian children of the same age with considerably less antigenic exposure (39,58). This study has confirmed that the salivary IgA and specific IgA antibody levels in PNG children are lower than those previously reported in Australian children (39,50). This observation provides further support for the hypothesis that early colonization may result in a 'high zone' tolerance to antigens that children are exposed to early in life.

Our data showed that undernourished children had significantly lower serum levels of NTHi OMP and *E. coli* antibodies than nourished children in 8 results distributed across the age groups (Table 1). In the remainder, in 21 cases there was no difference attributable to nutritional status. There were 3 exceptions where the levels were increased in the serum of undernourished children: for NTHi OMP-specific IgA antibody at 49-60 months and IgM antibody at 7-12 months, and for *E. coli*-specific IgM antibody at 25-36 months of age.

In saliva, IgA levels remain relatively constant until there is exposure to increased antigenic loads (43). The age profiles for both NTHi OMP and *E. coli* antibody in nourished children (Table 2) were similar to each other but different from that observed for IgA levels. In this group of children both specific antibody levels in saliva peaked at 7-12 months; however, only with the *E. coli*-specific IgA antibody was the peak level significantly higher. The level of *E. coli*-specific IgA antibody was significantly higher in nourished children than in undernourished children at 7-12 months. Interestingly, at 7-12 months there was a dramatic rise in the proportion of children with detectable *E. coli*-specific IgA antibodies in both undernourished and nourished groups with virtually all children having detectable antibody in their saliva (data not shown) with a gradual decline in these numbers with age. The decline in concentration of NTHi OMP- and *E. coli*-specific IgA antibody levels after 7-12 months may follow the action of immune control mechanisms, with suppression of an initial cooperative interaction between T and B lymphocytes, in response to differing bacterial colonization patterns.

In severely malnourished children with respiratory and enteric infections, it has been demonstrated that they have a lower

proportion of peripheral blood B cells (10,16) and decreased number of IgA-containing cells in the jejunal mucosa (59,60) compared with well-nourished children with infection. It is therefore not unreasonable to assume that the ontogeny of humoral immunity may also be compromised. This study is consistent with these observations since, in a number of age intervals studied, lower levels of IgG, IgM, IgA and specific antibody were observed in undernourished children. It is indeed this failure in malnourished children to increase the proportion of B lymphocytes during infection (16) that may offer some explanation for the observation in our study that at age 7-12 months, despite a greater than two-fold increase in the proportion of undernourished and nourished children with detectable antibody to *E. coli* (data not shown), the nourished children were able to mount an antibody response that was significantly greater than that in the undernourished children.

This is the most comprehensive study undertaken that examines undernourished children at specific ages from 6 weeks to 60 months. These observations, while generally suggesting that undernutrition may compromise both mucosal and systemic immunity in children with pneumonia, are not fully consistent between age groups. Clearly, the effect of undernutrition on the immune system is complex and modified by the age of the child, whether or not infection is present, the history of recurrent infections of different kinds, and the degree of malnutrition.

The influence of nutritional status on the outcome of pneumonia is profound (32). This study confirms that the mechanisms of this influence involve perturbations of mucosal and systemic humoral immunity. However, the evidence from both the literature and this study leads to the conclusion that these mechanisms are not to be found simply in the cross-sectional levels of humoral immune factors.

ACKNOWLEDGEMENTS

We thank Simone Barnish, Sebeya Lupiwa and other members of the staff of the Papua New Guinea Institute of Medical Research for their excellent assistance in the conduct of this study. We dedicate this manuscript to the infants and children and their families who participated in the study, and to the memory of our esteemed colleague Helena Vrbova.

REFERENCES

- 1 **Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, Mathers C, Rivera J, Maternal and Child Undernutrition Study Group.** Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;371:243-260.
- 2 **World Health Organization.** The World Health Report 2000 – Health Systems: Improving Performance. Geneva: World Health Organization, 2000.
- 3 **Pelletier DL, Frongillo EA Jr, Schroeder DG, Habicht JP.** The effects of malnutrition on child mortality in developing countries. *Bull World Health Organ* 1995;73:443-448.
- 4 **Rice A, Sacco L, Hyder A, Black RE.** Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries. *Bull World Health Organ* 2000;78:1207-1221.
- 5 **Chandra RK.** Serum complement and immunoconglutinin in malnutrition. *Arch Dis Child* 1975;50:225-229.
- 6 **Chandra RK.** Fetal malnutrition and postnatal immunocompetence. *Am J Dis Child* 1975;129:450-454.
- 7 **McMurray DN, Loomis SA, Casazza LJ, Rey H, Miranda R.** Development of impaired cell-mediated immunity in mild and moderate malnutrition. *Am J Clin Nutr* 1981;34:68-77.
- 8 **McMurray DN, Watson RR, Reyes MA.** Effect of renutrition on humoral and cell-mediated immunity in severely malnourished children. *Am J Clin Nutr* 1981;34:2117-2126.
- 9 **Neumann CG, Lawlor GJ Jr, Stiehler ER, Swenseid ME, Newton C, Herbert J, Ammann AJ, Jacob M.** Immunologic responses in malnourished children. *Am J Clin Nutr* 1975;28:89-104.
- 10 **Rikimaru T, Taniguchi K, Yartey JE, Kennedy DO, Nkrumah FK.** Humoral and cell-mediated immunity in malnourished children in Ghana. *Eur J Clin Nutr* 1998;52:344-350.
- 11 **Haller L, Zubler RH, Lambert PH.** Plasma levels of complement components and complement haemolytic activity in protein-energy malnutrition. *Clin Exp Immunol* 1978;34:248-252.
- 12 **Ozkan H, Olgun N, Sasmaz E, Abacioglu H, Okuyan M, Cevik N.** Nutrition, immunity and infections: T lymphocyte subpopulations in protein-energy malnutrition. *J Trop Pediatr* 1993;39:257-260.
- 13 **Seth V, Chandra RK.** Opsonic activity, phagocytosis, and bactericidal capacity of polymorphs in undernutrition. *Arch Dis Child* 1972;47:282-284.
- 14 **Zaman K, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE.** Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. *Acta Paediatr* 1997;86:923-927.
- 15 **Nájera O, González C, Cortés E, Toledo G, Ortiz R.** Effector T lymphocytes in well-nourished and malnourished infected children. *Clin Exp Immunol* 2007;148:501-506.
- 16 **Nájera O, González C, Toledo G, López L, Ortiz R.** Flow cytometry study of lymphocyte subsets in malnourished and well-nourished children with bacterial infections. *Clin Diagn Lab Immunol* 2004;11:577-580.
- 17 **Nájera O, González C, Toledo G, López L, Cortés E, Betancourt M, Ortiz R.** CD45RA and CD45RO isoforms in infected malnourished and infected well-nourished children. *Clin Exp Immunol* 2001;126:461-465.
- 18 **Bell RG, Turner KJ, Gracey M, Suharjono, Sunoto.** Serum and small intestinal immunoglobulin levels in undernourished children. *Am J Clin Nutr* 1976;29:392-397.
- 19 **Dülger H, Arik M, Sekeroglu MR, Tarakcioglu M, Noyan T, Cesur Y, Balahoroglu R.** Pro-inflammatory cytokines in Turkish children with protein-energy malnutrition. *Mediators Inflamm* 2002;11:363-365.
- 20 **Chandra RK, Wadhwa M.** Nutritional modulation of intestinal mucosal immunity. *Immunol Invest* 1989;18:119-126.
- 21 **Sirisinha S, Suskind R, Edelman R, Asvapaka C, Olson RE.** Secretory and serum IgA in children with protein-calorie malnutrition. *Pediatrics* 1975;55:166-170.
- 22 **McMurray DN, Rey H, Casazza LJ, Watson RR.** Effect of moderate malnutrition on concentrations of immunoglobulins and enzymes in tears and saliva of young Colombian children. *Am J Clin Nutr* 1977;30:1944-1948.
- 23 **Nagao AT, Carneiro-Sampaio MM, Carlsson B, Hanson LA.** Antibody titre and avidity in saliva and serum are not impaired in mildly to moderately undernourished children. *J Trop Pediatr* 1995;41:153-157.
- 24 **Nahani J, Nik-Aeen A, Rafii M, Mohagheghpour N.** Effect of malnutrition on several parameters of the immune system of children. *Nutr Metab* 1976;20:302-306.
- 25 **Reddy V, Raghuramulu N, Bhaskaram C.** Secretory IgA in protein-calorie malnutrition. *Arch Dis Child* 1976;51:871-874.
- 26 **Suskind R, Sirisinha S, Vithayasai V, Edelman R, Damrongsak D, Charupatana C, Olson RE.** Immunoglobulins and antibody response in children with protein-calorie malnutrition. *Am J Clin Nutr* 1976;29:836-841.
- 27 **Watson RR, McMurray DN, Martin P, Reyes MA.** Effect of age, malnutrition and renutrition on free secretory component and IgA in secretions. *Am J Clin Nutr* 1985;42:281-288.
- 28 **Earland J, Harrison M, Rubert P.** Nutrition for Papua New Guinea. In: Earland J, Harrison M, Rubert P, eds. Nutrition for Papua New Guinea, Third edition. Port Moresby: Nutrition Section, Department of Health, Papua New Guinea, 1995:189-198.
- 29 **Duke T, Michael A, Mgone J, Frank D, Wal T, Sehuko R.** Etiology of child mortality in Goroka, Papua New Guinea: a prospective two-year study. *Bull World Health Organ* 2002;80:16-25.
- 30 **Lehmann D, Heywood P.** Effect of birthweight on pneumonia-specific and total mortality among infants in the highlands of Papua New Guinea. *PNG Med J* 1996;39:274-283.
- 31 **Spooner V, Barker J, Tulloch S, Lehmann D, Marshall TF, Kajoi M, Alpers MP.** Clinical signs and risk factors associated with pneumonia in children admitted to Goroka Hospital, Papua New Guinea. *J Trop Pediatr* 1989;35:295-300.
- 32 **Lehmann D, Howard P, Heywood P.** Nutrition and morbidity: acute lower respiratory tract infections, diarrhoea and malaria. *PNG Med J* 1988;31:109-116.
- 33 **Shann F, Gratten M, Germer S, Linnemann V, Hazlett D, Payne R.** Aetiology of pneumonia in children in Goroka Hospital, Papua New Guinea. *Lancet* 1984;2:537-541.

- 34 **Anga G, Vince JD, Kaupa M.** Early introduction of solids and pneumonia in young infants in Papua New Guinea: a case control study. *J Trop Pediatr* 2008;54:192-195.
- 35 **Witt CS, Alpers MP.** Impaired cell-mediated immunity in Papua New Guinean infants. *PNG Med J* 1991;34:90-97.
- 36 **Barker J, Gratten M, Riley I, Lehmann D, Montgomery J, Kajoi M, Gratten H, Smith D, Marshall TF, Alpers MP.** Pneumonia in children in the Eastern Highlands of Papua New Guinea: a bacteriologic study of patients selected by standard clinical criteria. *J Infect Dis* 1989;159:348-352.
- 37 **Riley ID.** Pneumonia vaccine trials at Tari. *PNG Med J* 2002;45:44-50.
- 38 **Gratten M, Gratten H, Poli A, Carrad E, Raymer M, Koki G.** Colonisation of *Haemophilus influenzae* and *Streptococcus pneumoniae* in the upper respiratory tract of neonates in Papua New Guinea: primary acquisition, duration of carriage, and relationship to carriage in mothers. *Biol Neonate* 1986;50:114-120.
- 39 **Clancy RL, Cripps AW, Yeung S, Standish-White S, Pang G, Gratten H, Koki G, Smith D, Alpers MP.** Salivary and serum antibody responses to *Haemophilus influenzae* infection in Papua New Guinea. *PNG Med J* 1987;30:271-276.
- 40 **Heywood P, Hiles S, Cogill B, Clarke LJ.** Growth patterns of highland children and some possible implications for assessment of nutritional status. *PNG Med J* 1981;24:45-49.
- 41 **Keeble R, Keeble J.** Nutritional study of the 1-4 year old population of the Lower Jimi Valley, Western Highlands Province, Papua New Guinea. *PNG Med J* 2006;49:156-161.
- 42 **Gleeson M, Cripps AW, Clancy RL, Husband AJ, Hensley MJ, Leeder SR.** Ontogeny of the secretory immune system in man. *Aust NZ J Med* 1982;12:255-258.
- 43 **Gleeson M, Cripps AW, Clancy RL, Wlodarczyk JH, Dobson AJ, Hensley MJ.** The development of IgA-specific antibodies to *Escherichia coli* O antigen in children. *Scand J Immunol* 1987;26:639-643.
- 44 **Cunningham-Rundles S, McNeeley DF, Moon A.** Mechanisms of nutrient modulation of the immune response. *J Allergy Clin Immunol* 2005;115:1119-1128.
- 45 **Maggini S, Wintergerst ES, Beveridge S, Hornig DH.** Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *Br J Nutr* 2007;98 Suppl 1:S29-S35.
- 46 **Solon JA, Morgan G, Prentice A.** Mucosal immunity in severely malnourished Gambian children. *J Pediatr* 2006;149(Suppl):S100-S106.
- 47 **Cripps AW, Gleeson M.** Ontogeny of mucosal immunity and aging. In: Mestecky J, Bienenstock J, Lamm ME, Strober W, McGhee JR, Mayer L, eds. *Mucosal Immunology*, 3rd edition. Boston: Elsevier Academic Press, 2005:305-322.
- 48 **Chandra RK.** Nutrition and the immune system from birth to old age. *Eur J Clin Nutr* 2002;56(Suppl 3):S73-S76.
- 49 **Welsh FK, Farmery SM, MacLennan K, Sheridan MB, Barclay GR, Guillou PJ, Reynolds JV.** Gut barrier function in malnourished patients. *Gut* 1998;42:396-401.
- 50 **Gleeson M, Cripps AW, Clancy RL, Hensley MJ, Dobson AJ, Firman DW.** Breast feeding conditions a differential development pattern of mucosal immunity. *Clin Exp Immunol* 1986;66:216-222.
- 51 **Ouwehand A, Isolauri E, Salminen S.** The role of the intestinal microflora for the development of the immune system in early childhood. *Eur J Nutr* 2002;41(Suppl 1):I32-I37.
- 52 **Yoshida M, Kobayashi K, Kuo TT, Bry L, Glickman JN, Claypool SM, Kaser A, Nagaishi T, Higgins DE, Mizoguchi E, Wakatsuki Y, Roopenian DC, Mizoguchi A, Lencer WI, Blumberg RS.** Neonatal Fc receptor for IgG regulates mucosal immune responses to luminal bacteria. *J Clin Invest* 2006;116:2142-2151.
- 53 **Friesen H, Vince J, Boas P, Danaya R, Mokela D, Ogle G, Asuo P, Kemiki A, Lagani W, Rongap T, Varughese M, Saweri W.** Infant feeding practices in Papua New Guinea. *Ann Trop Paediatr* 1998;18:209-215.
- 54 **Brown KH, Black RE, Lopez de Romaña G, Creed de Kanashiro H.** Infant-feeding practices and their relationship with diarrheal and other diseases in Huascar (Lima), Peru. *Pediatrics* 1989;83:31-40.
- 55 **Rahman MZ, Begum BA.** Serum total protein, albumin and A/G ratio in different grades of protein energy malnutrition. *Mymensingh Med J* 2005;14:38-40.
- 56 **Klar A, Shoseyov D, Berkun Y, Brand A, Braun J, Shazberg G, Jonathan M, Gross-Kieselstein E, Revel-Vilk S, Hurvitz H.** Intestinal protein loss and hypoalbuminemia in children with pneumonia. *J Pediatr Gastroenterol Nutr* 2003;37:120-123.
- 57 **Prais D, Kuzmenko E, Amir J, Harel L.** Association of hypoalbuminemia with the presence and size of pleural effusion in children with pneumonia. *Pediatrics* 2008;121:e533-538.
- 58 **Cripps AW, Gleeson M, Clancy RL.** Ontogeny of the mucosal response in children. In: Mestecky J, Blair C, Ogra PL, eds. *Immunology of Milk and the Neonate*. New York: Plenum Press, 1991:87-92.
- 59 **Green F, Heyworth B.** Immunoglobulin-containing cells in jejunal mucosa of children with protein-energy malnutrition and gastroenteritis. *Arch Dis Child* 1980;55:380-383.
- 60 **Kaschula RO, Gajjar PD, Mann M, Hill I, Purvis J, Blake DR, Bowie MD.** Infantile jejunal mucosa in infection and malnutrition. *Isr J Med Sci* 1979;15:356-361.

Alpha⁺-thalassaemia and malaria in Melanesia: epidemiological perspectives

FREYA J.I. FOWKES¹ AND KAREN P. DAY²

Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia and
Department of Medical Parasitology, New York University School of Medicine, USA

SUMMARY

In 1948 Haldane first proposed that the high frequencies of thalassaemias in malaria-endemic regions were due to natural selection by malaria. Some of the highest frequencies of α^+ -thalassaemia are found in the Pacific region of Melanesia. Consequently, Melanesia has provided a unique opportunity for an extensive study of the association between α^+ -thalassaemia and malaria. Here we review the emergence of α^+ -thalassaemia in this region and the research that has been carried out, both from the historical perspective and the most recent developments, which may give insight into the selection of α^+ -thalassaemia by malaria.

Introduction

The heritable haemoglobinopathy α -thalassaemia is one of the most common monogenic disorders of humans (1). There are two main forms of α -thalassaemia, α^+ - and α^0 -thalassaemia, characterized by the deletion of one or two, respectively, of the duplicated α -globin genes ($\alpha\alpha/\alpha\alpha$) on chromosome 16 (1,2). The α^+ -thalassaemias can be further divided into $-\alpha^{3.7I}$, $-\alpha^{3.7II}$, $-\alpha^{3.7III}$ and $-\alpha^{4.2}$. The $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions represent two different gene rearrangements resulting from interchromosomal crossing-over following mispairing during meiosis (3,4). Individuals with α^+ -thalassaemia have a mild hypochromic microcytic anaemia compared with normal individuals (1).

In 1948 at the International Congress of Genetics Haldane first proposed that the high frequencies of thalassaemias in malaria-endemic regions were due to natural selection by malaria (5). However, the first haemoglobin (Hb) surveys in Melanesia in the 1950s were undertaken to determine whether Hb S, common in African populations, was present in the Pacific. It was not (6). The first Melanesian variant to be found was Hb

JTongariki (6) and the observation that JTongariki was associated with α -thalassaemia first suggested that α -thalassaemia may be widespread in this region (7). At around the same time a blood survey for Hb Barts (γ_4) in the Papuan Gulf also suggested that α^+ -thalassaemia was common there. Subsequent DNA studies demonstrated high frequencies of α^+ -thalassaemia in Vanuatu (26%) (8,9) and on the north coast of Papua New Guinea (PNG) and its adjacent islands (>65%) (10-13). Molecular genetic analyses showed that individual mutations are extraordinarily specific to particular populations. The two most common deletions in Melanesia, $-\alpha^{4.2}$ and $-\alpha^{3.7III}$, are rare and absent outside of Oceania (6). Within Melanesia, variation in subtypes has been shown. On the north coast of PNG the most common variant is $-\alpha^{4.2}$, while the $-\alpha^{3.7I}$ deletion is predominant in south coastal PNG (11). In Vanuatu and most of island Melanesia the commonest type of α^+ -thalassaemia is $-\alpha^{3.7III}$ (10). The compartmentalized distribution of these mutations implies that they arose independently in Melanesia, in discrete areas after the division and migration of the indigenous human population (14-16).

¹ Walter and Eliza Hall Institute of Medical Research, IG Royal Parade, Parkville, Victoria 3052, Australia
fowkes@wehi.edu.au

² Department of Medical Parasitology, New York University School of Medicine, 341 East 25th Street, New York, NY 10010, USA

Selection of α^+ -thalassaemia by malaria

A detailed study by Flint et al. demonstrated that the frequency of α^+ -thalassaemia exhibited both altitude- and latitude-dependent correlation with malaria endemicity throughout Melanesia (10). The lack of such a correlation for other unlinked polymorphisms (hypervariable regions on chromosome 11) in Melanesia supported the hypothesis that this deleterious allele was indeed selected for by malaria, rather than a population founder effect or genetic drift (10). It has been estimated with data from Vanuatu that within 4000 years from the archipelago first being populated, a novel haplotype arose, and became elevated in frequency by selection (17). The multiple origins of α^+ -thalassaemia deletions implied a local selection pressure, perhaps *Plasmodium vivax*, which was the dominant *Plasmodium* spp. in PNG until the 1970s (16). It is therefore possible that *P. vivax*, which has often placed a heavy burden of mortality on the populations it afflicted (18-21), was the principal agent of selection before the arrival of *P. falciparum* within these populations.

Alpha⁺-thalassaemia and parasite exposure

Although geographical studies pointed to an association between α^+ -thalassaemia and malaria, the mechanism by which α^+ -thalassaemia conferred protection was unknown. It became dogma that the protective mechanism was due to a direct interaction between the parasite and the altered thalassaemic erythrocyte resulting in a reduced parasite load (22). In vitro experiments attempted to define this interaction but they proved largely inconclusive (23-40). Various hypotheses were tested such as reduced invasion and growth of *P. falciparum* in thalassaemic erythrocytes, immune-mediated clearance of infected thalassaemic erythrocytes and rosetting. Epidemiological studies in asymptomatic Melanesian children have failed to demonstrate any protection against *P. falciparum* parasite density (41-44), nor age-specific seroconversion to variant surface antigens (45). This compelling observation makes it difficult to reconcile how either an altered physical interaction between the parasite and the erythrocyte or enhanced immune responses to the infected erythrocyte explains the protective mechanism.

Alpha⁺-thalassaemia and uncomplicated malaria

Initial epidemiological studies attempted to define the association between α^+ -thalassaemia and uncomplicated malaria. Studies by Oppenheimer et al. in 1987 (41) and Williams et al. in 1996 (8), in PNG and Vanuatu respectively, showed a greater frequency of malaria in young children homozygous for α^+ -thalassaemia. During a controlled trial of iron dextran prophylaxis in PNG, Oppenheimer et al. (41) performed clinical and haematological examination of infants in two cross-sectional studies at 6 and 12 months of age. Infants were divided according to Hb Bart's levels found at birth into 3 groups corresponding to putative genotypes. No difference in parasite density among α^+ -thalassaemia phenotypes was demonstrated, but children homozygous for α^+ -thalassaemia had raised spleen and parasite rates compared to heterozygous and normal children. Williams et al. (8) conducted a survey of the incidence of mild malaria on children living on the island of Espiritu Santo, Vanuatu, and showed that homozygous children aged 0-4 years had nearly twice the incidence of both falciparum and vivax malaria, as well as splenomegaly, compared to normal and heterozygous children.

These initial findings of increased splenomegaly and febrile illness in children with α^+ -thalassaemia did not fit with the concept of malaria as a selective pressure on α^+ -thalassaemia. Williams et al. suggested that increased susceptibility to malaria in homozygous children may lead to improved immunity in later life. The authors speculated that the high expression of malarial antigens on α^+ -thalassaemia erythrocytes (38) might facilitate the rapid development of protective immunity. However, if this were true, then individuals with α^+ -thalassaemia should be protected against mild malaria within older age groups, but this was not the case. Another interpretation of data incorporates a role for *P. vivax*. Williams et al. proposed that α^+ -thalassaemia may be associated with some degree of ineffective erythropoiesis and reduced erythrocyte survival resulting in a higher proportion of circulating young erythrocytes. As *P. vivax* preferentially invades the youngest, most metabolically active erythrocytes and reticulocytes this would lead to an increased incidence of malaria. *P. vivax* was particularly increased

in homozygous children aged less than 30 months in their study and it was proposed that this may act as a natural vaccine against *P. falciparum* through cross-species immunity. The one set of data that demonstrated the effect of improved cross-protective immunity with age/exposure was limited and there was no convincing statistical evidence.

While these early studies provided some interesting observations and hypotheses, neither study has been reproduced nor validated. A major criticism of these studies is that they did not adjust for the significant spatial and temporal heterogeneity of exposure to malaria reported in their study populations (46,47). A subsequent study by Allen et al. showed a non-significant tendency toward reduced mild malaria in children homozygous for α^+ -thalassaemia compared to normal children after adjusting for confounders (42). In addition, we recently analyzed data from a longitudinal morbidity study in children from the Amele population of rural Madang, PNG, and found no conclusive evidence of a protective effect of α^+ -thalassaemia homozygosity against non-severe malarial morbidity, using a number of clinical outcomes and adjusting for spatial and temporal heterogeneity (FJI Fowkes, unpublished data). Given that no protection of α^+ -thalassaemia has been observed against parasite density it is not surprising that protection is not seen against non-severe malaria, which is characterized by febrile illness associated with high parasite densities.

Alpha⁺-thalassaemia and severe malaria

To date, there has only been one study in Melanesia examining the association of α^+ -thalassaemia with severe malaria. A prospective, matched case-control study by Allen et al. (42) in 1997 took place in Madang Province, PNG, where α^+ -thalassaemia affects more than 90% of the population (10,11,13). Malaria transmission is intense (48) and an estimated 11% of deaths in children under 10 years were attributable to malaria (49). Because death from malaria in hospital is uncommon, admission to hospital with severe malaria was used as a surrogate for malaria mortality. The authors showed that the risk of developing severe malaria was 0.40 (95%CI 0.22- 0.74, $p = 0.003$) in children homozygous for α^+ -thalassaemia and 0.66 (95%CI 0.37- 1.20, $p = 0.2$) in heterozygous children compared to children of normal genotype. No differences in parasite burden

among α^+ -thalassaemia genotypes was observed.

The strongest protective effect of α^+ -thalassaemia homozygosity was observed against the complications of severe malarial anaemia (SMA; haemoglobin [Hb] <50 g/l), hyperlactataemia and metabolic acidosis rather than other clinical sequelae. The lowest odds ratios for children homozygous for α^+ -thalassaemia were in the acidosis and hyperlactataemia clinical subgroups, which are the strongest predictors of mortality in PNG (50). Comparison of clinical features and biochemical indices in children admitted to hospital with malaria indicated that α^+ -thalassaemia did not appear to alter the course of disease once it had developed to a sufficient degree to require hospital admission (42). This suggests that α^+ -thalassaemia most likely prevents rapidly fulminating disease before children reach the hospital.

The importance of protection against SMA, the most common clinical consequence of malaria in PNG (50), as a survival advantage is not altogether clear. Although SMA did not predict mortality during the hospital admission, and tends to improve rapidly without specific treatment, it may be a significant risk factor for death before treatment or in the short period after discharge. In Madang, children with SMA are more likely to be hyperlactataemic, but no association between SMA and acidosis has been noted (50). The interaction of SMA, hyperlactataemia and acidosis with the protective mechanism of α^+ -thalassaemia remains to be fully elucidated. However, a recent haematological analysis revealed a haematological mechanism of protection of α^+ -thalassaemia against SMA and possibly other clinical sequelae.

Alpha⁺-thalassaemia provides a haematological advantage against malaria

We recently hypothesized that the lower concentration of Hb per erythrocyte and the larger population of erythrocytes associated with α^+ -thalassaemia homozygosity may be a biologically advantageous host strategy against a pathogen that significantly lowers erythrocyte count. Modeling observed data, we showed that, for a given amount of erythrocyte reduction, children homozygous for α^+ -thalassaemia will lose less Hb than normal children as they have lower mean cell

Hb (51). In addition, homozygous children with increased erythrocyte counts would have to lose 10% more erythrocytes than normal children to reach the definition of SMA. We therefore concluded that a higher microcytic erythrocyte count in children homozygous for α^+ -thalassaemia enables them to maintain their Hb concentration above the 50 g/l threshold, thereby reducing the risk of SMA. Indeed, considering the degree of malaria haemolysis associated with SMA in normal children, children who were homozygous for α^+ -thalassaemia would be 48% less likely to develop SMA than those of normal genotype (51). This suggested that microcytosis and increased erythrocyte count contributes considerably to the 66% protection against SMA observed in this population in children homozygous for α^+ -thalassaemia (42).

As mentioned, it is unclear how SMA may be related to other clinical pathologies but a key role for pro-inflammatory cytokines has been implicated in all clinical subgroups (52). Hb is an extremely toxic molecule and outside the erythrocyte and its constituent antioxidant defence systems, the oxidative potential of Hb can cause substantial oxidative tissue damage and release of pro-inflammatory cytokines such as TNF- α (53,54). It is plausible that children homozygous for α^+ -thalassaemia release less Hb during haemolysis and thereby do not stimulate pro-inflammatory responses as readily as those of normal genotype. Interestingly, children homozygous for α^+ -thalassaemia have also been shown to be protected against severe non-malarial disease in this population (42). Lower Hb concentrations per cell may reduce inflammation during any disease-related haemolysis.

The major assumption of this haematological mechanism is that erythrocyte loss is similar among α^+ -thalassaemia genotypes. This assumption is reasonable in the context of parasite density which is similar among α^+ -thalassaemia genotypes (42,43,55,56). However, further studies are required to elucidate whether anaemia is due to a standardized degree of erythrocyte loss. The cause of SMA is multifactorial so there is potential for other processes to contribute to anaemia, such as destruction of unparasitized erythrocytes and dyserythropoiesis (57). It has been proposed that individuals with α^+ -thalassaemia have increased phagocytosis of erythrocytes (58-60) and expanded erythroid marrow (61), but

the balance between erythrocyte survival and production among α^+ -thalassaemia genotypes is unknown, particularly during a malaria infection.

Alpha⁺-thalassaemia and complement receptor 1 (CR1)

CR1 is an immune regulatory protein found on the surface of erythrocytes and CR1 count per erythrocyte is under control of high (H) and low (L) copy number (62). This expression polymorphism is common in Pacific regions but is not present in African populations (63,64). We have recently demonstrated that the α^+ -thalassaemia genotype is associated with the CR1 genotype in the Amele region of Madang Province, PNG (65). Children who were homozygous for α^+ -thalassaemia were more likely to be of LL genotype and those of normal genotype were more likely to be HH. Thus it would appear that there is a selective advantage of being homozygous and LL. Alpha⁺-thalassaemia is also associated with CR1 deficiency, independently of CR1 copy number, in Melanesian adults (66). CR1 has been implicated in rosetting, the phenomenon whereby malaria-infected erythrocytes bind to uninfected erythrocytes (67). Reduced expression of CR1 on α -thalassaemic cells may circumstantially explain why α -thalassaemic cells are less likely to form rosettes than normal erythrocytes (37).

The frequency of HH/aa/aa in this population is very low (~1%) so current studies have had insufficient power to examine the interaction of these two polymorphisms with protection against clinical disease. In the case-control study by Allen et al. in Madang Province of PNG, resistance to severe malaria (SMA and cerebral complications only) was associated more with the HL and LL genotypes than the HH genotype, independently of α^+ -thalassaemia (66). It has been proposed that individuals with high levels of CR1 may be more likely to form rosettes, bind immune complexes and sequester in the brain capillaries, stimulating inflammation and leading to cerebral malaria (68,69). However, the protective mechanism against SMA is unclear. A role for rosetting in SMA has been proposed by some data (70), but this remains difficult to resolve pathophysiologically. Under normal physiological conditions, CR1 is continuously lost from the surface of the erythrocyte (71-73) making older erythrocytes more

susceptible to complement-mediated destruction (74). It is possible that erythrocytes with low CR1 (ie, LL and α/α) would be more likely to be removed from the circulation. A response to this may be increased erythrocyte turnover in these individuals. We propose that both polymorphisms could act synergistically to minimize the reduction in erythrocyte count during acute disease.

Summary and future research

The relationship between α^+ -thalassaemia and malaria has been extensively researched in Melanesia over the past 50 years. Epidemiological studies have shown that α^+ -thalassaemia has been selected for at high frequencies due to the protective effect of α^+ -thalassaemia against severe malaria, rather than non-severe malaria or parasite density per se. Recent work from Africa concurs with Melanesian observations.

Interestingly, an increase in *P. vivax* infection (42) and febrile illness (8) has been noted in children homozygous for α^+ -thalassaemia and it is noteworthy that the highest frequencies of α^+ -thalassaemia are found in areas where *P. vivax* is prevalent (1,75). These tantalizing observations may highlight an important interaction between α^+ -thalassaemia and *vivax* malaria, yet no one has tested for the protective effect of α^+ -thalassaemia specifically against *P. vivax*. The case-control study by Allen et al. only examined falciparum malaria. *P. vivax* was traditionally the predominant species of *Plasmodium* in PNG but a recent study on the north coast of PNG showed that *P. vivax* accounted for 4.5% of febrile malarial illness compared to 92.8% by *P. falciparum* (76). The contribution of *P. vivax* to severe disease in this area is currently unknown but in other parts of PNG is significant (77). Needless to say, very large studies will be required to have sufficient power to examine the association between α^+ -thalassaemia and *P. vivax*.

The striking bias favouring α^+ -thalassaemia and the CR1-L allele in PNG may make it difficult to conduct any statistical testing of relationships with malaria morbidity and mortality outcomes. However, the additive effect of these genes in protection against malaria in this region requires further investigation. Moreover, severe malaria is a complex, multisystem disease with several somewhat distinct disease mechanisms involved. How these mechanisms vary in

these different genetic environments remains to be elucidated. Once this crucial and complex question is answered it will provide an enhanced understanding of the selection of α^+ -thalassaemia, and other polymorphisms, by malaria.

ACKNOWLEDGEMENTS

We thank Leanne Robinson for helpful comments on the manuscript. We dedicate this paper to the memory of Helena Vrbova.

REFERENCES

- 1 Weatherall DJ, Clegg JB. The Thalassaemia Syndromes, 4th edition. Oxford: Blackwell Science, 2001.
- 2 Bernini LF, Hartevelde CL. Alpha-thalassaemia. *Baillieres Clin Haematol* 1998;11:53-90.
- 3 Lauer J, Shen CK, Maniatis T. The chromosomal arrangement of human alpha-like globin genes: sequence homology and alpha-globin gene deletions. *Cell* 1980;20:119-130.
- 4 Embury SH, Miller JA, Dozy AM, Kan YW, Chan V, Todd D. Two different molecular organizations account for the single alpha-globin gene of the alpha-thalassaemia-2 genotype. *J Clin Invest* 1980;66:1319-1325.
- 5 Haldane JBS. The rate of mutation of human genes. Proceedings of the Eighth International Congress of Genetics. *Hereditas* 1949;35(Suppl):267-273.
- 6 Hill AVS, O'Shaughnessy DF, Clegg JB. Haemoglobin and globin gene variants in the Pacific. In: Hill AVS, Serjeantson SW, eds. The Colonization of the Pacific: A Genetic Trail. Oxford: Clarendon Press, 1989:246-285.
- 7 Old JM, Clegg JB, Weatherall DJ, Booth PB. Haemoglobin J Tongariki is associated with alpha thalassaemia. *Nature* 1978;273:319-320.
- 8 Williams TN, Maitland K, Bennett S, Ganczakowski M, Peto TE, Newbold CI, Bowden DK, Weatherall DJ, Clegg JB. High incidence of malaria in alpha-thalassaemic children. *Nature* 1996;383:522-525.
- 9 Bowden DK, Pressley L, Higgs DR, Clegg JB, Weatherall DJ. Alpha-globin gene deletions associated with Hb J Tongariki. *Br J Haematol* 1982;51:243-249.
- 10 Flint J, Hill AVS, Bowden DK, Oppenheimer SJ, Sill PR, Serjeantson SW, Bana-Koiri J, Bhatia K, Alpers MP, Boyce AJ, Weatherall DJ, Clegg JB. High frequencies of alpha-thalassaemia are the result of natural selection by malaria. *Nature* 1986;321:744-750.
- 11 Yenchitsomanus PT, Summers KM, Board PG, Bhatia KK, Jones GL, Johnston K, Nurse GT. Alpha-thalassaemia in Papua New Guinea. *Hum Genet* 1986;74:432-437.
- 12 Yenchitsomanus PT, Summers KM, Bhatia KK, Cattani J, Board PG. Extremely high frequencies of alpha-globin gene deletion in Madang and on Kar Kar Island, Papua New Guinea. *Am J Hum Genet* 1985;37:778-784.
- 13 Oppenheimer SJ, Higgs DR, Weatherall DJ, Barker J, Spark RA. Alpha thalassaemia in Papua New Guinea. *Lancet* 1984;1:424-426.

- 14 **Carter R, Mendis KN.** Evolutionary and historical aspects of the burden of malaria. *Clin Microbiol Rev* 2002;15:564-594.
- 15 **Flint J, Harding RM, Boyce AJ, Clegg JB.** The population genetics of the haemoglobinopathies. *Baillieres Clin Haematol* 1998;11:1-51.
- 16 **Hill AV.** Molecular epidemiology of the thalassaemias (including haemoglobin E). *Baillieres Clin Haematol* 1992;5:209-238.
- 17 **Harding RM, Clegg JB.** Molecular population genetic studies of the island peoples of the South Pacific. *Am J Hum Biol* 1996;8:587-597.
- 18 **Dobson MJ.** Malaria in England: a geographical and historical perspective. *Parassitologia* 1994;36:35-60.
- 19 **Sinton J.** What malaria costs India, nationally, socially and economically. *Rec Mal Surv India* 1935-1936;5:223-264, 413-489.
- 20 **James S.** The disappearance of malaria from England. *Proc R Soc Med* 1929;23:71-87.
- 21 **Mendis K, Sina BJ, Marchesini P, Carter R.** The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 2001;64:97-106.
- 22 **Nagel RL, Roth EF Jr.** Malaria and red cell genetic defects. *Blood* 1989;74:1213-1221.
- 23 **Geary TG, Delaney EJ, Klotz IM, Jensen JB.** Inhibition of the growth of *Plasmodium falciparum* in vitro by covalent modification of hemoglobin. *Mol Biochem Parasitol* 1983;9:59-72.
- 24 **Yuthavong Y, Butthep P, Bunyaratvej A, Fucharoen S, Khushmith S.** Impaired parasite growth and increased susceptibility to phagocytosis of *Plasmodium falciparum* infected alpha-thalassaemia or hemoglobin Constant Spring red blood cells. *Am J Clin Pathol* 1988;89:521-525.
- 25 **Senok AC, Li K, Nelson EA, Yu LM, Tian LP, Oppenheimer SJ.** Invasion and growth of *Plasmodium falciparum* is inhibited in fractionated thalassaemic erythrocytes. *Trans R Soc Trop Med Hyg* 1997;91:138-143.
- 26 **Pattanapanyasat K, Yongvanitchit K, Tongtawe P, Tachavanich K, Wanachiwanawin W, Fucharoen S, Walsh DS.** Impairment of *Plasmodium falciparum* growth in thalassaemic red blood cells: further evidence by using biotin labeling and flow cytometry. *Blood* 1999;93:3116-3119.
- 27 **Luzzi GA, Torii M, Aikawa M, Pasvol G.** Unrestricted growth of *Plasmodium falciparum* in microcytic erythrocytes in iron deficiency and thalassaemia. *Br J Haematol* 1990;74:519-524.
- 28 **Schrier SL.** Thalassaemia: pathophysiology of red cell changes. *Annu Rev Med* 1994;45:211-218.
- 29 **Schrier SL, Rachmilewitz E, Mohandas N.** Cellular and membrane properties of alpha and beta thalassaemic erythrocytes are different: implication for differences in clinical manifestations. *Blood* 1989;74:2194-2202.
- 30 **Destro B, Giardina B, Sansonetti B, Spedini G.** Interaction between oxidised hemoglobin and the cell membrane: a common basis for several falciparum malaria-linked genetic traits. *Yearbook Phys Anthropol* 1996;39:137-159.
- 31 **Friedman MJ.** Oxidant damage mediates variant red cell resistance to malaria. *Nature* 1979;280:245-247.
- 32 **Senok AC, Li K, Nelson EA, Arumanayagam M, Li CK.** Flow cytometric assessment of oxidant stress in age-fractionated thalassaemic trait erythrocytes and its relationship to in vitro growth of *Plasmodium falciparum*. *Parasitology* 1998;116(Pt 1):1-6.
- 33 **Destro-Bisol G, D'Aloja E, Spedini G, Scatena R, Giardina B, Pascali V.** Brief communication: resistance to falciparum malaria in alpha-thalassaemia, oxidative stress, and hemoglobin oxidation. *Am J Phys Anthropol* 1999;109:269-273.
- 34 **Senok AC, Nelson EA, Li K, Ismaeel AR, Olliaro P, Oppenheimer SJ.** Ultrastructural assessment of *Plasmodium falciparum* in age-fractionated thalassaemic erythrocytes. *Parasitol Res* 2006;98:381-384.
- 35 **Luzzi GA, Merry AH, Newbold CI, Marsh K, Pasvol G.** Protection by alpha-thalassaemia against *Plasmodium falciparum* malaria: modified surface antigen expression rather than impaired growth or cytoadherence. *Immunol Lett* 1991;30:233-240.
- 36 **Ifediba TC, Stern A, Ibrahim A, Rieder RF.** *Plasmodium falciparum* in vitro: diminished growth in hemoglobin H disease erythrocytes. *Blood* 1985;65:452-455.
- 37 **Carlson J, Nash GB, Gabutti V, Al-Yaman F, Wahlgren M.** Natural protection against severe *Plasmodium falciparum* malaria due to impaired rosette formation. *Blood* 1994;84:3909-3914.
- 38 **Luzzi GA, Merry AH, Newbold CI, Marsh K, Pasvol G, Weatherall DJ.** Surface antigen expression on *Plasmodium falciparum*-infected erythrocytes is modified in alpha- and beta-thalassaemia. *J Exp Med* 1991;173:785-791.
- 39 **Udomsangpetch R, Todd J, Carlson J, Greenwood BM.** The effects of hemoglobin genotype and ABO blood group on the formation of rosettes by *Plasmodium falciparum*-infected red blood cells. *Am J Trop Med Hyg* 1993;48:149-153.
- 40 **Williams TN, Weatherall DJ, Newbold CI.** The membrane characteristics of *Plasmodium falciparum*-infected and -uninfected heterozygous alpha⁰-thalassaemic erythrocytes. *Br J Haematol* 2002;118:663-670.
- 41 **Oppenheimer SJ, Hill AV, Gibson FD, Macfarlane SB, Moody JB, Pringle J.** The interaction of alpha thalassaemia with malaria. *Trans R Soc Trop Med Hyg* 1987;81:322-326.
- 42 **Allen SJ, O'Donnell A, Alexander NDE, Alpers MP, Peto TEA, Clegg JB, Weatherall DJ.** Alpha+ thalassaemia protects children against disease caused by other infections as well as malaria. *Proc Natl Acad Sci USA* 1997;94:14736-14741.
- 43 **Imrie H, Fowkes FJL, Michon P, Tavul L, Hume JCC, Piper KP, Reeder JC, Day KP.** Haptoglobin levels are associated with haptoglobin genotype and α^+ -thalassaemia in a malaria-endemic area. *Am J Trop Med Hyg* 2006;74:965-971.
- 44 **Ganczakowski M, Bowden DK, Maitland K, Williams TN, O'Shaughnessy D, Viji J, Lucassen A, Clegg JB, Weatherall DJ.** Thalassaemia in Vanuatu, south-west Pacific: frequency and haematological phenotypes of young children. *Br J Haematol* 1995;89:485-495.
- 45 **Fowkes FJL, Michon P, Pilling L, Ripley RM, Tavul L, Imrie HJ, Woods CM, Mgone CS, Luty AJ, Day KP.** Host erythrocyte polymorphisms and exposure to *Plasmodium falciparum* in Papua New Guinea. *Malar J* 2008;7:1.
- 46 **Maitland K, Williams TN, Bennett S, Newbold CI, Peto TE, Viji J, Timothy R, Clegg JB, Weatherall DJ, Bowden DK.** The interaction between *Plasmodium falciparum* and *P. vivax* in children on Espiritu Santo island, Vanuatu. *Trans R Soc Trop Med Hyg* 1996;90:614-620.
- 47 **Oppenheimer SJ, MacFarlane SB, Moody JB, Bunari O, Williams TE, Harrison C, Hendrickse RG.** Iron and infection in infancy – report on field studies in Papua New Guinea: 1. Demographic

- description and pilot surveys. *Ann Trop Paediatr* 1984;4:135-143.
- 48 **Cattani JA, Tulloch JL, Vrbova H, Jolley D, Gibson FD, Moir JS, Heywood PF, Alpers MP, Stevenson A, Clancy R.** The epidemiology of malaria in a population surrounding Madang, Papua New Guinea. *Am J Trop Med Hyg* 1986;35:3-15.
 - 49 **Moir JS, Garner PA, Heywood PF, Alpers MP.** Mortality in a rural area of Madang Province, Papua New Guinea. *Ann Trop Med Parasitol* 1989;83:305-319.
 - 50 **Allen SJ, O'Donnell A, Alexander NDE, Clegg JB.** Severe malaria in children in Papua New Guinea. *QJM* 1996;89:779-788.
 - 51 **Fowkes FJL, Allen SJ, Allen A, Alpers MP, Weatherall DJ, Day KP.** Increased microerythrocyte count in homozygous alpha⁺-thalassaemia contributes to protection against severe malarial anaemia. *PLoS Med* 2008;5:e56.
 - 52 **Mackintosh CL, Beeson JG, Marsh K.** Clinical features and pathogenesis of severe malaria. *Trends Parasitol* 2004;20:597-603.
 - 53 **McFaul SJ, Bowman PD, Villa VM.** Hemoglobin stimulates the release of proinflammatory cytokines from leukocytes in whole blood. *J Lab Clin Med* 2000;135:263-269.
 - 54 **Alayash AI.** Hemoglobin-based blood substitutes and the hazards of blood radicals. *Free Radic Res* 2000;33:341-348.
 - 55 **Wambua S, Mwangi TW, Kortok M, Uyoga SM, Macharia AW, Mwacharo JK, Weatherall DJ, Snow RW, Marsh K, Williams TN.** The effect of alpha⁺-thalassaemia on the incidence of malaria and other diseases in children living on the coast of Kenya. *PLoS Med* 2006;3:e158.
 - 56 **Williams TN, Wambua S, Uyoga S, Macharia A, Mwacharo JK, Newton CR, Maitland K.** Both heterozygous and homozygous alpha⁺-thalassemias protect against severe and fatal *Plasmodium falciparum* malaria on the coast of Kenya. *Blood* 2005;106:368-371.
 - 57 **Menendez C, Fleming AF, Alonso PL.** Malaria-related anaemia. *Parasitol Today* 2000;16:469-476.
 - 58 **Bunyaratvej A, Fucharoen S, Butthep P, Sae-ung N, Kamchonwongpaisan S, Khuhapinant A.** Alterations and pathology of thalassemic red cells: comparison between alpha- and beta-thalassemia. *Southeast Asian J Trop Med Public Health* 1995;26(Suppl 1):257-260.
 - 59 **Chinprasertsuk S, Wanachiwanawin W, Pattanapanyasat K, Tatsumi N, Piankijagum A.** Relation of haemolytic anaemia and erythrocyte-bound IgG in alpha- and beta-thalassaemic syndromes. *Eur J Haematol* 1997;58:86-91.
 - 60 **Rachmilewitz EA, Treves A, Treves AJ.** Susceptibility of thalassemic red blood cells to phagocytosis by human macrophages in vitro. *Ann NY Acad Sci* 1980;344:314-322.
 - 61 **Rees DC, Williams TN, Maitland K, Clegg JB, Weatherall DJ.** Alpha thalassaemia is associated with increased soluble transferrin receptor levels. *Br J Haematol* 1998;103:365-369.
 - 62 **Krych-Goldberg M, Atkinson JP.** Structure-function relationships of complement receptor type 1. *Immunol Rev* 2001;180:112-122.
 - 63 **Xiang L, Rundles JR, Hamilton DR, Wilson JG.** Quantitative alleles of CR1: coding sequence analysis and comparison of haplotypes in two ethnic groups. *J Immunol* 1999;163:4939-4945.
 - 64 **Thomas BN, Donvito B, Cockburn I, Fandeur T, Rowe JA, Cohen JH, Moulds JM.** A complement receptor-1 polymorphism with high frequency in malaria endemic regions of Asia but not Africa. *Genes Immun* 2005;6:31-36.
 - 65 **Fowkes FJL, Woods CM, Imrie HJ, Michon P, Tavul L, Reeder JC, Day KP.** Complement receptor 1 polymorphism is associated with alpha⁺-thalassaemia genotype in a malaria-endemic region of Papua New Guinea. Abstract 2510 in Program and Abstracts of the Fifty-fifth Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, Georgia, 12-16 Nov 2006.
 - 66 **Cockburn IA, Mackinnon MJ, O'Donnell A, Allen SJ, Moulds JM, Baisor M, Bockarie M, Reeder JC, Rowe JA.** A human complement receptor 1 polymorphism that reduces *Plasmodium falciparum* rosetting confers protection against severe malaria. *Proc Natl Acad Sci USA* 2004;101:272-277.
 - 67 **David PH, Handunnetti SM, Leech JH, Gamage P, Mendis KN.** Rosetting: a new cytoadherence property of malaria-infected erythrocytes. *Am J Trop Med Hyg* 1988;38:289-297.
 - 68 **Rowe JA, Moulds JM, Newbold CI, Miller LH.** *P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. *Nature* 1997;388:292-295.
 - 69 **Stoute JA.** Complement-regulatory proteins in severe malaria: too little or too much of a good thing? *Trends Parasitol* 2005;21:218-223.
 - 70 **Newbold C, Warn P, Black G, Berendt A, Craig A, Snow B, Msobo M, Peshu N, Marsh K.** Receptor-specific adhesion and clinical disease in *Plasmodium falciparum*. *Am J Trop Med Hyg* 1997;57:389-398.
 - 71 **Cohen JH, Lutz HU, Pennaforte JL, Bouchard A, Kazatchkine MD.** Peripheral catabolism of CR1 (the C3b receptor, CD35) on erythrocytes from healthy individuals and patients with systemic lupus erythematosus (SLE). *Clin Exp Immunol* 1992;87:422-428.
 - 72 **Moldenhauer F, Botto M, Walport MJ.** The rate of loss of CR1 from ageing erythrocytes in vivo in normal subjects and SLE patients: no correlation with structural or numerical polymorphisms. *Clin Exp Immunol* 1988;72:74-78.
 - 73 **Ripoche J, Sim RB.** Loss of complement receptor type 1 (CR1) on ageing of erythrocytes. Studies of proteolytic release of the receptor. *Biochem J* 1986;235:815-821.
 - 74 **Petz LD, Garratty G.** Acquired Immune Hemolytic Anemias. New York: Churchill Livingstone, 1980.
 - 75 **Guerra CA, Snow RW, Hay SI.** Mapping the global extent of malaria in 2005. *Trends Parasitol* 2006;22:353-358.
 - 76 **Michon P, Cole-Tobian JL, Dabod E, Schoepflin S, Igu J, Susapu M, Tarongka N, Zimmerman PA, Reeder JC, Beeson JG, Schofield L, King CL, Mueller I.** The risk of malarial infections and disease in Papua New Guinean children. *Am J Trop Med Hyg* 2007;76:997-1008.
 - 77 **Genton B, D'Acremont V, Rare L, Baea K, Reeder JC, Alpers MP, Müller I.** *Plasmodium vivax* and mixed infections are associated with severe malaria in children: a prospective cohort study from Papua New Guinea. *PLoS Med* 2008;5:e127.

Does Integrated Management of Childhood Illness (IMCI) make a difference to the assessment of sick children in Papua New Guinea?

M. MOTI¹ AND J.D. VINCE²

Division of Health, Mendi, Southern Highlands Province, Papua New Guinea and
Discipline of Child Health, School of Medicine and Health Sciences, University of
Papua New Guinea, Port Moresby

SUMMARY

Two provinces, one of which had introduced the Integrated Management of Childhood Illness (IMCI) policy to some degree and one in which there was no IMCI program, were selected to compare health workers' assessment of children attending provincial hospitals, district hospitals and health centres. 23 health workers were observed during 373 child assessments to determine their ability to detect the symptoms and signs detailed in the IMCI 10-step checklist. Health workers in the province that had introduced IMCI performed significantly better than their counterparts in 11 of the 24 criteria studied. These criteria included asking about 'too sick symptoms' ($p < 0.001$ for asking about vomiting and feeding and $p < 0.012$ for asking about convulsions), counting respiratory rate and checking for chest indrawing in children presenting with cough ($p < 0.001$), checking skin elasticity in children presenting with diarrhoea ($p < 0.02$), checking for neck stiffness in those presenting with fever ($p < 0.001$), checking for pallor ($p < 0.001$) and accurately plotting the child's weight on the weight graph ($p < 0.001$). Children in this province were more likely to be fully vaccinated (OR 1.96 [1.25-3.08]) than those in the province in which no attempt had been made to introduce IMCI. The facilities were ranked by the proportion of children correctly assessed. The best facility was the health centre which had been a pilot site for the introduction of IMCI in the province several years before the study. The results of the study, which clearly demonstrate that IMCI does make a difference, are in accordance with data from many parts of the resource-poor world and strongly support the Department of Health's decision to implement IMCI in the country. Every effort should be made to ensure that all provinces introduce the program and support its continuation as part of the Strategic Package for Child Survival.

Introduction

Papua New Guinea (PNG) has the worst child health indices in the Pacific Islands Region and the second worst in the World Health Organization (WHO) Western Pacific Region (1). The Demographic and Health Surveillance (DHS) Survey of 1996 reported under-5 mortality and infant mortality rates of 112 and 74 per 1000 live births (2). The National Department of Health reported rates of 102 and 73 in 2000 (3) and the most recent values of 64 and 49 are from the 2006 DHS Survey (4). Whilst these latest figures are encouraging, the country still has a long way

to go before reaching the 4th Millennium Development Goal of reducing the under-5 mortality rate to two-thirds of the 1990 rate by the year 2015 (5). The major causes of child deaths in the country are pneumonia, meningitis and malaria. Low birthweight, prematurity, birth injuries, neonatal sepsis, diarrhoeal diseases and tuberculosis are also important causes of death, with deaths from HIV/AIDS (human immunodeficiency virus/acquired immune deficiency syndrome) rapidly increasing (6).

The Integrated Management of Childhood Illness (IMCI) program was introduced by

¹ Division of Health, PO Box, Mendi, Southern Highlands Province 251, Papua New Guinea

² Discipline of Child Health, Division of Clinical Sciences, School of Medicine and Health Sciences, PO Box 5623, Boroko, National Capital District 111, Papua New Guinea
johndvince@gmail.com
Corresponding author

WHO in the mid-1990s in an effort to improve child health outcomes in resource-poor countries (7). It has three components:

1. Improving health worker skills in identifying and treating sick children
2. Improving family and community health practices
3. Strengthening health systems support for IMCI.

The first component is based on training health workers to assess sick children through a checklist. It depends on a simple systematized but comprehensive step-by-step history and examination and includes assessing the nutritional and immunization status of the child. Initially a 10-step checklist was used for children up to five years old. An 8-step checklist for infants of less than two months of age was added more recently. The original concept of a 10-step checklist was first introduced in PNG in the early 1990s as part of the USAID-funded Child Survival Strategy and was incorporated into the Paediatric Standard Treatment Book in 1993. The concept was subsequently adopted and modified by WHO.

IMCI requires considerable training and ongoing support. Its introduction and distribution require careful planning and attention to detail and a significant investment in time and finance. Nevertheless, early reports (8-10) suggested that IMCI had the potential to improve health care for children, and by 2004 it had been introduced to more than 80 countries throughout the world (11). Although there have been significant problems with implementing all three components of the IMCI program in many countries, there is now a considerable body of evidence that, when applied, the program results in improved management of children at health care facilities (11-17). IMCI is now one of the 7 evidence-based strategies in the WHO/UNICEF (United Nations Children's Fund) Essential Package for Child Survival (18).

In PNG the first training on IMCI was carried out in Madang in 2001. It was envisaged that the IMCI program would roll out to all districts in all provinces over the next few years. Unfortunately such a roll-out has been very slow and there are still areas of the country where the program has not been

introduced.

The aim of the present study was to see if IMCI is making a difference to the assessment and treatment of children in the PNG context.

Methods

This cross-sectional study employed quantitative and qualitative methods to compare the assessment and management of sick children in rural and hospital settings. With Henganofi as one of the initial pilot sites, and with some IMCI training having been carried out for other Eastern Highlands Province (EHP) health workers, it was felt that EHP could be regarded as an 'IMCI' province. In contrast, although a small number of staff from Southern Highlands Province (SHP) had undergone the IMCI training course, there has been no roll-out of the program within the province and it was regarded as a 'non-IMCI' province.

The provincial hospital, one district hospital and one health centre were chosen from each province, primarily by virtue of their accessibility by road. In the EHP, data were collected at Goroka Base Hospital, Kainantu District Hospital and Henganofi Health Centre. In the SHP, data were collected at Mendi General Hospital, Ialibu District Hospital and Pangia Health Centre.

The study population included all children attending the health facilities who were aged between 2 months and 5 years. The initial plan was to sample 400 assessments of children from 2 months to 5 years of age, 100 assessments from each provincial hospital, 50 assessments from each district hospital and 50 assessments from each health centre.

Systematic sampling was used to select every second child that came into the health facility as the subject for the observed assessment by outpatient department staff. There were no exclusion criteria.

The following methods were used to record data from this study.

1. Child assessment checklist – This was used by the observer, and at one facility by a trained assistant, to record data as the outpatient staff assessed the sick children.

2. Exit interviews – Exit interviews were conducted in Tok Pisin in a comfortable setting in the vicinity of the clinic by the principal researcher (MM) or an assistant after staff attended to the child and necessary treatments and vaccinations, if any, were administered. Consent was obtained before conducting the interviews. Clients were assured of confidentiality. Information on regularity of breastfeeding and how often the child was being fed with other food was collected. Vaccination status of children was collected from the child's health record book.
3. Essential items checklist – This checklist was used to collect data on the availability of some items necessary for the full implementation of the IMCI. The information was acquired mainly through interviewing the nursing staff at each facility.

The checklists were modified from the

WHO Health Facility Survey (19).

Data were collected between January and February of 2007 spanning four weeks in each province. Two weeks were spent in each hospital with one week in each district hospital and health centre. No variation in disease prevalence was expected since the disease patterns of childhood illnesses are similar in the highlands provinces.

The variables that were used to compare and describe performances of staff among the study facilities (Table 1) were essentially those in the 10-step checklist. The observer noted the content of the histories taken and the examinations performed. The observer also noted whether the child was admitted, referred or treated according to the diagnosis the staff had made and whether treatments prescribed were consistent with treatment protocols adapted by the National Department of Health.

Pre-testing of the patient assessment

TABLE 1

LIST OF VARIABLES IN THE CHILD ASSESSMENT CHECKLIST

Condition	Symptom variables	Sign variables
Too sick child	Not feeding or eating, vomiting and convulsions	Convulsions, drowsy and unconscious
Cough	Cough	Fast breathing, chest indrawing, pulse of 160 per minute, enlarged liver and auscultation
Fever	Fever	Temperature check and neck stiffness
Diarrhoea	Diarrhoea and blood or mucus in stool	Sunken eyes, skin elasticity and unable to drink
Anaemia		Pallor in conjunctiva, hand palms and fingernails
Ear problems	Pain and discharge	Mastoid swelling, discharge and auroscope examination of the ear
Weight chart		Child weighed, plotting the graph, oedema and wasting
Breastfeeding	Breastfeeding, number of times and other food given	
Vaccinations		Being fully immunized

checklist was carried out, with permission, at the Six Mile Urban clinic in Port Moresby, resulting in minor changes to the section on breastfeeding and nutrition in the exit interview data collection form.

Data were checked each evening. All data, except for immunization and breastfeeding that were analyzed manually, were entered into and analyzed using the Epi Info 6 computer software. Yates corrected chi-squared tests were used for comparable data sets, unless figures were less than 5, when Fisher's exact test was used. Percentages were rounded to the nearest whole number. Proportions were used to rank facilities in terms of performance of staff. The odds ratio (OR) and 95% confidence interval were used to compare the vaccination coverage of the children from the two provinces.

The study was approved by the University of Papua New Guinea School of Medicine and Health Sciences Research Committee and permission to carry out the study was obtained from managers of the participating health facilities in the two provinces. The only financial support for the study came from Mendi Provincial Hospital, which provided K1500 for accommodation costs.

Results

Health workers' assessments of 373 children were observed in the six health facilities. Only 24 children were seen at Pangia instead of the 50 anticipated. The overall sex ratio of the children seen was 1.3:1, with a higher ratio in EHP than in SHP (1.46:1 vs 1.14:1). Figure 1 shows the age profile of the children; 45% of the children were less than 1 year of age and 28% were aged between 1 and 2 years.

49% of the children seen presented with cough (53% in EHP and 46% in SHP), 24% presented with fever (21.5% and 27%) and 13% with diarrhoea (13.5% and 13%). Cough, fever and diarrhoea accounted for 87% (323/373) of the presentations.

Observations of 23 health workers were made, 9 in SHP and 14 in EHP. 16 (8 in each province) were nursing officers, 2 in EHP were health extension officers and the remaining 5 were community health workers (4 in EHP, 1 in SHP).

The staff performance in assessing the children is shown by province in Table 2. Staff in EHP facilities performed significantly better

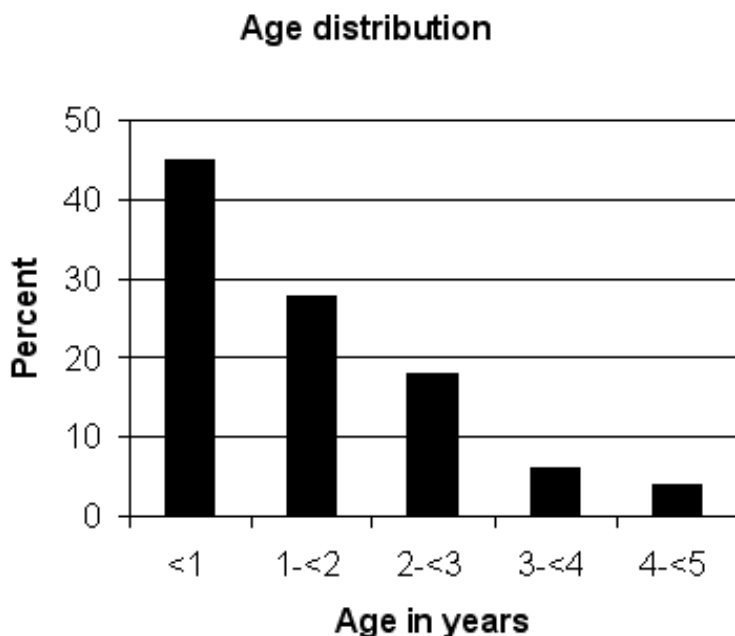


Figure 1. Age distribution of the study children.

TABLE 2

COMPARISON OF STAFF PERFORMANCE BETWEEN THE PROVINCES

Indicator	Eastern Highlands Province (n=200)	Southern Highlands Province (n=173)	p value
Too sick symptoms			
Asked about feeding/eating	156	75	<0.001
Asked about vomiting	88	37	<0.001
Asked about convulsions	18	4	<0.012
Too sick signs			
Came in with convulsions	0	0	
Came in unconscious	0	0	
Cough and pneumonia			
Cough	106	79	ns
Checked for fast breathing	42	7	<0.001
Checked for chest indrawing	72	9	<0.001
Did auscultations	53	73	<0.002
Fever (malaria and meningitis)			
Complain of fever	43	44	ns
Taken temperature	116	123	<0.012
Neck stiffness	21	2	<0.001
Diarrhoea			
Complain of diarrhoea	27	24	ns
Checked dysentery	22	22	ns
Checked skin elasticity	6	0	<0.02
Thirsty to drink	3	0	ns
Checked for sunken eyes	6	2	ns
Anaemia			
Checked conjunctiva, palm and fingernail beds	64	1	<0.001

Ear problems

Complain of ear pain	6	4	ns
Checked for discharge	112	9	<0.001
Checked for mastoid swelling	1	1	ns
Checked with auroscope	31	5	<0.001

Nutrition assessment

Weighed the child	185	153	ns
Plotted the weight	135	54	<0.001

ns = difference not significant

than those in the SHP in 11 of the 24 criteria studied and these differences were present at all levels. These criteria included asking about 'too sick symptoms', counting respiratory rate and checking for chest indrawing in children presenting with cough, checking skin elasticity in children presenting with diarrhoea, checking for neck stiffness in those presenting with fever, checking for pallor, checking for ear discharge and accurately plotting the child's weight on the weight graph. In 2 criteria (auscultation and taking the child's temperature) the staff in SHP facilities performed better.

Facilities were ranked in relation to staff performance (Table 3). Staff at Henganofi outperformed those in other facilities, with those at Goroka and Kainantu ranked second and third overall.

Vaccination coverage of children in the study is shown in Figure 2. 57.5% of children less than 1 year of age were up to date with their immunization and 64% of children aged less than 18 months were fully vaccinated. Of the children aged between 24 months and 5 years, 63% had been fully immunized. Children attending the EHP facilities were more likely than those attending SHP facilities to be up to date with their vaccinations (141/200 (71%) vs 95/173 (55%); OR 1.96 [1.25-3.08]).

The proportion of children who were still breastfeeding by age is shown in Figure 3. 96% of the children who were aged 2 to 17 months were breastfed. Breastfeeding rates

were similar between the two provinces with almost universal breastfeeding up to 6 months and 90% from 7 to 17 months. Over 18 months of age breastfeeding rates dropped, but 57% of children were still breastfeeding in the 18 to 23 months age group.

All facilities had vaccine storage fridges and vaccines but all vaccines in Pangia had been frozen. All facilities had chloramphenicol suspension and injection, paraldehyde, diazepam, half-strength Darrow's solution and oral rehydration salts. All had artemether injection but there were no supplies of amodiaquine (or chloroquine) or artesunate tablets at Kainantu. All facilities had both foot scales and hanging scales, but there was no auroscope at either of the district hospitals or health centres. Kainantu had no thermometers and although Henganofi had 3 initially all three were broken during the study.

Discussion

There were a number of limitations to this study. Time and financial constraints meant that only facilities accessible by road were chosen. By-passing of the 'lower-level' health facilities by those who could afford transport to the provincial hospital may have biased the samples. The total number of assessments observed was less than planned in SHP. Observer-induced bias may have occurred, particularly as female staff were being observed by a male, but staff had been informed of the nature of the study, and were aware that the data would be 'anonymous' –

TABLE 3**RANKING OF FACILITIES BY STAFF PERFORMANCE**

Indicator	Best	Second	Third
Too sick symptoms (Not feeding/eating, vomiting and convulsions)	Henganofi (90%)	Pangia (79%)	Goroka (78%)
Cough (Fast breathing and chest indrawing)	Henganofi (97%)	Goroka (65%)	Kainantu (36%)
Fever (Neck stiffness)	Henganofi (100%)	Goroka (30%)	lalibu (7%)
Diarrhoea (Sunken eyes, loss of skin elasticity and thirstiness)	Henganofi (44%)	Goroka (29%)	Mendi (13%)
Plotting of weights	Henganofi (98%)	Goroka (86%)	Mendi (55%)
Ear problems (Ear pain, check discharge and mastoid swelling)	Henganofi (92%)	Goroka (64%)	Kainantu (6%)
Anaemia (Check for pallor signs)	Henganofi (84%)	Goroka (20%)	Kainantu (4%)
Breastfeeding (Child below two years continuously breastfed)	lalibu and Goroka (75%)	Kainantu (67%)	Henganofi and Mendi (62%)
Immunizations (Up to date with immunization at 11 months)	Goroka (78%)	Henganofi (70%)	Kainantu (57%)

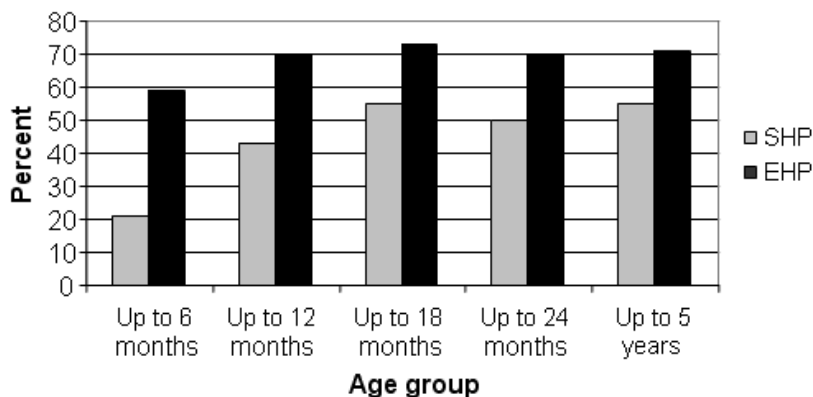
Immunization coverage

Figure 2. Immunization coverage (up to date or completed) in Southern Highlands Province and Eastern Highlands Province.

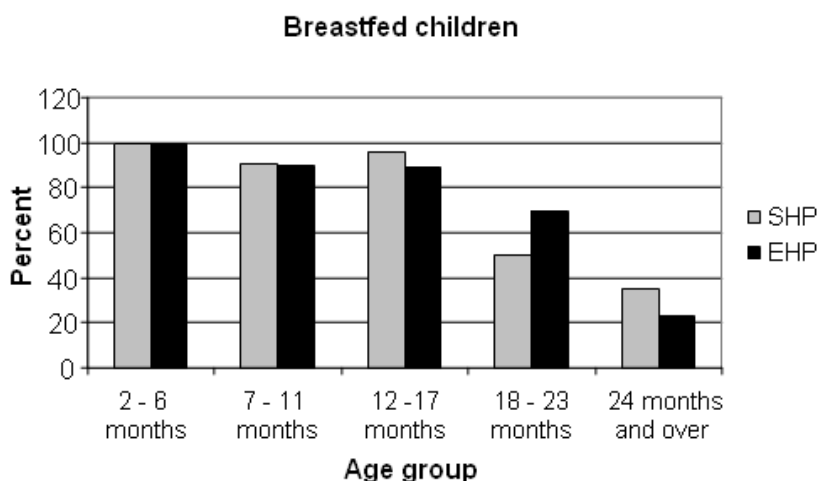


Figure 3. Comparison of breastfeeding between the two provinces.

and such bias would be likely to apply across all facilities. The use of an assistant in one facility might have introduced some inter-observer bias, but care was taken in the training of the observer and such bias is thought to be minimal.

The initial broad assumption that all health workers and health facilities in EHP were practising IMCI was incorrect. Nevertheless, Henganofi had been one of the initial pilot sites and staff there were largely adhering to the IMCI method. At Goroka, although the nurses screening the children were new recruits and had not been through IMCI training, each of the tables at which they were stationed had a 10-step flip chart which they had been advised to follow. In addition Goroka Hospital had two paediatricians who had been trained in IMCI and who championed the cause of IMCI in the province. In contrast, no training courses had been carried out in SHP, there was no paediatrician in the province, and none of the staff had been through an IMCI training course.

The initial sampling size had been determined by what was felt to be practical within the study time frame. Sample size calculations performed after the completion of the study were based on the ability of the study to demonstrate a 25% difference between health workers in EHP and those in SHP health facilities in detection of subcostal recession in children with cough and in the

detection of 'too sick symptoms' in presenting children (assuming baseline rates of 20% and 40% in the non-IMCI province). The calculations for a study power of 90% and a significance of <0.05 indicated sample sizes of 80 and 90 respectively. The study found differences that were considerably greater than 25%.

There would seem to be little doubt that in facilities in which IMCI is being either almost completely or at least partly implemented or where there has been some exposure of health workers to the IMCI program, assessment of children was considerably better than in those facilities where IMCI was not practised at all. This finding is entirely consistent with the studies from other countries.

It was encouraging to find that with the exception of Kainantu, at which there were no oral antimalarial drugs, the health facilities were appropriately stocked with pharmaceuticals. It should be remembered, however, that they were all accessible by road, facilitating drug procurement. Alarming, all the vaccines at Pangia were frozen, indicating either a poor understanding of vaccine storage requirements or lack of attention to these requirements. Auroscopes were present only at the provincial hospitals. The availability of thermometers, regarded by most health workers as an essential piece of equipment, varied. There were none at

Kainantu, those at Henganofi were broken during the study, and, interestingly, nurses at Goroka, much to their credit, had purchased their own.

Selection of the 11 children for hospital admission (including 3 at Goroka and 7 at Mendi) was appropriate. However, standard treatment guidelines were not always followed. At Goroka, children with a fever and cough, but no raised respiratory rate or intercostal or subcostal recession, were given a stat dose of benzyl penicillin and a shower, and sent home on oral amoxicillin when their temperature had fallen. This almost certainly represents an overuse of antibiotics for viral infections – a problem which is almost universal.

Only 57.5% of children below the age of 1 year were appropriately vaccinated. Whilst there was a 'catch up' to 64% by 18 months, this was still well below the national target. Vaccination coverage in EHP was consistently better than that in SHP at all ages. It is possible that differences in the magnitude of law and order problems between the provinces and the relationship of such problems to access to health facilities and to immunization services through maternal and child health outreach activities may have contributed to this difference.

The introduction and practice of IMCI is only one of the strategies proposed in the Strategic Package for Child Survival to enable countries to meet the 4th Millennium Development Goal. Relatively few countries are on target to reach the goal. One of those that is on target – in spite of significant problems – is Tanzania. In a recent assessment of the factors contributing to this progress, it was noted that the practice of IMCI had increased from 19% to 73% of districts between surveys in 1999 and 2004-2005 (20). Other strategies had also improved – the administration of vitamin A by 71% and the use of insecticide-treated bed nets by 25%. Exclusive breastfeeding in children aged less than 2 months had increased from 58% to 70% and from 32% to 41% in children less than 6 months. Although the present study did not determine the rate of exclusive breastfeeding at various ages, and previous studies have shown that solids are introduced well before 6 months in a high proportion of Papua New Guinean infants, particularly in the highland provinces (21-22), it was reassuring to find that some

breastfeeding was almost universal in the first 6 months and was continued for 96% of children up to 17 months.

Although the present study did not examine the financial implications of IMCI, the international literature indicates that IMCI is highly cost-effective (23-27). Part of the financial benefit is likely to result from improved drug, particularly antibiotic, prescribing (28).

Whilst the use of the IMCI algorithms has been clearly shown to improve the assessment and management of children at various levels of health facility, a number of problems with the implementation of all three components of the IMCI package have become apparent. Without the simultaneous attention to health care facility and the community involvement components, IMCI is unlikely to achieve its potential benefits. A multi-country evaluation of IMCI has reported significant programmatic difficulties in 'rolling out' the program from the initial starting sites (29) and it is recognized that, once established, the program requires ongoing support. Concern has been expressed that IMCI is not reaching those who most need it, the poorest and most geographically isolated populations (30,31). PNG is experiencing its own problems in the roll-out of the IMCI program to all provinces and districts. Much remains to be done to ensure that the benefits of IMCI are evenly and efficiently distributed.

In conclusion, this study has clearly demonstrated the benefits of the IMCI approach to the assessment and management of sick children in the Papua New Guinea context. It does make a difference. The results give added impetus to efforts to roll out the IMCI program to all provinces and all facilities and to provide ongoing encouragement and support to all health workers in their use of this important and efficient tool in their day-to-day practice.

ACKNOWLEDGEMENTS

We thank the Eastern and Southern Highlands Provincial Health authorities for allowing the study to take place, the health workers assessed in this study for their willing participation, and the mothers and children for their involvement. We also thank Prof. Trevor Duke for assistance with sample size calculations.

REFERENCES

- 1 **World Health Organization.** Western Pacific Country Health Information Profiles 2005 Revision. Manila: Regional Office for the Western Pacific, 2005.
- 2 **National Statistical Office.** Demographic and Health Surveillance Survey. Papua New Guinea National Statistical Office, Port Moresby, 1996.
- 3 **Papua New Guinea Department of Health.** Papua New Guinea National Health Plan 2001-2010, Volume III, Part One. Port Moresby: Department of Health, Aug 2000:34-35.
- 4 **National Statistical Office.** Demographic and Health Surveillance Survey. Papua New Guinea National Statistical Office, Port Moresby, 2006.
- 5 **United Nations Children's Fund.** The State of the World's Children 2008: Child Survival. New York: UNICEF, 2007:152.
- 6 **Papua New Guinea Department of Health.** Leading causes of mortality, summary health statistics, 2004. Papua New Guinea Department of Health, Port Moresby, 2004.
- 7 **Nicoll A.** Integrated management of childhood illness in resource-poor countries: an initiative from the World Health Organization. *Trans R Soc Trop Med Hyg* 2000;94:9-11.
- 8 **Kalter HD, Schillinger JA, Hossain M, Burnham G, Saha S, de Wit V, Khan NZ, Schwartz B, Black RE.** Identifying sick children requiring referral to hospital in Bangladesh. *Bull World Health Organ* 1997;75(Suppl 1):65-75.
- 9 **Kolstad P, Burnham G, Kalter HD, Kenya-Mugisha N, Black RE.** The integrated management of childhood illness in western Uganda. *Bull World Health Organ* 1997;75(Suppl 1):77-85.
- 10 **Shah D, Sachdev HP.** Evaluation of the WHO/UNICEF algorithm for integrated management of childhood illness between the age of two months to five years. *Indian Pediatr* 1999;36:767-777.
- 11 **Armstrong Schellenberg J, Bryce J, de Savigny D, Lambrechts T, Mbuya C, Mgalula L, Wilczynska K, Tanzania IMCI Multi-Country Evaluation Health Facility Survey Study Group.** The effect of Integrated Management of Childhood Illness on observed quality of care of under-fives in rural Tanzania. *Health Policy Plan* 2004;19:1-10.
- 12 **El Arifeen S, Blum LS, Hoque DM, Chowdhury EK, Khan R, Black RE, Victora CG, Bryce J.** Integrated Management of Childhood Illness (IMCI) in Bangladesh: early findings from a cluster-randomised study. *Lancet* 2004;364:1595-1602.
- 13 **El Arifeen S, Bryce J, Gouws E, Baqui AH, Black RE, Hoque DM, Chowdhury EK, Yunus M, Begum N, Akter T, Siddique A.** Quality of care for under-fives in first-level health facilities in one district of Bangladesh. *Bull World Health Organ* 2005;83:260-267.
- 14 **Armstrong Schellenberg JR, Adam T, Mshinda H, Masanja H, Kabadi G, Mukasa O, John T, Charles S, Nathan R, Wilczynska K, Mgalula L, Mbuya C, Mswia R, Manzi F, de Savigny D, Schellenberg D, Victora C.** Effectiveness and cost of facility-based Integrated Management of Childhood Illness (IMCI) in Tanzania. *Lancet* 2004;364:1583-1594.
- 15 **Bryce J, Gouws E, Adam T, Black RE, Schellenberg JA, Manzi F, Victora CG, Habicht JP.** Improving quality and efficiency of facility-based health care through Integrated Management of Childhood Illness in Tanzania. *Health Policy Plan* 2005;20(Suppl 1):i69-i76.
- 16 **Chopra M, Patel S, Cloete K, Sanders D, Petersen S.** Effect of an IMCI intervention on quality of care across four districts in Cape Town, South Africa. *Arch Dis Child* 2005;90:397-401.
- 17 **Zhang Y, Dai Y, Zhang S.** Impact of implementation of Integrated Management of Childhood Illness on improvement of health system in China. *J Paediatr Child Health* 2007;43:681-685.
- 18 **World Health Organization, United Nations Children's Fund.** Regional Child Survival Strategy: Accelerated and Sustained Action Towards MDG 4. Manila: World Health Organization Regional Office for the Western Pacific, 2006.
- 19 **World Health Organization.** Health Facility Survey. Tool to Evaluate Quality of Care Delivered to Sick Children Attending Outpatients Facilities. Geneva: World Health Organization, 2003.
- 20 **Massanja H, de Savigny D, Smithson P, Schellenberg J, John T, Mbuya C, Upunda G, Boerma T, Victora C, Smith T, Mshinda H.** Child survival gains in Tanzania: analysis of data from demographic and health surveys. *Lancet* 2008;371:1276-1283.
- 21 **Friesen H, Vince J, Boas P, Danaya B, Mokela D, Ogle G, Asuo P, Kemiki A, Lagani W, Rongap T, Varughese M, Saweri W.** Infant feeding practices in Papua New Guinea. *Ann Trop Paediatr* 1998;18:209-215.
- 22 **Anga G, Vince JD, Kaupa M.** Early introduction of solids and pneumonia in young infants in Papua New Guinea: a case control study. *J Trop Pediatr* 2008;54:192-195.
- 23 **Boulanger LL, Lee LA, Odhacha A.** Treatment in Kenyan rural health facilities: projected drug costs using the WHO-UNICEF Integrated Management of Childhood Illness (IMCI) guidelines. *Bull World Health Organ* 1999;77:852-858.
- 24 **Adam T, Manzi F, Schellenberg JA, Mgalula L, de Savigny D, Evans DB.** Does the Integrated Management of Childhood Illness cost more than routine care? Results from the United Republic of Tanzania. *Bull World Health Organ* 2005;83:369-377.
- 25 **Bishai D, Mirchandani G, Pariyo G, Burnham G, Black R.** The cost of quality improvement due to Integrated Management of Childhood Illness (IMCI) in Uganda. *Health Econ* 2008;17:5-19.
- 26 **Wammanda RD, Ejemi CL, Iorliam T.** Drug treatment costs: projected impact of using the integrated management of childhood illnesses. *Trop Doct* 2003;33:86-88.
- 27 **Kolstad PR, Burnham G, Kalter HD, Kenya-Mugisha N, Black RE.** Potential implications of the Integrated Management of Childhood Illness (IMCI) for hospital referral and pharmaceutical usage in western Uganda. *Trop Med Int Health* 1998;3:691-699.
- 28 **Gouws E, Bryce J, Habicht JP, Amaral J, Pariyo G, Schellenberg JA, Fontaine O.** Improving antimicrobial use among health workers in first-level facilities: results from the multi-country evaluation of the Integrated Management of Childhood Illness strategy. *Bull World Health Organ* 2004;82:509-515.
- 29 **Bryce J, Victora CG, Habicht JP, Black RE, Scherpbier RW, MCE-IMCI Technical Advisors.** Programmatic pathways to child survival: results of a multi-country evaluation of Integrated Management of Childhood Illness. *Health Policy Plan* 2005;20(Suppl 1):i5-i17.
- 30 **Gwatkin DR.** IMCI: what can we learn from an innovation that didn't reach the poor? *Bull World*

- Health Organ* 2006;84:768.
- 31 **Victora CG, Huicho L, Amaral JJ, Armstrong-Schellenberg J, Manzi F, Mason E, Scherpbier R.** Are health interventions implemented where they are most needed? District uptake of the Integrated Management of Childhood Illness strategy in Brazil, Peru and the United Republic of Tanzania. *Bull World Health Organ* 2006;84:792-801.

***Glycophorin C* Δ^{exon3} is not associated with protection against severe anaemia in Papua New Guinea**

L. TAVUL¹, I. MUELLER¹, L. RARE¹, E. LIN¹, P.A. ZIMMERMAN², J. REEDER³, P. SIBA⁴ AND P. MICHON^{1,5}

Papua New Guinea Institute of Medical Research, Madang and Goroka, Papua New Guinea, Case Western Reserve University, Cleveland, United States of America and Burnet Institute of Medical Research, Melbourne, Australia

SUMMARY

The high frequencies of mutant haemoglobin and erythrocyte surface proteins in malaria-endemic regions have indicated that polymorphisms in human genes have been under selection pressure by severe malarial disease. Glycophorin C (GYPC) is a major surface erythrocyte protein and also a receptor for the *Plasmodium falciparum* erythrocyte-binding antigen 140 (EBA-140, also known as BAEBL). There is no binding to GYPC in Gerbich-negative (deletion of exon 3 in GYPC gene: *GYPC* Δ^{exon3}) erythrocytes by EBA-140, hence limiting invasion of erythrocytes by certain *P. falciparum* lines. The *GYPC* Δ^{exon3} allele reaches high frequencies in two areas of Papua New Guinea (PNG) where malaria is highly endemic. There is, however, no indication that Gerbich negativity protects against malaria-related illness. Using archival blood samples collected from children (<6 years of age) in the Wosera District, East Sepik Province, PNG, we investigated *GYPC* Δ^{exon3} as a possible genetic component of protection against severe malarial anaemia (SMA). The frequency of this human genetic polymorphism was found to be in accordance with previous studies. However, our result showed no association between SMA and *GYPC* Δ^{exon3} . Until such an association is clearly shown with severe malaria outcomes, these results raise questions regarding the role of malaria as a selective force for Gerbich negativity.

Introduction

Malaria is a major infectious disease in tropical areas and continues to be a burden in developing and poor countries. Annually, malaria parasites infect 10% of humanity and malaria disease is responsible for two to three million deaths per year and 400 to 900 million episodes of clinical illness throughout the world (1). In Papua New Guinea (PNG), malaria is the most frequent diagnosis at outpatient departments (2). With an annual incidence of 303 per 1000 people at risk,

malaria is a leading cause of admission into health facilities in endemic areas of PNG (3). To determine the factors leading to the development of severe malaria, significant research has focused on parasite virulence phenotypes and host genetics as two of the major contributing factors.

The proposal that variations in the host susceptibility to disease, during epidemics or after longer exposure to infectious agents, might have a genetic basis and could be a selectable trait is not new to science.

¹ Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea

² Center for Global Health and Disease, Case Western Reserve University, Cleveland, Ohio 44106-7286, United States of America

³ International Health Research Strategy, Burnet Institute of Medical Research, PO Box 2284, Melbourne, Victoria 2001, Australia

⁴ Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, Eastern Highlands Province 441, Papua New Guinea

⁵ Corresponding author
pmichon@datec.net.pg

Haldane suggested that the geographical distribution and frequency of thalassaemias, which are haemoglobin deficiencies, were due to a selective pressure of malaria on the maintenance of this genetic condition in human populations (4), and initiated what later became known as the 'malaria hypothesis'. The presence of several erythrocyte polymorphisms at high frequencies in malaria-endemic areas such as PNG provides support for this hypothesis. Genetic polymorphisms characterizing human populations exposed to malaria in parts of PNG include Southeast Asian ovalocytosis (SAO), α^+ -thalassaemia and Gerbich negativity. SAO (5,6) and α^+ -thalassaemia (7) have already been shown to confer protection against severe malaria. The Gerbich-negative phenotype is caused by a deletion of exon 3 in the glycophorin C (GYPC) gene on chromosome 2. Frequencies of this mutation were observed at approximately 15% on the North Coast of Madang, where SAO is present (7.4%), and up to 46.3% in the Wosera region (East Sepik Province), where SAO is virtually absent (8). Nevertheless, the same study showed no association between Gerbich negativity (or SAO alterations) in the prevalence or density of asymptomatic blood-stage malaria infection. Likewise there was no negative interaction between SAO and the Gerbich-negative alleles.

Glycophorins are abundant proteins present on the surface of human erythrocytes. Various polymorphisms have been described involving highly conserved GYP A, B and C either following recombination between GYP genes or single nucleotide substitutions defining the MNS and S/N blood group antigens (on GYPA and GYPB, respectively) (9). The high rate of non-synonymous substitutions and frequent interlocus conversions between these 3 genes suggested that they were under intense positive selection, possibly as a means to evade malaria parasites (10). GYPA and GYPB play an important role in the invasion of red blood cells (RBCs) by *Plasmodium falciparum*. GYPA is the receptor of a binding ligand of *P. falciparum*, the 175-kD erythrocyte-binding antigen (EBA-175) mediating a sialic-acid-dependent invasion pathway (11,12). GYPB is involved in invasion and it has been shown in West Africa that GYPB-deficient (also known as S-s-U) RBCs are resistant to invasion in vitro (12). The *P. falciparum* ligand EBA-140 (also known as BAEBL) (13) binds to GYPC and

conversely does not bind to Gerbich-negative erythrocytes suggesting that *P. falciparum* uses an alternative ligand for invading Gerbich-negative RBCs (11). These *P. falciparum* ligands are part of a multigene family of paralogous merozoite molecules (14) thought to be involved in alternative invasion pathways, which provide a certain redundancy in the invasion process that could prove beneficial to *P. falciparum* and allow it to escape host gene polymorphisms and immune responses (15,16).

Little is known about the effect of these glycophorin polymorphisms on severe malaria, and in particular on severe malarial anaemia (SMA), one of its main components in PNG. However, we postulated that there would be a strong association between exon 3 deletion in the GYPC gene and SMA. To test this we investigated the association of Gerbich negativity with SMA in a case-control study using SMA cases and clinical controls from the Wosera District, East Sepik Province, PNG. We also assessed the gene frequency of Gerbich negativity in the population of this area of intense malaria transmission.

Methods

Study site and sample collection

For this study, archival blood samples that had been collected in preparation for malaria vaccine trials were used, as part of the Wosera Demographic Surveillance System (DSS), a member of the INDEPTH network – an International Network of field sites with continuous Demographic Evaluation of Populations and Their Health. These samples were obtained between 1996 and 2002 from over 16,000 people presenting with presumptive malaria during routine morbidity surveillance at two health subcentres (Kaugia and Kunjingini, Wosera District, East Sepik Province, PNG). Approximately 12% of samples lacking haemoglobin readings were excluded. For the remaining samples (including 1141 from children aged over 6 months and less than 6 years) age, sex, blood slide readings and haemoglobin measurements were available. In order to test for the effect of the selected red cell polymorphism (*GYPC* Δ^{exon3}) on severe malarial anaemia, children 6 months to 6 years of age with haemoglobin levels of <50 g/l (World Health Organization recommended cut-off for severe anaemia) were selected as

cases. A control group of children with haemoglobin >50 g/l was selected within the matching criteria of age, sex and time (± 1 year, most within 3 months), with the closest time match available being chosen in most cases. After damaged or lost samples were excluded, 138 matched pairs of specimens were available for testing the association between Gerbich negativity and SMA.

DNA extractions and storage

A QIAamp®96 DNA Blood Kit was used to extract DNA from 310 packed-cell pellet field samples from the Wosera. DNA was re-suspended in 200 μ l of TE (Tris-EDTA) buffer given by the manufacturer and stored at -80°C for long-term storage and 4°C for current use.

Glycophorin C DNA analysis

DNA samples were amplified by PCR (polymerase chain reaction) in a reaction mixture containing: PCR buffer provided by the manufacturer, 1.5 mM $MgCl_2$, 200 μ M of each dNTP, 0.5 μ M of oligonucleotide primers GYPCin1/2-F (ACA ACA AGA GTC CCT GCC TTC ATA C) and GYPCex3-R (TGG GGG TGG AGG TCT CC), 0.3 μ M of oligonucleotide primers GYPCex2-F (GGG GAT GGC CTC TGC CTC) and GYPCin3-R (CAG ACA CGT TAG AAT CAT ACC CCA GG), and 0.025 U/ μ l Taq polymerase (Invitrogen, New Zealand). Touchdown PCR conditions included 6 cycles of 95°C for 30 seconds, 70°C for 30 seconds (with a decrease of 1°C at each cycle) and 72°C for 2 minutes. The remaining 30 cycles were 95°C for 30 seconds, 65°C for 30 seconds and 72°C for 2 minutes. PCR products were loaded in a 1.5% agarose gel (prepared in 1X TE buffer and containing 0.5 μ g/ml ethidium bromide).

Statistical analysis

Gene frequencies for SMA cases, clinical controls and published community frequencies were compared using chi-squared tests and logistic regression.

Results

Out of the 138 paired samples 49% (68/138) were males and the mean age (2.9 or 2.8 years) was similar for both the cases and controls (Table 1). The mean haemoglobin for the cases was 42.2 g/l and 88.3 g/l for the controls. *P. falciparum* infections assessed

by light microscopy were 67.4% in cases and 37.0% in the controls.

DNA from all archival samples was analyzed by PCR for Gerbich negativity. Expected PCR products were a 401 bp fragment for the wild type allele (normal, non-deleted *GYPC*) and a 1460 bp fragment for the exon 3-deletion allele (Δ^{exon3}) (Figure 1). The following frequencies were obtained for SMA cases: 28.3% (39/138) normal *GYPC*, 22.5% (31/138) homozygous for *GYPC* Δ^{exon3} and 49.3% (68/138) heterozygous. For the clinical controls 23.2% (32/138) were normal *GYPC*, 25.4% (35/138) homozygous *GYPC* Δ^{exon3} and 51.4% (71/138) heterozygous. The distribution of genotypes between cases and controls was similar ($\chi^2 = 1.00$, $p = 0.61$) (Table 2). The genotype frequency in cases was also similar to the one observed in a general population survey as published in a previous study (Table 2): cases vs community survey in the Wosera, $\chi^2 = 0.08$, $p = 0.96$ (8). Consequently, the *GYPC* Δ^{exon3} was not associated with protection against SMA, in neither the homozygous nor heterozygous state (Table 2). This lack of association was observed both in relation to matched clinical controls (the present study) and in relation to the general population.

Discussion

We found no association between *GYPC* Δ^{exon3} genotypes and protection against SMA and therefore our hypothesis that *GYPC* Δ^{exon3} confers protection was rejected. It could be argued that the lack of association was due to our choice of controls. The controls were all children attending the same health centre with a non-respiratory febrile illness as opposed to healthy community controls in the same age groups. As many of them were suffering from uncomplicated malaria, this could have led to an underestimation of any potential effect of Gerbich negativity on SMA, and possibly against uncomplicated malaria illness. However, frequencies for *GYPC* Δ^{exon3} that causes Gerbich negativity, for both SMA cases and controls, were similar in our studies to previous observations made in the general population in the same area (8,17). So our observation is unlikely to be related to our choice of controls but to a true lack of protection by *GYPC* Δ^{exon3} against SMA.

It has been proposed that malaria may

TABLE 1

COMPARISON OF CHARACTERISTICS OF CASES AND CONTROLS

	Cases Hb <50 g/l N = 138	Controls Hb >50 g/l N = 138
Male	68 (49.3%)	68 (49.3%)
Mean age (years)	2.87	2.84
Mean haemoglobin (g/l)	42.2	88.3
Malaria microscopy		
<i>Plasmodium falciparum</i>	93 (67.4%)	51 (37.0%)
<i>Plasmodium vivax</i>	15 (10.9%)	24 (17.4%)
<i>Plasmodium malariae</i>	0 (0%)	4 (2.9%)
<i>Plasmodium ovale</i>	0 (0%)	0 (0%)
Mixed infections*	4 (2.9%)	6 (4.3%)

*Mixed infections included 4 *Plasmodium falciparum*/*Plasmodium vivax* infections among cases and 4 *Plasmodium falciparum*/*Plasmodium vivax*, 1 *Plasmodium falciparum*/*Plasmodium malariae* and 1 *Plasmodium falciparum*/*Plasmodium vivax*/*Plasmodium malariae* among controls

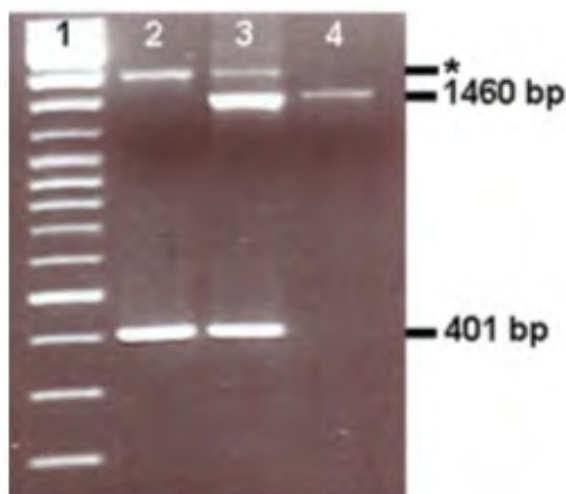


Figure 1. PCR band pattern for *GYPC* genotyping. lane 1: DNA molecular marker; lane 2: wild type; lane 3: heterozygous *GYPC*Δ^{exon3}; lane 4: homozygous *GYPC*Δ^{exon3}; *: extra 1789 bp PCR fragment not used for diagnostic purposes.

have been the selective force driving the mutation in Melanesia. This is because the deletion of exon 3 in the glycoporphin C gene ($GYPC\Delta^{exon3}$) is common in people living in malaria-hyperendemic areas of coastal PNG but virtually absent in populations living in the malaria-free highlands (8,17). Recent laboratory studies suggested that $GYPC\Delta^{exon3}$ could affect invasion of the red blood cell by *P. falciparum* merozoites. GYPC has been identified as the receptor for EBA140, a *P. falciparum* ligand involved in mediating one of the multiple invasion pathways of the merozoite into human erythrocytes (13,18). In Gerbich-negative erythrocytes, binding of EBA140 to its erythrocyte receptor GYPC is abrogated, and *P. falciparum* cannot invade such cells using this invasion pathway (11). It is also important to note that *P. falciparum* is more complex than *P. vivax* in its binding phenotype to erythrocyte receptors like GYPC. *P. falciparum* expresses four members of the erythrocyte binding-like (EBL) antigen family (14). A single point mutation in any of the four antigens in the family (like BAEBL/EBA140) can affect the binding

phenotype of *P. falciparum* parasites. This means that a single point mutation may give rise to several *P. falciparum* variants, which may result in different binding behaviour to either wild type or mutant GYPC.

Previous studies also failed to show in vivo protection by $GYPC\Delta^{exon3}$ against asymptomatic *P. falciparum* or *P. vivax* malaria infection (8,17). Severe anaemia is the most common form of severe malarial illness in PNG (19) and the lack of protection by $GYPC\Delta^{exon3}$ against this malaria-related condition raises the question: is malaria the selective force responsible for the high frequencies of this mutation in Melanesia? This study did not address other possible explanations for the presence of the $GYPC\Delta^{exon3}$ mutation in PNG. It would be interesting to test whether the $GYPC\Delta^{exon3}$ polymorphism is associated with cerebral malaria or other severe malaria outcomes. Due to the role of GYPC in merozoite invasion, GYPC mutants may have driven *P. falciparum* to use a different invasion pathway from the one using GYPC/EBA140 in the

TABLE 2

ASSOCIATION OF $GYPC\Delta^{EXON3}$ GENOTYPE WITH SEVERE MALARIAL ANAEMIA (SMA)

	Cases Hb <50 g/l N = 138	Clinical controls Hb >50 g/l N = 138	Community survey N = 742
GYPC genotypes			
wt	39 (28.3%)	32 (23.2%)	211 (28.4%)
het $GYPC\Delta^{exon3}$	68 (49.3%)	71 (51.4%)	372 (50.1%)
hom $GYPC\Delta^{exon3}$	31 (22.5%)	35 (25.4%)	159 (21.4%)
		$\chi^2 = 1.00$ p = 0.61	$\chi^2 = 0.08$ p = 0.96
Odds ratios			
wt vs het		0.79 [0.42-1.45] p = 0.41	0.99 [0.62-1.56] p = 0.96
wt vs hom		0.73 [0.35-1.50] p = 0.35	1.05 [0.61-1.82] p = 0.31

wt = wild type
het = heterozygous
hom = homozygous

area. Efforts could be directed towards analyzing *falciparum* parasite variants present in the study area and investigating how this mutation may also have affected *P. falciparum* populations.

Given that Gerbich negativity does not protect against the development of SMA, further studies need to be conducted to understand the significance of the high prevalence of *GYPC* Δ^{exon3} in PNG. Possible avenues of research would be (i) investigating *GYPC* Δ^{exon3} in the context of other severe malaria-related or non-malarial illnesses, (ii) testing more specifically the molecular interaction between *P. falciparum* and *GYPC*, and (iii) studying the effect that this interaction and the involvement of *GYPC* in *P. falciparum* invasion pathways has on local *P. falciparum* populations.

ACKNOWLEDGEMENTS

The Medical Research Advisory Committee (MRAC) of Papua New Guinea gave approval for the study. Funding came from AusAID.

This study would not have been possible without the participation of children from the Wosera area, East Sepik Province and consent from their parents. We thank the Maprik Institute of Medical Research staff for their assistance in retrieving the archived samples and for microscopy. Sandra Laney and Mata Mellombo provided assistance with the PCR assays.

REFERENCES

- 1 **Breman JG.** The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg* 2001;64(1-2 Suppl):1-11.
- 2 **Papua New Guinea Department of Health.** Papua New Guinea National Health Plan, 2001-2010. Port Moresby: Department of Health, Aug 2000.
- 3 **Müller I, Bockarie M, Alpers M, Smith T.** The epidemiology of malaria in Papua New Guinea. *Trends Parasitol* 2003;19:253-259.
- 4 **Haldane JBS.** The rate of mutation of human genes. Proceedings of the Eighth International Congress of Genetics. *Hereditas* 1949;35(Suppl):267-273.
- 5 **Genton B, Al-Yaman F, Mgone CS, Alexander N, Paniu MM, Alpers MP, Mokela D.** Ovalocytosis and cerebral malaria. *Nature* 1995;378:564-565.
- 6 **Allen SJ, O'Donnell A, Alexander NDE, Mgone CS, Peto TEA, Clegg JB, Alpers MP, Weatherall DJ.** Prevention of cerebral malaria in children in Papua New Guinea by Southeast Asian ovalocytosis band 3. *Am J Trop Med Hyg* 1999;60:1056-1060.
- 7 **Allen SJ, O'Donnell A, Alexander NDE, Alpers MP, Peto TEA, Clegg JB, Weatherall DJ.** Alpha+ thalassemia protects children against disease caused by other infections as well as malaria. *Proc Natl Acad Sci USA* 1997;94:14736-14741.
- 8 **Patel SS, King CL, Mgone CS, Kazura JW, Zimmerman PA.** Glycophorin C (Gerbich antigen blood group) and band 3 polymorphisms in two malaria holoendemic regions of Papua New Guinea. *Am J Hematol* 2004;75:1-5.
- 9 **Palacajornsuk P.** Review: molecular basis of MNS blood group variants. *Immunohematology* 2006;22:171-182.
- 10 **Black CG, Wang L, Wu T, Coppel RL.** Apical location of a novel EGF-like domain-containing protein of *Plasmodium falciparum*. *Mol Biochem Parasitol* 2003;127:59-68.
- 11 **Duraisingh MT, Maier AG, Triglia T, Cowman AF.** Erythrocyte-binding antigen 175 mediates invasion in *Plasmodium falciparum* utilizing sialic acid-dependent and -independent pathways. *Proc Natl Acad Sci USA* 2003;100:4796-4801.
- 12 **Pasvol G, Jungery M, Weatherall DJ, Parsons SF, Anstee DJ, Tanner MJ.** Glycophorin as a possible receptor for *Plasmodium falciparum*. *Lancet* 1982;2:947-950.
- 13 **Lobo CA, Rodriguez M, Reid M, Lustigman S.** Glycophorin C is the receptor for the *Plasmodium falciparum* erythrocyte binding ligand P1EBP-2 (baeb1). *Blood* 2003;101:4628-4631.
- 14 **Adams JH, Blair PL, Kaneko O, Peterson DS.** An expanding ebl family of *Plasmodium falciparum*. *Trends Parasitol* 2001;17:297-299.
- 15 **Baum J, Richard D, Healer J, Rug M, Krnajska Z, Gilberger TW, Green JL, Holder AA, Cowman AF.** A conserved molecular motor drives cell invasion and gliding motility across malaria life cycle stages and other apicomplexan parasites. *J Biol Chem* 2006;281:5197-5208.
- 16 **Preiser P, Kaviratne M, Khan S, Bannister L, Jarra W.** The apical organelles of malaria merozoites: host cell selection, invasion, host immunity and immune evasion. *Microbes Infect* 2000;2:1461-1477.
- 17 **Patel SS, Mehlotra RK, Kastens W, Mgone CS, Kazura JW, Zimmerman PA.** The association of the glycophorin C exon 3 deletion with ovalocytosis and malaria susceptibility in the Wosera, Papua New Guinea. *Blood* 2001;98:3489-3491.
- 18 **Pasvol G.** How many pathways for invasion of the red blood cell by the malaria parasite? *Trends Parasitol* 2003;19:430-432.
- 19 **Allen SJ, O'Donnell A, Alexander NDE, Clegg JB.** Severe malaria in children in Papua New Guinea. *QJM* 1996;89:779-788.

Is a 'convenience' sample useful for estimating immunization coverage in a small population?

JEAN E. WEIR¹ AND CARRIE JONES¹

SIL Clinic, Ukarumpa, Papua New Guinea

SUMMARY

Rapid survey methodologies are widely used for assessing immunization coverage in developing countries, approximating true stratified random sampling. Non-random ('convenience') sampling is not considered appropriate for estimating immunization coverage rates but has the advantages of low cost and expediency. We assessed the validity of a convenience sample of children presenting to a travelling clinic by comparing the coverage rate in the convenience sample to the true coverage established by surveying each child in three villages in rural Papua New Guinea. The rate of DTP3 immunization coverage as estimated by the convenience sample was within 10% of the true coverage when the proportion of children in the sample was two-thirds or when only children over the age of one year were counted, but differed by 11% when the sample included only 53% of the children and when all eligible children were included. The convenience sample may be sufficiently accurate for reporting purposes and is useful for identifying areas of low coverage.

Introduction

In order to assess the immunization coverage in the SIL Clinic catchment area, we have collected data from the health records of children presenting at travelling clinic points over the past several years. Such a sample constitutes a 'convenience' sample of the population of interest (children under 5 years of age in each of 3 villages) and is a more expedient and rapid sampling method than a true random or stratified random sample. The question is whether such data correctly represent the true coverage in the communities serviced and provide a reliable basis for decisions regarding service provision.

The established World Health Organization (WHO) rapid survey methodology (the '30 x 7' cluster method) is intended to approximate the true immunization coverage by $\pm 10\%$. This method involves sampling 7 children from each of 30 population clusters chosen by probability proportionate to size from all non-overlapping clusters within the population of interest. Variations of the 30 x 7 method have been assessed for attaining similar

accuracy while maintaining the assets of rapid survey methodologies: minimal training requirements and cost-effectiveness (1). The 30 x 7 method is only appropriate for monitoring coverage in the population as a whole (2) and is less suitable for comparing vaccination levels in different areas of the same country (3). The 30 x 7 method needs to be modified if it is to be used for other purposes (4). In many countries, the 30 x 7 methodology has resulted in lower immunization coverage estimates than official government-reported figures (5).

A second method, lot quality assurance sampling (LQAS), may be more appropriate for monitoring in small areas of high heterogeneous coverage (6) and comparing coverage rates of different areas within a country. The LQAS involves sampling a given number of children from each non-overlapping cluster within the population of interest for a binary variable such as completed immunization, in order to rate the 'lot' or area as having acceptable or unacceptable immunization coverage. The 30 x 7 method possibly overestimates coverage compared to the LQAS

¹ SIL Clinic, PO Box 222, Ukarumpa, Eastern Highlands Province 444, Papua New Guinea

methodology (6).

We wished to assess immunization coverage in the SIL Clinic catchment area and to compare coverage between villages. Clinic staff felt that the immunization rate was well below target values and the government-reported 61% DTP3 coverage (7), and that low rates were pocketed in certain villages. Village leaders would like to initiate programs to improve health in the catchment area but require specific information in order to target an awareness program.

The data collected from children's health books at the travelling clinics in the villages of interest represented 66% to 90% of the estimated population. This large a sample could be expected to be statistically similar to the larger population but the concern still remained as to whether there was bias in favour of children whose mothers brought them to the clinic points regularly and therefore were likely to achieve full immunization. Alternatively, the sample may over-represent infrequent attenders who were late in achieving full immunization. A further concern was whether this sample was biased by not including children immunized at other clinics.

In order to address these issues, we undertook a community-wide survey to record all immunizations in children aged under 5 years and compared these data to the convenience sample obtained at regular travelling clinics.

This survey was undertaken with the participation of the Community Health Board of Yomunka area, Obura-Wanenara District in Eastern Highlands Province of Papua New Guinea (PNG), and with the permission and support of the Obura-Wananera District Health Extension Officer and local Member of Parliament.

Background

Yomunka is a rural highlands area populated by Gadsup-speaking subsistence and market farmers. Health care is provided primarily by the SIL Clinic at Ukarumpa, approximately 12 km distant by road. The nearest hospital is at the town of Kainantu 20 km away. Travelling clinics visit 5 villages in the area once monthly from February to November unless prevented by heavy rains or clan fighting.

Methods

Three villages participated in this survey. Community Health Board volunteers from the three villages formed a focus group to discuss health issues in the area and identified 4 areas of concern: immunization rates, the number of disabled persons, barriers to health care, and unrecognized tuberculosis cases. We developed a questionnaire to assess these concerns and conducted training sessions for Board members before conducting the survey. Expatriate SIL volunteers were recruited to assist with recording and logistics.

Teams consisting of one Community Health Board member and one expatriate recorder visited each house in each village. All children aged under 5 years normally resident in the home were recorded whether at home at the time or not. The health record was examined and birthdate and the date of each immunization were recorded. Every attempt was made to extract usable data from the health record. Missing or completely illegible cards were recorded as unusable. The adults present then were questioned regarding any disabilities, chronic cough and perceived barriers to health care in their village. The community survey was carried out in October 2006.

The data recorded from the travelling clinics for August through November 2006 were used for comparison. The data for each child presenting for immunization were transcribed from the health record, including any immunizations given on that day. Duplicate records were eliminated during the analysis phase and the latest record used for comparison. The resultant group of records is the 'convenience sample' for the purposes of this study.

'Completed immunization' is defined as the 13 primary immunization shots mandated by the Papua New Guinea Department of Health for administration before 1 year of age. DTP3 coverage was calculated using two methods: firstly, the number of children over the age of 6 months who had received the 3rd DTP immunization before their first birthday divided by the total number of children over the age of 6 months at the time of the survey (ie, those eligible for having received three DTP immunizations). Secondly, we used the WHO 30 x 7 method of including only the children over the age of 12 months at the time of the survey who had received their 3rd DTP

immunization before 12 months of age.

The estimated number of children aged under 5 years in each village was obtained from the Obura-Wanenara District Health Extension Officer using the 2002 National Census, an estimated annual birth rate of 3% and estimated proportion of population less than 5 years of age of 13.2%.

All immunization data were entered into a database and analyzed using SPSS. Due to data that were not normally distributed and unequal sample variances and sizes, we used Mann-Whitney U nonparametric analysis rather than t-tests to compare 'mean age at completion' between sampling methodologies. We used Fisher's Exact Test to compare proportions of children receiving their third DTP immunization by 12 months as measured by the two methodologies in each of the three villages.

Results from the questions regarding disabilities, barriers to health care and case finding for tuberculosis will be reported elsewhere.

Results

Table 1 shows the estimated number of

children aged under 5 years, the actual number found at the community survey, number of usable records and number of records transcribed at travelling clinics.

Immunization coverage data are presented in Table 2. The convenience sample accurately estimated mean age at completed immunization for two of three villages, but was inaccurate by 3.6 months (which was not considered statistically significant) in the third village. There were no significant differences in DTP3 coverage at one year between the convenience sample and community survey ($p > 0.05$ for all results). The convenience sample consistently overestimated the true DTP3 coverage. The overestimation was within 10% in two villages but 11% in one village when all children over the age of 6 months were included. The overestimation was within 10% for all villages when only those children over 1 year of age at the time of survey were included.

Discussion

The number of children less than 5 years of age found in the community survey was in excess of the census-based estimate of number of children in all three villages. Two possibilities may account for the difference:

TABLE 1

COMPARISON OF NUMBER OF CHILDREN BY CENSUS ESTIMATE, CONVENIENCE SAMPLE AND COMMUNITY SURVEY

	Village		
	Amomonta	Onamuna	Akuna
Census-based estimate of number of children under 5 years of age	86	90	107
Total number of children under 5 years of age recorded by community survey	97	114	139
Number of usable records	89	90	124
(% of total number of children)	(91.8%)	(78.9%)	(89.2%)
Number of children recorded at travelling clinic ('convenience sample')	65	60	97
(% of total number of children)	(67.0%)	(52.6%)	(69.8%)

TABLE 2

COMPARISON OF COVERAGE STATISTICS

	Village		
	Amomonta	Onamuna	Akuna
Mean age at completion of primary immunization series, community survey (months)	14.6	21.3	15.0
Mean age at completion of primary immunization series, convenience sample (months)	14.2	24.9	14.5
Children receiving DTP3 before 12 months of age, community survey (of those over 6 months at time of recording)	71 of 82 (87%)	47 of 80 (59%)	89 of 102 (87%)
Children receiving DTP3 before 12 months of age, convenience sample (of those over 6 months at time of recording)	59 of 63 (94%)	44 of 63 (70%)	85 of 94 (90%)
Children receiving DTP3 before 12 months of age, community survey (of those over 12 months at time of recording)	60 of 71 (85%)	38 of 71 (54%)	72 of 85 (85%)
Children receiving DTP3 before 12 months of age, convenience sample (of those over 12 months at time of recording)	39 of 43 (91%)	30 of 49 (61%)	61 of 70 (87%)

$p > 0.05$ for all vertical comparisons, $p < 0.05$ for differences between Onamuna and the other two villages

either the 2002 census did not record all residents, or the annual population growth rate and proportion of children under 5 years of age are in excess of the national average. This number undoubtedly under-represents the numbers of births during the same time period as PNG under-5 mortality rates are estimated between 65 and 93 per 1000 live births (8,9).

The convenience sample of under-5 children seen at the travelling clinics was two-thirds of the total number of children in two of the three villages, but only slightly over half the total number of children in the third village (Onamuna). DTP3 coverage was accurately assessed by the convenience sample (within 10% of the true coverage as assessed by the community survey) when only children over the age of one year were counted or when the convenience sample proportion was high (67% and 70%). The DTP3 coverage assessment differed by 11% when all children eligible for having received their 3rd DTP (those over 6 months old) were included and the convenience sample proportion was lower

(53%), which, though not statistically significant at the 0.05 level, is outside the acceptable 10% margin of accuracy established by WHO.

The convenience sample accurately represented a statistically significant difference in DTP3 coverage and mean age at completion of the primary immunization series in Onamuna as compared to the other two villages ($p < 0.05$).

DTP3 coverage rates could be expected to be higher when recorded at the travelling clinic as immunizations given at that visit are reported whereas no immunizations were given during the community survey. Even with this apparent increase in DTP3 coverage created by the methodology, the coverage rate estimate by the convenience sample is close to that found during the community survey.

This study could have resulted in apparent improved immunization rates at travelling clinic points during the course of the fieldwork

due to increased awareness following the community-wide survey. This possibility is consistent with the higher DTP3 coverage rate found by the travelling clinic one month after the community survey at the village with lowest DTP3 coverage (Onamuna). The travelling clinic visited one of the villages with high DTP3 coverage before the community survey (Akuna) and one after (Amomonta). There was no difference in the proportion of children in any of the villages attending the travelling clinics before and after the community survey.

Conclusions

Although convenience sampling is prone to bias, the advantages in expense and expediency make it an attractive methodology for assessing immunization coverage in areas where immunizations may be recorded as part of regular travelling clinic activities. Where the proportion of children sampled was relatively high (two-thirds or more) and the WHO age cutoffs were utilized, the estimate of true immunization coverage was within WHO rapid-assessment parameters. Where the proportion of children was 53% and all children eligible for having received the immunization in question were included, the coverage rate estimated from the convenience sample was not sufficiently accurate for reporting purposes. The data accurately reflect local differences in immunization rates and are suitable for use in targeting supplementary immunization programs and community awareness programs to villages with lower immunization coverage.

Contrary to Clinic staff perception, DTP3 coverage at 1 year of age was in excess of government estimates in two of three villages. Since under-5 mortality rates in Papua New Guinea are significantly influenced by immunization (10), full immunization of all children is an important strategy in health care. We suggest that community surveys may be a useful strategy for improving immunization rates in areas of low coverage.

ACKNOWLEDGEMENTS

We thank Justin Wase, District Health Extension Officer for Obura-Wanenara District, for his support and encouragement. This study would not have been possible without the enthusiasm and participation of the Yomunka Health Board members and SIL volunteers.

REFERENCES

- 1 **Lwanga S, Sapirie S, Steinglass R, Stroh G, Wylie A.** Immunization Coverage Cluster Survey – Reference Manual. Geneva: World Health Organization, 2005.
- 2 **Hoshaw-Woodard S.** Description and Comparison of the Methods of Cluster Sampling and Lot Quality Assurance Sampling to Assess Immunization Coverage. Geneva: World Health Organization, 2001.
- 3 **Bennett S, Woods T, Liyanage WM, Smith DL.** A simplified general method for cluster-sample surveys of health in developing countries. *World Health Stat Q* 1991;44:98-106.
- 4 **Brogan D, Flagg EW, Deming M, Waldman R.** Increasing the accuracy of the Expanded Programme on Immunization's cluster survey design. *Ann Epidemiol* 1994;4:302-311.
- 5 **Murray CJ, Shengelia B, Gupta N, Moussavi S, Tandon A, Thieren M.** Validity of reported vaccination coverage in 45 countries. *Lancet* 2003;362:1022-1027.
- 6 **Singh J, Jain DC, Sharma RS, Verghese T.** Evaluation of immunization coverage by lot quality assurance sampling compared with 30-cluster sampling in a primary health centre in India. *Bull World Health Organ* 1996;74:269-274.
- 7 **World Health Organization.** Immunization Surveillance, Assessment and Monitoring. Geneva: World Health Organization, 2008. www.who.int/immunization_monitoring/en/globalsummary/timeseries/tscoverageotp3.htm
- 8 **United Nations Children's Fund, World Health Organization.** Immunization Summary. A Statistical Reference Containing Data through 2007. New York: UNICEF, 2009. www.childinfo.org/files/Immunization_Summary_2009.pdf
- 9 **World Health Organization.** Mortality Country Fact Sheet 2006 – Papua New Guinea. Manila: World Health Organization Regional Office for the Western Pacific, 2006. www.who.int/whosis/mort/profiles/mort_wpro_png_papuanewguinea.pdf
- 10 **Lehmann D, Vail J, Firth MJ, de Klerk NH, Alpers MP.** Benefits of routine immunizations on childhood survival in Tari, Southern Highlands Province, Papua New Guinea. *Int J Epidemiol* 2005;34:138-148.

List of Medical Research Projects in Papua New Guinea

Approved or Noted

By the Medical Research Advisory Committee in 2007

Differences in neonatal immune regulation in the 'developing' and 'developed' world: implications for neonatal vaccinations?

Dr Danielle Stanisic, Dr Anita van den Biggelaar and Dr Suparat Phuanukoonnon (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

Evaluation of new fluorescence bead based assay to measure antibodies to *P. vivax* merozoite surface protein 1 (MSP1)

Dr Hernando del Portillo, Dr Danielle Stanisic, Dr Harin Karunajeewa and Dr Ivo Mueller (Centre for International Health, Hospital Clinic, Universitat de Barcelona, Rossello 132, 4-2, 08036, Barcelona, Spain)

Expanding HIV/AIDS treatment for infants of the Eastern Highlands Province

Mr Sarthak Das, Dr Andy Carmone and Prof. Peter Siba (Clinton HIV/AIDS Initiative, PNG Rural Initiative Office, PO Box 599, Goroka, EHP 441, Papua New Guinea)

Growth and nutrition of the Mountain Ok: a follow-up study

Dr Jessica Schwartz, Dr Robert Brumbaugh and Prof. Peter Siba (Department of Molecular & Integrative Physiology, University of Michigan, 6815 Med Sci II, Box 0622, Ann Arbor, MI 48109-0622, USA)

Assessing the impact of a sector wide approach on the national malaria control program in PNG

Mr Rishabh Singh and Dr P. Ngabung (C/- Rohit Singh, Policy & Co-ordination Division, Department of Prime Minister & NEC, PO Box 639, Waigani, NCD 131, Papua New Guinea)

Prevalence of oncogenic HPVs within cervical cancer and high grade cervical smear cases in PNG

Prof. Glen Mola and Prof. Susanne Garland (Obstetrics & Gynaecology Division, School of Medicine and Health Sciences, University of Papua New Guinea, PO Box 5623, Boroko, NCD 111, Papua New Guinea)

Meningoencephalitis among children in PNG: defining the burden of Japanese encephalitis and other pathogens, and looking for clinical indicators of specific diagnoses

Prof. John Vince and Dr Nakapi Tefuarani (School of Medicine and Health Sciences, University of Papua New Guinea, PO Box 5623, Boroko, NCD 111, Papua New Guinea)

Genotyping of drug metabolizing enzymes in archival samples from the WHO standard treatment trial

Prof. Ken Ilett and Dr Ivo Mueller (Pharmacology Unit (M510), School of Medicine and Pharmacology, QE II Medical Centre, M Block, The University of Western Australia, Crawley, WA 6009, Australia)

Collection of blood samples for in vitro culture of *Plasmodium falciparum* and *Plasmodium vivax*

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

Cost effectiveness assessment of Intermittent Preventive Treatment of Malaria in infants (IPTi) in Papua New Guinea

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

The acquisition of anti-malarial immunity in early childhood

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

Impact of HIV/AIDS-stigma and discrimination on the access to VCT and other health services in selected populations of the National Capital District, Papua New Guinea

Dr J. A. K. Lauwo, Mr Isu Aluvula, Dr G. Tau, Dr Esorom Daoni and Dr I. Kitur (School of Medicine and Health Sciences, PO Box 5623, Boroko, NCD 111, Papua New Guinea)

Management of antiretrovirals (ARVs) in

the area medical store – Badili

Dr J. A. K. Lauwo and Ms Lessi Bronywn
(School of Medicine and Health Sciences, PO
Box 5623, Boroko, NCD 111, Papua New
Guinea)

A prospective study of clinical and
demographic predictors of paediatric HIV
infection in PNG

Dr Mobumo Kiromat and Prof. John Vince
(School of Medicine and Health Sciences, PO
Box 5623, Boroko, NCD 111, Papua New
Guinea)

Childhood tuberculosis in Papua New
Guinea: a baseline evaluation of available
data from national and health facility registries
(2001-2007)

Dr Irwin Law and Prof. John Vince (Centre
for International Child Health, Department of
Paediatrics, Royal Children's Hospital,
Flemington Road, Parkville, Victoria 3052,
Australia)

Papua New Guinea observational clinical
database

Dr Goa Tau (Port Moresby General
Hospital, Free Mail Bag, Boroko, NCD 111,
Papua New Guinea)

Systematic establishment of local capacity
for diagnosis of pulmonary tuberculosis and
TB drug resistance in sentinel hospitals in
PNG

Dr Suparat Phuanukoonnon and Prof.
Peter Siba (Papua New Guinea Institute of
Medical Research, PO Box 60, Goroka, EHP
441, Papua New Guinea)

Evaluation and alleviation of environmental
burden due to subsistence transition in Papua
New Guinea – elucidation of health impact
(EASTPNG)

Dr Masahiro Umezaki and Dr Suparat
Phuanukoonnon (Department of Human
Ecology, Graduate School of Medicine,
University of Tokyo, 7-3-1 Hongo, Bunkyo-ku,
Tokyo, 113-0033, Japan)

Indoor air pollution from biomass
combustion and increased risk of acute
respiratory infection (ARI) in infants in
highlands Papua New Guinea

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

Analysis of the phylogenetic structure and

geographic distribution of the gastric and oral
pathogenic bacteria *Helicobacter pylori* and
Streptococcus mutans in people from Papua
New Guinea

Dr Mark Achtman, Dr Suparat
Phuanukoonnon and Dr John Irima
(Department of Molecular Biology, Max
Planck Institute for Infection Biology,
Chariteplatz 1, 10117 Berlin, Germany)

Investigation of the prevalence of
neutralizing antibodies for measles and
rubella in patients presenting with a febrile
episode at the outpatient clinic in a semi-rural
hospital in Papua New Guinea

Dr Nicolas Senn, Prof. Peter Siba and Dr
Chris Morgan (Papua New Guinea Institute
of Medical Research, PO Box 378, Madang,
Madang Province 511, Papua New Guinea)

Expression of Duffy antigen on SAO red
cells

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

Betelnut chewing during pregnancy

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

Pharmacokinetics of azithromycin in
pregnancy

Prof. Tim Davis, Dr Ivo Mueller and Dr
Harin Karunajeewa (Department of Medicine,
University of Western Australia, Nedlands,
Western Australia 6009, Australia)

Prospective study on the contribution of
dengue fever to the burden of febrile illnesses
in a semi-rural hospital in Papua New Guinea
(Madang Province)

Dr Nicolas Senn, Prof. John McBride and
Prof. Peter Siba (Papua New Guinea Institute
of Medical Research, PO Box 378, Madang,
Madang Province 511, Papua New Guinea)

Exposure of young children to antimalarial
treatment in a hyperendemic area of PNG –
rationale for a new approach in first line and
emergency malaria treatment

Dr Andreas Schultz, Dr Ulla Schultz and
Prof. Francis Hombhanje (Braun Memorial
Hospital, Finschhafen, Morobe Province 435,
Papua New Guinea)

The experiences of people and families

living with HIV/AIDS in the National Capital District, Papua New Guinea

Ms Lily Lesley (School of Medicine and Health Sciences, PO Box 5623, Boroko, NCD 111, Papua New Guinea)

An ethnicity-based analysis of genomic polymorphism and susceptibility to infectious diseases in Papua New Guinea

Dr Peter Zimmerman, Prof. Peter Siba and Mr Tony Lupiwa (Centre for Global Health and Diseases, Case School of Medicine, Wolstein Research Building, 10900 Euclid Avenue, Cleveland, Ohio 44106-7286, USA)

Changing dynamics of anopheline transmission of malaria and filariasis in Papua New Guinea

Dr Ivo Mueller, Prof. Charles King, Dr Peter Zimmerman, Prof. James Kazura and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Papua New Guinea/The Global Fund Malaria Control Program Evaluation Plan

Dr Ivo Mueller, Dr Daniel Tisch, Mr Leo Makita and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Cellular immunity to *Plasmodium vivax* in early childhood

Dr Louis Schofield (The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Victoria 3050, Australia)

Epidemiology study of TB: treatment follow-up study at Modilon Hospital, Madang, Papua New Guinea

Dr Suparat Phuanukoonnon and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

PNG Australia Sexual Health Improvement Program (PASHIP), PNGIMR baseline research in pilot sites around PNG

Mr Tony Lupiwa, Ms Geraldine Maibani and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

P. vivax vaccine baseline and immunology cohort study in PNG

Dr Inoni Betuela, Dr Danielle Stanicic and Dr Ivo Mueller (Papua New Guinea Institute

of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Immunogenetic epidemiology of severe and uncomplicated malaria in PNG children

Dr Ivo Mueller, Dr Louis Schofield and Dr Stephen Rogerson (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

The art of living: the social impacts of ART for PLWHA in PNG

Dr Angela Kelly, Dr Heather Worth and Prof. Peter Siba (Papua New Guinea Institute of Medical Research, PO Box 60, Goroka, EHP 441, Papua New Guinea)

Is routine lumbar puncture indicated in a PNG child with febrile convulsion?

Dr Moses Laman, Prof. John Vince and Dr Laurens Manning (Papua New Guinea Institute of Medical Research, PO Box 378, Madang, Madang Province 511, Papua New Guinea)

The prevalence of HIV in pregnant women attending antenatal clinic in Gulf Province

Mr Robert Saliau and Dr Isimel Kitur (Division of Public Health, School of Medicine and Health Sciences, PO Box 5623, Boroko, NCD 111, Papua New Guinea)

A study into the prevalence of HIV in adult TB patients at Nonga Hospital, East New Britain Province, Papua New Guinea

Dr Peniel Boas and Dr Daoni Esorom (Unit 55, Building 5, Hospital Accommodation Complex, North Street, Westmead, NSW 2145, Australia)

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 2007.

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

PUBLICATIONS OF RELEVANCE TO PAPUA NEW GUINEA AND MELANESIA

Bibliographic Citation List generated from MEDLARS

- 1 **Allison WE, Iobuna V, Kalebe V, Kiromat M, Vince J, Schaefer M, Kaldor J.**
Attitudes to HIV testing among carers of children admitted to Port Moresby General Hospital, Papua New Guinea.
J Paediatr Child Health 2008 Nov;44(11):618-621. Epub 2008 Aug 19.
AIM: To assess the acceptability of voluntary counselling and testing among the carers of children admitted to hospital in Papua New Guinea. METHODS: Forty semistructured interviews were carried out between February and April 2007. RESULTS: All the carers interviewed were women, mostly from Port Moresby. Virtually all of them attended primary school. About half of them attended secondary school but none completed it. Half of them knew an adult or child with HIV. Three quarters of the women interviewed would consent to having a child in their care tested for HIV, and over half of those who had never been tested would agree to be tested themselves. Correct answers to more than half the HIV knowledge questions posed were significantly related to agreement to an HIV test. CONCLUSIONS: This study supports the need for further evaluation of knowledge about HIV/AIDS and opportunities for health promotion in this group of women, particularly in view of the implication for voluntary counselling and testing and prevention of mother-to-child HIV transmission programmes in Papua New Guinea.
- 2 **Alpers MP.**
Review. The epidemiology of kuru: monitoring the epidemic from its peak to its end.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3707-3713.
Kuru is a fatal transmissible spongiform encephalopathy restricted to the Fore people and their neighbours in a remote region of the Eastern Highlands of Papua New Guinea. When first investigated in 1957 it was found to be present in epidemic proportions, with approximately 1000 deaths in the first 5 years, 1957-1961. The changing epidemiological patterns and other significant findings such as the transmissibility of kuru are described in their historical progression. Monitoring the progress of the epidemic has been carried out by epidemiological surveillance in the field for 50 years. From its peak, the number of deaths from kuru declined to 2 in the last 5 years, indicating that the epidemic is approaching its end. The mode of transmission of the prion agent of kuru was the local mortuary practice of transumption. The prohibition of this practice in the 1950s led to the decline in the epidemic, which has been prolonged into the present century by incubation periods that may exceed 50 years. Currently, the epidemiological surveillance is being maintained and further studies on human genetics and the past mortuary practices are being conducted in the kuru-affected region and in communities beyond it.
- 3 **Alpers MP.**
Some tributes to research colleagues and other contributors to our knowledge about kuru.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3614-3617.
- 4 **Anderson WH.**
Early perceptions of an epidemic.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3675-3678.
This article surveys some descriptions of the Fore people made on early contact in the 1950s by patrol officers, social anthropologists and medical doctors. Sorcery accusations and cannibalism initially impressed these outside observers, though gradually they came to realize that a strange and fatal condition called kuru was a major affliction of the Fore, especially women and children. Fore attributed kuru to sorcery, anthropologists speculated on psychosomatic causes and medical officers began to wonder if it was a mysterious encephalitis.
- 5 **Anua A.**
'My late husband Mr Anua was a hard-working man'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3618.
- 6 **Asher DM.**
Kuru: memories of the NIH years.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3618-3625.
- 7 **Bavasa A.**
'My adopted daughter and then my second wife died of kuru'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3626.
- 8 **Beasley A.**
Richard Hornabrook's first impressions of kuru and Okapa.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3626-3627.
- 9 **Benfante R.**
Reminiscences of an aspiring graduate student in the 1970s who worked on kuru-related projects with Dr Gajdusek.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3628.
- 10 **Bennett JH.**
Family and population studies by the Adelaide Kuru Team, 1957-1965.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3629.
- 11 **Benton KW.**
Saints and sinners: training Papua New Guinean (PNG) Christian clergy to respond to HIV and AIDS using a model of care.

J Relig Health 2008 Sep;47(3):314-325. Epub 2008 Jan 12.

Papua New Guinea has experienced a growing HIV/AIDS epidemic. The Christian churches have played a vital role in responding to HIV, through community support, encouragement and social change. Strong, effective church leadership can help create safe environments of care and support for those infected and for prevention of HIV. *Method.* A series of trainings in capacity development for clergy were undertaken by the National AIDS Council Secretariat (NACS)/National HIV/AIDS Support Project (NHASP). *Results.* A model 'Church's Response to HIV and AIDS in a Care Continuum' was developed to assist the training. This paper discusses the model and the lessons learned.

- 12 **Berlioz-Arthaud A, Barny S, Yvon JF, Roque-Afonso AM, Dussaix E.**

Laboratory based hepatitis A surveillance in New Caledonia: from an endemic to an epidemic pattern (1986-2007). [Fr]

Bull Soc Pathol Exot 2008 Oct;101(4):336-342.

This study aimed at describing the evolution of the epidemiological pattern of hepatitis A in New Caledonia since 1986 and the recent epidemic which occurred in 2005-2006, regarding particularly its demographic and virological aspects and the public health response implemented. The annual or monthly activity records for hepatitis A sero-diagnostics performed at the Pasteur Institute of New Caledonia were processed in a retrospective analysis (9723 samples tested for the detection of IgM to hepatitis A). Over the 2004-2006 period, a phylogenetic study of representative strains from New Caledonia and other Pacific islands was carried out by the French National Reference Laboratory for hepatitis A (Paul-Brousse Hospital, Villejuif, France). **RESULTS:** The continuous improvement of hygiene that occurred in New Caledonia during the last two decades led to a dramatic drop in the frequency of hepatitis A among patients tested, ranging from an average value of 79 cases (14%) for the 1986-1999 period to 0 case from 2002. However, in 2005, a strong increasing number of confirmed cases was notified, mainly among young people (78% were under the age of 20). In 2006, this epidemic reached the island of Futuna where it involved more than 1% of the total population (56 cases). The phylogenetic study has confirmed the clonality of the virus circulating during this epidemic, not related to other regional strains (Fiji, Vanuatu, New Zealand) nor with a New Caledonian strain from the previous endemic period. This transition situation, with persistence of a high epidemic risk, should encourage the health authorities to implement adapted response strategies, based in particular on systematic case detection and targeted immunisation programmes.

- 13 **Boone K.**

An account of the last autopsy carried out on a kuru patient.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3630.

- 14 **Brandner S, Whitfield J, Boone K, Puwa A, O'Malley C, Linehan JM, Joiner S, Scaravilli F, Calder I, Alpers MP, Wadsworth JDF, Collinge J.** Central and peripheral pathology of kuru: pathological analysis of a recent case and comparison with other forms of human prion

disease.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3755-3763.

While the neuropathology of kuru is well defined, there are few data concerning the distribution of disease-related prion protein in peripheral tissues. Here we report the investigation of brain and peripheral tissues from a kuru patient who died in 2003. Neuropathological findings were compared with those seen in classical (sporadic and iatrogenic) Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD). The neuropathological findings of the kuru patient showed all the stereotypical changes that define kuru, with the occurrence of prominent PrP plaques throughout the brain. Lymphoreticular tissue showed no evidence of prion colonization, suggesting that the peripheral pathogenesis of kuru is similar to that seen in classical CJD rather than vCJD. These findings now strongly suggest that the characteristic peripheral pathogenesis of vCJD is determined by prion strain type alone rather than route of infection.

- 15 **Breitling LP, Wilson AJ, Raiko A, Lagog M, Siba P, Shaw MA, Quinnell RJ.**

Heritability of human hookworm infection in Papua New Guinea.

Parasitology 2008 Oct;135(12):1407-1415.

Hookworms infect approximately 740 million humans worldwide and are an important cause of morbidity. The present study examines the role of additive genetic effects in determining the intensity of hookworm infection in humans, and whether these effects vary according to the sex of the host. Parasitological and epidemiological data for a population of 704 subjects in Papua New Guinea were used in variance components analysis. The 'narrow-sense' heritability of hookworm infection was estimated as 0.15 ± 0.04 ($P < 0.001$), and remained significant when controlling for shared environmental (household) effects. Allowing the variance components to vary between the sexes of the human host consistently revealed larger additive genetic effects in females than in males, reflected by heritabilities of 0.18 in females and 0.08 in males in a conservative model. Household effects were also higher in females than males, although the overall household effect was not significant. The results indicate that additive genetic effects are an important determinant of the intensity of human hookworm infection in this population. However, despite similar mean and variance of intensity in each sex, the factors responsible for generating variation in intensity differ markedly between males and females.

- 16 **Cantin AM.**

Childhood pneumonia and oxygen treatment.

Lancet 2008 Oct 11;372(9646):1278-1280. Epub 2008 Aug 15.

- 17 **Cathala F.**

Why I joined the research laboratory of Prof. D. Carleton Gajdusek in 1968.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3631-3632.

- 18 **Ciarleglio CM, Ryckman KK, Servick SV, Hida A, Robbins S, Wells N, Hicks J, Larson SA, Wiedermann JP, Carver K, Hamilton N, Kidd KK, Kidd JR, Smith JR, Friedlaender J, McMahon DG, Williams SM, Summar ML, Johnson CH.**

Genetic differences in human circadian clock genes among worldwide populations.

J Biol Rhythms 2008 Aug;23(4):330-340.

The daily biological clock regulates the timing of sleep and physiological processes that are of fundamental importance to human health, performance, and well-being. Environmental parameters of relevance to biological clocks include (1) daily fluctuations in light intensity and temperature, and (2) seasonal changes in photoperiod (day length) and temperature; these parameters vary dramatically as a function of latitude and locale. In wide-ranging species other than humans, natural selection has genetically optimized adaptiveness along latitudinal clines. Is there evidence for selection of clock gene alleles along latitudinal/photoperiod clines in humans? A number of polymorphisms in the human clock genes *Per2*, *Per3*, *Clock*, and *AANAT* have been reported as alleles that could be subject to selection. In addition, this investigation discovered several novel polymorphisms in the human *Arntl* and *Arntl2* genes that may have functional impact upon the expression of these clock transcriptional factors. The frequency distribution of these clock gene polymorphisms is reported for diverse populations of African Americans, European Americans, Ghanaians, Han Chinese, and Papua New Guineans (including 5 subpopulations within Papua New Guinea). There are significant differences in the frequency distribution of clock gene alleles among these populations. Population genetic analyses indicate that these differences are likely to arise from genetic drift rather than from natural selection.

- 19 **Clark BR, Engene N, Teasdale ME, Rowley DC, Matainaho T, Valeriote FA, Gerwick WH.**

Natural products chemistry and taxonomy of the marine cyanobacterium *Blennothrix cantharidosmum*.

J Nat Prod 2008 Sep;71(9):1530-1537. Epub 2008 Aug 13.

A Papua New Guinea field collection of the marine cyanobacterium *Blennothrix cantharidosmum* was investigated for its cytotoxic constituents. Bioassay-guided isolation defined the cytotoxic components as the known compounds lyngbyastatins 1 and 3. However, six new acyl proline derivatives, tumonoic acids D-I, plus the known tumonoic acid A were also isolated. Their planar structures were defined from NMR and MS data, while their stereostructures followed from a series of chiral chromatographies, degradation sequences, and synthetic approaches. The new compounds were tested in an array of assays, but showed only modest antimalarial and inhibition of quorum sensing activities. Nevertheless, these are the first natural products to be reported from this genus, and this inspired a detailed morphologic and 16S rDNA-based phylogenetic analysis of the producing organism.

- 20 **Cliffe SJ, Tabrizi S, Sullivan EA, Pacific Islands Second Generation HIV Surveillance Group.**

Chlamydia in the Pacific region, the silent epidemic. *Sex Transm Dis* 2008 Sep;35(9):801-806.

BACKGROUND: Second generation surveillance of HIV infection and sexually transmitted infections (STIs) among pregnant women in 6 Pacific Island countries and territories were undertaken to improve knowledge and to make recommendations on future prevention and

management of STIs. **METHODS:** Cross-sectional studies, using standardized questionnaire, laboratory tests, and protocols were undertaken in Fiji, Kiribati, Samoa, Solomon Islands, Tonga, and Vanuatu between 2004 and 2005. For each country, between 200 and 350 pregnant women aged 15 to 44 years were consecutively recruited from antenatal clinics located in the main hospital of the major urban centre of each Pacific Island country and territory. Consenting participants were interviewed about their socio-demographic characteristics and their sexual behavior, and were tested for HIV, chlamydia, syphilis (*Treponema pallidum* antibody seroactivity), and gonorrhoea. **RESULTS:** Amongst the 1618 pregnant women studied, the most prevalent STI was chlamydia with 26.1% of women under 25 and 11.9% of women aged 25 years and over being positive. Highest infection was detected in single teenage women with 38.1% positive for chlamydia. The overall prevalence of gonorrhoea and syphilis was 1.7% and 3.4%, respectively. No case of HIV was detected. Chlamydia infection was independently associated with younger age, being nulliparous, single status, multiple lifetime sexual partners, and commercial sex activity. **CONCLUSION:** In a population of young women, chlamydia infection was endemic. Regional leadership is needed to implement strategies to prevent the spread of chlamydia and to implement HIV and STI prevention and management.

- 21 **Collinge J, Whitfield J, McKintosh E, Frosh A, Mead S, Hill AF, Brandner S, Thomas D, Alpers MP.**

A clinical study of kuru patients with long incubation periods at the end of the epidemic in Papua New Guinea.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3725-3739.

Kuru is so far the principal human epidemic prion disease. While its incidence has steadily declined since the cessation of its route of transmission, endocannibalism, in Papua New Guinea in the 1950s, the arrival of variant Creutzfeldt-Jakob disease (vCJD), also thought to be transmitted by dietary prion exposure, has given kuru a new global relevance. We investigated all suspected cases of kuru from July 1996 to June 2004 and identified 11 kuru patients. There were four females and seven males, with an age range of 46-63 years at the onset of disease, in marked contrast to the age and sex distribution when kuru was first investigated 50 years ago. We obtained detailed histories of residence and exposure to mortuary feasts and performed serial neurological examination and genetic studies where possible. All patients were born a significant period before the mortuary practice of transumption ceased and their estimated incubation periods in some cases exceeded 50 years. The principal clinical features of kuru in the studied patients showed the same progressive cerebellar syndrome that had been previously described. Two patients showed marked cognitive impairment well before preterminal stages, in contrast to earlier clinical descriptions. In these patients, the mean clinical duration of 17 months was longer than the overall average in kuru but similar to that previously reported for the same age group, and this may relate to the effects of both patient age and PRNP codon 129 genotype. Importantly, no evidence for lymphoreticular colonization with prions, seen

uniformly in vCJD, was observed in a patient with kuru at tonsil biopsy.

22 Collinge J.

Review. Lessons of kuru research: background to recent studies with some personal reflections. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3689-3696.

The widespread exposure of the UK population to bovine spongiform encephalopathy prions, and the potential consequences for public health, led to a renewed interest in kuru, the principal example of epidemic human prion disease. Kuru research in Papua New Guinea was expanded to study the range of incubation periods possible in human prion infection, to investigate maternal and other possible natural routes of transmission, to characterize genetic susceptibility and resistance factors and to gain insights into the peripheral pathogenesis of orally acquired prion disease in humans. Although now essentially over, the kuru epidemic continues to provide important lessons.

23 Collinge J, Alpers MP.

Reminiscences and reflections on kuru, personal and scientific. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3613.

24 Collinge J, Alpers MP.

Introduction. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3607-3612.

25 Curry C.

A perspective on developing emergency medicine as a specialty. *Int J Emerg Med* 2008 Sep;1(3):163-167. Epub 2008 Sep 25.

AIMS: A rapidly increasing number of countries are developing their capacities to respond to acute illness and injury and organizing emergency medicine training programs. This article offers some insight into the way emergency medicine has undergone development in the Australasian region. METHODS: The perspective is built from experience in Australia, New Zealand and Papua New Guinea. CONCLUSION: The challenges are many, but with persistence can be surmounted. Lessons derived from these diverse environments are presented.

26 Curtain CC.

We see what we are trained to see, or must we? Some personal lessons from a brush with kuru research. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3633-3634.

27 D'Ombrain MC, Robinson LJ, Stanisic DI, Taraika J, Bernard N, Michon P, Mueller I, Schofield L.

Association of early interferon-gamma production with immunity to clinical malaria: a longitudinal study among Papua New Guinean children. *Clin Infect Dis* 2008 Dec 1;47(11):1380-1387.

BACKGROUND: Elucidating the cellular and molecular basis of naturally acquired immunity to *Plasmodium falciparum* infection would assist in developing a rationally based malaria vaccine. Innate, intermediate, and adaptive immune mechanisms are all likely to contribute to immunity. Interferon-gamma (IFN-gamma) has been implicated in both protection against and the

pathogenesis of malaria in humans. In addition, considerable heterogeneity exists among rapid IFN-gamma responses to *P. falciparum* in malaria-naïve donors. The question remains whether similar heterogeneity is observed in malaria-exposed individuals and whether high, medium, or low IFN-gamma responsiveness is differentially associated with protective immunity or morbidity. METHODS: A 6-month longitudinal cohort study involving 206 school-aged Papua New Guinean children was performed. Peripheral blood mononuclear cells collected at baseline were exposed to live *P. falciparum*-infected erythrocytes. Early IFN-gamma responses were measured, and IFN-gamma-expressing cells were characterized by flow cytometry. IFN-gamma responsiveness was then tested for associations with parasitological and clinical outcome variables. RESULTS: Malaria-specific heterogeneity in early IFN-gamma responsiveness was observed among children. High-level early IFN-gamma responses were associated with protection from high-density and clinical *P. falciparum* infections. Parasite-induced early IFN-gamma was predominantly derived from gamma delta T cells (68% of which expressed the natural killer marker CD56) and alpha beta T cells, whereas natural killer cells and other cells made only minor contributions. The expression of CD56 in malaria-responsive, IFN-gamma-expressing gamma delta T cells correlated with IFN-gamma responsiveness. CONCLUSIONS: High, early IFN-gamma production by live parasite-stimulated peripheral blood mononuclear cells is a correlate of immunity to symptomatic malaria in Papua New Guinean children, and natural killer-like gamma delta T cells may contribute to protection.

28 du Toit R, Ramke J, Palagyi A, Brian G.

Spectacles in Fiji: need, acquisition, use and willingness to pay. *Clin Exp Optom* 2008 Nov;91(6):538-544. Epub 2008 Jun 5.

BACKGROUND: Little information is available regarding the perceived need, previous acquisition, use and willingness to pay for spectacles in Fiji, on which to base spectacle provision services. METHODS: Using a rapid appraisal technique, semi-structured interviews were conducted with 174 urban and rural households in Fiji's Central Province to assist in planning eye-care services. RESULTS: Problems with distance and/or near vision comprised 85.8 per cent of reported eye problems and started between the ages of 40 and 64 years for 54.8 per cent of people surveyed. Of these vision problems, no treatment was sought for 24.2 per cent and of the remainder, spectacles were the treatment for 65.5 per cent. At least one person in 51.7 per cent of households previously or currently used spectacles, and 90 per cent of these reported using them for near tasks. Spectacle usage occurred in more urban (61.8 per cent) than rural (47.1 per cent) households. The majority (54.0 per cent) were willing to pay over FJD10 for spectacles in the future, although more rural (21.8 per cent) than urban (7.3 per cent) households were willing to pay less than FJD10 (USD 4.70). Where spectacles had been received at no cost in the past, 89.5 per cent were prepared to pay FJD10 or more for these in the future. CONCLUSIONS: Given the high number of reported visual problems, it should be a priority to construct a sustainable spectacle system for Fiji. This will require further consultation with the

community and government but it should be possible to design a system responsive to the financial and other needs of urban and rural Fijians.

- 29 **Duke T.**
HIV in Papua New Guinea: the need for practical action, and a focus on human resources and health systems for women and children.
J Paediatr Child Health 2008 Nov;44(11):611-612.

- 30 **Duke T, Wandt F, Jonathan M, Matai S, Kaupa M, Saavu M, Subhi R, Peel D.**
Improved oxygen systems for childhood pneumonia: a multihospital effectiveness study in Papua New Guinea.
Lancet 2008 Oct 11;372(9646):1328-1333. Epub 2008 Aug 15.

BACKGROUND: In rural hospitals of developing countries, oxygen supplies are poor and detection of hypoxaemia is difficult. Oxygen concentrators and pulse oximeters might help to manage the disease; however, use of such technology in developing countries needs comprehensive assessment. We studied the effect of an improved oxygen system on death rate in children with pneumonia in Papua New Guinea. **METHODS:** We installed an improved oxygen system in five hospitals in Papua New Guinea, and assessed its use in more than 11 000 children with pneumonia (2001-07) and compared case-fatality rates. Admissions between January, 2001, and December, 2004, formed the pre-intervention group, and those between July, 2005, and October, 2007, formed the post-intervention group. Oxygen concentrators and pulse oximeters were introduced in the five hospitals, and a protocol for detection of hypoxaemia and clinical use of oxygen was supplied. All children admitted had their oxygen saturation measured; if it was less than 90%, oxygen was delivered via nasal prongs at a starting flow rate of 0.5-1 L/min. We recorded all costs associated with the establishment and maintenance of this system. The study was approved by the Medical Research Advisory Committee of Papua New Guinea, number MRAC 04.02. **FINDINGS:** Before the use of this system, 356 of 7161 children admitted in the five hospitals for pneumonia died (case-fatality rate 4.97% [95% CI 4.5-5.5]), whereas 133 of 4130 children died in the 27 months after the introduction of the system (3.22% [2.7-3.8]). After the improved system was introduced, the risk of death for a child with pneumonia was 35% lower than that before the project began (risk ratio 0.65 [0.52-0.78], $p < 0.0001$). Mortality rates varied between hospitals. The estimated costs of this system were US\$51 per patient treated, US\$1673 per life saved, and US\$50 per disability-adjusted life-year (DALY) averted. **INTERPRETATION:** Pulse oximetry and oxygen concentrators can alleviate oxygen shortages, reduce mortality, and improve quality of care for children with pneumonia in developing countries. The cost-effectiveness of this system compared favourably with that of other public-health interventions. **FUNDING:** The Papua New Guinea National Department of Health; WHO, Papua New Guinea and Western Pacific Regional Office; AirSep Corporation, Buffalo, NY, USA; the Ross Trust, VIC, Australia; AusAID; Jacques Gostelli, Switzerland; and a grant from the University of Melbourne.

- 31 **East IJ, Hamilton S, Sharp LA, Garner MG.**
Identifying areas of Australia at risk for H5N1 avian

influenza infection from exposure to nomadic waterfowl moving throughout the Australo-Papuan region.

Geospat Health 2008 Nov;3(1):17-27.

Since 2003, highly pathogenic avian influenza (HPAI) due to the H5N1 virus has been reported from both domestic poultry and wild birds in over 60 countries and this has resulted in the direct death or slaughter of over 250 million birds. The potential for HPAI to be introduced to Australian commercial poultry via migratory shorebirds returning from Asia has previously been assessed as a low risk. However, introduction of HPAI from areas to the immediate north of Australia via nomadic waterfowl that range throughout the Australo-Papuan region provides a second potential pathway of entry. Surveillance programmes provide an important early warning for Australia's estimated 2,000 commercial poultry farms but to be efficient they should be risk-based and target resources at those areas and sectors of the industry at higher risk of exposure. In order to address this need, this study compared the distribution and movement patterns of native waterfowl to identify regions where the likelihood of HPAI incursion and establishment was highest. Analysis of bird banding records provided information on the maximum distances moved and dispersal patterns of the species of waterfowl of interest. Introduction via Cape York was found to be most likely and all poultry farms in Queensland were found to be within range of waterfowl that can shed H5N1 virus for up to 17 days. The final analysis showed that the area at greatest risk of HPAI introduction is the Atherton tableland near Cairns.

- 32 **Farquhar J.**
The expanded Laboratory of Collaborative and Field Research.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3634-3635.

- 33 **Fernandez LS, Jobling MF, Andrews KT, Avery VM.**
Antimalarial activity of natural product extracts from Papua New Guinea and Australian plants against *Plasmodium falciparum*.
Phytother Res 2008 Oct;22(10):1409-1412.

In the search for new antimalarial compounds, a subset of a natural product extract library prepared from plant samples collected from Papua New Guinea and Australia was screened for in vitro activity against the chloroquine-sensitive 3D7 and chloroquine-resistant Dd2 strains of *Plasmodium falciparum*. Using the incorporation of (³H)-hypoxanthine into parasite nucleic acid as a marker of growth, 93 of the 794 extracts screened displayed >40% inhibition against 3D7 infected erythrocytes at 312 microg/mL. Antimalarial activity was confirmed in 48 of these extracts against both 3D7 and Dd2 infected erythrocytes at concentrations between 78 and 390 microg/mL, 14 of which caused >90% growth inhibition of 3D7 at the lowest concentration screened. Extracts were also tested for mammalian cell cytotoxicity to evaluate selectivity of action.

- 34 **Gajdusek DC.**
Review. Kuru and its contribution to medicine.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3697-3700.

The solution of kuru led us to the solution of Creutzfeldt-Jakob disease and to the elucidation,

in humans and other species, of previously unknown mechanisms of infection. These require very close three-dimensional matching, which determines infectious nucleant or prion activity. Evidence for nucleation processes is found widely in the organic and inorganic worlds and in the interactions between them: in the formation of amyloid fibrils; in the biochemistry of silicon; in cave formations deep in the Earth; and in outer space. Kuru in its location in Papua New Guinea has also led to an understanding of the cultural achievements of the Palaeo-Melanesians, with deep roots in human history.

35 **Gajdusek DC.**

Early images of kuru and the people of Okapa. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3636-3643.

36 **Garland SM, Brotherton JM, Skinner SR, Pitts M, Saville M, Mola G, Jones RW.**

Human papillomavirus and cervical cancer in Australasia and Oceania: risk-factors, epidemiology and prevention.

Vaccine 2008 Aug 19;26(Suppl 12):M80-M88.

The region encompassing Australasia and Oceania, including Australia, New Zealand, Fiji and Papua New Guinea, is a diverse one with respect to ethnicities, cultures and behaviours. It includes countries with comprehensive cervical cytology screening programmes which can be credited with significant reductions in cervical cancer incidence and mortality, and countries with no prevention programmes and significantly higher incidence and mortality. As elsewhere in the world, human papillomavirus (HPV)-16 and 18 are the commonest high-risk types, with the highest rates in women under 25 years of age. These two high-risk HPV types are found most frequently in cervical cancers and high-grade dysplasias, although there are minimal data for many countries in Oceania. In April 2007, Australia became the first country worldwide to commence a government funded universal HPV vaccine programme. The school-based programme targets 12-year old females in an ongoing schedule, with a catch-up programme up to 26 years of age, to be completed in mid-2009. Vaccine introduction has been comprehensively rolled out, with around 75% uptake of the complete vaccine schedule among school-girls in the first year of this initiative. This represents a successful model for other countries. We present data on cervical cancer, risk factors and prevention strategies, including epidemiology of HPV and HPV vaccine strategies.

37 **Genton B.**

Malaria vaccines: a toy for travelers or a tool for eradication?

Expert Rev Vaccines 2008 Jul;7(5):597-611.

The demonstration of efficacy of two candidate malaria vaccines in children living in malaria-endemic areas, namely RTS,S from the circumsporozoite protein that reduced infection and clinical malaria in Mozambique, and an asexual blood-stage vaccine combining MSP1/MSP2/RESA that reduced parasite density in Papua New Guinea, allows one to believe that a malaria vaccine will be available for the fight against malaria in the next decade. Even if long-lasting impregnated bednets and indoor residual spraying have proven to be effective in reducing malaria transmission, these interventions may not be sufficient in the long-run since they rely on too few compounds and are, thus,

vulnerable to the emergence of resistance. New tools, such as malaria vaccines, may, therefore, provide an added value to achieve the goal of local elimination and subsequent eradication of malaria. A promising candidate for that purpose would be a highly efficacious multicomponent vaccine that includes at least a sexual-stage antigen, the appropriate initial setting would be an area with low endemicity and limited population exchange, and the most suitable mode of delivery would be mass vaccination. For nonimmune populations, such as travelers visiting malaria-endemic areas, the usefulness of the first generation of malaria vaccine(s) will be limited, since the level of protection that is foreseen is unlikely to achieve that of malaria chemoprophylaxis. Only long-term travelers, expatriates and soldiers might realistically benefit from a pre-erythrocytic and/or blood-stage vaccine with an intermediate level of efficacy.

38 **Gomea T.**

'We had to climb mountains and cross fast-flowing rivers'.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3643.

39 **Greenhill AR, Blaney BJ, Shipton WA, Frisvad JC, Pue A, Warner JM.**

Mycotoxins and toxigenic fungi in sago starch from Papua New Guinea.

Lett Appl Microbiol 2008 Oct;47(4):342-347.

AIMS: To assay sago starch from Papua New Guinea (PNG) for important mycotoxins and to test fungal isolates from sago for mycotoxin production in culture. METHODS AND RESULTS: Sago starch collected from Western and East Sepik Provinces was assayed for aflatoxins, ochratoxin A, cyclopiazonic acid, sterigmatocystin, citrinin and zearalenone and all 51 samples were negative. Frequently isolated species of *Penicillium* (13), *Aspergillus* (five) and *Fusarium* (one) were cultured on wheat grain, and tested for the production of ochratoxin A, cyclopiazonic acid, sterigmatocystin, citrinin, patulin and penicillic acid. All 12 isolates of *P. citrinin* and one of two *A. flavipes* isolates produced citrinin. A single isolate of *A. versicolor* produced sterigmatocystin. No other mycotoxins were detected in these cultures. CONCLUSIONS: No evidence was found of systemic mycotoxin contamination of sago starch. However, the isolation of several mycotoxigenic fungi shows the potential for citrinin and other mycotoxins to be produced in sago stored under special conditions. SIGNIFICANCE AND IMPACT OF THE STUDY: Sago starch is the staple carbohydrate in lowland PNG and the absence of mycotoxins in freshly prepared sago starch is a positive finding. However, the frequent isolation of citrinin-producing fungi indicates a potential health risk for sago consumers, and food safety is dependent on promoting good storage practices.

40 **Habgood PJ, Franklin NR.**

The revolution that didn't arrive: a review of Pleistocene Sahul.

J Hum Evol 2008 Aug;55(2):187-222. Epub 2008 May 15.

There is a 'package' of cultural innovations that are claimed to reflect modern human behaviour. The introduction of the 'package' has been associated with the Middle-to-Upper Palaeolithic transition and the appearance in Europe of modern humans. It

has been proposed that modern humans spread from Africa with the 'package' and colonised not only Europe but also southern Asia and Australia (McBrearty and Brooks, 2000; Mellars, 2006a). In order to evaluate this proposal, we explore the late Pleistocene archaeological record of Sahul, the combined landmass of Australia and Papua New Guinea, for indications of these cultural innovations at the earliest sites. It was found that following initial occupation of the continent by anatomically and behaviourally modern humans, the components were gradually assembled over a 30,000-year period. We discount the idea that the 'package' was lost en route to Sahul and assess the possibility that the 'package' was not integrated within the material culture of the initial colonising groups because they may not have been part of a rapid colonisation process from Africa. As the cultural innovations appear at different times and locations within Sahul, the proposed 'package' of archaeologically visible traits cannot be used to establish modern human behaviour. Whilst the potential causal role of increasing population densities/pressure in the appearance of the 'package' of modern human behaviour in the archaeological record is acknowledged, it is not seen as the sole explanation because the individual components of the 'package' appear at sites that are widely separated in space and time.

- 41 **Hadlow WJ.**
Kuru likened to scrapie: the story remembered.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3644.
- 42 **Hasan AU, Suguri S, Fujimoto C, Itaki RL, Harada M, Kawabata M, Bugoro H, Albino B.**
Genetic diversity in two sibling species of the *Anopheles punctulatus* group of mosquitoes on Guadalcanal in the Solomon Islands.
BMC Evol Biol 2008 Nov 24;8:318.
BACKGROUND: The mosquito *Anopheles irenicus*, a member of the *Anopheles punctulatus* group, is geographically restricted to Guadalcanal in the Solomon Islands. It shows remarkable morphological similarities to one of its sibling species, *An. farauti* sensu stricto (*An. farauti* s.s.), but is dissimilar in host and habitat preferences. To infer the genetic variations between these two species, we have analyzed mitochondrial cytochrome oxidase subunit II (COII) and nuclear ribosomal internal transcribed spacer 2 (ITS2) sequences from Guadalcanal and from one of its nearest neighbours, Malaita, in the Solomon Islands. **RESULTS:** *An. farauti* s.s. was collected mostly from brackish water and by the human bait method on both islands, whereas *An. irenicus* was only collected from fresh water bodies on Guadalcanal Island. *An. irenicus* is distributed evenly with *An. farauti* s.s. (Phi SC = 0.033, 0.38%) and its range overlaps in three of the seven sampling sites. However, there is a significant population genetic structure between the species (Phi CT = 0.863, $P < 0.01$; Phi ST = 0.865, $P < 0.01$ and FST = 0.878, $P < 0.01$). Phylogenetic analyses suggest that *An. irenicus* is a monophyletic species, not a hybrid, and is closely related to the *An. farauti* s.s. on Guadalcanal. The time estimator suggests that *An. irenicus* diverged from the ancestral *An. farauti* s.s. on Guadalcanal within 29,000 years before present (BP). *An. farauti* s.s. expanded much earlier on Malaita (texp = 24,600 BP) than the populations on Guadalcanal (texp = 16,800 BP for *An. farauti* s.s. and 14,000 BP for *An. irenicus*). **CONCLUSION:** These findings suggest that *An. irenicus* and *An. farauti* s.s. are monophyletic sister species living in sympatry, and their populations on Guadalcanal have recently expanded. Consequently, the findings further suggest that *An. irenicus* diverged from the ancestral *An. farauti* s.s. on Guadalcanal.
- 43 **Hermosura MC, Cui AM, Go RC, Davenport B, Shetler CM, Heizer JW, Schmitz C, Mocz G, Garruto RM, Perraud AL.**
Altered functional properties of a TRPM2 variant in Guamanian ALS and PD.
Proc Natl Acad Sci USA 2008 Nov 18;105(46):18029-18034. Epub 2008 Nov 12.
Two related neurodegenerative disorders, Western Pacific amyotrophic lateral sclerosis (ALS) and parkinsonism-dementia (PD), originally occurred at a high incidence on Guam, in the Kii peninsula of Japan, and in southern West New Guinea more than 50 years ago. These three foci shared a unique mineral environment characterized by the presence of severely low levels of Ca^{2+} and Mg^{2+} , coupled with high levels of bioavailable transition metals in the soil and drinking water. Epidemiological studies suggest that genetic factors also contribute to the etiology of these disorders. Here, we report that a variant of the transient receptor potential melastatin 2 (TRPM2) gene may confer susceptibility to these diseases. TRPM2 encodes a calcium-permeable cation channel highly expressed in the brain that has been implicated in mediating cell death induced by oxidants. We found a heterozygous variant of TRPM2 in a subset of Guamanian ALS (ALS-G) and PD (PD-G) cases. This variant, TRPM2(P1018L), produces a missense change in the channel protein whereby proline 1018 (Pro(1018)) is replaced by leucine (Leu(1018)). Functional studies revealed that, unlike WT TRPM2, P1018L channels inactivate. Our results suggest that the ability of TRPM2 to maintain sustained ion influx is a physiologically important function and that its disruption may, under certain conditions, contribute to disease states.
- 44 **Hill LA, Davis JB, Hapgood G, Whelan PI, Smith GA, Ritchie SA, Cooper RD, van den Hurk AF.**
Rapid identification of *Aedes albopictus*, *Aedes scutellaris*, and *Aedes aegypti* life stages using real-time polymerase chain reaction assays.
Am J Trop Med Hyg 2008 Dec;79(6):866-875.
In 2005, a widespread infestation of *Aedes albopictus* was discovered in the Torres Strait, the region between northern Australia and New Guinea. To contain this species, an eradication program was implemented in 2006. However, the progress of this program is impeded by the difficulty of morphologically separating *Ae. albopictus* larvae from the endemic species *Aedes scutellaris*. In this study, three real-time TaqMan polymerase chain reaction assays that target the ribosomal internal transcribed spacer 1 region were developed to rapidly identify *Aedes aegypti*, *Ae. albopictus*, and *Ae. scutellaris* from northern Australia. Individual eggs, larvae, pupae, and adults, as well as the species composition of mixed pools were accurately identified. The assay method was validated using 703 field-collected specimens from the Torres Strait.
- 45 **Hodel EM, Marfurt J, Müller D, Rippert A, Borrmann S, Müller I, Reeder JC, Siba P, Genton**

B, Beck HP.

Lack of multiple copies of *pfmdr1* gene in Papua New Guinea.

Trans R Soc Trop Med Hyg 2008 Nov;102(11):1151-1153. Epub 2008 Jul 2.

We describe here the results of an analysis of *Plasmodium falciparum* multidrug resistance protein 1 (*pfmdr1*) gene copy number from 440 field isolates from Papua New Guinea. No multiple copies of the gene were found, which corresponds to the lack of usage of mefloquine. These data extend regional knowledge about the distribution of multidrug-resistant *P. falciparum*.

46 Hsu HL, Woad KJ, Woodfield DG, Helsby NA.

A high incidence of polymorphic *CYP2C19* variants in archival blood samples from Papua New Guinea. *Hum Genomics* 2008 Sep;3(1):17-23.

There is considerable inter-ethnic variability in the incidence of *CYP2C19* genetic poor metabolisers (*var/var*). About 3 per cent of Caucasians are *CYP2C19 var/var*. By contrast, an extremely high incidence (70 per cent) is observed in the Melanesian island of Vanuatu. The colonisation of the Pacific Islands is believed to have involved migration through Papua New Guinea (PNG), and hence a high incidence may also be expected in this population. The reported incidence in PNG was only 36 per cent, however. PNG is a country of extensive ethnic diversity, and the incidence of the *CYP2C19 var/var* in other regional populations of PNG is currently not established. In this study, restriction fragment length polymorphism-polymerase chain reaction analysis of archival blood serum samples was used to determine the prevalence of the *CYP2C19*2* and **3* variant alleles in three different ethnic and geographically isolated populations of PNG. In the largest population studied (Iruana), the frequency of both variant *CYP2C19* alleles was high (0.37 and 0.34, respectively). Specifically, the frequency of the *CYP2C19*3* allele was significantly higher than in the PNG (East Sepik) population reported previously (0.34 vs 0.16; $p < 0.0001$). In the Iruana population, 48.9 per cent of the samples were homozygous variants for *CYP2C19*2* or **3*, which although higher was not statistically different from the East Sepik population (36 per cent). The results of this study indicated that other regional populations of PNG also have a relatively high incidence of the *CYP2C19* genetic polymorphism compared with Caucasian populations. The high incidence reported in Vanuatu, however, may be due to genetic drift rather than a PNG founder population, as the Vanuatu population is dominated by the *CYP2C19*2* allele, with a lower contribution from the **3* allelic variant.

47 Hunley K, Dunn M, Lindström E, Reesink G, Terrill A, Healy ME, Koki G, Friedlaender FR, Friedlaender JS.

Genetic and linguistic coevolution in Northern Island Melanesia.

PLoS Genet 2008 Oct;4(10):e1000239. Epub 2008 Oct 31.

Recent studies have detailed a remarkable degree of genetic and linguistic diversity in Northern Island Melanesia. Here we utilize that diversity to examine two models of genetic and linguistic coevolution. The first model predicts that genetic and linguistic correspondences formed following population splits and isolation at the time of early range expansions into the region. The second is

analogous to the genetic model of isolation by distance, and it predicts that genetic and linguistic correspondences formed through continuing genetic and linguistic exchange between neighboring populations. We tested the predictions of the two models by comparing observed and simulated patterns of genetic variation, genetic and linguistic trees, and matrices of genetic, linguistic, and geographic distances. The data consist of 751 autosomal microsatellites and 108 structural linguistic features collected from 33 Northern Island Melanesian populations. The results of the tests indicate that linguistic and genetic exchange have erased any evidence of a splitting and isolation process that might have occurred early in the settlement history of the region. The correlation patterns are also inconsistent with the predictions of the isolation by distance coevolutionary process in the larger Northern Island Melanesian region, but there is strong evidence for the process in the rugged interior of the largest island in the region (New Britain). There we found some of the strongest recorded correlations between genetic, linguistic, and geographic distances. We also found that, throughout the region, linguistic features have generally been less likely to diffuse across population boundaries than genes. The results from our study, based on exceptionally fine-grained data, show that local genetic and linguistic exchange are likely to obscure evidence of the early history of a region, and that language barriers do not particularly hinder genetic exchange. In contrast, global patterns may emphasize more ancient demographic events, including population splits associated with the early colonization of major world regions.

48 Kakulas BA.

Personal reflections on the neuropathology of kuru.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3645-3646.

49 Karunajeewa HA, Mueller I, Senn M, Lin E, Law I, Gomorrai PS, Oa O, Griffin S, Kotab K, Suano P, Tarongka N, Ura A, Lautu D, Page-Sharp M, Wong R, Salman S, Siba P, Ilett KF, Davis TM.

A trial of combination antimalarial therapies in children from Papua New Guinea.

N Engl J Med 2008 Dec 11;359(24):2545-2557. Epub 2008 Dec 8.

BACKGROUND: Malaria control is difficult where there is intense year-round transmission of multiple plasmodium species, such as in Papua New Guinea. **METHODS:** Between April 2005 and July 2007, we conducted an open-label, randomized, parallel-group study of conventional chloroquine-sulfadoxine-pyrimethamine and artesunate-sulfadoxine-pyrimethamine, dihydroartemisinin-piperaquine, and artemether-lumefantrine in children in Papua New Guinea 0.5 to 5 years of age who had falciparum or vivax malaria. The primary end point was the rate of adequate clinical and parasitologic response at day 42 after the start of treatment with regard to *Plasmodium falciparum*, after correction for reinfections identified through polymerase-chain-reaction (PCR) genotyping of polymorphic loci in parasite DNA. Secondary end points included the rate of adequate clinical and parasitologic response at day 42 with regard to *P. vivax* without correction through PCR genotyping. **RESULTS:** Of 2802 febrile children screened, 482 with falciparum malaria and 195 with vivax malaria were included. The highest rate of adequate clinical

and parasitologic response for *P. falciparum* was in the artemether-lumefantrine group (95.2%), as compared with 81.5% in the chloroquine-sulfadoxine-pyrimethamine group ($P=0.003$), 85.4% in the artesunate-sulfadoxine-pyrimethamine group ($P=0.02$), and 88.0% in the dihydroartemisinin-piperaquine group ($P=0.06$). The rate of adequate clinical and parasitologic response for *P. vivax* in the dihydroartemisinin-piperaquine group (69.4%) was more than twice that in each of the other three treatment groups. The in vitro chloroquine and piperaquine levels that inhibited growth of local *P. falciparum* isolates by 50% correlated significantly ($P<0.001$). Rash occurred more often with artesunate-sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine than with chloroquine-sulfadoxine-pyrimethamine ($P=0.004$ for both comparisons). **CONCLUSIONS:** The most effective regimens were artemether-lumefantrine against *P. falciparum* and dihydroartemisinin-piperaquine against *P. vivax*. The relatively high rate of treatment failure with dihydroartemisinin-piperaquine against *P. falciparum* may reflect cross-resistance between chloroquine and piperaquine.

- 50 **Karyana M, Burdarm L, Yeung S, Kenangalem E, Wariker N, Maristela R, Umana KG, Vemuri R, Okoseray MJ, Penttinen PM, Ebsworth P, Sugiarto P, Anstey NM, Tjitra E, Price RN.** Malaria morbidity in Papua, Indonesia, an area with multidrug resistant *Plasmodium vivax* and *Plasmodium falciparum*. *Malar J* 2008 Aug 2;7:148.

BACKGROUND: Multidrug resistance has emerged to both *Plasmodium vivax* and *Plasmodium falciparum* and yet the comparative epidemiology of these infections is poorly defined. **METHODS:** All laboratory-confirmed episodes of malaria in Timika, Papua, Indonesia, presenting to community primary care clinics and an inpatient facility were reviewed over a two-year period. In addition information was gathered from a house-to-house survey to quantify the prevalence of malaria and treatment-seeking behaviour of people with fever. **RESULTS:** Between January 2004 and December 2005, 99,158 laboratory-confirmed episodes of malaria were reported, of which 58% (57,938) were attributable to *P. falciparum* and 37% (36,471) to *P. vivax*. Malaria was most likely to be attributable to pure *P. vivax* in children under one year of age (55% 2,684/4,889). In the household survey, the prevalence of asexual parasitaemia was 7.5% (290/3,890) for *P. falciparum* and 6.4% (248/3,890) for *P. vivax*. The prevalence of *P. falciparum* infection peaked in young adults aged 15-25 years (9.8% 69/707), compared to *P. vivax* infection which peaked in children aged 1 to 4 years (9.5% 61/642). Overall 35% (1,813/5,255) of people questioned reported a febrile episode in the preceding month. Of the 60% of people who were estimated to have had malaria, only 39% would have been detected by the surveillance network. The overall incidence of malaria was therefore estimated as 876 per 1,000 per year (range: 711-906). **CONCLUSION:** In this region of multidrug-resistant *P. vivax* and *P. falciparum*, both species are associated with substantial morbidity, but with significant differences in the age-related risk of infection.

- 51 **Kayser M, Choi Y, van Oven M, Mona S, Brauer S, Trent RJ, Suarkia D, Schiefenhövel W, Stoneking M.**

The impact of the Austronesian expansion: evidence from mtDNA and Y chromosome diversity in the Admiralty Islands of Melanesia. *Mol Biol Evol* 2008 Jul;25(7):1362-1374. Epub 2008 Apr 3.

The genetic ancestry of Polynesians can be traced to both Asia and Melanesia, which presumably reflects admixture occurring between incoming Austronesians and resident non-Austronesians in Melanesia before the subsequent occupation of the greater Pacific; however, the genetic impact of the Austronesian expansion to Melanesia remains largely unknown. We therefore studied the diversity of nonrecombining Y chromosomal (NRY) and mitochondrial (mt) DNA in the Admiralty Islands, located north of mainland Papua New Guinea, and updated our previous data from Asia, Melanesia, and Polynesia with new NRY markers. The Admiralties are occupied today solely by Austronesian-speaking groups, but their human settlement history goes back 20,000 years prior to the arrival of Austronesians about 3,400 years ago. On the Admiralties, we found substantial mtDNA and NRY variation of both Austronesian and non-Austronesian origins, with higher frequencies of Asian mtDNA and Melanesian NRY haplogroups, similar to previous findings in Polynesia and perhaps as a consequence of Austronesian matrilocality. Thus, the Austronesian language replacement on the Admiralties (and elsewhere in Island Melanesia and coastal New Guinea) was accompanied by an incomplete genetic replacement that is more associated with mtDNA than with NRY diversity. These results provide further support for the 'Slow Boat' model of Polynesian origins, according to which Polynesian ancestors originated from East Asia but genetically mixed with Melanesians before colonizing the Pacific. We also observed that non-Austronesian groups of coastal New Guinea and Island Melanesia had significantly higher frequencies of Asian mtDNA haplogroups than of Asian NRY haplogroups, suggesting sex-biased admixture perhaps as a consequence of non-Austronesian patrilocality. We additionally found that the predominant NRY haplogroup of Asian origin in the Admiralties (O-M110) likely originated in Taiwan, thus providing the first direct Y chromosome evidence for a Taiwanese origin of the Austronesian expansion. Furthermore, we identified a NRY haplogroup (K-P79, also found on the Admiralties) in Polynesians that most likely arose in the Bismarck Archipelago, providing the first direct link between northern Island Melanesia and Polynesia. These results significantly advance our understanding of the impact of the Austronesian expansion and human history in the Pacific region.

- 52 **Kimura R, Ohashi J, Matsumura Y, Nakazawa M, Inaoka T, Ohtsuka R, Osawa M, Tokunaga K.** Gene flow and natural selection in Oceanic human populations inferred from genome-wide SNP typing. *Mol Biol Evol* 2008 Aug;25(8):1750-1761. Epub 2008 Jun 3.

It is suggested that the major prehistoric human colonizations of Oceania occurred twice, namely, about 50,000 and 4,000 years ago. The first settlers are considered as ancestors of indigenous people in New Guinea and Australia. The second settlers are Austronesian-speaking people who dispersed by voyaging in the Pacific Ocean. In this study, we performed genome-wide single-nucleotide polymorphism (SNP) typing on an indigenous

Melanesian (Papuan) population, Gidra, and a Polynesian population, Tongans, by using the Affymetrix 500K assay. The SNP data were analyzed together with the data of the HapMap samples provided by Affymetrix. In agreement with previous studies, our phylogenetic analysis indicated that indigenous Melanesians are genetically closer to Asians than to Africans and European Americans. Population structure analyses revealed that the Tongan population is genetically originated from Asians at 70% and indigenous Melanesians at 30%, which thus supports the so-called Slow train model. We also applied the SNP data to genome-wide scans for positive selection by examining haplotypic variation and identified many candidates of locally selected genes. Providing a clue to understand human adaptation to environments, our approach based on evolutionary genetics must contribute to revealing unknown gene functions as well as functional differences between alleles. Conversely, this approach can also shed some light onto the invisible phenotypic differences between populations.

53 Kivita I.

'Today I am so happy to see friends I once worked with many years ago'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3646.

54 Klitzman R.

Kuru fieldwork in 1981 ... and beyond.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3646-3647.

55 Kuzma J, Atua V.

Conservative management of splenic injury in the tropics.

Trop Doct 2008 Oct;38(4):210-213.

We undertook this study in order to determine whether the conservative management of splenic injuries is a safe practice in a low-volume tropical hospital. We evaluated 69 consecutive patients with splenic injury prospectively. The outcome measures were morbidity and mortality rates, overall hospital stay and blood transfusion requirements. Spleen preservation was achieved in 85% (59) of cases. Of the 16 patients who underwent splenic surgery, six had splenorrhaphy (38%). The overall mortality was 4.3% (3) and the deaths were not related to the conservative management. Our findings suggest that not only is the conservative management of splenic injuries safe, but also that the repair of an enlarged spleen (splenorrhaphy) is safe and feasible in tropical hospital settings. The findings in this study provide further evidence that the conservative management of splenic injury in a tropical hospital without computed tomography scan is a safe practice.

56 Kuzma J.

Randomized clinical trial to compare the length of hospital stay and morbidity for early feeding with opioid-sparing analgesia versus traditional care after open appendectomy.
Clin Nutr 2008 Oct;27(5):694-699. Epub 2008 Sep 10.

BACKGROUND & AIMS: Fast track protocols have been successfully used in abdominal surgery but there are no randomized trials on fast track after appendectomy. The aim of this study was to evaluate the safety and feasibility of fast track

perioperative care protocol including early feeding with opioid-sparing analgesia after open appendectomy. **METHODS:** We randomly allocated 62 consecutive patients who underwent appendectomy to an early feeding with opioid-sparing analgesia and traditional care group. The study was not blinded regarding the mode of postoperative rehabilitation. Clinical primary endpoint was length of postoperative hospital stay. Secondary endpoints were morbidity rate, time to bowel sounds and passage of flatus or stools, tolerance of solid diet and facial visual pain score. **RESULTS:** The mean length of primary hospital stay was significantly shorter in the early feeding with opioid-sparing analgesia than in traditional care group (2.2 versus 4.0 days, $p < 0.001$). No significant differences were seen between groups regarding demographics, degree of pathological changes in the appendix, and in the secondary endpoints such as morbidity, frequency of vomiting, visual facial pain score, time to first flatus or stools, resumption of bowel sounds and toleration of solid diet. **CONCLUSIONS:** This study indicates that early feeding and opioid-sparing analgesia after open appendectomy is safe and reduces length of hospital stay without deterioration of pain control.

57 Lasme P, Davrieux F, Montet D, Lebot V.

Quantification of kavalactones and determination of kava (*Piper methysticum*) chemotypes using near-infrared reflectance spectroscopy for quality control in Vanuatu.

J Agric Food Chem 2008 Jul 9;56(13):4976-4981. Epub 2008 Jun 10.

Kava (*Piper methysticum* Forst f., Piperaceae) has anxiolytic properties and the ability to promote a state of relaxation without the loss of mental alertness. The rapid growth of the nutraceutical market between 1998 and 2000 has been stopped by a ban in Europe and Australia because of some suspicion of liver toxicity. It is now important to develop a fast, cheap, and reliable quality test to control kava exports. The aim of this study is to develop a calibration of the near-infrared reflectance spectroscopy (NIRS) using partial least-squares (PLS) regression. Two hundred thirty-six samples of kava roots, stumps, and basal stems were collected from the Vanuatu Agricultural Research and Technical Centre germplasm collection and from four villages. These samples, representing 45 different varieties, were analyzed using NIRS to record their absorption spectra between 400 and 2500 nm. A set of 101 selected samples was analyzed for their kavalactone content using HPLC. The results were used for PLS calibration of the NIRS. The NIRS prediction of the kavalactone content and the dry matter were in agreement with the HPLC results. There were good correlations between these two series of results, and coefficients (r^2) were all close to 1. The measurements were reproducible and had repeatability on par with the HPLC method. The NIRS system has been calibrated for the six major kavalactone content measurements, and it is suggested that this method could be used for quality control in Vanuatu.

58 Le Hello S, Falcot V, Lacassin F, Baumann F, Nordmann P, Naas T.

Molecular epidemiology of carbapenem-resistant *Acinetobacter baumannii* in New Caledonia.
Clin Microbiol Infect 2008 Oct;14(10):977-981.

Carbapenem-resistant *Acinetobacter baumannii*

(CR-Ab) ranked third, with a frequency of 24.8%, among 202 strains of multidrug-resistant bacteria isolated from clinical samples in the main hospital of New Caledonia in 2004. All CR-Ab isolates were analysed by isoelectric focusing, conjugation, pulsed-field gel electrophoresis and PCR for the presence of carbapenemase genes. Fifty CR-Ab isolates produced carbapenemase OXA-23. The isolates belonged to a single clone presenting several subtypes, suggesting an endemic situation. This study further illustrates the widespread prevalence of carbapenemase OXA-23-producing CR-Ab isolates in the South Pacific.

59 **Le Hello S, Page S, Garin B.**

Fluoroquinolone resistance in a clinical isolate of *Streptococcus pneumoniae* in the South Pacific. *Int J Antimicrob Agents* 2008 Jul;32(1):91-92. Epub 2008 May 20.

60 **Lindenbaum S.**

Review. Understanding kuru: the contribution of anthropology and medicine. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3715-3720.

To understand kuru and solve the problems of its cause and transmission required the integration of knowledge from both anthropological and medical research. Anthropological studies elucidated the origin and spread of kuru, the local mortuary practices of endocannibalism, the social effects of kuru, the life of women and child-rearing practices, the kinship system of the Fore and their willingness to incorporate outsiders into it, the myths, folklore and history of the Fore and their neighbours, sorcery as a powerful social phenomenon and way of explaining the causation of disease, and concepts of the treatment of disease. Many scientists from different disciplines, government officers and others have contributed to this chapter of medical history but it is the Fore people who have contributed the most, through their suffering, their cooperative and reliable witness to kuru, and their participation, in various ways, in the research process itself.

61 **Lindenbaum S.**

First impressions of the Fore. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3648-3649.

62 **Lusida MI, Nugrahaputra VE, Soetjipto, Handajani R, Nagano-Fujii M, Sasayama M, Utsumi T, Hotta H.**

Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. *J Clin Microbiol* 2008 Jul;46(7):2160-2166. Epub 2008 May 7.

Eight genotypes (A to H) and nine subtypes (adw2, adw4, ayw1, ayw2, ayw3, ayw4, adrq+, adr- and ayr) of hepatitis B virus (HBV) have been identified worldwide. They appear to be associated with geographical distribution, virological characteristics, and possibly clinical outcomes. We performed sequence analysis of part of the S gene and the entire precore/core gene of HBV isolates obtained from HBsAg-positive blood donors in Papua Province, Indonesia. Phylogenetic analysis of the S gene sequences revealed that 23 (85.2%) of the 27 HBV isolates tested belonged to genotype C (HBV/C) and 2 (7.4%) each to HBV/B and HBV/D. Interestingly, 19 (82.6%) of the 23 isolates of HBV/C clustered in a branch that was distinct from

the previously reported subgenotypes C1 to C5 (HBV/C1 to HBV/C5). Similarly, two isolates of HBV/D clustered in a branch distinct from the reported subgenotypes HBV/D1 to HBV/D5. Phylogenetic analysis of the entire precore/core gene confirmed the consistent presence of the distinct branches in HBV/C and HBV/D. We therefore propose novel subgenotypes designated HBV/C6 and HBV/D6. The majority of HBV/C6 isolates in Papua had alanine at positions 159 and 177 (A159/A177) in the HBsAg. A159/A177 is different from the determinants for adrq+ (A159/V177), found throughout Asia, and adr- (V159/A177), found in New Caledonia and Polynesia, possibly representing a unique antigenic group (provisionally referred to as adr- indeterminate). In conclusion, we have identified two novel HBV subgenotypes, HBV/C6 and HBV/D6, the first of which is the most prevalent subgenotype of HBV in Papua, Indonesia.

63 **Mabage K.**

'The people in every village they visited were so often mourning the dead'. *Philos Trans R Soc Lond B Biol Sci* 2008 Nov 27;363(1510):3649-3650.

64 **Marfurt J, de Monbrison F, Brega S, Barbolat L, Müller I, Sie A, Goroti M, Reeder JC, Beck HP, Picot S, Genton B.**

Molecular markers of in vivo *Plasmodium vivax* resistance to amodiaquine plus sulfadoxine-pyrimethamine: mutations in *pvdhfr* and *pvmr1*. *J Infect Dis* 2008 Aug 1;198(3):409-417.

BACKGROUND: Molecular markers for sulfadoxine-pyrimethamine (SP) resistance in *Plasmodium vivax* have been reported. However, data on the molecular correlates involved in the development of resistance to 4-aminoquinolines and their association with the in vivo treatment response are scarce. **METHODS:** We assessed *pvdhfr* (F57L/I, S58R, T61M, S117T/N, and I173F/L) and *pvmr1* (Y976F and F1076L) mutations in 94 patients who received amodiaquine (AQ) plus SP in Papua New Guinea (PNG). We then investigated the association between parasite genotype and treatment response. **RESULTS:** The treatment failure (TF) rate reached 13%. Polymorphisms in *pvdhfr* F57L, S58R, T61M, and S117T/N and in *pvmr1* Y976F were detected in 60%, 67%, 20%, 40%, and 39% of the samples, respectively. The single mutant *pvdhfr* 57 showed the strongest association with TF (odds ratio [OR], 9.04; $p = .01$). The combined presence of the quadruple mutant *pvdhfr* 57L+58R+61M+117T and *pvmr1* mutation 976F was the best predictor of TF (OR, 8.56; $p = .01$). The difference in TF rates between sites was reflected in the genetic drug-resistance profile of the respective parasites. **CONCLUSIONS:** The present study identified a new molecular marker in *pvmr1* that is associated with the in vivo response to AQ+SP. We suggest suitable marker sets with which to monitor *P. vivax* resistance against AQ+SP in countries where these drugs are used.

65 **Martiniuk AL, Millar HC, Malefoasi G, Vergeer P, Garland T, Knight S.**

Cooperation, integration, and long-term commitment: what Solomon Islanders and development workers say about health sector aid. *Asia Pac J Public Health* 2008;20(4):287-297. Epub 2008 Aug 12.

INTRODUCTION: The Solomon Islands is

experiencing instability and insecurity and also a concomitant increase in aid. This article aims to address the need for theoretical coordination frameworks to be further informed by the actual experiences, requirements, and views of the recipients of aid. **METHODS:** Qualitative research techniques were used to better understand governmental and nongovernmental leaders' views of health sector aid in the Solomon Islands. Data were collected using previously published literature, government and nongovernmental documents, and in-person interviews. **RESULTS:** Two key themes emerged from the interviews: the need for coordination and integration of aid and the need for this integration to occur over the long-term. These themes are presented using quotations from key informants. **CONCLUSION:** Themes and quotations arising from the analyses may assist in understanding theoretical frameworks for coordination, particularly in postconflict states. Future needs regarding mechanisms of collaboration in the Solomons are also discussed.

- 66 **Mathews C.**
Family life at Okapa as a 'missus bilong dokta bilong kuru'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3650-3651.

- 67 **Mathews JD.**
Review. The changing face of kuru: a personal perspective.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3679-3684.

The epidemic of kuru is now known to have been transmitted among the Fore by ritual consumption of infected organs from deceased relatives. As cannibalism was suppressed by government patrol officers during the 1950s, most transmission had ceased by 1957, when the kuru research programme first commenced. As predicted in the 1960s, the epidemic has waned, with progressive ageing of kuru-affected cohorts over the years to 2007. The few cases seen in the twenty-first century, with the longest incubation periods, were almost certainly exposed as children prior to 1960. Although the research programme had almost no role in bringing the kuru epidemic to an end, it did provide important knowledge that was to help the wider world in controlling the later epidemics of iatrogenic and variant Creutzfeldt-Jakob disease and bovine spongiform encephalopathy.

- 68 **McLean CA.**
The neuropathology of kuru and variant Creutzfeldt-Jakob disease.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3685-3687.

A comparison of the pathological profiles of two spongiform encephalopathies with a similar presumptive route of infection was performed. Archival kuru and recent variant Creutzfeldt-Jakob disease (vCJD) cases reveal distinct lesional differences, particularly with respect to prion protein, suggesting that the strain of agent is important in determining the phenotype. Genotype analysis of the polymorphism on codon 129 reveals (in conjunction with updated information from more kuru cases) that all three genotypes (VV, MV and MM (where M is methionine and V is valine)) are detected in kuru with some preference for MM homozygosity. The presence of valine does not therefore appear

to determine peripheral selection of PrP^{CJD}. vCJD remains restricted to date to MM homozygosity on codon 129. It remains to be determined whether this genotype is dictating a shorter incubation period.

- 69 **Mead S, Whitfield J, Poulter M, Shah P, Uphill J, Beck J, Campbell T, Al-Dujaily H, Hummerich H, Alpers MP, Collinge J.**

Genetic susceptibility, evolution and the kuru epidemic.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3741-3746.

The acquired prion disease kuru was restricted to the Fore and neighbouring linguistic groups of the Papua New Guinea highlands and largely affected children and adult women. Oral history documents the onset of the epidemic in the early twentieth century, followed by a peak in the mid-twentieth century and subsequently a well-documented decline in frequency. In the context of these strong associations (gender, region and time), we have considered the genetic factors associated with susceptibility and resistance to kuru. Heterozygosity at codon 129 of the human prion protein gene (PRNP) is known to confer relative resistance to both sporadic and acquired prion diseases. In kuru, heterozygosity is associated with older patients and longer incubation times. Elderly survivors of the kuru epidemic, who had multiple exposures at mortuary feasts, are predominantly PRNP codon 129 heterozygotes and this group show marked Hardy-Weinberg disequilibrium. The deviation from Hardy-Weinberg equilibrium is most marked in elderly women, but is also significant in a slightly younger cohort of men, consistent with their exposure to kuru as boys. Young Fore and the elderly from populations with no history of kuru show Hardy-Weinberg equilibrium. An increasing cline in 129V allele frequency centres on the kuru region, consistent with the effect of selection in elevating the frequency of resistant genotypes in the exposed population. The genetic data are thus strikingly correlated with exposure. Considering the strong coding sequence conservation of primate prion protein genes, the number of global coding polymorphisms in man is surprising. By intronic resequencing in a European population, we have shown that haplotype diversity at PRNP comprises two major and divergent clades associated with 129M and 129V. Kuru may have imposed the strongest episode of recent human balancing selection, which may not have been an isolated episode in human history.

- 70 **Mortimer-Jones SM, Phillips ND, La T, Naresh R, Hampson DJ.**

Penicillin resistance in the intestinal spirochaete *Brachyspira pilosicoli* associated with OXA-136 and OXA-137, two new variants of the class D beta-lactamase OXA-63.

J Med Microbiol 2008 Sep;57(Pt 9):1122-1128.

Penicillin resistance mediated by beta-lactamase activity has been reported previously in the anaerobic intestinal spirochaete *Brachyspira pilosicoli*, and a novel class D beta-lactamase (OXA-63) hydrolysing oxacillin was described recently in a resistant human strain from France. In the current study, 18 *B. pilosicoli* strains from Australia and Papua New Guinea were tested for ampicillin and oxacillin susceptibility, and investigated for the presence of the class D beta-lactamase gene *blaOXA-63* using PCR. PCR products were

amplified from seven human and four porcine strains that were penicillin resistant, but not from seven penicillin-sensitive strains. Sequence analysis of the whole gene amplified from seven of the resistant strains from humans and pigs revealed only minor nucleotide differences among them, but there were significant differences compared with *blaOXA-63*. The predicted amino acid sequence of the enzyme from all seven strains had the same key structural motifs as the previously reported OXA-63, but two variants with 94-95% identity with OXA-63 were identified. OXA-136 had an additional amino acid and 12 other consistent amino acid substitutions compared with OXA-63. OXA-137 had the same differences compared with OXA-63 as OXA-136, but had an additional amino acid substitution at position 16. No structures consistent with integrons or transposons were found in the nucleotide sequences in the vicinity of *blaOXA-136* in partially sequenced *B. pilosicoli* strain 95/1000, and the GC content (25.2 mol%) of the gene was similar to that of the whole genome. The gene encoding OXA-136 from *B. pilosicoli* strain Cof-10 conferred penicillin resistance on *Escherichia coli*. This study shows that penicillin resistance in human and porcine *B. pilosicoli* strains from Australia is associated with the production of two variants of OXA-63, and that susceptible strains lack the genes encoding OXA-63 or the variants.

71 Negin J.

Australia and New Zealand's contribution to Pacific Island health worker brain drain.

Aust NZ J Public Health 2008 Dec;32(6):507-511.

OBJECTIVE: The paper aims to quantify Australia and New Zealand's contribution to the brain drain of Pacific Island health workers and to contribute firm evidence to the ongoing, highly-contested health professional migration issue. **METHODS:** The study uses the Australian and New Zealand 2006 census data to examine the number of Pacific Island born health professionals living in Australia and New Zealand and uses World Health Organization data to compare it against the numbers of health workers in Pacific Island countries. **RESULTS:** Six hundred and fifty-two Pacific Island born doctors and 3,467 Pacific Island born nurses and midwives are working in Australia and New Zealand, more than half of whom are from Fiji with significant numbers from Papua New Guinea, Samoa and Tonga as well. There are almost as many Fiji-born doctors in Australia and New Zealand as there are in Fiji. There are more Samoa, Tonga and Fiji-born nurses and midwives in Australia and New Zealand than in the domestic workforce. **CONCLUSIONS:** Migration of Pacific Island health professionals to Australia and New Zealand is very high and contributes to the shortage of health workers in Pacific Island countries. **IMPLICATIONS:** Australia and New Zealand are encouraged to actively address the issue in collaboration with Pacific Island partners with a number of solutions proposed.

72 Nomura Y, Lavu EK, Muta K, Niino D, Takeshita M, Hirose S, Nakamura S, Yoshino T, Kikuchi M, Ohshima K.

Histological characteristics of 21 Papua New Guinean children with high-grade B-cell lymphoma, which is frequently associated with EBV infection. *Pathol Int* 2008 Nov;58(11):695-700.

The aim of the present study was to confirm the histopathological features of aggressive B-cell

lymphoma in Papua New Guinea (PNG) – an EBV endemic region. The immunophenotypic features and expression of EBV-encoded proteins and RNA in B-cell lymphomas were analyzed in 21 PNG children, and compared to the corresponding features of 17 Japanese children with Burkitt lymphoma (BL). Histological diagnosis of the lymphomas from the PNG children was BL in nine patients; atypical Burkitt/Burkitt-like variant of BL (BLL) in three; diffuse large B-cell lymphoma (DLBCL) in four; and B-lymphoblastic lymphoma (B-LBL) in five. The lymphomas from the PNG children had a high positive rate on EBV-RNA in situ hybridization (EBV-ISH; 66.7%). With regard to the histological typing, 10 of 12 patients (83%) with BL/BLL, one of four (25%) with DLBCL, and three of five (60%) with B-LBL were positive for EBV-ISH. The findings of EBV-positive B-LBL were surprising because it is commonly considered that lymphoblastic lymphoma is not associated with EBV. EBV positivity was not detected in the 12 Japanese patients who were available for the EBV-ISH evaluation. It is concluded that it is possible that a proportion of DLBCL and B-LBL besides BL/BLL are associated with EBV in endemic regions.

73 Noro JC, Barrows LR, Gideon OG, Ireland CM, Koch M, Matainaho T, Piskaut P, Pond CD, Bugni TS.

Tetrahydroxysqualene from *Rhus taitensis* shows antimycobacterial activity against *Mycobacterium tuberculosis*.

J Nat Prod 2008 Sep;71(9):1623-1624. Epub 2008 Aug 19.

Tuberculosis has become a major health problem, in particular with the emergence of extremely drug resistant tuberculosis (XDRTB). In our search for new therapeutic leads against TB, we isolated a new triterpene (1) from the plant *Rhus taitensis* collected in Papua New Guinea. Tetrahydroxysqualene (1) was isolated using bioassay-guided fractionation of the methanolic extract of *R. taitensis* leaves and twigs. The structure of tetrahydroxysqualene (1) was elucidated on the basis of HRESIMS and 1D and 2D NMR spectra. Tetrahydroxysqualene (1) exhibited antituberculosis activity with an MIC of 10.0 microg/mL, while showing only modest cytotoxicity.

74 Ombeya P.

'We were only allowed to perform an autopsy on those patients we had taken good care of'.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3652.

75 Pako WH.

The work of the Kuru Field Unit, Kuru Research Project of the Papua New Guinea Institute of Medical Research and MRC Prion Unit.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3652.

76 Pattison DA, Speare R.

Strongyloidiasis in personnel of the Regional Assistance Mission to Solomon Islands (RAMSI). *Med J Aust* 2008 Aug 18;189(4):203-206.

OBJECTIVE: To investigate the first reported cases of strongyloidiasis in the Solomon Islands, and to establish whether this disease poses a risk to personnel of the Regional Assistance Mission to Solomon Islands (RAMSI). **DESIGN, SETTING AND PARTICIPANTS:** Retrospective review of the

- pathology database of the RAMSI Medical Facility in Honiara, Solomon Islands, for the period 1 July 2006-30 September 2007. **MAIN OUTCOME MEASURES:** Number and clinical features of confirmed cases of *Strongyloides stercoralis* infestation, as diagnosed by serological tests or faecal microscopy. **RESULTS:** Fourteen confirmed cases of strongyloidiasis in previously healthy RAMSI participants were identified. Of 13 patients with notes available, symptoms documented at presentation included epigastric pain (10 patients), diarrhoea (7) and urticaria (4). Clinical disease in all patients responded to oral anthelmintic therapy (albendazole or ivermectin). **CONCLUSIONS:** Strongyloidiasis is endemic in the Solomon Islands and a risk for RAMSI personnel. Australian medical professionals should be aware of this potentially fatal and lifelong infestation, particularly the importance of an occupation history, appropriate diagnostic tests, effective treatment and adequate follow-up to document cure. We recommend implementation of a postdeployment screening program for strongyloidiasis.
- 77 **Pekiyeva T.**
'The children of those who died of kuru are today still alive'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3653.
- 78 **Poki K.**
'We agreed that they could conduct an autopsy on my brother'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3653.
- 79 **Prusiner SB.**
Reflections on kuru.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3654-3656.
- 80 **Puwa A.**
'Most people still believe that kuru is caused by sorcery'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3656.
- 81 **Rees SJ, van de Pas R, Silove D, Kareth M.**
Health and human security in West Papua.
Med J Aust 2008 Dec 1-15;189(11-12):641-643.
Recent publications have highlighted the impact of human rights violations, poverty and extraction of natural resources on the health status of the indigenous people of West Papua. However, the Australian medical literature has so far remained silent on this issue. Long-standing allegations of violence being perpetrated against Papuan civil society are supported by accounts given by West Papuan refugees involved in an Australian-based study. Health data collected by Médecins du Monde and other sources provide an insight into the poor health and lack of health care in the province, with high rates of infant mortality and morbidity, maternal mortality, and HIV/AIDS. Extraction of natural resources is causing major disruptions to the traditional livelihoods of indigenous Papuans, as a result of environmental degradation, mass displacement and an influx of migrant workers. Australian health professionals are urged to assist in remediating this dire situation, in keeping with our tradition of contributing to the health care of societies in our region.
- 82 **Reid LMH.**
Memories of kuru while at Okapa, Papua New Guinea in 1957.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3657-3659.
- 83 **Roberts JA, Grant KA, Ibrahim A, Thorley BR.**
Annual report of the Australian National Poliovirus Reference Laboratory, 2007.
Commun Dis Intell 2008 Sep;32(3):308-315.
In July 2007, wild poliovirus type 1 was isolated from a patient suffering from poliomyelitis in Melbourne, Australia with onset in Pakistan. The imported case of polio demonstrates the ongoing risk faced by polio-free countries until the global certification of polio eradication. The poliovirus was detected by the National Poliovirus Reference Laboratory (NPRL) for Australia accredited by the World Health Organization (WHO). The NPRL acts as the national laboratory for the Pacific Islands, Brunei Darussalam and Papua New Guinea. Additionally, the NPRL functions as a regional reference laboratory for the WHO Western Pacific Region. The NPRL, in collaboration with the Australian Paediatric Surveillance Unit, co-ordinates surveillance for acute flaccid paralysis (AFP), a major clinical presentation of poliovirus infection. After classification of AFP cases by the Polio Expert Committee, the non-polio AFP rate for Australia in 2007 was 0.65 per 100,000 children aged less than 15 years, below the performance indicator of 1.0 per 100,000 set by the WHO. Adequate faecal sample collection totalled 48% (13/27) of eligible AFP notifications, below the 80% performance indicator recommended by the WHO. During 2007, 119 specimens were referred to the NPRL, 70 from AFP cases and 49 from other sources, including contacts of the wild poliovirus importation, all negative for poliovirus infection. Coxsackievirus A4 was isolated from 1 case and adenovirus from 2 cases. During 2007, 1313 cases of poliomyelitis due to wild poliovirus infection were reported worldwide: 1207 occurring in the 4 remaining polio-endemic countries and 106 cases reported in 5 non-endemic countries.
- 84 **Saito-Nakano Y, Tanabe K, Kamei K, Iwagami M, Komaki-Yasuda K, Kawazu S, Kano S, Ohmae H, Endo T.**
Genetic evidence for *Plasmodium falciparum* resistance to chloroquine and pyrimethamine in Indochina and the Western Pacific between 1984 and 1998.
Am J Trop Med Hyg 2008 Oct;79(4):613-619.
Plasmodium falciparum resistance to chloroquine and pyrimethamine is widely distributed in malaria-endemic areas. The origin and geographic spread of this drug resistance have been inferred mainly from records of clinical resistance (treatment failure). Identification of the *Plasmodium falciparum* chloroquine resistance transporter (pfcrt) gene and the dihydrofolate reductase (dhfr) gene as target genes of chloroquine and pyrimethamine, respectively, has made it possible to trace the history of genetic resistance to these two drugs. However, evidence for genetic resistance has been limited because of scarcity of archival specimens. We examined genotypes of *pfcrt* and *dhfr* in Indochina (Thailand, Myanmar, and Laos) and the Western Pacific (the Philippines, Indonesia, and Papua New Guinea) between 1984 and 1998 by testing samples obtained from malaria cases imported to Japan.

- Results show that 96% (28 of 29) and 77% (20 of 26) of samples had resistant genotypes of *pfcr* and *dhfr*, respectively, substantiating the inferred history of clinical resistance in these geographic areas during this period.
- 85 **Saweri A.**
Reminiscences about kuru and the people of Okapa.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3660.
 - 86 **Scragg RF.**
Kuru memories from 1957.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3661-3663.
 - 87 **Scrimgeour EM.**
Some recollections about kuru in a patient at Rabaul in 1978, and subsequent experiences with prion diseases.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3663-3665.
 - 88 **Simpson DA.**
The Adelaide Kuru Team in 1957-1959.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3665.
 - 89 **Steer AC, Vidmar S, Ritika R, Kado J, Batzloff M, Jenney AW, Carlin JB, Carapetis JR.**
Normal ranges of streptococcal antibody titers are similar whether streptococci are endemic to the setting or not.
Clin Vaccine Immunol 2009 Feb;16(2):172-175. Epub 2008 Dec 3.
Group A streptococcal (GAS) serology is used for the diagnosis of post-streptococcal diseases, such as acute rheumatic fever, and occasionally for the diagnosis of streptococcal pharyngitis. Experts recommend that the upper limits of normal for streptococcal serology be determined for individual populations because of differences in the epidemiology of GAS between populations. Therefore, we performed a study to determine the values of the upper limit of normal for anti-streptolysin O (ASO) and anti-DNase B (ADB) titers in Fiji. Participants with a history of GAS disease, including pharyngitis or impetigo, were excluded. A total of 424 serum samples from people of all ages (with a sample enriched for school-aged children) were tested for their ASO and ADB titers. Reference values, including titers that were 80% of the upper limit of normal, were obtained by regression analysis by use of a curve-fitting method instead of the traditional nonparametric approach. Normal values for both the ASO titer and the ADB titer rose sharply during early childhood and then declined gradually with age. The estimated titers that were 80% of the upper limit of normal at age 10 years were 276 IU/ml for ASO and 499 IU/ml for ADB. Data from our study are similar to those found in countries with temperate climates, suggesting that a uniform upper limit of normal for streptococcal serology may be able to be applied globally.
 - 90 **Stöcklin WH.**
My kuru adventure.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3666-3667.
 - 91 **Tada Y, Okabe N, Kimura M.**
Travelers' risk of malaria by destination country: a study from Japan.
Travel Med Infect Dis 2008 Nov;6(6):368-372. Epub 2008 Sep 23.
BACKGROUND: Country-specific information on the incidence of malaria in travelers provides the most reliable data on which to base the pre-travel risk assessment. Some such studies have been conducted among Western travelers; however, to our knowledge, there have been no reports on Japanese travelers. METHODS: Malaria cases that were diagnosed between April 1999 and December 2005 and were reported to the national infectious disease surveillance body were used as the numerators after being grouped into countries of disease acquisition. The denominators, the numbers of Japanese travelers visiting individual countries, were derived from the recipient countries and obtained through a Japanese organization. RESULTS: In addition to the well-documented high risks in sub-Saharan countries, our study showed that travelers to Papua New Guinea were exposed to a significantly high risk of malaria. In Asia, Myanmar had the highest risk. Generally, malaria incidence rates among Japanese travelers were lower than those previously reported for Western travelers. However, the rates were rather comparable to the data obtained recently. CONCLUSIONS: These malaria incidence data in travelers should be taken into consideration for pre-travel risk assessment. They need to be constantly updated, and at the same time limitations in data interpretation that are inherent in various study methodologies should also be clarified.
 - 92 **Taguse T.**
'I was selected to be trained as an aid post orderly'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3668.
 - 93 **Tarr PI.**
The late 1970s: a lull in the action on kuru.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3668-3671.
 - 94 **Tasa K.**
'Collecting human samples was very hard owing to the fear of sorcery'.
Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3671.
 - 95 **Taylor WR, Widjaja H, Basri H, Ohrt C, Taufik T, Tjitra E, Baso S, Fryauff D, Hoffman SL, Richie TL.**
Changes in the total leukocyte and platelet counts in Papuan and non-Papuan adults from north-east Papua infected with acute *Plasmodium vivax* or uncomplicated *Plasmodium falciparum* malaria.
Malar J 2008 Dec 18;7(1):259. [Epub ahead of print]
BACKGROUND: There are limited data on the evolution of the leukocyte and platelet counts in malaria patients. METHODS: In a clinical trial of chloroquine vs chloroquine plus doxycycline vs doxycycline alone against *Plasmodium vivax* (n=64) or *Plasmodium falciparum* (n=98) malaria, the total white cell (WCC) and platelet (PLT) counts were measured on Days 0, 3, 7 and 28 in 57 indigenous Papuans with life-long malaria exposure and 105 non-Papuan immigrants from other parts of Indonesia with limited malaria exposure. RESULTS: The mean Day 0 WCC (n=152) was 6.492 (range 2.1-13.4) $\times 10^9/L$ and was significantly lower in the Papuans compared to the non-Papuans: $5.77 \times 10^9/L$.

L vs $6.86 \times 10^9/L$, $D = -1.09$ [95% CI -0.42 to -1.79 $\times 10^9/L$], $P = 0.0018$]. 14 (9.2%) and 9 (5.9%) patients had leukopenia ($<4.0 \times 10^9/L$) and leukocytosis ($>10.0 \times 10^9/L$), respectively. By Day 28, the mean WCC increased significantly ($P = 0.0003$) from 6.37 to $7.47 \times 10^9/L$ (73 paired values) and was similar between the two groups. Ethnicity was the only WCC explanatory factor and only on Day 0. The mean Day 0 platelet count ($n = 151$) was 113.0 (range $8.0-313.0$) $\times 10^9/L$ and rose significantly to $186.308 \times 10^9/L$ by Day 28 ($p < 0.0001$). There was a corresponding fall in patient proportions with thrombocytopenia ($<150 \times 10^9/L$): $119/151$ (78.81%) vs $16/73$ (21.92%, $p < 0.00001$). Papuan and non-Papuan mean platelet counts were similar at all time points. Only malaria species on Day 0 was a significant platelet count explanatory factor. The mean D0 platelet counts were significantly lower ($P = 0.025$) in vivax ($102.022 \times 10^9/L$) vs falciparum ($122.125 \times 10^9/L$) patients. **CONCLUSIONS:** Changes in leukocytes and platelets were consistent with other malaria studies. The Papuan vs non-Papuan difference in the mean Day 0 WCC was small but might be related to the difference in malaria exposure.

- 96 **Thomas SJ, Harris R, Ness AR, Taulo J, MacLennan R, Howes N, Bain CJ.**

Betel quid not containing tobacco and oral leukoplakia: a report on a cross-sectional study in Papua New Guinea and a meta-analysis of current evidence.

Int J Cancer 2008 Oct 15;123(8):1871-1876.

Leukoplakia is an asymptomatic, potentially malignant change in the oral mucosa. Previous studies have reported that smoking and betel quid chewing are associated with increased risk of leukoplakia; few studies have reported on these associations in populations where betel quid does not contain tobacco. We conducted a case-control study nested in a cross-sectional study in Papua New Guinea and a systematic review of studies that included chewers of betel quid without tobacco. Our study recruited 1,670 adults. We recorded betel quid chewing and smoking. The prevalence of leukoplakia was 11.7%. In the nested case-control study of 197 cases and 1,282 controls, current betel chewing was associated with increased risk of leukoplakia with an adjusted odds ratio for current chewers of 3.8 (95% CI 1.7-8.4) and in the heaviest chewers of 4.1 (95% CI 1.8-9.1) compared to non-chewers. Current smoking was associated with an increased risk of leukoplakia with an adjusted odds ratio for current smokers of 6.4 (95% CI 4.1-9.9) and amongst heaviest smokers of 9.8 (95% CI 5.9-16.4) compared to non-smokers. The systematic review identified 5 studies examining risk of leukoplakia associated with betel quid chewing in populations where betel quid did not contain tobacco and that controlled for smoking. In studies that adjusted for smoking, the combined random effect odds ratio was 7.9 (95% CI 4.3-14.6) in betel quid chewers. The results of this study and systematic review of similar studies provide evidence of the role of betel quid not containing tobacco and leukoplakia.

- 97 **Velickovic M, Velickovic Z, Panigoro R, Dunckley H.**

Diversity of killer cell immunoglobulin-like receptor genes in Indonesian populations of Java, Kalimantan, Timor and Irian Jaya.

Tissue Antigens 2009 Jan;73(1):9-16. Epub 2008 Oct 24.

Killer cell immunoglobulin-like receptors (KIRs) regulate the activity of natural killer and T cells through interactions with specific human leucocyte antigen class I molecules on target cells. Population studies performed over the last several years have established that KIR gene frequencies (GFs) and genotype content vary considerably among different ethnic groups, indicating the extent of KIR diversity, some of which have also shown the effect of the presence or absence of specific KIR genes in human disease. We have determined the frequencies of 16 KIR genes and pseudogenes and genotypes in 193 Indonesian individuals from Java, East Timor, Irian Jaya (western half of the island of New Guinea) and Kalimantan provinces of Indonesian Borneo. All 16 KIR genes were observed in all four populations. Variation in GFs between populations was observed, except for *KIR2DL4*, *KIR3DL2*, *KIR3DL3*, *KIR2DP1* and *KIR3DP1* genes, which were present in every individual tested. When comparing KIR GFs between populations, both principal component analysis and a phylogenetic tree showed close clustering of the Kalimantan and Javanese populations, while Irianese populations were clearly separated from the other three populations. Our results indicate a high level of KIR polymorphism in Indonesian populations that probably reflects the large geographical spread of the Indonesian archipelago and the complex evolutionary history and population migration in this region.

- 98 **Vernel-Pauillac F, Nandi S, Nicholas RA, Goarant C.**

Genotyping as a tool for antibiotic resistance surveillance of *Neisseria gonorrhoeae* in New Caledonia: evidence of a novel genotype associated with reduced penicillin susceptibility. *Antimicrob Agents Chemother* 2008 Sep;52(9):3293-3300. Epub 2008 Jun 30.

Antibiotic resistance in *Neisseria gonorrhoeae* continues to be a major concern in public health. Resistance of *N. gonorrhoeae* bacteria to penicillin G is widespread in most developed countries, which has necessitated a change to newer drugs for treatment of gonococcal infections. Recent reports indicate that resistance to these newer drugs is increasing, highlighting the need for accurate therapeutic recommendations. In some countries or communities, however, *N. gonorrhoeae* isolates are still susceptible to penicillin, so the use of this antibiotic for single-dose treatment of medically under-resourced patients is beneficial. In order to evaluate the adequacy and sustainability of this treatment approach, we explored the presence and prevalence of chromosomally mediated resistance determinants in *N. gonorrhoeae* isolates collected from 2005 to 2007 in New Caledonia. We developed two new real-time PCR assays targeting the *penB* and *mtrR* determinants, to be used together with a previously described duplex assay targeting the *penA* and *ponA* determinants. The results of this study provided evidence that neither the most-common *mtrR* determinants nor the most-resistance-associated *penB* alleles are currently circulating in New Caledonia, suggesting that penicillin should still be considered a valuable treatment strategy. Additionally, using our genotyping assay, we observed an unexpected *penB* genotype at a relatively high frequency that was

associated with a decreased susceptibility to penicillin (average MIC, 0.15 µg/ml). Sequencing revealed that this genotype corresponded to an A102S mutation in the *penB* gene. The molecular tools developed in this study can be used successfully for prospective epidemiological monitoring and surveillance of penicillin susceptibility.

- 99 **Wadsworth JD, Joiner S, Linehan JM, Asante EA, Brandner S, Collinge J.**

Review. The origin of the prion agent of kuru: molecular and biological strain typing.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3747-3753.

Kuru is an acquired human prion disease that primarily affected the Fore linguistic group of the Eastern Highlands of Papua New Guinea. The central clinical feature of kuru is progressive cerebellar ataxia and, in sharp contrast to most cases of sporadic Creutzfeldt-Jakob disease (CJD), dementia is a less prominent and usually late clinical feature. In this regard, kuru is more similar to variant CJD, which also has similar prodromal symptoms of sensory disturbance and joint pains in the legs and psychiatric and behavioural changes. Since a significant part of the clinicopathological diversity seen in human prion disease is likely to relate to the propagation of distinct human prion strains, we have compared the transmission properties of kuru prions with those isolated from patients with sporadic, iatrogenic and variant CJD in both transgenic and wild-type mice. These data have established that kuru prions have prion strain properties equivalent to those of classical (sporadic and iatrogenic) CJD prions but distinct from variant CJD prions. Here, we review these findings and discuss how peripheral routes of infection and other factors may be critical modifiers of the kuru phenotype.

- 100 **Wall JD, Cox MP, Mendez FL, Woerner A, Severson T, Hammer MF.**

A novel DNA sequence database for analyzing human demographic history.

Genome Res 2008 Aug;18(8):1354-1361. Epub 2008 May 20.

While there are now extensive databases of human genomic sequences from both private and public efforts to catalog human nucleotide variation, there are very few large-scale surveys designed for the purpose of analyzing human population history. Demographic inference from patterns of SNP variation in current large public databases is complicated by ascertainment biases associated with SNP discovery and the ways that populations and regions of the genome are sampled. Here, we present results from a resequencing survey of 40 independent intergenic regions on the autosomes and X chromosome comprising ~210 kb from each of 90 humans from six geographically diverse populations (i.e., a total of ~18.9 Mb). Unlike other public DNA sequence databases, we include multiple indigenous populations that serve as important reservoirs of human genetic diversity, such as the San of Namibia, the Biaka of the Central African Republic, and Melanesians from Papua New Guinea. In fact, only 20% of the SNPs that we find are contained in the HapMap database. We identify several key differences in patterns of variability in our database compared with other large public databases, including higher levels of nucleotide

diversity within populations, greater levels of differentiation between populations, and significant differences in the frequency spectrum. Because variants at loci included in this database are less likely to be subject to ascertainment biases or linked to sites under selection, these data will be more useful for accurately reconstructing past changes in size and structure of human populations.

- 101 **Warner JM, Pelowa DB, Gal D, Rai G, Mayo M, Currie BJ, Govan B, Skerratt LF, Hirst RG.**

The epidemiology of melioidosis in the Balimo region of Papua New Guinea.

Epidemiol Infect 2008 Jul;136(7):965-971. Epub 2007 Aug 22.

The distribution of *Burkholderia pseudomallei* was determined in soil collected from a rural district in Papua New Guinea (PNG) where melioidosis had recently been described, predominately affecting children. In 274 samples, 2.6% tested culture-positive for *B. pseudomallei*. Pulsed-field gel electrophoresis using *SpeI* digests and rapid polymorphic DNA PCR with five primers demonstrated a single clone amongst clinical isolates and isolates cultured from the environment that was commonly used by children from whom the clinical isolates were derived. We concluded that individuals in this region most probably acquired the organism through close contact with the environment at these sites. *Burkholderia thailandensis*, a closely related *Burkholderia* sp., was isolated from 5.5% of samples tested, an observation similar to that of melioidosis-endemic areas in Thailand. This is the first report of an environmental reservoir for melioidosis in PNG and confirms the Balimo district in PNG as melioidosis endemic.

- 102 **Weil GJ, Kastens W, Susapu M, Laney SJ, Williams SA, King CL, Kazura JW, Bockarie MJ.**

The impact of repeated rounds of mass drug administration with diethylcarbamazine plus albendazole on bancroftian filariasis in Papua New Guinea.

PLoS Negl Trop Dis 2008 Dec;2(12):e344. Epub 2008 Dec 9.

BACKGROUND: This study employed various monitoring methods to assess the impact of repeated rounds of mass drug administration (MDA) on bancroftian filariasis in Papua New Guinea, which has the largest filariasis problem in the Pacific region. **METHODOLOGY/PRINCIPAL FINDINGS:** Residents of rural villages near Madang were studied prior to and one year after each of three rounds of MDA with diethylcarbamazine plus albendazole administered per World Health Organization (WHO) guidelines. The mean MDA compliance rate was 72.9%. Three rounds of MDA decreased microfilaremia rates (Mf, 1 ml night blood by filter) from 18.6% pre-MDA to 1.3% after the third MDA (a 94% decrease). Mf clearance rates in infected persons were 71%, 90.7%, and 98.1% after 1, 2, and 3 rounds of MDA. Rates of filarial antigenemia assessed by card test (a marker for adult worm infection) decreased from 47.5% to 17.1% (a 64% decrease) after 3 rounds of MDA. The filarial antibody rate (IgG4 antibodies to Bm14, an indicator of filarial infection status and/or exposure to mosquito-borne infective larvae) decreased from 59.3% to 25.1% (a 54.6% decrease). Mf, antigen, and antibody rates decreased more rapidly in children <11 years of age

(by 100%, 84.2%, and 76.8%, respectively) relative to older individuals, perhaps reflecting their lighter infections and shorter durations of exposure/infection prior to MDA. Incidence rates for microfilaremia, filarial antigenemia, and antifilarial antibodies also decreased significantly after MDA. Filarial DNA rates in *Anopheles punctulatus* mosquitoes that had recently taken a blood meal decreased from 15.1% to 1.0% (a 92.3% decrease). **CONCLUSIONS/SIGNIFICANCE:** MDA had dramatic effects on all filariasis parameters in the study area and also reduced incidence rates. Follow-up studies will be needed to determine whether residual infection rates in residents of these villages are sufficient to support sustained transmission by the *An. punctulatus* vector. Lymphatic filariasis elimination should be feasible in Papua New Guinea if MDA can be effectively delivered to endemic populations.

103 West P.

Tourism as science and science as tourism: environment, society, self, and other in Papua New Guinea.

Curr Anthropol 2008 Aug;49(4):597-626.

The experience of villagers in Maimafu, in the Crater Mountain Wildlife Management Area of the Eastern Highlands of Papua New Guinea, calls attention to two forms of social interaction between rural people and outsiders that have been little examined in the anthropological literature. One of these is scientific research and the other is scientific tourism, a form of ecotourism that is linked not to science but to self-fashioning and individual gain. Scientific tourists may be seeking an educational adventure that they can turn into symbolic capital on their return home, a way into the world of science, or an experience that can be turned into economic capital through publication in popular magazines. For both researchers and scientific journalists, New Guinea combines the exotic, the about-to-be-lost, the primitive, the untouched, and the spectacular and is therefore a powerful space for imaginary and representational practice.

104 Westermarck P, Westermarck GT.

Review. Reflections on amyloidosis in Papua New Guinea.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3701-3705.

The amyloidoses comprise a heterogeneous group of diseases in which 1 out of more than 25 human proteins aggregates into characteristic beta-sheet fibrils with some unique properties. Aggregation is nucleation dependent. Among the

known amyloid-forming constituents is the prion protein, well known for its ability to transmit misfolding and disease from one individual to another. There is increasing evidence that other amyloid forms also may be transmissible but only if certain prerequisites are fulfilled. One of these forms is systemic AA-amyloidosis in which an acute-phase reactant, serum AA, is over-expressed and, possibly after cleavage, aggregates into amyloid fibrils, causing disease. In a mouse model, this disorder can easily be transmitted from one animal to another both by intravenous and oral routes. Also, synthetic amyloid-like fibrils made from defined small peptides have this property, indicating a prion-like transmission mechanism. Even some fibrils occurring in the environment can transmit AA-amyloidosis in the murine model. AA-amyloidosis is particularly common in certain areas of Papua New Guinea, probably due to the endemicity of malaria and perhaps genetic predisposition. Now, when kuru is disappearing, more interest should be focused on the potentially lethal systemic AA-amyloidosis.

105 Whitfield JT, Pako WH, Collinge J, Alpers MP.

Mortuary rites of the South Fore and kuru.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3721-3724.

This paper is part of a wider study to explain the historical spread and changing epidemiological patterns of kuru by analysing factors that affect the transmission of kuru. Part of the study has been to look at the mortuary feasts that were the means of transmission of the kuru agent. This paper shows the complexity of Fore eschatology, and the variations and contradictions of human behaviour in relation to mortuary rites and the transmission of kuru. It also confirms that oral ingestion was the primary route of inoculation though some cases of parenteral inoculation may have occurred. The exclusion of alternative routes of transmission is of importance owing to the dietary exposure of the UK and other populations to bovine spongiform encephalopathy prions.

106 Whitfield JT.

Work among the people of the Okapa area from 1996 to the present.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3671-3672.

107 Zu Rhein GM.

My 'brush' with kuru research.

Philos Trans R Soc Lond B Biol Sci 2008 Nov 27;363(1510):3672-3673.

Papua New Guinea Institute of Medical Research Monograph Series

ISSN 0256 2901

1. Growth and Development in New Guinea. A Study of the Bundi People of the Madang District.
L.A. Malcolm. ISBN 9980 71 000 4, 1970, 105p.
2. Endemic Cretinism.
B.S. Hetzel and P.O.D. Pharoah, Editors. ISBN 9980 71 001 2, 1971, 133p.
3. Essays on Kuru.
R.W. Hornabrook, Editor. ISBN 9980 71 002 0 (also 0 900848 95 2), 1976, 150p.
4. The People of Murapin.
P.F. Sinnett. ISBN 9980 71 003 9 (also 0 900848 87 1), 1977, 208p.
5. A Bibliography of Medicine and Human Biology of Papua New Guinea.
R.W. Hornabrook and G.H.F. Skeldon, Editors. ISBN 9980 71 004 7, 1977, 335p. (with 1976 Supplement, 36p.)
6. Pigbel. Necrotising Enteritis in Papua New Guinea.
M.W. Davis, Editor. ISBN 9980 71 005 5, 1984, 118p.
7. Cigarette Smoking in Papua New Guinea.
D.E. Smith and M.P. Alpers, Editors. ISBN 9980 71 006 3, 1984, 83p.
8. Village Water Supplies in Papua New Guinea.
D.E. Smith and M.P. Alpers, Editors. ISBN 9980 71 007 1, 1985, 94p.
9. The Health of Women in Papua New Guinea.
Joy E. Gillett. ISBN 9980 71 008 X, 1990, 180p.
10. National Study of Sexual and Reproductive Knowledge and Behaviour in Papua New Guinea.
The National Sex and Reproduction Research Team and Carol Jenkins. ISBN 9980 71 009 8, 1994, 147p.

Monographs 1-5 are case-bound, 6-10 are paperbacks.

Monographs may be obtained from

The Librarian,
Papua New Guinea Institute of
Medical Research
PO Box 60, Goroka, EHP 441,
Papua New Guinea

Cost of each Monograph (see below for Postage and Handling):

1,2.....	K	5.00
3,4.....	K	8.00
5.....	K	12.00
6,7,8,9.....	K	6.00
10.....	K	12.00

Applications for free copies of any monograph should be sent to the Director at the above address.

	Postage and Handling (PNG Kina)				
	SURFACE MAIL	AIRMAIL			
	Within PNG	Within PNG	Zone 1	Zone 3/4	Zone 6
1,2,10	7.00	10.00	20.00	60.00	75.00
3,4,5	14.00	20.00	40.00	90.00	105.00
6,7,8,9	3.50	5.00	10.50	17.50	17.50

K=PGK=Kina. Please make payment in Kina. If payment is made in any other currency, please add sufficient funds to cover all bank charges.

THE MEDICAL SOCIETY OF PAPUA NEW GUINEA

Society Membership and Journal Subscription

Membership of the Medical Society of Papua New Guinea is open to all health workers whether resident in Papua New Guinea or overseas. Members of the Society receive four issues of the Papua New Guinea Medical Journal each year. The Society organizes an annual symposium and other activities.

Membership dues are:-

Papua New Guinea residents:

Members – K150

Associate (Student) Members – K20

Overseas residents: K200; AU\$120; US\$100

I wish to join the Medical Society of Papua New Guinea as a

Full Member

☐

Please indicate your category

Medical Officer []

Scientific Officer []

Pharmacist []

Health Extension Officer []

Nursing Officer []

Laboratory Technologist []

Radiographer []

Social Health Worker []

Other (Please specify) []

OR a Student Member

☐

(for full-time students)

Medical Student []

Other Student (Please specify) []

I enclose my membership fee of

K.....for the year(s).....

Name:

Title:

Address:

.....

.....

Telephone:

Fax:

Email:

(Forward to the Membership Secretary,
Medical Society of Papua New Guinea, PO
Box 60, Goroka, EHP 441, Papua New
Guinea)

INFORMATION FOR AUTHORS

The Papua New Guinea Medical Journal invites submission of original papers and reviews on all aspects of medicine. Priority will be given to articles and subjects relevant to the practice of medicine in Papua New Guinea and other countries in the South Pacific.

Manuscripts are accepted for publication only with the understanding that they have not been published nor submitted for publication elsewhere. All manuscripts will be sent out for referees' comments as part of the peer review process.

Original Articles: Reports of original and new investigations or contributions.

Brief Communications and Case Reports: Contents similar to that of original articles but text should be no more than a total of 4 Journal pages including all figures and tables.

Reviews: Critical analysis of previously collected and published information.

Letters: Short reports of clinical experience or topics of interest. Text should not exceed 2 pages of the Journal.

Other types of manuscript may also be accepted for publication at the Editor's discretion.

Submitted manuscripts should conform to the instructions set out below. Manuscripts not conforming to these instructions will be returned.

MANUSCRIPTS

Submit the original with a virus-free electronic copy on disk as a word document or send by email to the Editorial Office. All sections of the manuscript, including text, references, tables and legends, should be in double spacing. Manuscripts should not be right justified. Each paper should include an informative Summary, Introduction, Patients/Materials and Methods, Results, Discussion and References. The title page should include the title, full names of all authors, names and addresses of institutions where the work has been done and full present address of the first or corresponding author.

References should be kept to a minimum, and must be in the Vancouver style. Authors should check all references against the original source. Sample references are shown below.

- 3 **Garner PA, Hill G.** Brainwashing in tuberculosis management. *PNG Med J* 1985;28:291-293.
- 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.

ILLUSTRATIONS

Tables and figures should be prepared on separate pages. Figures should be sent as separate jpeg or tiff images. Do not paste the images into Word. Photographs should be glossy prints, either 7 cm or 14.5 cm in width. Photomicrographs should have internal scale markers. Each table should have a heading and footnotes which make it understandable without reference to the text. Each figure should have a legend; figure legends should be typed together on a separate sheet.

Abbreviations: Standard abbreviations and units should be used.

Drug Names: Generic names of drugs should be used.

Orthography: The Shorter Oxford English Dictionary is followed.

EDITORIAL MAIL

Manuscripts and other editorial communications should be forwarded to:

The Editor,
Papua New Guinea Medical Journal,
PO Box 60, Goroka, EHP 441,
Papua New Guinea
Email: pngmedj@pngimr.org.pg

SUBSCRIPTIONS AND ADVERTISEMENTS

Communications relating to advertisements or subscriptions should be addressed to the Journal as above. Matters related to the Society should be addressed to the *Medical Society of Papua New Guinea*, PO Box 6665, Boroko, NCD 111, Papua New Guinea.

Subscriptions: Members of the Medical Society of Papua New Guinea receive the Journal as part of their annual subscription. Others may subscribe and should contact the subscription secretary for a price.

CONTENTS

MEMORIAL TO DR HELENA VRBOVA

EDITORIAL

- A memorial tribute to Helena Vrbova *M.P. Alpers* 73

TRIBUTES

- Helena Vrbova: a very special person *G. Vrbova* 80
- Helena Vrbova – a personal tribute *D.J. Jolley* 86
- Helena Vrbova, malaria epidemiologist *B.A. Darlow* 90
- Helena Vrbova: a reminiscence *S.J. Oppenheimer* 92
- Dr Helena Vrbova – a pioneer in malaria research *J. Stace* 94
- Helena Vrbova – an appreciation *A.O. Lucas* 95
- Personal observations on the characteristics of scientists *J. Taime* 96
- Tribute from Gonoa village, sent to her family at the time of Helena's death
Gonoa Village and J.S. Moir 98
- Bibliography of Helena Vrbova 99

ORIGINAL ARTICLES

- Women's groups and the marketing of health interventions – a Tanzanian experience *D. Charlwood* 102
- Effective diagnostic tests and anthelmintic treatment for *Strongyloides stercoralis* make community control feasible *J.M. Shield and W. Page* 105
- The relationship between undernutrition and humoral immune status in children with pneumonia in Papua New Guinea *A.W. Cripps, D.C. Otczyk, J. Barker, D. Lehmann and M.P. Alpers* 120
- Alpha⁺-thalassaemia and malaria in Melanesia: epidemiological perspectives *F.J.I. Fowkes and K.P. Day* 131

ORIGINAL ARTICLES

- Does Integrated Management of Childhood Illness (IMCI) make a difference to the assessment of sick children in Papua New Guinea? *M. Moti and J.D. Vince* 138
- Glycophorin C* Δ^{exon3} is not associated with protection against severe anaemia in Papua New Guinea *L. Tavul, I. Mueller, L. Rare, E. Lin, P.A. Zimmerman, J. Reeder, P. Siba and P. Michon* 149
- Is a 'convenience' sample useful for estimating immunization coverage in a small population? *J.E. Weir and C. Jones* 155

MEDICAL RESEARCH PROJECTS IN PAPUA NEW GUINEA 160

MEDLARS BIBLIOGRAPHY 163