2003 Annual Report

10th Anniversary

WINDREF Mission Statement:

Founded in 1994, WINDREF seeks to advance health and environmental development through multi-disciplinary research and education programs.

WINDREF strives for program excellence by promoting collaborative relationships between internationally recognized scholars and regional scientists and by adhering to the highest ethical and academic standards in the design and conduct of research.

1.0 Introduction

Ten years ago the Windward Islands Research and Education Foundation (WINDREF) was registered in the USA as a 501 (C) (3) educational non-profit foundation. WINDREF was also registered in Grenada as an NGO and as a charitable trust in the UK. In 2001 WINDREF established an office in St Vincent. The goal of creating an institute facilitating the development of research in this region has been made possible by the many people, foundations and institutions who have contributed to WINDREF during its first decade. We thank them for their continuous support.

We report with great sadness the sudden passing in Grenada in July of Ambassador Bart Lawson (MD hons) one of our board members who had served WINDREF with great distinction since joining us in 1999. We will miss his inspiration, guidance and participation in our activities. Our thoughts and prayers are with his wife Karen and his three children BJ (6), Mackenzie (4) and Katia (2). He will be missed by many in Grenada to whom he was a very good friend.

Ambassador Stanislaus, Hon Minister of Tourism, Brenda Hood, Dr Macpherson, Mrs Otway-Noel and Ms Catacutan bid farewell to Ambassador Lawson at Point Salines airport.

WINDREF's main areas of focus: lymphatic filariasis in Guyana, dengue, intestinal parasites and rheumatic fever in Grenada and cystic hydatid disease (CHD) in Morocco and Uganda continued. Six students accompanied Dr Macpherson over the summer to work on CHD in Morocco and four others accompanied him to Uganda to work on the same disease. Dr Shamdeo Persaud, MBBS, MPH, a WINDREF research fellow and PhD candidate, was appointed as the Regional Representative for PAHO to the lymphatic task force in WHO. In February WINDREF hosted a workshop on lymphatic filariasis in the Institute. Professor Molyneux, Lord Soulsby, members from UNICEF, the MOH and PAHO in Guyana attended. Yolanda Ng completed her studies on immunological aspects of lymphatic filariasis in Dr Modlin's molecular biology laboratory at UCLA over the summer. Trevor Noel completed his MPH degree from SGU and has started to collaborate with Dr Kreek (Rockefeller), Dave Alexander, Drug Avoidance Officer, Ministry of Education and Thorne Roberts. Director, Carlton House, Ministry of Health, on a project on genetic correlates of the addictive diseases: cocaine, alcohol and

marijuana. An additional new project on decompression sickness among the indigenous fishing population in Grenada was started in 2003. The project on medicinal plants and childhood diarrhea was completed. Details of all the projects and an electronic copy of this Annual Report are provided on our website at www.sgu.edu.

After nine years of activity WINDREF's IRB was replaced by a larger SGU IRB with an increased membership. This development was in recognition of the increased research activities at SGU. The new IRB is registered in the USA.

The Liverpool Support Center continued to support the lymphatic filariasis project and also supported two scholarship students, Drs Persaud and Singh, to work towards their PhD and MPH degrees respectively. Trevor Noel was supported by grants from Rockefeller through Drs Zabriskie and Mary-Jeanne Kreek. A number of small donations were received from other donors during the year and we would like to thank everyone for their contributions to our activities. Thanks also to our collaborators for their valuable input.

Professor David Molyneux pours champagne for President Jimmy Carter. Professor Ade Lucas, who delivered WINDREF's 2nd annual lecture, looks on.

The fourth WINDREF lecture was presented by Professor David Molyneux. The title of his lecture was *"Success and failure in parasitic disease control: Lessons learnt? "*.

In closing, I would like to extend my thanks to our many donors, Board of Directors, Board of Trustees, Scientific Advisory Board, Senior Research Fellows, Research Scientists, Staff, St. George's University faculty and other collaborators. I also thank the Government Ministries in Grenada, St. Vincent, Guyana, Morocco and Uganda for their contributions to our research activities during the year. We look forward to developing new links in the coming decade and hope it will be another decade to remember.

Calum Macpherson
Director

2.0 WINDREF's Research Institute

The WINDREF Research Institute is located on the strategically sited grounds of St. George's University at its True Blue campus in Grenada West Indies.

A summary of the over forty different research projects, on a variety of different topics, has been completed at the Institute. They are listed at the back of the 2003 Annual Report.

Research scientists outside the WINDREF Research Institute, True Blue, Grenada

2.1 The WINDREF Research Institute - Board of Directors

- Dr Keith B. Taylor, (President)
- Dr Calum N.L. Macpherson (Vice President)
- Ms Margaret Lambert (Secretary/ Treasurer)
- Dr Allen Pensick
- Ambassador Joseph Zappala

2.2. WINDREF St Vincent and the Grenadines Board of Directors

- Dr Ed Johnson, M.D., Director
- Sir Fredrick Ballantyne, M.D., Associate Director

2.3 The WINDREF Research Institute- Scientific Advisory Board

- Sir Frederick Ballantyne, MD
- John R. David, MD
- John J. Ferguson, MBChB, FRCGP
- Edmond Fischer, DSc
- C. James Hospedales, MB, BS, MSc
- Sir Malcolm MacNaughton, MD, LLD, FRCPG, FRAC
- Calum Macpherson, PhD, DIC
- Thomas W. Meade, CBE, DM, FRCP, FRS
- Graham Serjeant, MD, FRCP, CMG
- Malcolm Ferguson-Smith, MBChB, FRCP, FRCPath
- Sir Kenneth Stuart, MD, DSc (Hon)
- M.S. Swaminathan, DSc
- Keith Taylor, DM, FRCP
- John B. Zabriskie, MD

2.4 The WINDREF Research Institute- Research Fellows

The following investigators have been appointed to the Windward Islands Research Institute as research fellows and are currently conducting collaborative research projects.

2.4.1 Senior Research Fellows:

- Paul J Fields, PhD, Brigham Young University
- Michael Fisher, PhD, Merck Research Laboratories
- Paul Garner, PhD, Liverpool School of Tropical Medicine
- Mary Glenn, PhD, Humboldt State University
- Duane Gubler, ScD, CDC Fort Collins
- Ruth Milner, MSc, Vancouver Hospital
- Stephen Morse, PhD, Columbia University
- Leslie Ramsammy, PhD, Minister of Health, Guyana
- Stanley Weiss, MD, University of Medicine and Dentistry, New Jersey
- Alan Kocan, PhD, Oklahoma State University

2.4.2 Research Fellows

- Glennis Andall, PhD
- Michael Anson, PhD
- Charles Avgeris, MSc
- Orazio Giliberti, MD
- Svetlana Kotelnikova, PhD
- Theresa McCann, MPH, PhD
- Barrymore McBarnette, MD
- Craig McCarty, PhD
- Shamdeo Persaud, MD, MPH
- Shanti Singh, MD
- Richard Kabuusu, DVM, MPH

2.5 WINDREF Research Institute- Research Scientists

Research Scientists appointed to the Research Institute include: John Adamski, Sadiq Al-Tamini, Sumita Asthana, Yitzhack Asulin, Bishara Baddour, Jean-Pierre Barakat, Keith Bensen, Matthew Boles, Karen Brennan, Ella Cameron, Mmakgomo Coangae, Rae Connolly, David Evans, Scott Forman, Vamsi Guntur, Anthony Junck, Sebastian Krietschitz, Erik Lacy, Richard Lehman, Setshidi Makwinja, Paul Mancuso, Baher Maximos, John McCormack, David Melamed, Kirk Minkus, Jessica Morlok, Yolanda Ng, Michael Nillas, Trevor Noel, Andre Panagos, Barry Politi, Sandeep Pulim, Sean Ramsammy, Tarek Refaie, Alan Rhoades, Laura Robinson, Karin Schioler, Corey Schwartz, Christopher Skaff, David Steinberg, Derrick Tlhoiwe, David Tortugal, Sarah Treter, Nghia Truong, James Tsai, Frank Van Natta, Ru-Amir Walker, David Winokur, Colleen Wunderlich, Elliot Yung.

2.6 The WINDREF Research Institute - Administration

Mr. Trevor Noel was appointed as Assistant Director in 2003. Mrs. Isha English remained as Executive Secretary.

3.0 WINDREF (USA)

WINDREF (USA) was established to facilitate coordination of the USA activities and to administer charitable donations from the United States to the WINDREF Research Institute. As a non-profit organization, its goal is to enhance the development of WINDREF's research and educational programs. The offices are located on Long Island in New York to provide administrative and logistical support for the WINDREF Research Institute. Ms Donna Damm is the program coordinator in the New York Office.

4.0 WINDREF (UK)

WINDREF (UK) was set-up in Winchester, England in 1999 to promote collaboration between WINDREF scientists and academic centers of research in the United Kingdom. It is hoped that by reaching out to a larger scientific community, WINDREF will broaden its research opportunities by forming collaborations with scientists from the European community.

4.1 WINDREF (UK) - Board of Trustees

A Board of Trustees was appointed in 1999 to oversee the activities of WINDREF (UK). Our distinguished Board of Trustees members were selected for their scholarly academic accomplishments and international acclaim. Members include:

- Lord Soulsby of Swaffham Prior, MA, PhD, DSc, DVM, FRCVS (Chairman)
- Sir Kenneth Calman, KCB, FRCSE
- Richard Summerfield, MB, BChir, MA, FRCA
- Sir Kenneth Stuart, MD, DSc
- Keith B. Taylor, DM, FRCP
- Calum Macpherson, PhD, DIC (Ex Officio)

4.2 WINDREF (UK)-administration

Ms. Sue Huntington continues as Executive Secretary. Ms. Huntington provides the administrative support and expertise that is central to WINDREF's (UK) fundraising, administrative and collaborative activities.

5.0 Human Subjects Institutional Review Board (IRB)

The last meeting of the WINDREF IRB was held on 24 March 2003. At the conclusion of this meeting, the WINDREF IRB officially disbanded. Its functions will be replaced by the new St. George's University IRB, which has capacity to review an increased number of proposals. The SGU IRB is registered in the US. It began conducting business in September 2003. Members include the former WINDREF IRB members Sir Paul Scoon, Dr. Cheryl Cox Macpherson, Ms. Ann-Marie George, Mr. Lloyd Noel, and Dr. Calum Macpherson (ex officio), to provide continuity.

The following research proposals were reviewed and approved at the meeting:

- *Decompression Illness among the Indigenous Fishing Population in Grenada - Scott Forman.* To assess of the burden of decompression sickness, fishermen throughout the island will be questioned as to how often they dive, how deep, how fast they surface, time spent on the surface between dives, and the economic burden of the disease, etc., to develop dive profiles and learn of recent and previous injuries and progress from the original onset of the disease.

- *Evaluating the Effectiveness of Educational Methods on the Knowledge, Awareness and Practices of Grenadian Schoolchildren and Educators in Regards to Rheumatic Fever/Heart Disease - Bishara David Baddour.* Part of a very successful ongoing project, this project has the objective of educating of teachers and children regarding RF in hopes of reinforcing the dramatic downturn in the disease.

- *The Mediation of Filarial Infection by Dendritic Cells in Lymphatic Filariasis - Yolanda Ng.* Data will be collected in the Caribbean and analyzed in a laboratory study at the University of California in Los Angeles.

- *The Ultrasound Prevalence of Cystic Hydatid Disease in the Berber of South-eastern Morocco - Calum Macpherson, PhD, DIC*

- *A Study of Cultural and Behavioural Factors that Contribute to Echinococcus Granulosus Prevalence in Morocco - Ella Cameron*

  Dr. Macpherson will use ultrasound to screen persons in Morocco, who will be counseled and treated if they are found to have the disease. Ms. Cameron will assess factors that contribute to the dog-transmitted disease amongst pastoral communities

- *Prevalence of Intestinal Nematode Infections in Rural Grenadian*

Schoolchildren - Anthony D. Junck. A previous study (1998-2002) in schools in coastal areas found low prevalence of intestinal helminths (*Ascaris, Trichuris,* and hookworm). Mr. Junck will focus on schools in interior, rural areas with high rainfall for comparative purposes and to determine if there are any associations with socio-economic status. The root transmission of the disease is outside defecation. Helminths were very prevalent around the 1940s, but since 1982 and the introduction of albendazole, the problem has been greatly reduced.

At the March meeting, Dr Macpherson updated members on the outcomes of previously approved research and thanked all the members for their significant contributions made during their tenure with the institute's IRB.

6.0 Current Research Projects

WINDREF's core research projects on dengue (1995), rheumatic fever (2000) in Grenada, lymphatic filariasis (1999) in Guyana and cystic echinococcosis (1999) in Africa continued through 2003 and several other projects continued during the year. The projects currently being undertaken are briefly reviewed below.

6.1. Communicable Diseases

6.1.1 Rheumatic Fever (A1)

In Grenada, morbidity caused by rheumatic fever / rheumatic heart disease has been quite extensive, resulting in the public health task of reducing rheumatic fever cases through the use of primary and secondary prevention. The key concept is to prevent the progression of streptococcal infections in the pharynx to rheumatic fever and the potential worst case scenario of rheumatic heart disease.

Institution of a streptococcal surveillance program in which 50 children of different age groups (ages 5-15 years) from each of 27 primary schools were randomly selected, for collection of blood and throat swabbing. Throat swabs and 5.0 ml of blood were collected from each child. The blood samples and throat swabs were drawn by a qualified community nurse. Sera samples were then subjected to a rapid agglutination test for the qualitative measurement of antistreptolysin – O antibodies in serum. (streptolysin O is produced by groups A, G, and C streptococcal strains but not by any other *streptococci*). Positive and negative controls were run with every test performed.

Vigorous swabbing of the tonsillar pharyngeal region was done on each student. These swabs were then streaked under the flow hood on plates made with trypticase soy agar with 5% sheep blood. They were placed in an incubator at 35 degrees celsius for a twenty four hour period. Clear halos where hemolysis has occurred due to beta hemolytic *streptococci* presence is noted twenty four hours later. The samples responsible for this are selected and are further streaked and cultured to ensure that colonies that are positive can be further sub grouped.

The number of positive sera samples and throat swabs were then analyzed and represented in both tabular and figure form using Microsoft excel. Chi Square analysis was carried out to investigate the difference analysis between parishes.

Samples were further analyzed for sub grouping purposes. They were

separated into three groups, A, C and G. This process was carried out at Rockefeller University/ University of Minnesota.

The 1,388 schoolchildren (637 males, 751 females) that gave blood samples & throat swabs were selected from the six Grenadian parishes, Carriacou and Petit Martinique. (See figure 1)

The sera samples that were positive for anti – bodies to *streptococci* and the throat cultures that were positive for beta hemolytic *streptococci* in Grenada in 2001/2002 can be seen in figure 2.

The parish with the largest percentage of throat cultures that were positive for beta hemolytic *streptococci* was St. Patrick's, 21.62 % of the parish population sampled tested positive. The parish with the lowest percentage of positive throat cultures for beta hemolytic *streptococci* was Carriacou, one of the smaller islands which comprises Grenada, 5.68% of the population sampled tested positive.

Figure 1: Map of Grenada showing where samples were drawn.

Figure 2: Prevalence of positive, ASOT and beta hemolytic *streptococci* by parish in Grenada.

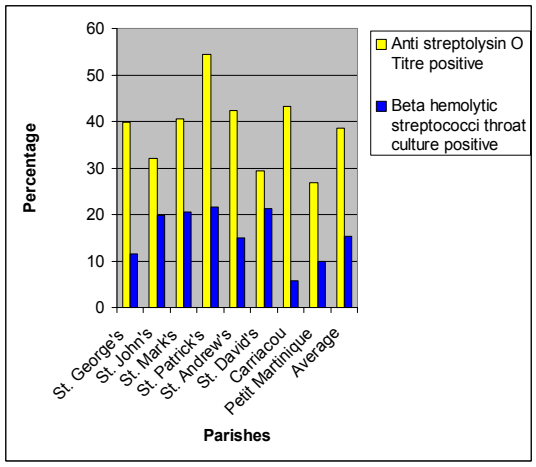
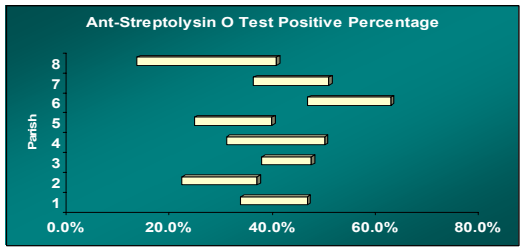


Figure 3: Anti – Streptolysin O test positive percentage by parish.



Parish 6 (St. Patrick's) has the highest ASOT positive percentage. Because the confidence interval for Parish 6 does not overlap with the confidence intervals for Parishes 2, 5 and 8 (St. David's, St. John's and Petit Martinique), we can say that the ASOT positive percentage for Parish 6 is significantly different from the positive percentage for parishes 2, 5 and 8.(See figure 3 above)

Therefore, the differences between St. Patrick's compared to St. David's, St. John's and Petit Martinique are probably not due to chance.

Parish 7 (Carriacou) has the lowest throat swab positive percentage. Because the confidence interval for Parish 7 does not overlap with the confidence intervals for Parishes 2, 3, 4,5 and 6. (St. David's, St. Andrew's, St. Mark's, St. John's and St.

Patrick's), The throat swab positive percentage for parish 7 is significantly different from the positive percentage for Parishes 2, 3, 4, 5 and 6. (see figure 4)

Figure 4: Throat Swab positive percentage by parish.

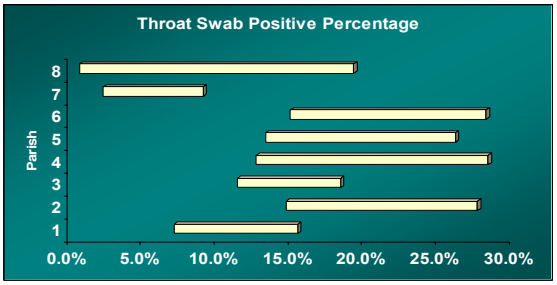
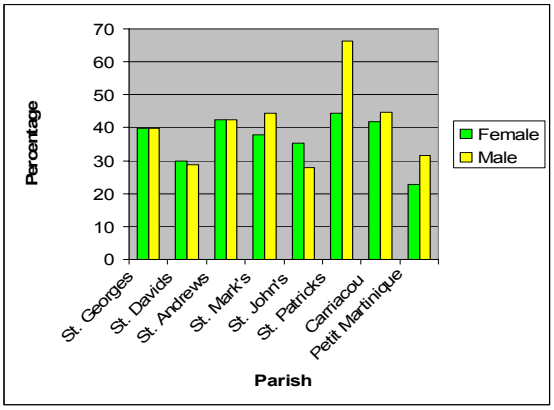


Figure 5: Prevalence of positive Anti streptolysin O titres.

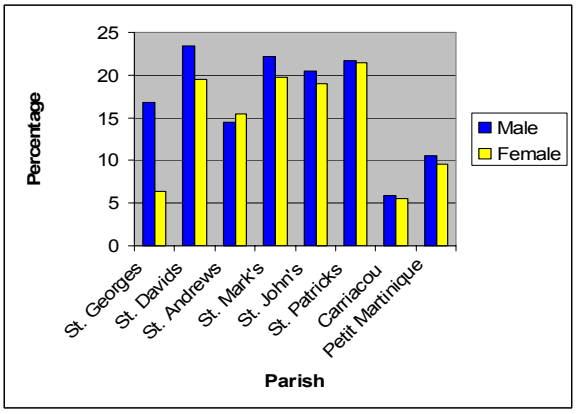


The differences between Carriacou compared to St. David's, St. Andrew's, St. Mark's, St. John's and St. Patrick's are probably not due to chance.

For ASOT, the positive percentage is significantly higher for males than for females with 99 % confidence only in St. Patrick's parish. This was seen using chi-square analysis with 1 degree of freedom at alpha =.01. The p value was .0084. (see figure 5)

For throat cultures, the positive percentage is significantly higher for males than for females with 98 % confidence only in St. George's parish. This was seen using chi-square analysis with 1 degree of freedom at alpha =.02. The p value was .0172. (see figure 6)

Figure 6: Prevalence of positive beta hemolytic streptococci.

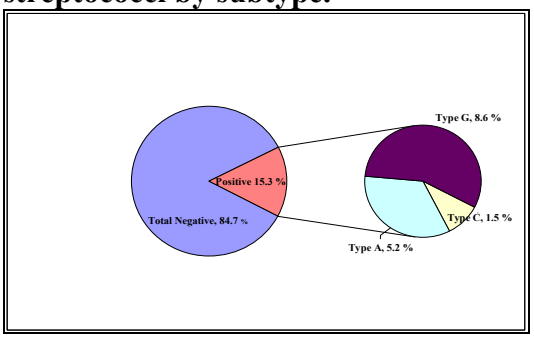


The average percentage of throat cultures that were positive for beta hemolytic *streptococci* for the entire country was 15.35 %.

Fifteen percent (15 %) of the throat cultures were positive for beta hemolytic *streptococci*. The fifteen percent (15 %) that were positive were divided into the following subtypes: type A thirty four percent (34 %), type C ten percent (10 %) and type G fifty six percent (56%). (see figure 7)

All children, screened through the target school surveillance, with positive ASO titers and throat swab samples were referred to the rheumatic fever (RF) clinic.

Figure 7: Positive beta hemolytic streptococci by subtype.



Two local pediatricians and a community nurse ran the RF clinic. This clinic was held on the fourth Thursday of every month. All children found positive for streptococcal infection in this study were treated at the RF clinic. Consultation and medication were given to each positive child free of cost through the clinic.

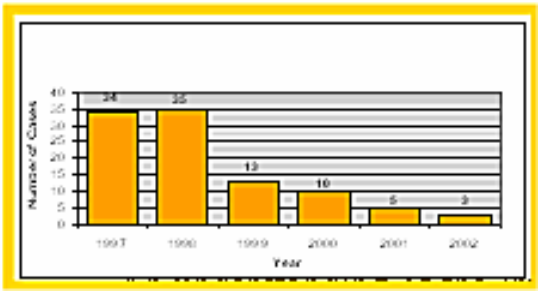
The results of this research allowed identification of potential high risk areas and provided a starting point for the educational program. The education program was introduced to various focus groups including the children, teachers and principals of 53 primary schools on the island of Grenada. The final schools included in the educational program were covered in the first quarter of this year.

The institution of a social mobilization program was considered a very important feature of this program. Descriptions in newspapers, television and on radio were used to convey the importance of the elimination of rheumatic fever by corrective treatment of streptococcal infection in the country.

The results of the study done in Grenada show the parish of St. Patricks as having both the greatest percentage of ASOT positive and beta-hemolytic streptococci positive. It has been noted in other parts of the world, Australia for example where the current high rates of rheumatic fever in one population, the Aboriginal population of the Top End of Australia are not related to ethnicity, but are likely to reflect high levels of exposure to group A streptococci, which, in turn, are related to overcrowding and continuing poor living conditions.

St. Patricks is a more rural part of the country, associated with low socio-economic status. This is also a highly probable cause for the high prevalence of *streptococci*. The noted significance both by parish and male gender may be attributable to levels of personal hygiene. It is possible that women are more prone to seeking medical attention in the Caribbean than men.

Figure 8: Annual incidence of acute rheumatic fever (1997–2002



An understanding of the impact of social relationships on health status, health behaviors, and health decision making, contributes to the design of effective interventions for preventing the onset of reducing the negative consequences of a wide array of diseases. Treatment of streptococcal diagnosis and educational intervention assists in a reduction in the occurrence of rheumatic fever. While there was an initial reduction prior to this study, the initial sensitization was carried out by members of our collaborative team. The continuing educational awareness has certainly contributed largely to the decline of index cases of rheumatic fever (See figure 8).

Similar success over the years, through primary and secondary prevention has been recorded in developing countries, such as Costa Rica, Trinidad and Tobago, Guadeloupe and Martinique.

During 2002 and the first quarter of this year, Nurse Geraldina Perrotte completed the contact tracing for all positive throat swab and ASOT students who were treated at the RF clinic. The contact tracing procedure involved the

testing of all persons that frequent the child's living environment (nuclear and extended family). It is extremely important in preventing recurring bouts of infection as well as identifying members of the family that could be potential carriers of streptococcal bacteria. Furthermore, contact tracing allows the RF team to monitor compliance with treatment for streptococcal infections.

Prospective WINDREF Plans include continuity with our evaluation of the role of surveillance in the student population studied and to continue to monitor the extent of the decline of the incidence of RF in Grenada.

The effectiveness of treating all streptococcal positive patients will be assessed for its overall contribution to the elimination program. While maintaining an overview of the future situation, this program has reverted to local governmental ownership. The feedback of results outlined above have been filtered to all the concerned ministerial and NGO personnel.

Submitted by Trevor Paul Noel
Assistant Director

6.1.2. Effectiveness of Educational Initiatives on the Prevention of Rheumatic Fever in Grenadian Schoolchildren (A2)

Rheumatic Fever (RF) has been coined "the chain that links the heart to the throat." This disease is the potential consequence of an untreated Group A streptococcal pharyngeal infection. The affected populations are mainly children ages 5-15. Rheumatic fever sequela rheumatic heart disease ultimately leads to debilitating heart valve complications with adverse consequences such as high healthcare expenditure, insult to quality of life and untimely death. Other sequelae include polyarthiritis, neurological and renal impairment.

Some factors that predispose to RF include positive family history, dampness of home, low education of mother, overcrowding, frequent sore throat and possibly socio-economic conditions. RF is easily preventable at early onset of pharyngitis with penicillin. If left untreated surgical intervention is chronic and costly. Prior 1950, RHD was one of the top factors for childhood morbidity in developed nations. Virtual elimination of RF as major health concern in industrialized nations today has been concomitant with rising economic standards and introduction of penicillin.

RF has been termed the "occupational disease of schoolchildren". Previous efforts at national awareness only penetrate as far as school. It has been shown that countries with low literacy rates can utilize students as vectors for control (India). Students demonstrate more enthusiasm for healthcare as well as enjoy friendships with non-school attending peers and therefore are suitable disseminators for educational motives. This has been proven in Indonesia where students imparted knowledge of oral re-hydration for diarrhea control to parents. Such educational initiatives have profoundly impacted other countries in regards to rheumatic fever. Costa Rica adopted such a program in 1970 resulting in a decrease in incidence from 98 to 6 (1984)

In Grenada the incidence of RF has dropped from 34 cases (1997) to 3 (2002), per (100,000). This has been attributed to involvement of many factors including a national awareness and eradication campaign described above.

The goals of this study are to…

1. Evaluate level of knowledge, awareness and prevention practices of

rheumatic fever in Grenadian:
Schoolchildren (ages 7-12)
Schoolteachers of this group
Parents of this group
2. Assess the progress of earlier and ongoing educational program by WINDREF involving:
Educational Pamphlet Distribution
PTA/Parish Hall Meetings
Blood Screening
3. Propose further methods to instill lasting and effective campaign aimed at eradication of RF from Grenada.
4. Contribute to the development of a finite model that will aid in the primary prevention of this and other easily preventable diseases the tri-island state of Grenada.

Tailored questionnaires were distributed to the following in the aforementioned schools according to parameters given:

- Schoolchildren (age 5-15)
- Parents of these children
- Respective Schoolteachers

Consent forms were included for quality assurance. Distribution of questionnaires was carried out by class and age level in the following distribution scheme: (sample size has increased since to 800 and anticipated 900)

Figure 1: Author with schoolchildren

Figure 2: School in St. John's Parish with previous intervention

Paraclete Government school in St. Andrews, this school won an award for highest combined scores on standardized exams. Whether this has any bearing on the relative knowledge of RF will be analyzed

- *17 Schools*
  - 30 Students from each school
  - 5 Educators from each school
  - 1 Parent for each child
  - Total sample size= 765 subjects
  - 5 Parishes, Carriacou and Petite Martinique

The aim of the questionnaires was to get a feel of the publics' knowledge, attitudes and practices in regards to rheumatic fever. Another aim set forth by the types of questions presented, was to gauge the effectiveness of previous educational initiatives aforementioned as well as to explore the willingness of subjects to take an active role in the control of this disease.

RF potential sequela RHD is a costly disease. Early detection is crucial (late diagnosis diminishes efficacy of secondary prophylaxis). RF is an easily preventable disease, requiring only a course of antibiotics upon presentation of pharyngitis. Therefore sound educational methods must be evaluated and practiced with the aims of recognizing the gravity of sore throat in those target populations. The hypothesis is that schools with

highest degree of intervention will have been best served by the previous initiatives and that pupil to pupil education can be enhanced via illustrating perceptions of the disease from the eyes of the children via drawings and colorings that subjectively show what they know.

Chi-squared tests will be run to determine significance values using Microsoft excel. Graphs and charts demonstrating trends in K.A.P. derived from objective questions. Subjective questions will be reported via essay. Data are under analyses at present.

Submitted by Bishara Baddour
Research Scientist

6.1.3. Dengue in Grenada. (A3)

Dengue is a vectorborne disease caused by one of four dengue viruses. Each of the viruses (termed serotype: DEN-1, DEN-2, DEN-3 and DEN-4) may cause a range of clinical manifestations, including mild undifferentiated fever, classic dengue fever (DF) and severe, potentially fatal dengue hemorrhagic fever (DHF) with dengue shock syndrome (DSS). Challenge by one serotype will only induce homotypic immunity, while sequential infections by all four serotypes may occur. The manifestation of severe DHF/DSS is often associated with repeated infections (especially in younger children), although viral determinants may play an equally important role. Treatment of DF is limited to supportive care, while DHF/DSS may require fluid replacement in serious cases of plasma loss. There are currently no prophylactic drugs or approved vaccines for prevention of dengue infection.

Aedes aegypti is recognized as the principal vector for transmission of the dengue viruses between humans. This peri-domestic mosquito persists throughout most of the pan-tropical regions of the world, favouring the urban environment as its main habitat. Most regions infested by *Aedes aegypti*, sustain endemic dengue activity with regular epidemic outbreaks following introduction of new serotypes or strains. The extended and continuous spread of the individual serotypes, are estimated to cause tens of millions of DF cases and more than 25,000 deaths due to DHF/DSS each year.

All four serotypes are currently found in Latin America and the Caribbean, where epidemics have become larger and more frequent during the past 20 years. Most countries in the region however, lack efficient laboratory-based surveillance of dengue. This is a critical problem, as health authorities must have access to timely information on circulating serotypes and activity levels, if incipient epidemics are to be predicted and challenged by appropriate control measures.

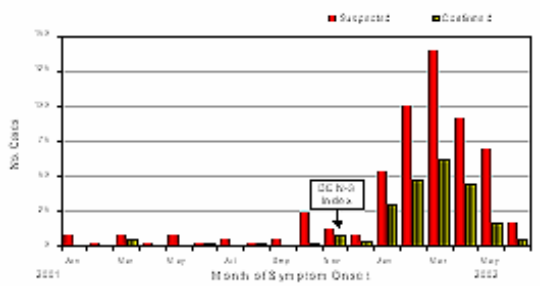
The aim of this project was to introduce an active surveillance system for early detection and characterisation of dengue cases, on the main island of Grenada. A central component of the surveillance efforts included the implementation of a laboratory service for diagnosis of clinically suspected cases. Detection of dengue viruses in patient serum was achieved by RT-PCR (Lanciotti *et al*.), while antibodies to dengue were identified using commercial IgM tests (PanBio Inc.) or standard IgG ELISA (Chungue *et al.* and Kuno *et al*). The alternative test methods were employed in order to ensure broadest possible diagnostic capacity for both acute (viral specimen: 0-5 days post symptom onset) and convalescent (dengue antibodies: 6 days or more post onset) phase samples.

The emphasis of the laboratory service was placed on rapid analysis and dissemination of test results, in order to enhance the predictive capability of the surveillance system. The diagnostic service was offered free-of charge to the entire health care sector for a period of approximately 18 months (January 2001-June 2002). Efforts to solicit serum samples from clinically suspected cases were maintained throughout the project, including visits to primary health care units, follow-up phone calls, presentations at medical meetings and regular reminders of the service via surveillance staff at the Ministry of Health (MOH). The project was launched as a parallel feature to the existing passive surveillance system, based on mandatory reporting to the Caribbean Epidemiology Centre (CAREC) in Trinidad. Following is a short resume of the general performance and a few of the central findings of the surveillance project.

The operation of the diagnostic laboratory was based on a simple system for sample referral, combined with the application of rapid laboratory assays. All test results were reported directly to the referring health professional, as well as the Division of Epidemiology, MOH. This system allowed for an average feed back of test result within 8 days of blood draw. The response time for the inter-island referral system (CAREC, Trinidad) was between 4 and 7 weeks in comparison.

The diagnostic laboratory received a total of 546 suspected cases for analyses, of which 220 were laboratory confirmed. The distribution of both suspected and confirmed cases, revealed two separate modes of disease dynamics in the order of low endemic transmission, followed by intense epidemic activity (see figure 1).

Figure 1: Distribution of suspected and laboratory confirmed cases by month



The sporadic cases observed during the endemic period, were attributed to DEN-2 (in circulation since 1997), while epidemic transmission was caused by DEN-3. Serotype 3 was reintroduced to the region in 1994 (after an absence of 17 years) but had never been identified in Grenada. The index case of DEN-3 was detected in the beginning of November 2001, almost eight weeks prior to overt epidemic activity.

The MOH was notified immediately upon DEN-3 recognition, with detailed information pertaining to the specific areas of activity. In response, the ministry issued a general alert to the medical community, while a special dengue task force was established in January 2002. The task force was assigned to monitor the distribution of disease transmission and to implement response measures, including public alerts in the national media and intensified vector control efforts in high transmission areas. St. George's University complimented these efforts by hosting a special dengue seminar for the medical community (CME session), with emphasis on dengue diagnosis and treatment of severe cases.

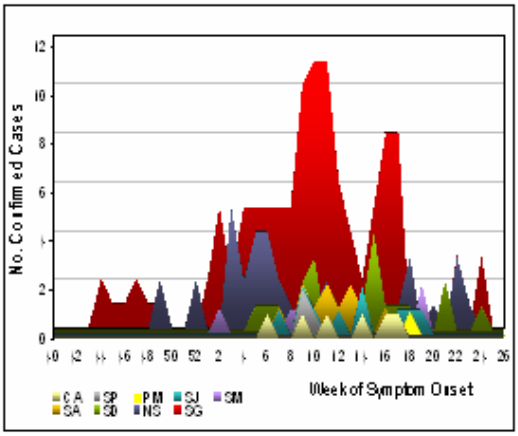
The early identification of DEN-3 underscored the predictive capability of the active surveillance system even if, in this case, epidemic activity was not averted. The two-month gap between the first report of DEN-3 circulation and the subsequent implementation of response

measures, left sufficient time for the virus to spread in epidemic proportions. It is evident from this experience that health authorities must have a prepared contingency plan for immediate activation, when notified of potential outbreaks by the surveillance operation.

The capital parish of St. George's (main urban development) represented the epicentre of the outbreak with 53% (117/219) of all confirmed cases including the DEN-3 index case. A lag phase of two or more weeks was observed between the epidemic onset in St. George's and the detection of DEN-3 cases in the other parishes and constituents (see figure 2).

Figure 2. Weekly distribution of confirmed cases by parish during the epidemic of DEN-3.

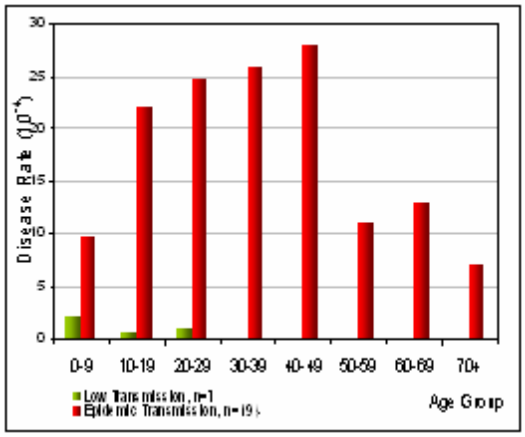
CA: Carriacou; SP: St. Patrick's; PM: Petite Martinique; SJ: St. John's; SM: St. Mark's; SA: St. Andrew's; SD: St. David; NS: not stated; SG: St. George's.



The incidence of DEN-3 infections was highest in the adult population (>15 years), whereas the majority of infections attributed to DEN-2 occurred in children less than 15 years of age. (see fig 3). The clinical presentation in the majority of confirmed cases, was classified as non-specific fever or classic DF, for all age groups. A single episode of non-fatal DHF was recorded in a 30-year old male, during the outbreak. The near absence of severe disease during the epidemic is curious, given the detection of previous exposure in 81% (116/143) of the analysed cases, and the general correlation between secondary infection and DHF/DSS. Phylogenetic analysis showed that the Grenadian DEN-3 virus was closely related to recent isolates from other Caribbean and Latin American outbreaks of DF (with DHF cases). The regional DEN-3 strains (genotype III) have been traced to Sri Lanka and India, where associated with genuine epidemics of hemorrhagic dengue.

Figure 3. Age-specific disease rate for the surveillance periods of low and epidemic disease activity.

(Low Transmission; January 2001 – September 2001, Epidemic Transmission; October 2001 – June 2002)



The observed activity of DEN-3, in terms of the geographic and demographic distribution of cases, should indicate the focus of general control efforts and future contingency plans. Grenada has yet to experience a major outbreak of DHF/DSS, but should nonetheless be prepared for an epidemic scenario, where severe cases will challenge the capacity of the health care system. One serotype with known potential for DHF/DSS propagation is

already established in Grenada - it seems at matter of time, only, before another is introduced.

Submitted by Karin Schioler
Research Scientist

6.1.4. Intestinal nematode infections in rural schoolchildren age 6 to 12 (A4)

Intestinal nematodes are one of the most common infections in humans, especially in tropical and sub tropical countries. The morbidity is greatest for children, with effects potentially including malnutrition, intestinal bleeding, reduced physical growth, iron deficiency anemia, diminished appetite, deficiencies in mental development and respiratory problems.

Intestinal nematode infections had been widely noted throughout the Caribbean Islands since the late 1700's and, at that time, Grenada was regarded as being the most afflicted. Intestinal nematodes were still common in the Caribbean only decades ago, but have more recently been found at generally low prevalence's due to improvements in chemotherapeutic treatments and sanitation.

A lot of progress has been made in the understanding of the epidemiology and ecology of these parasites. Previous studies have demonstrated that prevalence rates may vary depending on housing conditions, sanitary conditions, and socioeconomic status. Various other studies have examined climatic factors and have revealed regional prevalence differences associated with temperature and rainfall.

A recent WINDREF investigation on the island of Grenada demonstrated that the prevalence of intestinal nematodes in 6-12 year old children was low in Point Salines, a notably dry, costal area. Due to the dependence of nematode development on climatic factors, it seemed appropriate to further determine the prevalence within a region of high rainfall in order to generate a more complete understanding of island-wide prevalence. This study, as approved by the Grenadian Ministries of Health and Education, investigates the prevalence of intestinal nematode infections of school age children between 6 and 12 years of age in Grand Etang, a relatively sparsely populated area. The area of study was selected for its ecological characteristics of high rainfall and high altitude, as well as for its rural population distribution. Given the differing biotic and abiotic features of this area from those of the Point Salines area, differences in the prevalence rates of some nematode species were expected.

After informed consent was obtained, a single stool sample was collected from 236 participants. Prevalence's of the most common intestinal nematodes, *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm, *Enterobius vermicularis* and *Strongyloides stercoralis*, are being determined through analysis of the samples using salt flotation concentration techniques employing both sodium chloride and zinc chloride followed by direct identification of eggs by light microscopy.

Figure 1: Children at a standpipe in Vendome.

Additionally socio-economic and behavioral data are being gathered to examine for the existence of prevalence-associated risk factors which may support

alternate hypotheses for prevalence differences unrelated to climatic factors.

Children who are diagnosed with an infection were treated with a single dose of 400mg Albendazole. This chemotherapy has broad effectiveness in reducing the burden of infection for a wide variety of nematode species and has no known side effects in children. This medication was administered by the local district medical officer.

In addition to benefiting the population of children who participate in the study by eradicating their intestinal nematode infections, it is hoped that the prevalence findings will increase the understanding of the public health significance of intestinal nematode infections in Grenada. Potential also exists that our improved understanding of the climatic influence on prevalence in Grenada may also be generally applied to other tropical locations in an effort to more effectively control disease transmission on a wider scale.

Submitted by Anthony Junck
Research Scientist

6.1.5 Studies on *Echinococcus granulosus* in Africa. (A5)

A total of five students went to Morocco and four to Uganda to participate in studies on *Echinococcosis granulosus*, which causes hydatid disease: a public health and economic problem in both countries. These projects were planned in advance and processed through the Windward Islands Research and Education Foundation's Institutional Review Board and through the national committees in Morocco and Uganda respectively. Research clearance in each country was obtained, and the students were able to conduct their studies upon arrival in their study destinations. Many weeks of prior scientific preparation was carried out in Grenada, and subsequent seminar and abstract production was also performed in Grenada. Many aspects of the study were explained and studied in advance.

6.1.5.1 Morocco

The five students who went to Morocco (Ella Cameron, Garret Coleman, Jessica Hope Diebold, Maria Rodriguez and Katherine Wawronowicz), together with Trevor Noel, WINDREF Scientist, and Dr. Macpherson, worked very closely with local collaborators from the Institute Agronomie et Veterinaire, King Hassan II University in Rabat. Dr. Macpherson preceded the group with Maria Rodriguez in order to collect the ultrasound scanner for use for the surveys in London and to clear it through Moroccan Customs prior to the arrival of the other students in Morocco. Clearance of the scanner took three days as the Moroccan authorities have changed their importation procedures, during this period discussions were held with the Director of the Veterinary School in Rabat on the forthcoming collaborative studies. Prior to the arrival of the students, local collaborator Dr. Malika Kachani (Professor of Veterinary Parasitology) and her colleagues visited Ouarzazate, the study area, to prepare the local people and NGOs for our arrival. One NGO in particular, the Transhumants Project in Ouarzazate became very interested in our participation, as they have been supported by UNICEF, and the Save the Children amongst other NGOs to look at the lifestyles of the transhumant peoples in this area of Morocco. Part of their mandate was to look at the health of these people, but this had not yet been done, despite the project being ongoing for the previous two years. They

therefore completely facilitated the whole of the ultrasound survey and other aspects of the study during our time in Morocco. Upon arrival in Morocco, a few days were spent organizing for the trip in Casablanca and Marrakech, and once all the students had arrived, everyone traveled to Ouarzazate, the headquarters of the study.

Figure 1: The collaborative team in Ouarzazate, 2003.

An intense study was then carried out in Ouarzazate, and over 9,000 transhumant Berber people were screened for cystic hydatid disease. These peoples have a very low socio-economic status and survive by seasonally moving their animals to new grazing areas along set routes as the weather dictates. There are few schools, health care centers, and veterinary facilities. Piped water and sanitation are almost non existent and the people live close to oases as their source of potable water. The region is characterized as arid with light rainfall limited to short periods of the year.

Families are usually separated with the men herding the livestock into the hills in the summer and back to the plains where the women and children remain in the winter when snow covers the Atlas Mountains. The terrain is very rugged and daily temperatures are usually over 100^{o}F.

Figure 2: Moroccan children drawing water from the well.

Figure 3: Moroccan child awaits ultrasound screening.

Two ultrasound scanners, both portable machines, one called the Tringa, and the other was a Toshiba JustVision 200 were used with 3.5 megahertz sector and convex transducer probes respectively. It had been intended to carry out inter and intraobserver studies but the local radiologist was unable to participate in the study. A study on the advantages and disadvantages of the two machines was performed but the primary study was to screen as many people in the area as possible to obtain an indication of the importance of CHD in this area of Morocco. All infected individuals were

subsequently treated with Albendozole by the local physicians who collaborated in the study and were responsible for the follow-up treatment.

Figure 4: Moroccan physician advises on treatment. She will be responsible for following-up this patient.

The Transhumant Project Director was delighted with the outcome of the study, and two people, Mr. Trevor Noel, Dr. Macpherson's Research Assistant, and Dr. Macpherson, were presented by the King of Morocco's representative with medals for Services to Morocco. Plans are in place for the summer of 2004, and we hope that another team will be facilitated through MIRT to conduct a further study with transhumant peoples in the high Atlas Mountains, an area not previously investigated. Next year a team will comprise opthalmologists, dermatologists, veterinarians and scientists to try and provide a comprehensive health care coverage to these very disadvantaged people.

6.1.5.2 Uganda

Three students (Brian Butler, Alissa Melamud and Jared Morgan), together with Richard Kabuusu, went to Uganda in advance of Dr. Macpherson, Dr. Buckanamune and Mr. Brandon Francis. Upon arrival they found that the project in Karamoja district that was planned was not possible due to local activity in that area. It was therefore decided to work in Western Uganda in Queen Elizabeth National Park (QENP) and Lake Mburu National Park. The project was headed by Dr. Ludwig Sieffert, a Professor at Makerere University's Wildlife Department. The students conducted a number of studies at the Entebbe zoo and as part of the lion project in the QENP. These included using GPS to map the distribution of lions observed in the park.

Figure 6: Small boy peeps through the wall of the hut to look at the screening process.

On arrival of Brandon Francis and Dr. Macpherson, the ultrasound survey began looking at the prevalence of cystic echinococcus in the people who live in and around the national parks. Over 1,600 people were screened in this area and later in Karamoja for cystic hydatid disease. A few cases were found in Karamoja and none were found in Western Uganda, despite hospital records in Western Uganda which indicate that the disease is present.

The communities studied which live both in and around the parks were very happy about the surveys, and participation was high. The study provided the Uganda Wildlife Authority with much needed kudos for conducting the health surveys for the communities.

Figure 5: Major General James Kazini with SGU students and assistants at Lake Mburu NP, Uganda.

Our relationship with the Uganda Wildlife Authority could not be better, and we would very much like to return to this area next year to continue some longitudinal studies on the health of people in and around the parks, and looking at innovative methods of turning cattle ranches back into game ranches in accordance with the wishes of the surrounding communities.

Submitted by Calum Macpherson
Director

6.1.6 Human Cystic Echinococcosis in Morocco (A6)

Cystic echinococcus (CE) is considered a public health problem and a considerable contributor to morbidity in endemic areas of the world. Also known as hydatidosis, this disease is found on every major continent in the world. Human CE is a zoonotic parasitic disease caused by *Echinococcus granulosus*, a dog tapeworm, and its cystic larval stage (hydatid cyst). Echinococcus is a cyclozoonosis sustained by two vertebrate hosts, a definitive host and an intermediate host. The cycle is analogous to the predator/prey relationship, the carnivore (definitive host) harbors the adult tapeworm in the intestines eventually passing on eggs through feces where the herbivore (intermediate host) ingests the eggs and consequently becomes infected with the larval hydatid stage. Human infection is the result of ingestion of tapeworm eggs through direct or indirect contact, which develop into hydatid cysts.

Echinococcosis is a preventable disease that has a substantial deleterious economic and social impact on endemic populations. High morbidity results in loss for both the public health sector and animal husbandry industries. Successful programs have dramatically reduced the prevalence of echinococcus in humans, sheep, and dogs through a de-worming prophylaxis regiment. Before implementation of such a program an in-depth surveillance of the country and relevant hosts' prevalence should be conducted and specific factors targeted for intervention. There is adequate evidence to support the behavioral theory for disease transmission and depending on the specific parasite or disease certain trends emerge based on age, sex, and occupation etc.

The ultrasound surveillance study was conducted in Morocco, located in Northern Africa along the Strait of Gibraltar. The country is geographically

Situated 15 kilometers south of Spain between Algeria and Mauritania to the

South. The study was conducted at multiple sites located outside the southeast city of Ouarzazate in the surrounding High Altas mountain region. A team of physicians, scientists, veterinarians and students carried out the US survey which also used a questionnaire to examine risk factors. Over 9,000 individuals (Table 1) were screened (by CNLM) and eleven individuals were identified as having CE (0.1% prevalence for study population).

The WHO standardized classification of CE ultrasound images Was used to determine treatment options.

Table 1: Ultrasound Results by Age & Sex

Age	Male	Female	Total
0-5 years	511	356	867
6-15 years	2045	1598	3643
16-30 years	383	1244	1627
31-45 years	452	1036	1488
> 45 years	493	930	1423
Total	3884	5164	9048

Hospital documentation from 2000-2003 was collected and reviewed from the regional hospital. A total of 46 cystic hydatid cases were recorded for surgical intervention. Cases were reviewed and data was looked at for age, sex, location and year of hospital admittance.

Table 2: Surgical Cases by Location (Liver or Lung)

Location	Male	Female
Liver	5	22
Lung	8	12

- One male patient was diagnosed with cysts in both the liver and lung.

Age (years)	0-5.	6-15	16-30	31-45	> 45.	TOTAL
Male	1	2	6	2	1	12
Female	0	4	15	10	5	34
Total Cases	1	6	21	12	6	46

Table 3: Surgical Cases by Age and Sex

Table 4: Surgical Cases by Year of Hospital Admittance

Year	Number of Cases
2000	12
2001	15
2002	12
2003	7

- 2003 data was not complete (only data from January thru July)

Development in the field of diagnosis and treatment of CE has produced more successful techniques than ever; however, early diagnosis and education in high-risk populations is essential to break the cycle This study allowed for early diagnosis using the non-invasive technology of ultrasound while providing education about the disease to the populations screened.

Endemic countries have the ability to effectively combat CE by interrupting transmission and successfully limiting the number of infective definitive hosts. Education is essential to limiting human infection and more must be done to identify high-risk behaviors that result in increased burden of disease.

Submitted by Ella Cameron
Research Scientist

6.1.7 Epidemiology and public health importance of cystic hydatidosis in Uganda (A7)

Cystic echinococcosis (CE) is a parasitic disease that has impacted every continent. It is caused by the larvae of a tapeworm called *Echinococcus granulosus*. There are currently, four identified species of Echinococcus, namely *granulosus, multilocularis, oligarthrus,* and *vogeli*. All are pathogenic, causing hydatid cyst disease in different organs, with different definitive hosts, and they all specialize in slightly different parts of the world. *E. granulosus* is of particular interest, as it is one of the most widespread zoonoses known to man. This zoonosis is known to be an important public health and economic problem in Uganda, East Africa; it has been reported to occur most commonly in the Jie of North-Eastern Uganda. Additionally, wildlife cycles involving lions and jackals as the main definitive hosts and wildebeest, buffalo, zebra, warthog and other antelope as intermediate hosts, occur in a predator:prey cycle in game conservation areas and have been reported in Queen Elizabeth National Park (QE2) in western Uganda.

The hydatid cysts form in all organs of the body. The most common sites are the liver and the lungs; however, there are also cerebral cysts, ocular cysts, intramuscular cysts, and so on. This disease is most prevalent in peoples routinely and intimately involved with large wild carnivores. The people most affected are often nomadic herders and therefore, screening them is difficult because they can be hard to find.

The most common cysts, hepatic cysts, make up approximately 70% of cysts in most populations. There are, however, also lung cysts that make up between 20%-30% of the cysts. Considering that the liver and lungs are two different types of tissue, the screening measures would be different for each. Ultrasonography (US) is the diagnostic and screening technique of choice for hepatic cysts, however, for lung cysts, x-rays often prove more useful. Unfortunately, the x-rays for lung cysts are not reliable and more importantly, the people who are at risk (nomadic herders) are often nowhere near an x-ray facility. Furthermore, the cost of such a diagnostic or screening tools greatly limits access.

US is the best method for detecting liver cysts; liver cysts comprise approximately 70% of all CE. US is, therefore, the method of choice for screening CE; it is considered the gold standard.

An ultrasound prevalence study was conducted in July 2003, to ascertain the relative impact of CE on populations near several national parks in Uganda. A total of 1,605 people were screened using a portable, battery powered 50S Tringa ultrasound with a 3.5 MHz convex probe. Villages surrounding Queen Elizabeth, Lake Mburo and Kidepo Valley National Parks were selected. The organs screened for the presence of cysts were the liver and right kidney. There were no confirmed positive cases found during this preliminary study, however, both hospital records and reports by villagers indicate that the disease is present. These preliminary findings indicate that more work needs to be done to ascertain the complete impact of this disease in these and similar populations.

Submitted by Brandon Francis
Research Scientist

6.1.8 Studies on lymphatic filariasis in Guyana (A8)

As part of a PhD program at SGU, studies on the *in vivo* assessment of the effect of albendazole on adult worms in the spermatic cord will be assessed using longitudinal ultrasound studies. This program is being generously supported by the Liverpool support centre. Initial identification of the patients was carried out in 2003 through selecting suitable candidates identified in the sentinel sites set up for monitoring the elimination program. In 2004 the project will be submitted to the IRB and will hopefully start in April 2004. An initial ultrasound training program has been set-up with Dr Gerusa Dryer in Brazil, set for January 2004. It is intended that the study will take up to three years to complete.

Submitted by Shamdeo Persaud
Research Fellow

6.1.9 Immunologic studies on lymphatic filariasis. (A9)

Equally important to the eradication of lymphatic filariasis is the focus on the secondary effects of the primary parasitic infection, specifically, the infected patients who also develop lymphedema. Often found accompanying the lymphedema are secondary bacterial and fungal infections and acute attacks accompanied by dermatolymphangioadenitis (DLA) that can persist even though the microfilaremesis has been eliminated. It is found that most patients who develop lymphedema or elephantitis are usually amicrofilaremic and exhibit a hyperimmune response, while those who are appear to be asymptomatic are usually microfilaremic and exhibit a hypoimmune response.

It has been proposed that this differential immune response can be attributed to a Th1/Th2 paradigm. The same study also shows that the functions of the T lymphocytes of the patients are normal. A 17 year study shows that a down-regulated T lymphocyte response persists in people who were once microfilaremic, but at the end of the 17 years became amicrofilaremic after drug treatment. Thus they do not revert from a hyporesponsive state to a hyperresponsive state that seems to be dictated by their antigenic state.

Since a proliferative T lymphocyte response is dependent on proper presentation of antigen by APCs, there is a possibility for those who are experiencing hyporesponsiveness via a down-regulation of T lymphocytes have abnormal APC function. In addition, it has been shown that the down-regulation of T lymphocyte remains after microfilaremia have been cleared indicating a permanent change in immune response. Whether there is damage to those who develop a hyperimmune response is unknown, but if it does occur, it could lead to an explanation of why these patients do not develop a cellular immune response to antigen, but maintain a humoral response to all antigens.

In an attempt to gain more insight into the immunology behind a microfilarial infection, I decided to look at the response elicited by the dendritic cell (DC), which is the most potent APC, as well as monocytes and peripheral blood mononuclear cells (PBMCs). Another topic that I aimed to shed some light on was the induction of inflammation that leads to the disfiguring lymphedema. In many of the countries where LF is endemic, there is also the presence of other infectious diseases such as tuberculosis and leprosy. Polyparasitism of those affected with LF is not rare. Therefore, it is beneficial to study the effects of the presence of microfilaria on other infectious diseases.

Gratefully, I was able to carry out this kind of study in the laboratory of Dr. Robert Modlin at UCLA's Department of Microbiology, Immunology, Molecular Genetics.

Dr. Modlin's research is focused on the immunology of an intracellular microbial infection, using leprosy as a model. Not only was the supply of *Mycobacterium leprae*, the causative agent of leprosy, abundant, but it was also advantageous to have the availability of a large number of biotechnological tools.

Stimulation, *in vitro*, by the microfilaria itself did not elicit any kind of protective, or inflammatory response, but did induce production of the immunosuppressive cytokine, IL-10 from PBMCs and monocytes. DCs do not characteristically produce IL-10. Based on the cytokine profile seen in this data it is consistent with previously published data and suggests that the presence of microfilarial antigen alone cannot induce inflammation and therefore does not play a direct role in the lymphedema that we see in patients.

Figure 1: IL-10 production in response to microfilarial antigen

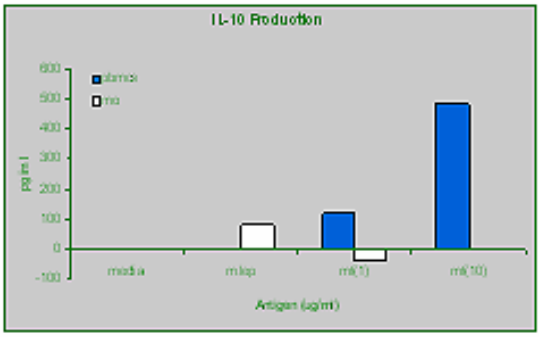
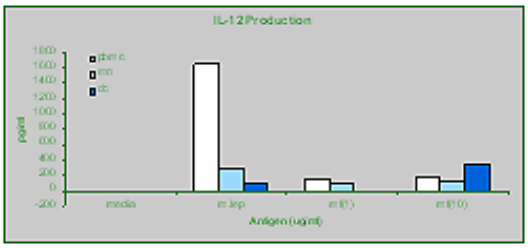


Figure 2: IL-12 production in response to microfilarial antigen



Therefore, a lack of overt symptoms is typically associated with the presence of microfilaria.

Co-infection, in vitro, of M.leprae with microfilarial antigen generated interesting results that could possibly give insight into Figure: 2 the cause of lymphedema. Normally, M.leprae alone can induce a protective inflammatory response by an immunologically healthy individual. This is determined by the presence of inflammatory cytokines, such as IL-12. As seen previously, the presence of microfilarial antigen alone is unable to elicit such a response. (Figure: 3a,b,c).

However, when the two antigenic agents are co-cultured, it is seen that enhancement of inflammatory cytokines is seen. In a couple instances, there was also depression of immunosuppressive cytokines (data not shown). This preliminary data suggests that there are other factors, and not the actual nematode itself, that contribute to the lymphedema or severe inflammation seen in lymphatic filariasis patients.

Figure 3a:

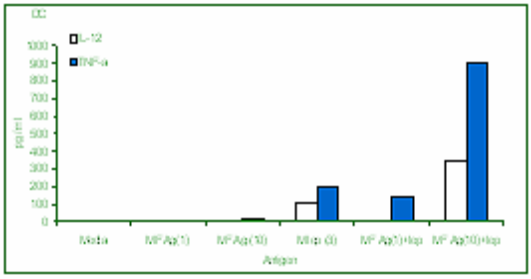


Figure 3b:

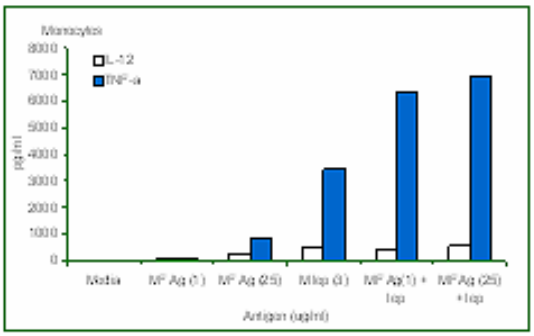
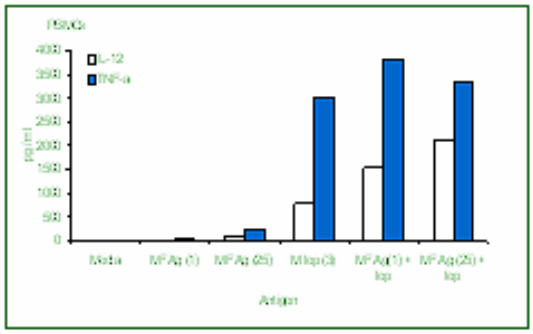


Figure 3c: Enhancement of IL-12 and TNF-α (inflammatory cytokines) enhancement in **A** PBMCs **B** Monocytes and **C** Dendritic cells



The presence of the worm, either adult or larvae, definitely exacerbates the situation via damage to the lymphatic vessels and nodes as well as via a manipulation of the immune system. Those who suffer from secondary bacterial and fungal infections are definitely at risk for the development of chronic lymphedema due to this relationship that is shown with co-infection of microfilaria and *M.leprae*.

It has already been shown that in the case of river blindness, which is caused by another filarial nematode, the inflammation seen in the eyes that contributes to the blindness is caused by a bacterial endosymbiont, *Wolbacchia*, and not the worm itself.

This is an important area of study and further investigation into the actual mechanism of inflammatory enhancement as well as other effects of polyparasitism and co-infection could be very beneficial towards the development of a more focused treatment for those who suffer from the disease.

Hopefully, it will also provide those who suffer with a little bit of insight and resolution as to why the disease manifests itself as it does.

Submitted by Yolanda Ng
Research Scientist

6.2 Investigation of Grenadian medicinal plants for antibacterial activity in childhood diarrhea (A 10)

Diarrhea is a major cause of infant morbidity worldwide, particularly in tropical, developing areas such as the Eastern Caribbean, where standard antimicrobial use is often limited by resistance and other factors. The purpose of this study was to initiate an investigation into Grenadian medicinal plants for relevant bioactivity. The research comprised of two parts: 1. field ethnobotany to determine what plants are used to treat childhood diarrhea, and 2. laboratory testing of the extracts of the plants determined in part 1 for antibacterial activity against common enteropathogens.

Prior research into the Caribbean tradition of *bush medicine* conducted in Grenada identified diarrhea as frequently being treated by local plants. This study built upon past work through additional interviews with traditional healers, focusing on use of medicinal

plants in pediatric diarrhea and their manner of preparation. The combined results identified 5 medicinal plants for laboratory investigation. These included; *Chamaesyce hirta* (malome); *Psidium guajava* (guava bud); *Petiveria alliacea* (cudjoe root); *Alysicarpus vaginalis* (masheate) and Desmodium *incanum* (kod a vyelon).

Aqueous extracts of the 5 medicinal plants were prepared as per indigenous prescriptions, and assayed at various concentrations for antibacterial activity against 3 microorganisms *Shigella sonnei* (ATCC #25931), *Salmonella typhimurium* (ATCC #14028), and nonpathogenic *E. coli* (ATCC #25922). Of the extracts tested, only guava leaf/bud (*Psidium guajava*) showed significant *in vitro* antibacterial activity; this nontoxic native proved bactericidal against *Shigella sonnei* at a concentration of 0.02 g/ml (dry herb/water). This study thus continues the investigation of Grenadian medicinal plants, providing one potential antimicrobial, and identifying several other plants for future bioassays.

Submitted by Charles C. Avgeris
Research Scientist

6.3 Non-Communicable Diseases

6.3.1 Decompression illness among fishermen divers of Grenada (A11)

Decompression illness (DCI) affects compressed air divers as a result of the combined effects on the body of breathing a pressurized gas mixture and the exposure of the body to the ambient pressure of water. Often debilitating and potentially deadly, DCI can refer to an aterial gas embolism; Type I decompression sickness (DCS), which is non-systemic; or Type II DCS, which involves the pulmonary, cardiovascular, and/or nervous systems.

For decades an enigmatic malady, research by the U.S. Navy beginning in the early 1900s through the 1960s showed that slowing the rate of ascent following exposure to elevated pressure could reduce or eliminate symptoms of DCS. Today it is generally accepted that the cause of DCS is nitrogen bubbles in the venous blood, musculoskeletal system or other body tissues. These bubbles form when a diver breathing compressed air ascends too quickly, given the depth and time of a dive. However, neither the precise pathophysiology of DCS nor the pathogenesis of associated sequelae is entirely understood. An arterial gas embolism (AGE) can also result from pulmonary barotraumas induced by the sudden expansion of air in the lungs upon too hasty an ascent.

In addition to the acute symptoms resulting from a diving accident, various long-term sequelae can manifest years after a diving accident or, in the absence of a known precipitating incident, from the cumulative effects of hyperbaric exposure. There is empirical evidence that sub-clinical insults from long-term deep diving may lead to subsequent reported cases of hearing problems, organ tissue damage, dysbaric osteonecrosis and cognitive difficulties in divers with no history of decompression sickness.

Compressed air divers are affected by pressure in two ways: through the breathing of a pressurized gas mixture and through the exposure of the body to the ambient pressure of the water. At sea level, the atmospheric pressure is 760 millimeters of mercury (mmHg), 1 atmosphere (atm) or 14.7 pounds per square inch (psi). Under water, absolute

pressure increases faster than in air: a foot of seawater exerts pressure of 0.445 pounds per square inch gauge (psig). At 33 feet under water, a depth gauge reads 14.7 psi (33 ft. x 0.445 psig); meaning that the absolute pressure (ATA) has doubled. Ambient pressure under water increases approximately linearly with depth, so an additional atmosphere of pressure (1 atm or 14.7 psi) is added with each subsequent 33 feet of depth in seawater. To illustrate the magnitude of the effect of the increase in pressure, a depth change of seven meters produces a change in ambient pressure commensurate with going from sea level to the top of Mt. Everest [29,035ft; 8,850m].

Significant research has resulted in improved knowledge of the physiology of DCI as well as intricate dive tables to help divers safely manage their ascents. However, this knowledge of physiology and ascent staging is not widespread among the fishermen divers of indigenous populations around the world. Hence, what has been termed an epidemic of paraplegia is occurring, as DCI takes its toll on fishermen and their families. Studies have attempted to document the extent of DCI related death and infirmity in several countries (Nicaragua, Honduras, Turkey, Thailand). However, there are no published studies of the effects of DCI (acute or chronic), or assessments of the burden of disease on the fishermen divers in Grenada or more broadly in the Caribbean.

The definitive treatment for the majority of clinical manifestations of DCI, whether DCS or AGE, is hyperbaric oxygen therapy. Objectives in HBO mirror those of ascent staging with the critical additional benefits of sustained or intermittent high-flow 100% oxygen administration. The closest hyperbaric treatment facility to Grenada is in Barbados.

Figure 1: Hyperbaric pressure chamber.

Objective

The objective of this work is to define and assess the burden of disease with respect to acute and chronic manifestations of decompression illness (DCI) among the fishermen divers in Grenada and to recommend potential approaches to ameliorate the problem commensurate with its magnitude.

The first phase of this study is assessment of the clinical incidence of DCI in Grenada based on hospital records and hyperbaric chamber records. The second phase is an appraisal of the field incidence of DCI based on data collected from personal observations, personal interviews, and questionnaires completed by fishermen divers. In addition to increasing the power in sample groups, the intent of the second phase is to assess sequelae and long-term outcomes related to initial onset of DCI; qualify and quantify diving practices and profiles; assess knowledge, attitudes and practices regarding DCI and appropriate medical care; and understand divers' risk perception and behavior with respect to chamber location. This information will be used to assess the burden of disease of the various manifestations of DCI and to analyze potential remedies including

education, government regulation of fishermen and/or compressed air suppliers and local provision of hyperbaric services.

Data from Dr. Brian Charles of the Barbados Defense Force shows that 26 fishermen divers from Grenada were treated at the BDF hyperbaric facility from January 1985 – July 2003. These numbers grossly understate the incidence of DCI among this population, as many fishermen with DCI insults do not receive hyperbaric treatment due to the high cost and difficult logistics of emergency evacuation and treatment, as well as reluctance to commit to definitive treatment.

Figure 2: Fisherman tank diver with speared fish and lambi. Calliste, St. George's, Grenada.

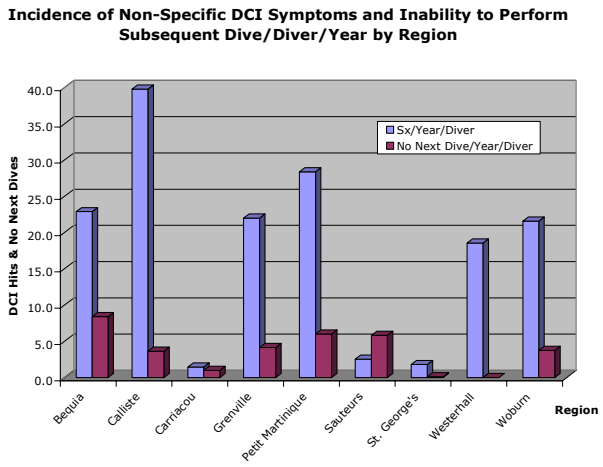
Information was gathered on 123 divers, including the receipt of detailed questionnaires from the 115 active and former divers on the islands of Grenada, Carriacou, Petit Martinique, Bequia and Union. Preliminary analysis of the data shows high morbidity and mortality associated with DCI. Roughly 61% of the divers in Grenada and the southern Grenadines that fish Grenadian waters have received a clinical diagnosis of DCI compared to a field reported incidence of approximately 86%. By comparison, in a recent study of indigenous fishermen divers in Thailand, the field reported incidence of DCI was 50%. Interestingly, other research estimates that Type I DCS afflicts 85% of the indigenous diving population in Thailand.

On average, a diver in the Grenada region reports more than four DCI hits, but this figure vastly understates the true incidence of the DCI. The same divers cumulatively report pain or paresthesias immediately after diving in excess of 2,000 times per year, or more than 16 incidents per diver per year; such pain or paresthesias following ascent from compressed air diving is generally classified as a decompression hit. The local fishermen divers do not term such hits DCI for a variety of reasons, likely associated with a combination of lack of education and denial.

The fishermen dive aggressive profiles, which put them at high risk for DCI. There appears to be some local variation, but across the region as a whole, fishermen dive on average 3-6 tanks per day, 5-7 days a week.

They dive to an average depth of 100 ft. and remain at depth for an average of 25 minutes.

Figure 3: Incidence of non-specific DCT symptoms and inability to perform subsequent dive\diver\year by region.

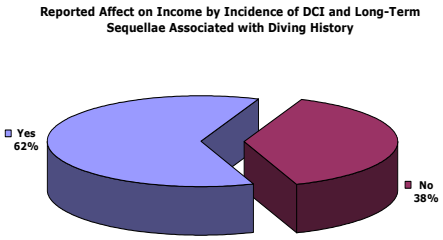


This is followed by a rapid ascent (nearly 17 ft./min), generally without decompression stops and a surface interval that averages only 11 minutes – generally long enough to haul in their catch and change tanks. Virtually all of the divers routinely violate the no-decompression limits set by the U.S. Navy Standard Air Decompression tables. Their exertion while under water and during their surface interval is extreme, exacerbating their risk of a DCI hit. Further analysis will seek to determine if there is a significant correlation between dive profile aggressiveness and incidence of DCI.

The fishermen dive aggressive profiles primarily for economic reasons. Although the majority of the fishermen divers in the study have a rudimentary idea about what causes DCI, they do not appear to have sufficient understanding of how their dive profiles contribute to the problem. In addition to insufficient education about what causes DCI, lack of regulations governing various aspects of the industry also appears to be a factor. Over 70% of the divers' state that they would dive less aggressively if they were informed about what causes DCI and how changing their dive profiles could reduce the likelihood they would be hit, even if it meant a smaller lobster or lambi catch. Counter to what studies of individuals involved in other high risk activities might suggest, relatively few of the divers (less than 7%) stated that they would dive more aggressively if a hyperbaric chamber was located on Grenada, making access to treatment easier and less costly.

In terms of burden of disease, more than half the diversi stated that their income was negatively affected by chronic health problems associated with DCI.

Figure 5: Reported effect on income by incidence of DCI.



When statistical analysis is complete, information about the prevalence and burden of disease of DCI among the fishermen divers of the region will be made available to the appropriate government ministries and other relevant organizations. Recommendations will also be made concerning options to reduce the incidence of DCI and alleviate its severity and long-term consequences.

Data gathered by Dr. Brian Charles of the Barbados Defense Force

and compiled by Scott Forman as well as preliminary findings of this study were presented in October of 2003 at an international CME conference on Caribbean health issues for general practitioners.

Submitted by Scott Forman
Research Scientist

6.3.2 Novel Antibiotics from Tropical Marine Environments (A12)

Recent studies, have shown that nosocomial (hospital acquired) infection rates of patients have increased dramatically over the past few years.

The increase in Oxacillin Resistant Staphylococcus aureus (MRSA) which has gone from thirty percent in 1989 to over sixty percent a little over a decade later in 2001.

Another study conducted by the CDC in 2000 confirms this finding as well as presenting other groups of pathogens whose resistance to antibiotics have risen. Noted among these microbes are K. pneumoniae which has increased in resistance to Cephalosporin 5%, enterococci which has increased in resistance to Vancomycin 31%, and P. aeruginosa which has increased in resistance to Quinoline 53% . These figures are alarming when taking into consideration that the time frame of these increases is five years. This presents a major problem in the field of medical science. C:\GetARef\Refs\marine antibiotics.ref #2;

So when the antibiotics of today, such as vancomycin, an essential antibiotic in the modern age, which is now reserved for use in patients who are gravely ill or for infections caused by organisms resistant to penicillin, cephalosporin, or other antibioticsC:\GetARef\Refs\marine antibiotics.ref #1; , fain in their efficacy, where do doctors and research scientists look to for
help?

The oceans are a unique resource that provides a diverse array of natural products, primarily from invertebrates such as sponges, tunicates, bryozoans, and molluscs, and from marine bacteria and cyanobacteria. Due to this diversity the marine environment provides novel leads against fungal, parasitic, bacterial, and viral diseases.

It is a new frontier in the fight against infection. Grenada offers a perfect location for such a study. The tropical waters surrounding the island exhibit a wide variety of marine life and an endless array of micro ecosystems on abiotic surfaces from which to sample new microbes.

Submitted by Nicholas D. Caputo
Research Scientist

6.4 Caribbean Environmental Research Initiative (A13)

The Caribbean Environmental Research Initiative (CERI) is a program within WINDREF, unifying a group of SGU faculty involved in environment-related research. The group, coordinated by Dr. Kotelnikova includes Drs. A. Qureshi, D. Lennon, Z. Ross, M. Anson, C. Morrall, J. Rayner, R. Sis, M. Ford, and Mr. T. Day, Ms. C. Bruno, and Mrs. S. Davis.

CERI aims to: bring all benefits from rationally used resources and target priorities which have emerged from a broad-based consultation with the political authorities, the scientific world, the business sector, and user representatives. coordinate framework programs based on international cooperation and beneficial services of its

citizens. integrate scientific competence, European industries and Caribbean needs.

attract research funds and visiting scientists to conduct a wide range of environmental projects. The use of the Marine Station is likely to be of great value to this research.

6.4.1 Monitoring of recreational aquatic environments in Grenada (A 14)

Clean and healthy atmosphere, aquatic and terrestrial entities is a pre-requisite for the existence of contemporary tourist industries in Caribbean. Extensive agricultural and tourist exploitation of the Windward Islands along with growing human population may lead to irreversible pollution and eutrophication. The regular monitoring and scrutiny of the natural environments (marine and fresh water) is invoked to prevent pollution problems leading to the global change of environment, rising infectious diseases and other problems of public health. CERI is designed to ensure that its unique island laboratory will find solutions to the problems facing our coastal areas.

Goal of this study was assessment of the potential environmental risk factors and microbial pathogenic agents (coliforms) in the sea and freshwater environments of Grenada.

The research project dealing with aquatic monitoring was initiated in May of 2003.

In the course of the project water was tested in a range of habitats including recreationally active beach areas on Caribbean and Atlantic sides of the island, estuary edging with fringing reef, river banks, and a volcanic lake at a height of 1800 feet. Water at these sites at varying depths was collected and analysed for numbers of CFU of coliforms, MPN of coliforms, biological oxygen demand (BOD), ferrous iron, dissolved oxygen, salinity, temperature, and pH. Pure cultures of coliforms were isolated from the tested waters and identified. Unique tropical aquatic environments represent sources of new bacterial species, genes, known opportunistic and non-opportunistic pathogens.

Figure 1. Grand Etang Lake.

The BOD values in rivers and lake water ranged between 0.96 and 14 mg/l. A water with BOD level of 1-2 mg/l is considered very clean. We observed such value in La Sagesse River. There will not be much organic waste present in the water supply. A water supply with a BOD level of 3-5 mg/l is considered moderately clean. The water in Prickly Bay next to the Calabash hotel, Grand Etang Lake (Figure 1) and water from La Sagesse Bay are moderately clean.

In water with a BOD level of 6-9 mg/l, the water is considered somewhat polluted because there is usually organic matter present and bacteria are decomposing this waste. The water in Grand Anse Bay next to Flamboyant Hotel, Flamboyant desalination plant water, Prickly Bay next to the Boatyard, Clark's Court Bay, St. John's River are somewhat polluted with organic matter.

Concentration of dissolved oxygen and pH negatively correlated with BOD values. Correlation coefficients were r^2=0.55 and r^2=0.84, respectively. Concentrations of dissolved ferrous iron in the tested marine, fresh waters and waste correlated positively with BOD values (r^2=0.60) Salinity did not correlate with BOD, r^2=0.28. Colony forming units of coliforms were generally high and ranged between 600 and 20000 cells per 100 ml. Most probable numbers of coliforms were between 6.2 and 2000 cells per ml and correlated positively with BOD values (r^2=0.76)

Eutrophication would lead to the death of marine organisms and complicate the socio-economic situation in the Caribbean region. The fact of fish-kill and coral reef death around Windward Islands is an indication of on-going environmental disturbance in the region. There is a clear indication that "Tropical Caribbean Paradise" is threatened by pollution of: sewage sludge, recalcitrants and household waste. There is a need for establishment of stationary station that would perform concise and regular monitoring of the water to show background condition, follow trends, find out reasons, and solve the problem..

Isolation of potentially pathogenic bacteria from the aquatic environment

Most frequent adverse health outcome associated with exposure to faecally contaminated recreational water is enteric illness. Children, elderly, immune-suppressed individuals and new exposed visitors comprise the populations that may be at higher risk. Unsafe water in Grenada could have potentially devastating effects on the economy in areas of tourism, lost wages and lost of educational days.

Increased outbreaks of diarrheal gastro-intestinal disorders in Grenada are usually observed during the rainy season. Can it be linked to the contamination of recreational waters? What kind of pathogenic microorganisms may thrive in the aquatic environments of the tropical island? Our project dealt with isolation and identification of microorganisms from aquatic environments around Grenada. The goal of the identification was to determine whether the organisms were pathogenic or just normal environmental bacteria.

Bacteria like *Escherichia coli, Enterobacter cloacae* and *Enterococcus faecalis* are normally part of normal microflora of gastro-intestinal tract, however these organisms survive in the environment without replication and therefore are used as organisms indicating fecal contaminations in water. For marine water, only intestinal enterococci 10^4 CFUs/100 ml, and 61 CFUs/100 ml for fresh water showed a dose/response relationship for GI illness (EPA). Seven sites were chosen: Prickly Bay (beach and Boatyard), Grand Anse Beach, La Sagesse (sea and river), Carenage Bay, and Grand Etang Lake.

Location of sites monitored was designated based on existing use of water, frequency/density of use of recreational sites and probable potential pollution sources/rain water discharges. Tested fresh water (May –July 2003) contained typical water associated heterotrophic bacteria that are related to *Serratia marcescens, Pseudomonas sepatica* and marine water - *Acinetobacter sp.*

Figure 2. S. Davis, collecting samples from Carenage Bay

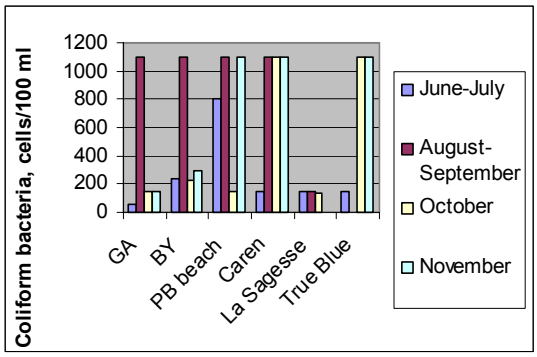


Figure 3: MPN counts of coliform bacteria in marine water in Grenada, May-November 2003.

Coliforms found in marine and freshwater were identified as *Shigella flexnery, Proteus spp., Pantea sp., Klebsiella ozonae, Enterobacter agglomerans, Enterobacter cloacae, Enterobacter agglomerans, Enterobacter amiguensis* and different strains of *Escherichia coli*. *E. coli* was found in Prickly Bay, True Blue Bay, Grand Anse Bay and La Sagesse in July of 2003. Marine water contaminated with sewage is an excellent environment for survival of gastro-intestinal pathogens in tropic climate and may be considered as a source of infection when concentrations of indicator organisms are higher than defined by EPA. Most probable numbering of indicator microorganisms for faecal coliforms showed that viable counts of opportunistic gastro-intestinal tract pathogens in marine bays and freshwater environments tested were higher than 200 cells/100ml in 80% of samples between May and October of 2003. Numbers of GI are higher after intensive rains due to watershed and sewage overflow.

Bacterial Isolates from fresh water of Grand Etang Lake (8/14/03) were identified as *Citrobacter freundi, Pantoea spp., Pseudomonas aeurginosa and Shigella spp.* while bacteria isolated 9/23/03 were identified as *Escherichia coli, Ochrobactrum anthropi, Pseudomonas aeurginosa, Pantoea spp. Chryseobacteria sp. and Pasteurella haemolytica.* Gram-negative bacteria isolated from marine water were less diverse. Different strains of *E.coli* were isolated from Prickly Bay in vicinity of the beach, from water in Grand Anse Beach area. Carenage Bay resulted both in *E.coli* and *E. vulnaris*. Marine water of La Sagesse contained *E.coli* and *Enterobacter cloacae.* Estuary of La Sagesse river showed presence of *E.coli* and *Cedeca sp.* Most cultures were identified. Unidentified organisms did not match with any known in the database and may represent new species.

Culture collection of 63 marine and freshwater microorganisms was created. Forty three (70%) of them were identified including 50% that were identified as opportunistic GI pathogens and 20% non-pathogenic, while the rest may represent new species.

E.coli was found in water of Grand Anse, True Blue, Prickly Bay, La Sagesse, Carenage Bay and Grand Etang Lake in August, September and October. *E. coli* makes up 95% of resident microflora of the intestines in humans

and other warm blooded animals. It is an indicator of faecal contaminations in the fresh water and some strains of *E.coli* may cause enteric disease or urinary tract infections due to production of enterotoxins or other virulence factors in different immune-compromised groups.

Submitted by Elizabeth Davis and Svetlana Kotelnikova

6.4.2 Antagonistic properties of reef fish microflora (A15)

Microbial biofilms cause systematic infections in humans and costly marine related damage and inefficiency. There is interest in development of stable, non-xenobiotic, anti-fouling agents for the marine environment. Marine eukaryotes have developed two strategies to protect themselves against fouling: the secretion of signaling compounds and housing of probionts. Both strategies interfere with signals regulating biofilm formation. Indigenous fish mucosal surface bacteria have been shown to have inhibitory effects on some bacteria. Twenty bacteria were isolated from the epidermal mucosal surfaces of coral reef fish (*Sparisoma ninidae* and *Lutjanus campechanus*). Morphology and physiology of 15 isolates were studied. Most of them were gram-positive cocci and rods, pigment-producing halotolerant heterotrophs, with variable products and patterns of hemolysis, variable presence of catalase and cytochrome oxidase. Isolated organisms were also screened for anti-microbial and bacteriocidal activities.

Fish external epithelial mucus accommodated capsule-producing strains P6-5 and P6-6. The microbial capsule material had anti-bacterial activities against *S. aureus, S. epidermidis, K. pneumoniae, and P. vulgaris* comparable to penicillin while the rest of tested fish probionts produced secondary metabolites with weak antibacterial activities.

Four strains were screened for anti-fouling activity using gels on glass slides exposed *in situ* to the seawater in True Blue Bay. The glass slides were screened for numbers of microbial cells, bryozoan and algal settlement under UV microscope. Microbial cells appeared orange or green after staining with akridine orange, while chlorophill-containing organisms were seen as red-colored. The resulting counts were compared to controls, free of extracts, using T-test. The *in situ* attachment of bacteria was significantly inhibited by extracts of three strains, P5-2 ($p=0.024$), P3-2 ($p=0.061$), and P4-4 ($p=0.099$). *In situ* algal colonization was significantly inhibited by extracts of one strain, P3-2 ($p=0.0006$), and by washed whole cells of two strains, P3-2 ($p=0.037$) and P4-4 ($p=0.063$).

The isolates that showed antagonistic anti-biofilm forming activities were identified using 16S rDNA sequencing and fatty acid identification. Most of them appeared to be new microbial taxa. Strain P4-4, isolated from parrotfish may represent a new genus, while isolates P1-5, P2-2, are new species, distantly related to *Desemzia incerta* and P3-2 may be a new species, distantly related to *Aerococcus viridans.* Our fish isolates were related to organisms that typically inhabit animal organs and surfaces.

Extracts from isolate P4-4 (64%), "*Aerococcus viridians"* P3-2 (82%), *Staphylococcus warneri* P5-2 (85%) inhibited both microbial and eukaryotic fouling in *in situ* experiments. Living cells of isolate P4-4 (36% reduction) and living cells (27%) and extracts of *Aerococcus viridans* P3-2 (42%)

inhibited micro-eukaryotic fouling in the *in situ* experiments. Association of anti-biofilm properties with weak antibacterial activities indicated chemical signaling nature of the exometabolites.

The tested isolates are part of a probiotic microbial community acting as a protective network. They have different properties creating a network that results in protection of fish from microbial settlement, infections, and macro-fouling. Some of them competed with non-indigenous and pathogenic microorganisms by producing secondary metabolites that inhibited growth of pathogens, some of them prevented settlement of bacterial cells, while other members of the probiotic network prevented algal and bryozoan settlement.

90% of antibiotic resistant medically important pathogens grow in biofilms on human organs and medical devices. An advantage of using natural compounds produced by the isolates is that they are less likely to be harmful to the body or environment. The ether solvable substance may be incorporated as an anti-fouling agent in non-polar coatings or used as anti-biofilm forming agent. In addition, the isolated probionts are robust halotolerant facultative anaerobes that showed wild-type phenotype, therefore, biotechnological usage will be simple and the genotype probably will not revert. However, it is not known whether the genes responsible for the antagonistic properties are associated with a chromosome or a plasmid.

Submitted by Cynthia Bruno and Svetlana Kotelnikova

6.4.3 CERI and Grenada Co-operative Nutmeg Association (GCNA) activities (A16)

Nutmeg (*Myristica fragrans)* is grown in Grenada and Indonesia. Nutmeg oil is clear to pale yellow oil containing aromatic fraction of ground nutmeg seeds that is produced by steam distillation. The nutmeg and nutmeg products are widely used as flavouring and fragrant seasoning (Muscat) in different foods and drinks including nutmeg oil, which is used as a main flavor-giving addition to coca-cola.

The Grenada Co-operative Nutmeg Association (GCNA) is the main industry exporting whole nutmeg seeds, mace and nutmeg oil and is one of the biggest producers of nutmeg worldwide. Two problems threaten the nutmeg industry: wilt disease, killing nutmeg trees, and slow degradation of waste of oil distillation. According to preliminary estimates, approximately 10-15% of east-northern plantations in Grenada are infected with wilt. The tree dies in 1 week by loosing leaves. Degradation of biomass resulting steam distillation of oil takes years. The accumulation of the nutmeg waste hinders production of the nutmeg oil.

Hypothesis

1. Wilt disease is caused by microbial succession in the rhyzosphere due to high humidity and low pH.
2. Microbial degradation of waste material of nutmeg biomass resulting steam distillation of essential oils is ineffective due to two reasons: first, lack of electron acceptors (oxygen, sulfate, nitrate, ferric iron) to oxidize the excess of organic material and, the second, due to the anti-microbial nature of the waste.

The objectives of the project were to:

1) Evaluate an association of pathogenic fungi with wilt diseased roots; 2) Characterize soil infected with wilt (humidity, chemical composition, pH,

salinity); 3) Quantify numbers of bacteria, actinomycetes and fungi in the soil infected with wilt; 4) Evaluate content of biodegradable organic substances in nutmeg wastes using bioassay; 5) Characterize nutmeg waste for presence of microorganisms and anti-microbial activities; 6) Screen nutmeg products for antimicrobial activity against human pathogens, environmental bacteria and soil originated fungi; Rizhosphere soil was analyzed in an area infected with Nutmeg wilt.

The sampled area was chosen because of high frequency of wilt. Hermitage is situated in climatic zone with 8 months rainfall, and we determined that the humidity of the H 1-1 soil at site H1-1 Hermitage was 22% and H 2-1 32%. These values were pilot estimates to be compared with further tests and give an idea whether the humidity provides a beneficial environment for the development of pathogenic fungi.

Chemical content of Hermitage soil (Belmont Clay Loam) was following (mg per kg): phosphorus, 10-30; nitrogen, 1200; potassium, 75-150; soil depth,cm, 0-150; organic matter,1500-2500, Rb, 0.02; Li, 0.045, Sr, 0.020; Ba, 0.300, V, 0.450, Cr, 0.550, Co, 0.09, Ni, 0.450; Zr, 0.350; Y, 0.02, Cu, 0.160; Mo, 0.004; Ga, 0.040, Pb, 0.050, Mn, 3.000, Fe, 300, 000. The soil contained more of vanadium, copper, gallium and manganese then other Grenadian soils. pH was between 5.76±0.13 and 5.97±0.04 and close to literature data of 5.7-5.9.

Microscopic observation of the soil samples showed Gram-positive cocci, no rods, a lot of fungal filament diameter of 6 micrometers in Hermitage 1-1 soil sample and fungal filaments, ultra-small gram-positive cocci of 3 micrometers in diameter and rods in Hermitage 2-1 sample.

Numbers (cell/g soli) of fungi (0.02-1 $x10^{10-}$ 2 $x10^{11}$), bacteria (2.6 $x10^{11-}$ 2 $x10^{14}$) and *Actynomycetes(*4 $x10^{8}$) in soil were estimated using serial dilutions and colony forming units (CFU) count on V8 tomato juice agar, water based agar, subaraud agar, nutrient agar and actynomycetes specific medium at 29C Microflora found in the soil around wilt infected trees consisted prevalently of bacteria and fungi. Microscopic **fungi** CFU counts on SDA were generally around 1000 times lower then **bacterial** CFU counts on NA. **Actinomycetes** CFU counts were 100 times lower then the fungal. Pathogenic fungi were associated with wilt diseased roots.

Degradation of nutmeg waste.

The project targets the improvement of the quality and safety and reduction of the pollutions derived from oil production systems.

Objectives: Characterization of BOD, pH, iron, presence of microorganisms and oxygen in the waste and investigation of antimicrobial effects of nutmeg waste from Malmount site 1 and Site 2, St. Patrick waste, nutmeg flesh extracts, nutmeg oil and the ground nutmeg seed.

BOD, oxygen, iron and microbial presence in the nutmeg waste from 4 different sites; oil distillery in Gouyave (11.03.2003), Malmount lake 1 and lake 2, St. David (26.06.2003) and Gouyave (03.07.2003).

The water in the lakes was black, with a few bubbles. The material was dry, cellulose rich and smelling with organic acids, probably myristic acid with pH (3.6-4.7), salinity (0), temperature (27-29C), dissolved oxygen (0-3 mg/l) of nutmeg waste was monitored in the liquid and solid nutmeg oil waste that has been disposed for around 2 years. Concentrations of ferrous iron in the waste were up to 300 mg a litre. BOD is

biological oxygen demand during 5 days. The water in Lake 1 showed BOD of $1.6x10^3$ mg/l, in the lake 2 it showed BOD of $2.1x10^3$ mg/l. Ferrous iron ranged between 2.75 and 300 mg/l while dissolved oxygen was 1.34-2.93 mg/l. Solid waste in Lake 1 showed BOD of $5.4x10^3$ mg/l, in the lake 2 it showed BOD values ranging between $7.6x10^4$ and $1.3x10^5$ mg/l. Gouyave's pond with non-treated waste (after distillation with BOD of $2.61x10^5$ mg/l) and the waste after 2 months of treatment with "Oppenheimer mixture" with BOD of $9.54x10^4$ mg/l. The waste was free of measurable oxygen at both last sampling occasions and sites.

At BOD levels of 100 mg/l or greater, the source is considered very polluted with organic waste. The results showed extreme high BOD of both treated and non-treated nutmeg waste. Generally, if the waste with the observed BOD will be disposed into the marine or river water it will lead to chemical reduction and microbial consumption of oxygen. Organisms that need higher oxygen levels (i.e. fish larvae, coral spongy and crustacean) will not survive. Besides, the Caricom recommended standards for industrial waste to be discharged to the public sewers require BOD below 500 mg/l, and pH of 6-9. No oil is allowed.

Main components of Grenadian nutmeg oil were elemecin, myriticin ($C_{11}H_{20}O_3$) and mescalin. These components were extracted effectively with hydrothermal extraction. The oxidized fraction of these compounds, as myrictic acid and trimyristic fatty acids, stay in the waste. The waste consisted at 8% of essential oils including 7% safrol, 14% starch, 30% fibre, 8% water and 40% fat. The fat contains mostly trimyristic fatty acids that may be potentially converted to soap by trans-etherification.

Microscopic observations showed that the nutmeg waste contained indigenous microorganisms, Nutmegtrix" threads 0.5x15 μm; rods, 0.8x4-8 μm, *Bacillus* with bilateral spores; cocci in chains 1x2 μm and chains of coccobacilli 0.8x1.0 μm. Similarity of microflora in the waste from Gouyave sampled the 11.03.2003 and from St David's Malmount lakes sampled in the 26.06.2003 and Gouyave 3.7.2003 indiicates that the nutmeg waste is slowly degraded by native nutmeg waste bacteria (Figure 1b). The specific microflora is adapted to grow at low pH while the "Oppenheimer's mixture" might experience difficulties because of the low pH. It may be a reason for high BOD of "treated waste". Our study showed that:

Minimal BOD was higher than 500 mg/l that that attests presence of high concentrations of organic materials after two years and indicates that degradation is extremely slow. Release of the nutmeg waste would be threatening for the environment. The waste was oxygen free and contained low ferric iron that confirm our hypothesis about lack of respiration acceptors.

The waste contains own original microbial community that is highly adjusted to the unique environment and therefore apparently is most efficient in degrading the waste. There is a perspective of development of effective methods for the nutmeg degradation using the original microflora that is adapted to low pH and specific substrates like myristicin, myristic acid, tri-myristic ether, starch, paraphin, oil, etc.

Antimicrobial activity of nutmeg waste

The effect of nutmeg waste on growth of microorganisms (fungal and bacterial) showed that nutmeg waste inhibited growth of *Ps. fluorescens* and *Kleb. pneumonia*. Anti-microbial properties of other such nutmeg products as ground

nutmeg and nutmeg fruit were studied along with the nutmeg waste as chemically derived extracts soaking sterile filters and placed on a Petri dish, pre-inoculated with a pathogen.

Inhibition zones from the waste were 50-66% smaller compared to penicillin. Antimicrobial activity was observed for all of the products tested against different pathogenic agents. **Ground nutmeg** was active against (*Staphilococcus aureus, S. epidermidis*), that are part of normal skin microflora of human and dangerous opportunistic pathogens forming biofilms. It was active against gastro-intestinal pathogens *Salmonella typhi, Shigella sonni, Escherichia coli* and *Enterobacter cloacae.* In addition, it was antifungal showing activities against *Phytophtora* F5 isolated from the wilt root in Hermitage soil.

Anti- microbial effects of **nutmeg fruit** was studied for the first time and our study demonstrated that different extraction procedures resulted in products with different targets active against *Klebsiella pneumonia, P. fluorescens, E. coli* and *Shigella sonn*i as well as wilt agent *Phytophtora* F5.

Interesting that three fungi F1, F2 and F3, isolated from rizhosphere of nutmeg tree were resistant both to ground nutmeg and fruit extracts.

We did not observe anti-bacterial effect of the nutmeg oil however antimicrobial activity of commercial nutmeg oils against various pathogenic fungi and bacteria were investigated previously. *Myristica fragrans* oil from the seeds and mace showed the most significant fungicidal activity against *Pseudallescheria boydii, A. niger*, *Candida albicans*, *Fusarium oxysporum* var. lycopersic , *Microsporum cani,s Pseudallescheria boydii*, and *Trichopyton simii*. Nutmeg oils from the seeds and mace were not active against *Trichopyton mentagrophytes* and *Aspergillus flavus,* respectively.

Our experiments with the effect of the **nutmeg waste** on growth of microorganisms (fungal and bacterial) showed that nutmeg waste inhibited growth of human skin and GI pathogens, soil fungi, wilt disease fungi and *Candida albicans*. It means that the waste might inhibit degrading bacteria as well. Nutmeg waste inhibited growth of fungi (*Candida albicans, Phytophtora sp., F4 and F1*) and pathogenic bacteria. This discovery has important implications for treatment of the wilt disease in Grenada without using toxic fungicides, but just leaving nutmeg waste under the trees. Leaving fruits and the introduction shells of nutmeg seeds may help to get rid of wilt disease of nutmeg.

Submitted by Svetlana Kotelnikova
Research Fellow

6.4.4 Iron-oxidising microorganisms in St Andrews Hot Springs in Grenada (A 17)

Microbial life is a crucial component of a hot spring's environment that is responsible for the cycling of elements and energy. Microbial photo- and chemosynthesis and decomposing of organic matters at high temperature constitute the basis for cycling of nutrients (C, N, S, P, O, and Fe) in the ecosystem. Hot springs contain diverse microbial communities. The microbial diversity of the hot solfataric springs situated near St. Patrick's in Grenada is totally unexplored. Today the diverse microorganisms from such unique ecosystems as hot soda springs, or geysers, are considered to be a source of biologically active compounds which could be used in future biotechnology.

Our project dealt with isolation and characterisation of hot soda spring water-borne facultative aerobic heterotrophic microorganisms possessing the ability to grow at high temperatures (55-60°C) and isolation of thermophilic iron-oxidising bacteria.

Our hypothesis was that hot springs support highly specific and diverse microflora. In order to survive under extreme conditions, the spring microflora possesses unique physiology.

The goal of the study was identification of water composition, isolation and identification of unique bacteria from the hot springs situated near St. Patrick 's.

The water and biofilms were sampled from the Soda Springs near St. Andrew's in March 2003. The spring is situated at altitude of 1810 feet in the middle of rain forest and nutmeg plantations. The springs had bright yellow coloured sediments. The water was clean and saturated with gases including carbon dioxide. The gas bubbles were issuing from the green-yellow biofilm. The resulting water was saturated with the gas.

Hydrothermal vents usually are found only in areas where there is volcanic activity and the magma is close enough to the surface to heat the fluids. Hydrothermal vents have very high temperature below the ground 300°C, under high pressure 500 bars. Stream of reduced minerals (Si, Fe, Cu, Mo, Ni), clays and gases (H_4S, H_2, CH_4, CO, $CO_{2)}$ make the composition of the hydrothermal liquid unique.

Temperature of the water was 50-52C, pH ranged between 6.0 and 6.1, salinity was slightly above 6 g/l, COD was 49 mg/l, conductivity 764 microSimens/cm, total number of microorganisms was defined using UV Carl Zeiss Axioscope with 420 nm light and acridine orange staining, ranged from 0 to 3.65 x 10^3 cells /ml. Water analysis for the dissolved $O_{2,}$ (COD) and ions (mg/l) Cl^-,28; SO_4^-,0.0; $PO4^{-3}$,2.08; NO_3^-,7.04; measurements of temperature, pH, alkalinity (HCO_3^-),272; hardness (Ca^{2+} 139, Mg^{2+},111), 250, Fe total,2.61; NH4, 1.24; conductivity and salinity were performed in SGUSOM and WINDREF in collaboration with the NAWASA Observatory, Mrs. Thomas and Mr Lambert.

The water contained high concentrations of magnesium, iron and calcium, phosphorus and ammonium. No sulphate was detected. The composition of the water was unique as composition of most of groundwaters. The composition of the water will be used to design a new medium for isolation of indigenous microorganisms. There is very big chance that these organisms are new.

Biofilms contained *Gallionella ferruginea* and iron precipitates but no sulphur. Iron is an element that is widely distributed in the Earth crust. It is the 4-th abundant element in the crusts after Si, O, and Mg in mantle rock, while Al, Ca, Na and K comprise 98% of the weight of 70 km crust. Iron is found in following soluble: ferric oxyhydroxide or limonite (FeOOH), goethite Fe_2O_3 H_2O, hematite $Fe_2O_{3,}$ magnetite $Fe_3O_{4,}$ ilmenite FeO TiO_2 and non-soluble minerals siderite ($FeCO_3$) and Pyrite FeS_2 or pyrrohotite Fe_5S_6

In water solution, ferric iron dissmutates to ferrous iron in accordance wit following reaction: $Fe^0 + 2Fe^{3+} \rightarrow 3 Fe^{2+.}$ Microorganisms are actively cycling iron in nature using it as source or energy or as electron acceptor. *Gallionella ferruginea* has mixed type of metabolism shifting between autotrophy and heterotrophy. *It* uses CO_2 as a source of carbon or use glucose or fructose or

sucrose as source carbon and energy when grows with oxygen. The rate of iron deposition is estimated to be around 2 mg a day per sq. cm. *Gallionella ferruginea* is recognized as organism possessing a twisted stalk containing iron hydroxide incrustated into excreted extracellular organic matrix. Twisted stalk is characteristic of *Galionella*. *Galionella* spp. have never been found in Grenada before. Our study is the first record for existence of thermophilic *Galionella* species in tropical environments. It often found in association with *Leptothrix* o*chracea*. and *Sphaerotilis*

Leptothrix ochracea and Sphaerotilus sp are **iron**-oxidizing sheathed beta-Proteobacteria. They grow in organic carbon rich or contaminated environments being heterotrophic, they use beef extract as a source of carbon when grow with oxygen. Lepthothrix ooxidizes both iron and manganese and have sheath consisting of $Fe(OH)_3$ and tannic or humic acids. When young swarmer cells will grow inside to 2 um in diameter and then leave the sheath

Conclusions

- The chemical composition of water and physical conditions in the springs were studied to design a new nutrient media to reveal bacteria with specific properties.
- New thermophilic microorganisms were isolated from the soda springs.
- A new discovered environment volcanic hydrothermal vents was added to earlier described habitats of *Galionella-Leptothrix* consortium.

Submitted by Svetlana Kotelnikova
Research Fellow

6.5 Genetic Correlates of the Addictive Diseases: Cocaine, Alcohol, and Marijuana Addiction – Grenada. (A18)

Conceptual discussions were held in Grenada in both February and August 2003. These feasibility discussions were held in Carlton House and the following persons were involved, Dr. Mary Jeanne Kreek (Rockefeller University), Trevor Noel (WINDREF), Mr. Thorne Roberts (Carlton House), Nurse Brenda Scott (Carlton House), Mr. Dave Alexander (Drug Avoidance Office) along with Nurse Luret Clarkson (Grenada Nursing School). Great enthusiasm was shown on the part of everyone concerned for the success of the potential implementation of this research program.

The project was to identify specific genetic factors underlying individual differences in susceptibility to drug addiction and chemical dependency, with a specific emphasis on cocaine, alcohol, and marijuana addiction or combination thereof.

Many treatment programs and paradigms have been developed to combat the problems of drug addiction and chemical dependency. Pharmacotherapies and behavioral treatments are effective for some drug addiction and chemical dependency.

The biological basis of addiction, particularly with respect to specific genetic or inherited individual differences which may influence susceptibility to drug addiction, have not been characterized for each addiction.

Figure 2: At the conclusion of conceptual research discussions in February 2003. Seated rt to lt: Calum Macpherson, Dirk Burkhardt, Mary Jeanne Kreek, Dave Alexander and Trevor Noel.

The knowledge of specific hereditary factors underlying individual predisposition to drug and alcohol addictions and related co-dependencies and co-morbid conditions could have a significant impact in both prevention and treatment of these problems. The proposal was submitted to the SGU internal review board and was approved on December 9th, 2003. In 2004, we intend to process this proposal through the Research Oversight Committee in the Ministry of Health, Grenada.

Submitted by Trevor Noel
Research Scientist

7.0 Acknowledgements

WINDREF works in close collaboration with a number of local and internationally based institutions and individuals. In Grenada, we would like to thank the Ministry of Health, Ministry of Education, Ministry of Agriculture and the National Parks and Protected Areas Department, Ministry of Tourism and the Forestry Department for their help and cooperation with the research projects. We would also like to thank Dr Satnarine Maharaj, CMO, MOH for his considerable input into project implementation and review during 2003.

7.1 Associated faculty, staff and collaborators

Collaborator	Project(s)
Mr. D. Alexander	A17
Mrs. M. Alexander	A1
Mr. X. Ameer	A1
Dr. A. Antoine	A1, A3, A4
Mrs. E. Baptiste	A1
Dr. S. Barada	A5
Mrs. J. Benoit	A1
Mr. J. Bernard	A9
Ms. J. Blau	A1
Ms. E. Cameron	A1
Dr. B. Charles	A10
Ms. E. Charles	A9
Mr. R. Charles	A9
Mrs. B. Christopher	A2
Ms. L. Clarkson	A17
Ms. B. Clarkson	A1, A17
Dr. C. Cox-Macpherson	All projects
Dr. C. Conoscenti	A10
Mrs. C. Crosby	A1
Mrs. A. David-Antoine	A1
Mrs. G. DeAllie	A1
Dr. M. DeSantis	A10
Mrs. G. Dolphin-Bond	A1, A3
Mrs. S. Duncan	A2
Ms. V. Edwards	A9
Ms. J. Fahy	A8
Mrs. C. Forman	A10
Dr. M. Forde	A13
Dr. S. Fry	A14
Dr. P. Garner	A3
Ms. I. Gomez	A3
Mr. H. Gordon	A9
Dr. L. Hallbeck	A8
Dr. P.J. Hotez	A9
Dr. P. Janson	A14
Mr. A. John	All projects
Mr. R. Johnson	A2
Dr. L. Joseph,	A2
Mr. D. Joules	A8
Dr. R. Kabuusu	A5, A7
Dr. M. Kachani	A5, A6
Dr. Ed Kaplan	A1
Dr. S. Kjelleberg	A14
Dr. W. Kolbinger	A10
Mr. J. Kopycinski	A1
Mrs. R. Kopycinski	A1
Dr. M.J. Kreek	A17
Dr. K. Kwon	A14
Mrs. M. Lord	A1
Dr. E. Lyagoubi	A5, A6
Ms. C. Lambert	A1
Ms. M. Lambert	All projects
Dr. P. Lammie	A8
Dr. D Lennon	A4,A12
Dr. C.N.L. Macpherson	All projects
Dr. S. Maharaj	All projects
Dr. T. McPherson	A8
Mrs. R.J.M. Mendez	A15
Dr. R. Milner	All projects
Dr. C. Modest-Curwen	All projects
Dr. R. Modlin	A8
Dr. D. Molyneux	A8
Dr. B. Nelson	A1
Dr. L. Nelson	A10
Dr. K. Newman	A14
Dr. B. Noel	A10
Ms. H. Owens	A1
Mrs. A. Otway-Noel	A1
Dr. M. Parsmore	A14
Mr. R. Patrice	A2

Dr. A. Pensick	A3, A10
Dr. S. Persaud	A8
Mr. C. Peters	A1
Dr. J. Pettus	A7
Mrs. J. Phillip	A1, A2
Dr. T. Poon-King	A1
Dr. L. Ramsammy	A8
Mr. T. Roberts	A17
Dr. Z. Ross	A9
Mr. S. Samerson	A1
Ms. B. Scott	A17
Dr. L. Sieffert	A5,A7
Mr. M. Simon	A10
Dr. S. Singh	A8
Dr. C. Subbarao	A3, A4,A9
Dr. K. Taylor	All projects
Dr. V. Vorndam	A3
Mr. A. Worme	A3,A13
Dr. J. Zabriskie	A1

7.2 Grants

We would like to thank all of the donors who have made WINDREF's work possible in 2003. These include:

- The Liverpool Support Center for their continued support of the lymphatic filariasis project in Guyana
- The Fogarty Foundation who supported students to work on the CE projects in Uganda and Morocco.
- The Danish Research Academy for support for Karin Schioler's work on dengue.
- Drs Zabriskie and Mary-Jeanne Kreek for their support of the RF and substance abuse projects.
- Pie Medical for the loan of the Tringa ultrasound used in Uganda and Morocco.

Dr. Orazio Giliberti and Dr. Calum Macpherson discuss a future glaucoma research project in Grenada. ALCON funded a dinner in NY to bring together world experts on glaucoma. Dr Giliberti is the first SGU alumni to develop a research project with WINDREF and to seek funding for its implementation.

8.0 Past, present and future research projects. (Present Research Projects bolded)

8.1 Non-communicable diseases

- Angiotensin converting enzyme and angiotensinogen gene polymorphisms in the Grenadian population: relation to hypertension
- Development of a decision rule for screening Obstructive Sleep Apnea and its epidemologic relevance to the people of Grenada
- Prevalence and associated risk factors of hypertension in a sample population of native Caribbean's in Grenada, West Indies
- Assessing the prevalence of diabetic complications by examining type I and type II adult diabetics for signs of retinopathy, neuropathy, nephropathy and dermatological changes associated with poor glucose control within the native Caribbean population of Grenada
- Hypertension management and control in two Caribbean countries
- Assessment of the effectiveness of broad-spectrum treatment to children with protozoan and nemathelminthic parasitic infections on diarrhea and school attendance
- The effects of iron-deficiency anemia on cognition and behavior in infants
- Diurnal variation of urinary endothelin-I and blood pressure: related hypertension
- Alcohol consumption in Grenada
- The incidence and mortality of cancer in Grenada over the ten year period: 1990-1999
- The prevalence of abnormal haemoglobin traits in Grenadian secondary school adolescents
- Knowledge, attitudes, beliefs and practices of Sickle Cell Anemia in Grenadian Primary and Secondary school children
- **Decompression sickness among the indigenous fishing population in Grenada: Assessing the burden of disease**

8.2 Infectious diseases

- Investigation of the prevalence of SIV in the mona monkey (*Cercopithcus mona*) in Grenada
- Seroprevalence of HIV-I and HIV-II in pregnant women in Grenada, W.I. Their knowledge of AIDS and their exposure hazards to the virus
- A cross sectional study of the current status of *Schistosoma mansoni* in St. Lucia by field surveys and supplementary data collection
- Identification and characterization of hantaviruses among the mammal population of Grenada
- HIV/AIDS health education and evaluation program in Grenada
- The seroprevalence of *Toxoplasma gondii* in a population of pregnant women and cats in Grenada, West Indies
- The efficiency of diagnosing women of *Toxoplasma gondii* using PCR techniques in comparison with ELISA
- Dengue virus in Grenada: seroprevalence and associated risk factors
- A current appraisal of dengue virus in Grenada - serotype analysis and vector assessment
- A site receptivity study determining the threat of reintroduction of malaria into Grenada through the study of Anopheline spp. mosquito vectors
- Chlamydial infection among STD clinic attenders in Grenada
- Fever in Grenada
- Mosquitoes and Tourism in Grenada

- Effectiveness of a formula feeding/weaning intervention program in preventing transmission of HTLV-1 from seropositive mothers to newborns in Grenada
- A multi-center longitudinal research study of the behavioral significance of the prevalence of HIV-1 infection in pregnant women and their babies on the islands of Grenada and St. Vincent
- A multi-center longitudinal research study of the ethical analysis of informed consent of the prevalence of HIV-1 infection in pregnant women and their babies on the islands of Grenada and St. Vincent
- Determining the role of IL-15 in mediating function of viral-specific CD8+ T cells in the myelopathogenesis of HTLV-1: Symptomatic versus asymptomatic patients
- Intestinal protozoan infections in 6-12 year old children in Grenada
- Intestinal helminth infections in 6-12 year old children in Grenada
- The prevalence of intestinal parasites in school children in rural Guyana
- The prevalence of filariasis and its effects on children aged 8-14 in the central corentyne region of rural Guyana
- **The prevalence of streptococcal infection in school children aged 5 – 15 years in Grenada, Carriacou and Petit Martinique**
- **Studies examining the elimination of lymphatic filariasis as a public health problem in Guyana**
- Seroprevalence of heartworm infection in dogs in Grenada.
- **Dengue in Grenada**
- Assessing the potential risk factors of dengue and dengue hemorrhagic fever in the tri-island state of Grenada, Carriacou and Petit Martinique
- A comparative study to find out if there is an association between sexual practices and knowledge in adult populations of Botswana and Grenada with the prevalence of HIV/AIDS
- HIV/AIDS in rural Botswana differentiating between informing and educating
- Rheumatic Fever in Grenada
- Isolating T cells from Rheumatic Fever positive blood: Immunofluorescent assay of T lymphocytes via fluorescently labeled monoclonal antibodies
- Possible genetic predisposition to Rheumatic Fever: Demonstrating the inheritance fashion of non-HLA B lymphocyte alloantigen D8/17, a marker for Rheumatic Fever
- ELISA antibody titres against group A streptococcal M protein moiety and cell wall N-Acetyl-D-Glucosamine in Grenadian Rheumatic Fever patients
- **Evaluating the effectiveness of educational methods in the prevention of Rheumatic Fever and K.A.P**
- **Prevalence of intestinal helminth infections in rural Grenadian school children**
- **Cystic echinococcosis in Morocco and Uganda**

8.3 Unique projects

- Characterization of five amphibians inhabiting Grenada and subsequent isolation and antimicrobial assay of potential antibiotics derived from their skin
- Mona Monkey studies in West Africa

- Investigation of medicinal plants in Grenada
- Use of medicinal plants in Grenada
- Medicinal drugs from the sea. What do Grenada's waters have to offer?
- Beekeeping in Grenada: Effects of the mite *Varroa jacobsoni* and its control
- Effects of Grenadian Medicinal Plants on Endemic Microbial causes of Diarrhoeal Diseases
- The neurobiological basis of hypoglycemia-associated autonomic failure
- Stimulation of angiotensin 4 in cardiac fibroblasts activates matrix metalloproteinases through MAP kinases pathways: A model for astrocytes
- REM sleep and memory
- End of life care in Grenada
- **Novel antibiotics from tropical marine environments**

9.0 Conferences/meetings/workshops

February Workshop on lymphatic filariasis held in WINDREF.

May-July Dr. C.N.L. Macpherson, T.P. Noel and a number of students from the Graduate Studies and MPH programs and the School of Veterinary Medicine went to Morocco & Uganda.

July Dr. C.N.L. Macpherson presented a paper on the use of ultrasound in screening for parasitic diseases at a CME in Pavia, Italy.

September Dr. C.N.L. Macpherson, Mr. T.P. Noel and a number of faculty from St. George's University, attended and participated in the Basic Research Skills Training Workshop which was held in the WINDREF Research Institute. This workshop was conducted by the Caribbean Health Research Council (CHRC) in collaboration with the Ministry of Health, Grenada and St. George's University.

Dr. Macpherson attended the first meeting on the formation of the Department of Ophthalmology for St. George's University at the Regency Hotel in New York City. This meeting was sponsored by ALCON Laboratories and also included the following:

- An overview of WINDREF
- The structuring and implementation of the Caribbean African Glaucoma study

10.0 Publications/papers/abstracts 2003

- ❖ Asulin, Y., McCann, T.J., McCarty, C.W., Hage, R., Rooney, P. and Macpherson, C.N.L. 2003. Cancer incidence and mortality in Grenada: 1990-2000. *West Indian Medical Journal*, (*in press*).
- ❖ Cox-Macpherson, C. 2004. Forum: The potential for IRBs in developing countries. *Lancet Oncology*, (submitted).
- ❖ Cox-Macpherson, C. 2004. Searching for consensus. *Bioethics*, (submitted).
- ❖ Kabuusu, R.M. McCann, T.J. and Sharma, R.N. 2003. Isolation of bacterial pathogens from table eggs in Grenada. *Journal of the Caribbean Veterinary Medical Association,* **1:** 21-25.
- ❖ Kachani, M., Macpherson, C.N.L., Lyagoubi, M., Berrada, M., Bouslikhane, M., Kachani F. and El Hasnaoui, M. 2003. Public health education/importance and experience from the field. Educational impact of community based ultrasound

screening surveys. *Acta Tropica*, **85:** 363-269.

❖ Kreitschitz, S. and Cox-Macpherson, C. 2003. End of life care: Perspectives from families and caregivers. *West Indian Medial Journal*, **52:** 269-274.

❖ Macpherson, C.N.L., Vuitton, D.A., Gharbi, H.A., Caremani, M., Frider, B., Brunettii, E., Perdomo, R., Schantz, P.M., Felice, C., Teggi, A., da Silva, A., Pawlowski, Z.S., Todorov, T., Pelaez, V., Salama, H., Tinelli, M., Guarnera, E., Lapini, L., Akhan, O. and Hao, W. 2003. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiology settings. *Acta Tropica*, **85:** 243-261.

❖ Macpherson, C.N.L., Kachani, M., Lyagoubi, M., Berrada, M., Bouslikhane, M., Shepherd, M., Fields, P.J. and El Hasnaoui, M. 2003. Cystic enchinococcosis in the Berber of the Mid Atlas Mountains, Morocco: new insights into the natural history of cystic hydatid disease in humans. *Annals of Tropical Medicine and Parasitology*, (submitted).

❖ Macpherson, C.N.L., Bartholomot, B. and Frider, B. 2003. Application of ultrasound in diagnosis, treatment, epidemiology, public health and control of *Echinococcus granulosus* and *E. multilocularis*. *Parasitology*, (*in press).*

❖ Macpherson, C.N.L. and Milner, R. 2003. Performance characteristics and quality control of community based ultrasound surveys for cystic and alveolar echinococcosis. *Acta Tropica*, **85:** 203-209.

❖ Panagos, A., Lacy, E. Macpherson, C.N.L. and Gubler, D. 2003. A knowledge, attitude, practice: Vector and seroprevalence survey of dengue fever in Grenada. *American Journal of Public Health*, (submitted)

❖ MacColl, A.D.C. and Stevenson, I.R. 2003. Stasis in the morph ratio cline in the banaquit on Grenada, West Indies. *The Condor*, **105:** 821-825.

10.1 Completed MSc thesis 2003

Avgeris, Charles. An investigation of Grenadian medicinal plants for antibacterial activity in childhood diarrhea.

Makwinja, Setshidi. HIV/AIDS in rural Botswana: Knowledge, practices and attitudes.

10.2 Seminars

❖ *International financial organizations and health care*. Dr. Steve Miles. 22nd January, 2003.

❖ *Integrating natural and socio-economic sciences to create disaster resilient communities in the OECS.* Ms Valma Jessamy. 29th January, 2003.

❖ *Physician assisted suicide.* Dr. Eliot Sorel. 12th February, 2003.

❖ *Lymphatic Filariasis in Guyana*. Dr. Shamdeo Persaud. 19th February, 2003.

❖ *Rheumatic Fever in Grenada.* Mr. Trevor Noel. 26th February, 2003.

❖ *Opportunities in research for dry forest biodiversity conservation*. Ms. Bonnie Rusk. 19th March, 2003.

❖ *Protein expression and production dendritic cells upon stimulation by filarial antigen.* Ms. Yolanda Ng. 26th March, 2003.

❖ *Decompression sickness among the indigenous fishing population in*

Grenada: Assessing the burden of disease. Mr. Scott Forman. 2nd April, 2003.

- *Determinants of non-communicable diseases in a Muslim population in Tanzania* Dr. Theresa McCann. 9th April, 2003.
- *Bovine neosporosis: Host parasite infections.* Dr. Diana Williams. 16th April, 2003.
- *Prevalence of intestinal helminth infections in rural Grenadian school children.* Mr. Anthony Junck. 23rd April, 2003.
- *Evaluating the effectiveness of educational methods in the prevention of Rheumatic Fever and K.A.P.* Mr. Bishara Baddour. 30th April, 2003.
- *The Zen of Botox*. Dr. David Lennon. 7th May, 2003.
- *The influence of cultural and behavioral factors on cystic echinococcosis in Morocco*. Ms. Ella Cameron. 14th May, 2003.
- *Raccoon roundworm as a cause of animal and human disease.* Dr. Kevin Kazacos. 27th August, 2003.
- *New Technologies and their use in learning.* Dr. Peter Abrahams. 10th September, 2003.
- *Prevention of fouling by reef fish microflora.* Ms. Cynthia Bruno. 17th September, 2003.
- *The World's Children.* Dr. Zuri Amuleru – Marshall. 24th September, 2003.
- *Decompression sickness among the indigenous fishing population in Grenada: Assessing the burden of disease*. Mr. Scott Forman. 15th October, 2003.
- *Lymphatic filariasis: An immunological impact.* Ms. Yolanda Ng. 22nd October, 2003.
- *Functional genomic characterization of alcohol metabolism in sulphate – reducing bacteria.* Dr. Shelley Haveman. 29th October, 2003.
- *The influence of cultural and behavioral factors on cystic echinococcosis in Morocco.* Mrs. Ella Cameron. 5th November, 2003.
- *Screening – Screening as a public health tool.* Mr. Brandon Francis. 12th November, 2003.
- *Plant lipoxygenases at full throttle.* Dr. Mark & Ms. Lydia Williams. 12th November, 2003.
- *Natural history of Borrelia lonestar in the southeastern United States: A newly recognized tick – borne disease.* Dr. Susan Little. 19th November, 2003.
- *Evaluating the effectiveness of educational methods in the prevention of Rheumatic Fever and K.A.P.* Mr. Bishara Baddour. 26th November, 2003.
- *What's for lunch?* Dr. Joanna Rayner. 3rd December, 2003.
- *Prevalence of intestinal helminth infections in rural Grenadian school children.* Mr. Anthony Junck. 10th December, 2003.

Medals were presented to Dr. Macpherson, Trevor Noel and Ella Cameron by the King's representative for services to Morocco.

11.0 Further information

For further information please contact:

Ms. Donna Damm
Administrative Assistant
WINDREF (USA)
1 East Main Street, Suite 154
Bayshore, New York, 11706
Tel: 1 (800) 899-6337
Fax: 1 (631) 665-2796
E-mail: ddamm@sgu.edu

Mrs. Isha English
Executive Secretary
WINDREF (Grenada)
P.O. Box 7, St. George's, GRENADA
Tel: 1 (473) 444-3997
Fax: 1 (473) 444-3041
E-mail: ienglish@sgu.edu

Mrs. Sue Huntington
Executive Secretary
WINDREF (UK)
Kingdon's Yard, Parchment Street
Winchester, Hampshire, SO 23 8AT
Tel: 01962 850650
Fax: 01962 850567
E-mail: Sue_Huntington@sgu.edu

Dr. Svetlana Kotelnikova
Director, CERI
P.O. Box 7, St. George's, GRENADA
Tel: 1 (473) 444-4175, ext. 2465
Fax: 1 (473) 439-1845
E-mail: skotelnikova@sgu.edu

Dr. Calum Macpherson
Director, **WINDREF (Grenada)**
P.O. Box 7, St. George's, GRENADA
Tel: 1 (473) 444-3068
Fax: 1 (473) 444-3041
E-mail: **cmacpherson@sgu.edu**

Mr. Trevor Noel
Assistant Director
WINDREF (Grenada)
P.O. Box 7, St. George's, GRENADA
Tel: 1 (473) 444-3997
Fax: 1 (473) 444-3041
E-mail: trevornoel@sgu.edu