Fighting infection

Dr Sara Irène Eyangoh discusses her research on Buruli ulcer disease in Cameroon, with a focus on its enigmatic transmission and infection pathogenesis. She also outlines the studies she has undertaken with French collaborators Drs Arnaud Fontanet, Laurent Marsollier and Jean François Guégan

Could you outline your background as a researcher? What influenced you to investigate Buruli ulcer disease (BUD)?

I am a microbiologist with extensive experience in mycobacteriology. My earlier work researching molecular epidemiology of tuberculosis in Cameroon gave me the insight to move to another mycobacteriological field – BUD. Furthermore, the technical platform already available at the Centre Pasteur du Cameroun for *Tuberculosis bacilli*, has facilitated the molecular diagnosis of *Buruli bacilli* and the development of a new research programme on this topic.

What is BUD and why has it become more prominent in the past 20 years?

BUD is a necrotising skin and soft tissue disease caused by bacteria called *Mycobacterium ulcerans*, which belong to the same family as those causing leprosy and tuberculosis. Though the disease is rarely fatal, victims are often left deformed with permanent scarring. Discovered over a century ago, the disease has become more prominent in the past 20 years, correlating with environmental changes such as dam constructions. Its prevalence influenced the World Health Organization (WHO) to create the Global Buruli Ulcer Initiative in 1998, dedicated to raising awareness of the disease while promoting research endeavours to develop better tools for treatment and prevention. The Initiative subsequently roused much interest among scientists who are passionate about investigating this important public health problem.

This affliction is the third most prevalent mycobacterial disease in the world, and has been reported in over 30 tropical and subtropical countries. Despite this, relatively little is known about BUD – why do you think this is?

Limited knowledge of BUD could be explained by the focal distribution of the disease and the fact that it affects mainly poor, rural communities in tropical countries, with the surprising exceptions of Australia and Japan. Furthermore, only a few laboratories in the world are working on this disease, which is the latest to be classified as a neglected tropical disease by WHO. Research into BUD has only been underway for around a decade, and research funding is not easy to mobilise.

Can you describe studies that you have carried out to identify risk factors associated with BUD? What were the results of these studies?

We conducted two case-control studies – one near the marshes of the Nyong River (Akonolinga, Ayos) at historical sites of infection, and another which is an emerging area for Buruli ulcer prevalence located in the Bankim region – which presents different geographical and cultural factors from those along the Nyong basin. During the investigation, characteristics and lifestyles of patients suffering from BUD were compared with those of healthy individuals. The main risk factors identified were: contact with stagnant water; wearing clothes that do not cover the entire body during farming activities; and improper care of skin wounds. One relevant and innovative result was the protection offered by mosquito nets. The understanding of the role of mosquitoes and other peri-domestic insects in the transmission of the disease opened up new research avenues, and this work is currently in progress.

What led to the initiation of your programme with the Institut Pasteur International Network to target this disease in Cameroon?

We initiated the programme to meet the demand of the Cameroon health authorities to facilitate the diagnosis of suspected BUD cases and to help improve mapping of endemic sites, because Centre Pasteur du Cameroun is the National Reference and Public Health Laboratory. As a member of the Institut Pasteur International Network, we quickly benefited from their support and implemented diagnostic strategies, subsequently launching research and training programmes.

How have you advanced understanding of the transmission and epidemiology of *Mycobacterium ulcerans*, the pathogen that causes this disease?

*M. ulcerans* transmission and epidemiology remains frustratingly difficult to understand. But gradually, the puzzle is being deciphered. Our work showed the presence of *M. ulcerans* in the saliva of water insects in natural conditions, reinforcing our hypothesis regarding their role in the transmission and/or spread of the pathogen. A large-scale study conducted in a non-endemic area and an endemic area described seasonal and regional dynamics of aquatic bugs correlating with the rate of infection of *M. ulcerans*. It was not, however, identified in non-endemic areas, suggesting that the detection of mycobacteria could be used as a marker for monitoring environmental risks related to the emergence of BUD.

In addition to your field work, which other activities were embedded in your programme?

In order to ensure the sustainability of Buruli ulcer control activities, we initiated a training programme on the microbiology of *M. ulcerans* for endemic countries of Buruli ulcer in Africa, supported by Institut Pasteur International Network, WHO and Fondation Sanofi Espoir. By the end of four training sessions, more than 50 technicians and scientists from 12 African countries (Benin, Cameroon, Congo, Ghana, Togo, CAR, Gabon, Ivory Coast, Nigeria, Mali, DRC and Gambia) were trained. The aim was to improve the quality of diagnosis in other endemic countries and to ease the use of diagnosis via polymerase chain reaction as a marker for monitoring environmental risks related to the emergence of BUD.
A battle in the tropics

A team from the Centre Pasteur du Cameroun, Cameroon, together with French researchers from the Institut Pasteur, INSERM and Institut de Recherche pour le Développement, are identifying the risk factors and exploring the underlying mechanisms that drive the development of Buruli ulcer disease

ONE OF THE World Health Organization’s (WHO) 17 neglected tropical diseases, Buruli ulcer disease (BUD) manifests itself as a debilitating skin and soft tissue infection caused by Mycobacterium ulcerans, leading to the development of large ulcers, scarring and, in some cases, long-term functional disability. Though the causative organism has been established as M. ulcerans, the precise transmission mechanism of the disease is not yet fully understood.

SEASONAL AND REGIONAL DYNAMICS
Dr Sara Irene Eyangoh, Head of the Mycobacteriology Unit and Scientific Director at the Centre Pasteur du Cameroun in Cameroon, aims to better understand the underlying mechanism of transmission and pathogenesis in BUD, as well as exploring prominent risk factors and effective prevention methods. The disease is commonly associated with stagnant and slow-flowing water and previous research work suggested that transmission could occur via water-borne insects. Dr Laurent Marsollier and his colleagues corroborated these findings with his colleagues corroborated these findings with a multidisciplinary approach combining inventory of aquatic invertebrates from different environments allowing a description of their spatio-temporal distribution and coexistence with M. ulcerans strains in the environment. Dr Guégan explains: “The reconstruction of environment-microbe communities based on real data collected during one year will permit us to reconstruct scenarios of disease agent persistence and spread, and better understand the environmental conditions that are more favorable for the transmission of the microbe from the environment to humans”. Additionally, the group welcomed five PhD students and one postdoctorate candidate to the framework. An inventory of aquatic bugs from various endemic and non-endemic sites in Cameroon have just identified nine families of water bugs on the basis of their morphological characteristics: Belostomatidae, Corixidae, Gerridae, Hydrochidae, Hydrodrometridae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae and Veliidae – all confirmed using molecular barcoding which involved sequencing the mitochondrial genome (COI, COII, 866pb, 102 sequences).

Furthermore, in collaboration with Dr Timothy Stinear of Melbourne University, Australia, an expert in genomics and molecular biology of mycobacteria, Eyangoh is utilising genomic studies of M. ulcerans to aid with the understanding of its evolution and spreading patterns: “This is done through whole-genome sequencing techniques to allow phylogenetic

AQUATIC INSECTS
To better understand the biology and ecology of aquatic bugs, and to decipher transmission dynamics of the pathogen, the group built a multidisciplinary approach combining inventory and classification of all aquatic invertebrates, as well as domestic insects. They followed up the presence of M. ulcerans in all collected micro- and macroinvertebrates from different environments allowing a description of their spatio-temporal distribution and coexistence with M. ulcerans strains in the environment. Dr Eyangoh elaborates. “The reconstruction of environment-microbe communities based on real data collected during one year will permit us to reconstruct scenarios of disease agent persistence and spread, and better understand the environmental conditions that are more favorable for the transmission of the microbe from the environment to humans”. Additionally, the group welcomed five PhD students and one postdoctorate candidate to the framework. An inventory of aquatic bugs from various endemic and non-endemic sites in Cameroon have just

RISK FACTORS
In order to identify the risk factors associated with the pathogenesis of BUD, Eyangoh conducted two case studies: the first near the marshes of the Nyong River in 2006 and the second in an emergent Buruli area in the region of Bankim in 2009.

Together with Marsollier and Fontanet, Eyangoh explored BUD risk factors in Cameroon using the largest ever face-to-face questionnaire published for the disease, contrasting confirmed cases with community-matched control individuals. Risk factors were monitored in 163 pairs, revealing low levels of education; frequent swamp wading; the wearing of short, lower body clothing while farming; housing near a cocoa plantation or woods; the use of adhesive bandages to treat wounds; and the use of mosquito coils as all affecting infection rate.

Many of the risks were correlated with exposure to water, and especially to the Nyong River swamp – identified from univariate community-matched and family-matched analyses. There was much colinearity, as wading (community-matched analysis) and swimming (family-matched analysis) being the highest associations.
matched analysis) in the Nyong River were established as risk factors, whereas fishing was not.

**PREVENTION AND TREATMENT**

Despite extensive diversity in geographic and cultural factors of the two Cameroonian regions studied, they both demonstrated the effective protection offered by mosquito nets against infection. Bed nets are primarily used in Cameroon to prevent malaria, an endemic disease in the country, and Eyangoh’s group observed that BUD was less common among families using bed nets than those without them. This finding thus corroborates the hypothesis of BUD transmission via insect vectors – however, similar studies conducted in Ghana have failed to demonstrate this link, signalling the need for further research.

In an independent investigation, Marsollier discovered that bites from *M. ulcerans*-free aquatic insects conferred some form of immunity against BUD infection. In the same vein, washing clothes daily in waters where these insects are prevalent was also observed to provide some protection. Adopting a natural curative approach – using leaves rather than adhesive bandages – was also shown to decrease infection somewhat, most likely due to the antiseptic or astringent active principles of leaves.

Eyangoh explains that, despite ongoing analyses of collected data, ecological indicators could certainly be developed for this pathogen: “Preliminary findings suggest that specific macroinvertebrate taxa may be used as aquatic biological indicators of *M. ulcerans* persistence and spread within aquatic environments. There are also some taxonomic or functional groups of aquatic organisms that could be used as biotic integrators (or tags) of *M. ulcerans* presence and concentration”. Clearly such indicators of disease prevalence in a region could be of great importance for understanding and preventing the infection in humans.

**A HEALTHCARE STRATEGY**

The key strategy for dealing with BUD, when prevention is not possible, is early detection and treatment before the disease progresses. To encourage patients to seek medical attention at an early stage, hygiene guidelines should be implemented to influence a change in behaviour, which can be challenging. Eyangoh explains that early presentation to the hospital for diagnosis is limited by stigma – communities often have superstitious beliefs about the causes of the disease and its management, and will preferentially seek treatment from traditional practitioners. There is also a lack of awareness when it comes to available treatments and their effectiveness, and poor access to health facilities and trained health staff.

In order to tackle people’s objections to treatment, the group has used the knowledge gained from their epidemiological investigations to develop an educational message to the public under the theme ‘Health and education: new weapons against Buruli ulcer’. With support from Cameroon’s Ministry of Health, and an eye-opening documentary film, Night Fire: Buruli ulcer and beliefs, funded by the French Ministry of Foreign Affairs through the Institute for Research and Development, they aim to tackle misconceptions about this affliction and make a real impact on its spread in communities.

**KEY COLLABORATORS**

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Dr Sara Irène Eyangoh received a PhD in microbiology from the Paris Diderot University-Paris 7, France, and since 2002 has been leading the Mycobacteriology Service of Centre Pasteur du Cameroun (CPC), National Reference laboratory for tuberculosis and Buruli ulcer. She was appointed scientific director of CPC in 2012 and defended her ‘Habilitation à Diriger les Recherches’ in 2013 at the Paris Diderot University-Paris 7, France.