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The fight against Malaria and other related mosquito-born Diseases

Results and proposed next Steps of the

Rotary Seminar at the University Prishtina (Kosovo)

2019



Bekämpfung von Malaria und weiteren von Moskitos übertragenen Krankheiten

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Thanks to all who contributed to the success of the Seminar



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Organizers of the Seminar in Pristina

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Foreword /Preface

The inter-country committee Germany-Turkey was founded in 1978, and since then has been involved in fostering relations between Turkish and German Rotary Clubs. To this end, apart from the annual meeting between the two groups, there have also been youth exchanges and workshops.

At the ICC annual congress in 2018 in Antalya it was decided that in addition to Polio plus, a further worldwide health problem would be taken on. There was also a wish to create an event format that would serve (to promote) peace in the Balkans.



The concensus of the conference was to take on the problem "Malaria and other mosquito-borne diseases", especially as the global warming expected in the next few years could well result in pandemics of this disease.

Fortunately, the Rotary Clubs of Radolfzell-Hegau and Şişli-Istanbul were prepared to take on the sponsorship of the malaria seminar together with the

ICC Germany-Turkey. President Ilir Krasniqi and Erik Mackinlay of The Rotary Club Pristina International were prepared to guide this international workshop as a "Hands-On Project".

We would like to thank our friend Matthias Feil for the implementation of the workshop with modern management methods. The results of the workshop impressed even experts in the field. The large creative force that can be generated by groups of different nationalities was shown by the young people from Greece, Turkey, Kosovo



and Germany. During this seminar, held in English, there was no trace of any differences of nationality among the participants – we were one big Rotary family. The workshop was thus a small contribution to peace in the world, especially in the Balkan region.

Dr. Wolfgang Uebel President ICC DE-TR & Prof. Dr. Manfred Raupp Secretary

Bernhard Hertrich: Liebe rotarische Freundinnen und Freunde, wir können dieses Jahr ein kleines Jubiläum feiern. Seit zehn Jahren bestehen unsere besonderen Kontakte zum Rotary Club Şişli - Istanbul. In diesen zehn Jahren hat sich ein freundschaftliches Verhältnis entwickelt, welches wir so am Anfang dieser Beziehungen noch nicht erkennen konnten.

Besonders erfreut bin ich darüber, dass sich diese Beziehung unserer beiden Clubs, ganz im Sinne von Rotary International, über alle politischen Turbulenzen hinweg immer weiterentwickelt hat.

Ein Höhepunkt dieser gemeinsamen Beziehungen und dieser gemeinsamen Aktivitäten sind sicherlich die von beiden Clubs gemeinsam zusammen mit dem Deutsch-Türkischen Länderausschuss durchgeführten Seminare und Workshops.

Diese dienen einerseits der Wissensvermittlung der jungen Generation, sie dienen andererseits aber auch und gerade der Festigung der Freundschaft zwischen unseren beiden Clubs.

All dies wäre natürlich nicht möglich gewesen ohne die treibende und energische Kraft unseres Freundes Wolfgang Uebel. Wolfgang hat mit Beharrlichkeit und Enthusiasmus diese Freundschaft unserer beiden Clubs vorangetrieben und vor allem auch die unendliche Mühe der Organisation dieser Treffen, insbesondere der Seminare und Workshops vorangetrieben. Ja, er ist sicherlich DER MOTOR in unserer gemeinsamen Beziehung, der uns immer wieder vorantreibt und voranbringt.

Sehr herzlich und ganz besonders möchte ich mich aber auch bei Matthias Feil bedanken. Dieser hat sich jedes Mal ohne zu zögern bereit erklärt, die Workshops zu organisieren und zu leiten. Wir sind stolz darauf, einen solchen Freund in unseren rotarischen Reihen zu haben.

Nicht zuletzt danken darf ich auch allen Freunden unseres Clubs, die bereit waren, dieses gemeinsame Projekt mit dem Rotary Club Şişli – Istanbul ideell zu unterstützen. Ich weiß, dass dies keine Selbstverständlichkeit ist. Ich wünsche uns allen, dass es gelingen möge, diese freundschaftlichen Bande mit dem Rotary Club Şişli – Istanbul weiter zu festigen und zu fördern und noch viele gemeinsame Projekte mit unseren Freunden durchzuführen.

Bernhard Hertrich Präsident des Rotary Club Radolfzell-Hegau

Hale Erel: RYLA: A great project of Rotary Inter-country Committee (ICC)

Germany/ Turkey planned to motivate an International RYLA project. We, Istanbul -Şişli RC (D2420), together with Radolfzell Hegau RC (D1930) happily took part in this inspiring project, where we both have been uniting our abilities for World Understanding and Cultural Exchange joint projects for more than years.

As of this sentence an international project, RYLA (Rotary Young Leadship Awards) education and leadership seminar was planned and held this year in Pristina.

In this period, where international acquaintance is very important, the participation of university students from Germany, Greece, Kosovo, and Turkey were truly pleasing.



RI President's Regional Representative PDG Murat Çelik and Rtn. Hulusi Gencay from RC of Şişli and Rtn. Wolfgang Uebel from RC of Radolfzell Hegau together with other ICC Members were involved with young people to create a very good friendship and education this year. Young people know each other and have made the beginning of a friendship and acquaintance that will continue in the years to come.

We thank Murat Çelik, Wolfgang Uebel and Hulusi Gencay as well as Rtc. Aysu Ozge from Şişli Rotaract Club and other attendees coming from different towns of Turkey and different countries who made this seminar an unforgetable one. With three days of training and a cultural trip, this adventure has been a lifelong memorable experience for the participants. I hope that in the years to come, we will continue our contribution by getting a chance to repeat this beautiful project because the youth in Rotary is the future. We also would like to thank to the Rector of University of Prishtina and to the President and Members of RC Pristina International for the facilities offered and their very warm hospitality.

Rtn. Hale Erel, Istanbul – Şişli RC President 2018 - 19

Ilir Krasniqi: From 10 – 14 April, 2019, the RYLA (Rotary Youth Leadership Awards) seminar was held in Pristina.

As Rotary Club Pristina International we were pleased to help in the organizing of this seminar, which brought together students from Turkey, Germany, Greece and Kosovo.

One of the guiding principles of Rotary is "to build goodwill and better friendships" and it was, therefore, a pleasure to see how well the students worked together.

The maturity and creativity of the ideas, which the students presented to us at the end of the seminar, were frankly amazing.

All in all it was a wonderful experience for everyone, also for us as host club.

I would also like to express my gratitude to all those who decided to bring the RYLA seminar to Kosovo as it gave us a chance to show the real Kosovo - a welcoming and attractive place to visit and study.



Ilir Krasniqi, Rotary Club Pristina International President 2018 - 19

Prof. Dr. Marjan Dema: Opening Speech of the Rector of the University Prishtina

Dear students, Dear experts, professors, and participants,

I am proud to be a member of such a wonderful organization Rotary International and to lead the most influential university in Kosovo, with our moto "Service above self", based on the four way test of the thing we



say and do: Is it the truth, Is it fair to all concerned, Will it build good will and better friendship and Will it be beneficial to all concerned.

It is my pleasure to greet you on behalf of University of Prishtina "Hasan Prishtina" in opening of the international seminar "*Neglected problems of Malaria and other mosquito related diseases*". At the same time, as rector of UP, I would like to give you a warm welcome at our university campus, wishing that the time passed here will form a great experience for you.

University of Prishtina joined the initiative of organizing this seminar since it was initially launched. I consider it significant, because the seminar touches one important topic to our community, a topic that has not been dealt with enough attention until now by our country's researchers. This seminar has drawn a lot of academic and public attention, gathering also selected students from Kosovo, Germany, Turkey, and Greece. I am convinced that this multicultural audience is the adequate one, being all the participants opened to study the problem o of mosquitos. I am sure the student's participants in the seminar will soundly contribute to reduction of malaria and other mosquito related diseases.

The student's participants in this seminar, including the ones coming from University of Prishtina, are engaged in voluntary work, promoting volunteering as one of the most salient values. Volunteering is one of the values I try to cultivate working with you as rector, and as a Rotarian. When it comes to valuing the volunteering work, I would like to cite one saying, which I particularly like: *"Volunteers do not get paid, not because their work is not worthy, but because their work is priceless".* It is probable that my appreciation to volunteering work derives from my Rotarian perspective; however, to me volunteering is not a usual activity; to me volunteering is a heart motivated activity. Volunteering enriches and fulfills the person in a way no other activity can do. I encourage each and every one present in this seminar to continue conducting volunteering work, to contribute to the well-being of the community, while not forgetting to include your friends and colleagues in such initiatives.

The seminar we are opening today has gathered in our campus participants from different countries in Europe. This strengthens the value of multiculturalism of our society. The seminar will contribute in exchanging of knowledge and experiences among the participants.

Since we are in the campus of the University of Prishtina, I am sure you would like to know more about our university. Allow me to inform you that University of Prishtina is confidently continuing the transformation path undertaken during the last three years. We should emphasize that UP is in the best conditions since its establishment almost 50 years ago. University of Prishtina has gone through very hard times especially since 1981. UP during year 2018 was included in well-known databases and was ranked the most highly since its existence. We have established and functionalized different supportive structures supporting research and other related activities. Innovation, teaching, and internationalization are other domains UP advanced significantly during the last year. UP's progress is based on fundamental values of higher education such as integrity, equal access, accountability, academic freedom, transparency, responsibility, and social responsibility.

Once again, I would like to express my pleasure of having the opportunity to be among you. I would also like to congratulate the organizers for the work done in organizing this wonderful seminar. At the end, I would also like to wish fruitful discussions, and ideas during the seminar.

Prof. Dr. Marjan Dema Rector **Murat Çelik** PDG Rotary Inernational Good Morning,

My name is Murat Çelik. I am the Past District Governor from Turkey and I am working since 5 years as the Rotary International Presidents representative for the Non Districted Clubs of Albania and Kosovo.

This is the first time we are cooperating a RYLA Seminar in conjunction with a Symposium. I am sure, with your help and involvement, it will be a success.

We will all benefit from this meeting. We will listen to experts and learn from them. We will meet new friends and enjoy their friendship. Your friendships



are very important for your future. In your future we will probably not be around but you and your friends will. Take good care and value it.

Nelson Mandela in one of his speeches said and I quote: The most powerful tool to change the world is education.



I wish you all the best for your time in Kosovo and enjoy every moment.

Assoc. Prof. Dr. Norbert Becker: Mosquitos and their control, Mosquitoes – their burden, systematics, vector biology and control

by PD Dr habil. Norbert Becker, Scientific Director of the German Mosquito Control Association (KABS); Executive Director of the European Mosquito Control Associtaion (EMCA); Associate Professor at the University of Heidelberg, Germany.



In his presentation Dr Becker highlighted that mosquitoes are the most dangerous organisms for human beings. Each minute a human is dying because of a mosquito bite, mainly children in the age group of 1 to5 years and mostly in Africa, south of the Sahara. Amongst the more than 3500 mosquito species known today, about 1000 species can transmit diseases and 60 species mainly anophelines (Malaria mosquitoes) are serious vectors. Still more than 3 billion people are at risk to be infected by mosquito-borne diseases. Each year the WHO counts about 216 million malaria infections and more than 500.000 deaths. Arboviruses like dengue, dengue haemorrhagic fever, Chikungunya, Zika and Japanese Encephalitis become more and more important when we consider that each year more than 390 million people are infected by arboviruses (arthropod-borne viruses). However, the case fatality rate is much lower than with malaria. It is estimated that less than 50.000 people die each

year due to a good case management. Lymphatic filariasis is not deadly but at least 120 million people are infected by nematode worms. Mosquitoes also changed the world-politics when we consider that Alexander the Great died (323 BC) on a mosquito bite in Bagdad in the age of 32 years when he almost ruled the known western world at that time.

Mosquitoes look back to a long evolutionary development. They are known since the Mesozoic period (>100 million years ago) and have already bitten dinosaurs. Human beings (Homo sapiens) are only 200.000 years on the globe, our ancestors about 5 million years. So mosquitoes are 500 times longer on the globe as human beings. As a result of this long evolutionary process mosquitoes adapt to a great variety of aquatic habitats and can be found in almost all kinds of standing water e.g. heavily organic polluted (cess pools) or unpolluted freshwater, small water collections (buckets, vases), temporarily flooded plains, swampy woodlands, rice fields, rainwater barrels, water catch basins, tree holes or rock pools, only to mention a few. We know at present 3.528 mosquito species, in Europe more than 100 species and in Germany 52 species. Since 1995 six new species were introduced to Germany by the globalisation favourite by climate change.

A result of the long-term evolution is frequent zoonosis. Usually mosquitoborne diseases are zoonosis, pathogens are transmitted from mosquitoes to animals, but in the course of co-evolution some of the disease pathogens spread to the human population. We know today more than 500 viruses (100 infect humans and 40 life stock) transmitted by mosquitoes. Five human protozonooses (Malaria parasites) are transmitted by mosquitoes and last but not least nematodes (worms) like *Wucheria, Brugia* or *Dirofilaria*. All diseases are very old: Malaria is known since more than 2500 years, dengue more than 1000 years and lymphatic filariasis more than 3100 years. The co-evolution of

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vectors, pathogens/parasites and humans resulted in a complex life cycles which are difficult to interrupt e.g. by the development of vaccines.

Frequently mosquitoes are diminishing the life quality as well, especially in river valleys with wide-spread inundation areas and floods in the summer time. Not seldom, more than 1000 females of the so-called floodwater mosquitoes can attack a person in less than 2 minutes e.g. in the Upper Rhine Valley and the consequence is that people cannot spend time outside their houses from late afternoon or restaurants are empty, people sell their properties which loose value due to the natural disaster with mosquitoes. In many areas the demand for mosquito control is great. The economic loss in the Upper Rhine valley alone amounts to 12 million Euro. Therefore, more than 20 organisations in almost all European countries conduct mosquito control dealing with more than 2 million hectares of breeding sites. These organisations are usually members of the "European Mosquito Control Association" (EMCA).

Before mosquito control operations are initiated studies on the biology of the mosquitoes has to be conducted. All mosquitoes need a water body for their development. About 4 days after the blood meal the mosquito females lay either single eggs (Floodwater mosquitoes, e.g. *Aedes vexans,* tiger mosquitoes, *Aedes albopictus*) above the water line or egg batches (e.g. house mosquitoes, *Culex pipiens*) on the surface for instance in water containers. Water and temperature are important factors for the development of the mosquitoes. The higher the temperature the faster is the development in the water. All mosquito species have 4 larval and one pupal instar in the water body before the adults are emerging from the pupae.

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Both sexes of the adults need nectar or other sugar containing fluids for their life activities. However, mosquito females need a blood meal to develop eggs. They need proteins from the blood source for the egg yolk development, therefore only mosquito females are biting. Before the blood meal the mosquitoes have to mate. Usually the males build swarms and attract the females by the sound of their body fibrations (ca. 600 Hertz). The females are flying into the male swarm and are caught by a male mosquito to transfer the sperm into spermateca. Now the female needs a blood meal to develop the eggs.

The females are attracted by the breath of the host for the blood meal especially the carbon dioxid as well as by lactic and butter acid and other substances as components of sweat. The female pierce the skin by 6 stylets into the blood vessel and suck about 3 times of its body weight to lay between 100 and 400 eggs. They can suck several times and lay several egg batches without a new copulation. Before they suck blood, they release saliva with proteins and histamine into the wound to avoid blood agglumination and to support the blood sucking process. Humans react with allergic reactions with a small inflammation against the saliva.

According to their biology we can differ between several mosquito groups:

a) **The floodwater mosquitoes:** Here the females lay their single eggs in depressions in the floodplains which are frequently flooded. When the eggs are flooded during increasing water levels the larvae are hatching and develop through 4 larval and one pupal instar to the adults. The number of floods influences the abundance of the floodwater mosquitoes. The adults of some species like *Aedes vexans* and *Ae. sticticus* can migrate long distances (up to more than 15 km) when they search for a victim for the blood meal (humans or animals). The floodwater mosquitoes are usually the species which cause

tremendous nuisances. Notseldom > 100 million larvae per hectare flooded area can be counted. The adult floodwater mosquitoes can live several weeks per generation. In autumn the adults are dying and the larvae overwinter in the egg shell. They can survive several years in the egg shell if no flood occurs. The tiger mosquitoes have a similar biology but they don't lay their eggs in ponds but usually in artificial breeding sites also above the water line such as rain water barrels or buckets with water. They hatch when the water level in the container raises e.g. due to rainfall or when people refill the barrels.

2) The house mosquitoes such as *Culex pipiens* or *Culiseta annulata*: These mosquitoes overwinter as females in stables or cellars where the frost is absent. In spring time usually in April in Central Europe they leave the hibernation places, suck blood usually inside the houses during night (sleeping rooms) and lay their eggs on the surface of water bodies, usually rain water containers in garden areas, but also in a large variety of different natural and artificial breeding sites. After two days of embryonic development the larvae hatch straight into the water body and develop via four larval and one pupal instar to the adults which usually search for a blood meal close to their breeding sites and frequently during nights in sleeping rooms when humans search for sleeping.

3) **The Fever mosquitos (***Anopheles species***).** In Germany we have 7 *Anopheles species.* Some of them such as *Anopheles messae* or *Anopheles daciae, An. atroparvus , An claviger* or *An. plumbeus* were transmitting malaria e.g. in the Upper Rhine Valley or along the coasts in northern Germany where they developed in marshes. The most common anophelines prefer semi-permanent or permanent water bodies with vegetation. They bite humans but some prefer frequently cattle as host for the blood meal. They overwinter also as adult mosquitoes and occur usually not in great numbers. *An. plumbeus* can be a

great pest in rural areas where abundant farming occurs with non-used cess pits as mass breeding sites of *An. plumbeus*. This species bites also during daytimes. Globally the most dangerous mosquito is a *Anopheles* species, namely *Anopheles gambiae* in Africa which kills each minute a person.

4) Beside the above described groups we have also more rare species such as *Coquillettidia*, *Uranotaenia* etc.

Major Mosquito-Borne Diseases

Malaria: The human malaria parasites are: *Plasmodium falciparum* (causing Malaria tropica); *Plasmodium vivax* (Malaria tertiana); *Plasmodium ovale* (Malaria tertiana); *Plasmodium malariae* (Malaria quartana) and *Plasmodium knowlesi*. The parasites possess a very complex life cycle in humans (in the liver and red blood cells) and in mosquitoes. We have more than 60 important malaria vectors, but the most serious one is *Anopheles gambiae* s.l., the main vector of the frequently deadly Malaria tropica which occurs in Africa south of the Sahara. Each year we have 216 mill. new clinical cases of which 80% occur in Africa. 500.000 people mainly children are dying each year according to the WHO and the costs for malaria control alone in Africa amounts to approximately 2 bill. US\$.

Arbovirosis: The main arbovirosis are caused by the so-called Flaviviruses (belonging to the Yellow fever group). The main diseases are dengue, dengue haemorrhagic fever, Zika and West-Nile fever. The main vectors of dengue and Zika are the tiger mosquitoes, *Aedes aegypti* and *Aedes albopictus* which breed predominately in human settlings (e.g. in water barrels etc).

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Most problematic is dengue haemorrhagic fever which is caused by a secondary infection with a different serotype than the first dengue infection which cause usually mild symptoms. However, the antibodies produced by the human immune system during the first infection are not able to neutralize the viruses of a serotype different of the one of the first infection. The viruses can proliferate during the second infection in epithelial cells of the blood vessels which become permeable and the victim is dying on bleeding to death.

West-Nile viruses are transmitted mainly by *Culex* mosquitoes like our house mosquito *Culex pipiens,* which occurs wide-spread in Europe and world-wide. Usually it is a zoonosis between birds and mosquitoes, however, humans and horses can also be infected and this can be deadly. In 2018 almost 1500 people were infected in Europe and almost 200 people died (e.g. in Greece: 45; Italy and Romania: each 42; Serbia 35).

Chikungunya viruses are alpha-viruses and also transmitted by tiger mosquitoes (*Aedes albopictus*). The first outbreak in Europe occurred in 2007 in Italy when about 300 people were infected and one person died. The vector was the Asian tiger mosquito *Aedes albopictus* which was imported as neozoen by used tires to Italy in 1990 and spread since that along the mediterranean coast and even to Germany as blind passengers in vehicles coming from Italy.

Lymphaitic filariasis is caused by a nematode, mostly *Wuchereria bancrofti* which is transmitted mainly by *Culex* mosquitoes. The adult worms develop in the lymph system and can block the lymph fluid which leads to swelling of legs etc (Elephantiasis).

The fight against Mosquitoes

The basis for the fight against this diseases was the understanding of the role of mosquitoes in the transmission of the pathogen/parasite. Walter Reed (1851-1902) proved conclusively that mosquitoes carry yellow fever from person to person. Sir Ronald Ross demonstrated in 1897 as military physician in India that anophelines transmit malaria. The discovery of Quinine extracted from the bark of the Cinchona tree was a breakthrough in the fight against malaria. Today we have some synthetic drugs or combination of them e.g. malarone which kill stages of the Malaria parasite (*Plasmodium* spp.) in the human body. Natural derivates such as artemisinin deriving from *Artemesia* are still very important weapons in the fight against malaria.

Control of the mosquito vectors

The fight against mosquitoes is a steady fight between "cerebral (human) and evolutionary (mosquito) intelligence. The development of resistance of mosquitoes is a good example how mosquito neutralize "human weapons". What we could learn from the past is that we have to use all weapons which we have to our disposal and that we have to use them in an integrated manner. Col. William Gorgas, 1904, head of the sanitary department in Panama demonstrated this when the Panama Canal was build. Only by the appropriate control of Malaria and Yellow fever and their vectors, the Panama Canal could be build. Gorgas approach comprised: drainage of the water, larviciding, brush and grass cutting, prophylactic quinine administration, screening of the patience and adult mosquito killing.

With the discovery of DDT (Dichlorodiphenyltrichloroethane) at the beginning of the 20th century the modern mosquito control started. A drawback was the

quick onset of resistance against DDT and the environmental residues and accumulation in the food chain (fat body). Therefore, DDT is banned in many countries in the second half of the 20th century in Europe and the search for alternative insecticides to the organochlorines started. The second generation was the organophosphates (developed 1932) which do not persist in the environment, but they are more toxic than e.g. DDT. The third and fourth generation were the carbamates (developed in the 1950s) and pyrethroids (in the 1960s). In the 1970s the search for biorational insecticides like "Insect Growths Regulators (IGRs)" and microbial control agents (*Bacillus thuringiensis israelensis* (B.t.i.) and *Lysinibacillus sphaericus* (L.s.) started.

In many programmes integrated approaches are undertaken favorited by the World Health Organisation (WHO) as in the "Roll Back Malaria Programme (RBM)" - an alliance of international, national governmental organisations and NGOs (e.g. WHO, Wold Bank, UNICEF, Bill and Melinda Gates foundation, PMI). The RBM programme is mainly based on the use of a) long-lasting insecticidal nets (LLINs) treated with pyrethroids, b) indoor residual spraying (walls are sprayed with insecticides as DDT, bendiocarb or pyrethroids) to kill resting anopheline femals inside the houses) and c) effective diagnosis and medical treatment of malaria cases. This strategy lead to a significant reduction of malaria cases especially in Africa.

However, there are also drawbacks of this strategy:

1) onset of resistance against pyrethroids;

2) human behaviour (people stay outside their houses during the transmission time for *Plasmodium* in the evening and have no protection by the nets);

3) The strategy targets endophagic and endophilic *Anopheles* species like *An. gambiae* which bite inside the houses and stay after the bite inside the houses.

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However, exophagic and exophilic species which bite outside the houses are not killed and still transmit malaria. Exophilic species have a positive selection pressure and become more abundant. Today the number of malaria cases is increasing in some areas again.

As a conclusion, the practised strategy had to be altered and should be more integrated and include also "Larval Source Management (LSM)" what is supported by WHO. LSM comprises source reduction (e.g. removal of breeding sites for anophelines and larviciding mainly with microbial control agents such as B.t.i..

Control programmes in Europe e.g. the programme of the German Mosquito Control Associtaion (KABS) can be an example for the successful implementation of integrated biological control strategies.

Mosquito Control in Germany as an example of an successful approach

The control of mosquitoes in Germany has a long history. In the 1920's and 1930's breeding sites were treated with petroleum oils. During the 1950's and 1960's adulticides were used. In the early 1970's, the mosquito population was extremely high because of frequent fluctuations of the water level of the Rhine. The people in the villages couldn't spend any length of time outside their houses. There was an attack rate of more than 1000 female mosquitoes per minute. As a reaction to this natural disaster 44 towns and communities in the Upper Rhine valley on both sides of the river Rhine merged their interest in a united mosquito control programme, the KABS (Kommunale Aktionsgemeinschaft zur Bekämpfung der Stechmückenplage e.V.) which was founded in 1976. Nowadays, 100 municipalities along a 310 kilometre stretch of the Upper Rhine River, with a total population of 2.7 million people, have joined forces to control the mosquitoes, mainly Ae. vexans over a breeding

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area of some 600 km² of the Rhine's flood-plain. The budget of the program is approximately 4 million Euros a year which results in overall costs per person per year of approximately 1.5 Euro.

The overall concept is integrated biological control (IBC) and to integrate the protection of humans against mosquitoes and the conservation of biodiversity. When the ecosystem is compared with a web and each group of organisms represents one mesh, the strategy of the KABS aims at the reduction of the mesh representing the floodwater mosquitoes without cutting other meshes in the "food web" and thus keeping the stability of the ecosystem.

This goal could only be reached in an optimum when biological control methods are used. The conservation and encouraging of predators is an important goal of the programme. Therefore, microbial and biological methods are integrated with environmental management (e.g. improving of the ditch system for regulation of the water level and providing of permanent habitats for aquatic predators such as fish).

The discovery of the gram-positive, endospore-forming soil bacterium, *Bacillus thuringiensis* subsp. *israelensis* (B.t.i.) in the Negev desert of Israel in 1976 by Yoel Margalit, has opened the door for the use of microbial control agents as B.t.i. The outstanding advantage of this control agent is its specificity. It kills exclusively mosquito and black fly larvae and few other Nematoceran flies. Thus the environmental impact is negligible. The strategy is also implemented by experienced biologists.

B.t.i. is a soil bacterium and can be found in almost each habitat world-wide as a part of the natural ecosystem. During sporulation the bacillus produces the so-called protein crystal harbouring protein toxins. The Bacillus can be fermented in huge 150.000 litres fermenters for two days at 28°C and oxygen supply. At the end of the fermentation process hundreds of kilograms of the fermentation substances can be harvested containing the toxins. This can be formulated into powders, water dispersible granules (WDGs) as well as to solid granules or fizzy tablets.

When the formulations are applied to the breeding sites the protein crystals are ingested by the mosquito larvae and activated in the mosquito larval gut by proteases. The activated polypeptides (toxins) bind specifically to glycoreceptors in the gut bio-membrane. Pores are built by the toxins in the membrane combined with an influx of water into the gut cell. The cell is swelling and bursting. Finally the mosquito larvae are dying and all other organisms except nematoceran flies (mosquitoes) are not harmed. The specificity of B.t.i. is based on the glycol- receptors which occur only in mosquitoes and some nematoceran flies.

For the successful implementation and use of microbial control agents the following prerequisites are necessary: entomological studies, precise mapping and numbering of all major breeding sites, assessment of the effective dosage in bioassays and in small field tests, adaptation of the application technique to the requirements in the field, design of the control strategy as well as training of the field staff and governmental application formalities.

For almost four decades *B. thuringiensis israelensis* have been successfully used in Germany as biological control agents against floodwater mosquitoes (e.g. *Ae. vexans*) and the so-called house mosquito *Culex* mosquitoes (e.g. *Cx. p. pipiens* biotype *molestus*) and since 2015 also against the Asian Tiger mosquito *Aedes albopictus*. Annually up to 250 km² of breeding areas (depending of the number and size of the floods) are treated with *B. thuringiensis israelensis*, resulting in a reduction of the mosquito population of more than 90% of the emerging population year by year.

The flood plains of the Rhine are usually inundated two and more times each summer. The extent of the flooding depends on the snow-melt in the Alps and on rainfall, and it is constantly necessary to monitor the water flow in the Rhine and in the flood plain. During flooding, *Ae. vexans* and other floodwater mosquito larvae hatch within minutes or hours at temperatures exceeding 8°C. Before control measures are to be conducted, the larval density and the larval stages are checked by means of sample scoops at representative breeding sites, in order to justify the action being undertaken and to establish the correct dosage and the best formulation used.

The treatment can be done by ground application when 500 grams of Bti-WDG is suspended in 10 liters of water and applied by pressurized knapsack sprayers. In areas with dense vegetation the helicopter applies Bti-ice-granules. The Bti-water suspension is dropped into fluid nitrogen and the resulting icy pearls containing the Bti toxins can be spread across the breeding sites containing a sufficient number of mosquito larvae by the helicopters. One day after application, spot sample scoops are taken at the reference breeding sites to check mosquito density and thereby establishing the efficacy of the treatment.

For the precise application and real time monitoring helicopters are GPS guided and 3D models are used for the precise assessment of the application areas.

Control of urban mosquito species is mainly carried out by householders or inhabitants. To assist with this, KABS provides information on the biology of container breeding mosquitoes such as the house mosquito *Cx. pipiens* and the Asian tiger mosquito *Aedes albopictus* as well as information on appropriate control measures. Bti-Culinex[®] tablets have been particularly successful. They kill mosquito larvae in water containers over a period of several weeks monitoring the program

Some 8% of the KABS budget is invested in monitoring mosquito populations, mosquito resistance and environmental impact. All the studies carried out to date have shown that the introduction of *B. thuringiensis israelensis* has reduced the numbers of nuisance mosquitoes to a tolerable level, but that the diversity of the ecosystem as a whole has not been damaged. So far no resistance occur as well.

Monitoring mosquito abundance: To monitor the abundance of adult mosquitoes, a sufficient number of CO2-baited traps are placed at comparable sites throughout the entire inundation area. These are monitored twice a month from April to September. On each occasion for a whole night, the mosquito density is sampled by means of carbon dioxide light-traps. Catches in areas where no control measures have been undertaken serve as points of reference (100% of the mosquito population) for catches from areas being treated, in order to determine the success of the measures (mortality rate in percent). It has been shown that since the widespread application of *B*.

thuringiensis israelensis in 1981, mass occurrences of mosquitoes have been successfully averted. Naturally, these control measures have had an extremely positive reception among the local people.

Monitoring the environmental impact: It has been essential to document the environmental impact of *B. thuringiensis israelensis*, in order to provide a scientific basis for rebuting the arguments commonly brought against mosquito control by its opponents. Before large-scale application of microbial control agents was undertaken, the most important members of various aquatic groups (*Cnidaria* to *Amphibia*) were screened in the laboratory and in small-scale field trials for their susceptibility to microbial control agents. This study showed that in addition to mosquitoes (Culicidae) and black flies (Simuliidae), only a few species of midges (Chironomidae) were affected by *B. thuringiensis israelensis*. For the most part, these midges were much less susceptible to *B. thuringiensis israelensis* than the target organisms or occur mainly in permanent water bodies where no floodwater are developing.

The development of insects in treated and untreated water is regularly monitored using emergence traps. The occurrence and abundance of insects in treated areas is assessed by regular light trap catches. All investigations have shown that while the numbers of *Aedes* mosquitoes are drastically reduced, all other insects continue to develop in the water and, as winged adults, provide a food resource for birds, amphibians and bats.

Monitoring the resistance: Mosquito populations are checked at regular intervals for the development of resistance. No resistance has been detected after 30 years of treatment with *B. thuringiensis israelensis*.

Invasion of exotic mosquitoes in Europe and their control

Out of the more than 3500 mosquito species worldwide, only about 30 species have begun to spread far beyond their original geographical borders. The most successful invasive species are *Aedes aegypti, Ae. albopictus, Ae. japonicus, Ae. koreicus, Ae. atropalpus* and *Ae. triseriatus.* Initially, these mosquitoes colonize small natural and artificial water bodies, such as water-filled tree holes, rock pools, phytothelms or artificial breeding sites as water barrels, vases, flower pots, buckets or frequently used tires. Usually, these small accumulations of water show a large variation of the size of the water body, in temperature and

other abiotic conditions which require special adaptations of these so-called "container breeding mosquitoes".

Overall, these exotic species possess a high ecological potency and can rapidly adapt to new habitats due to their genetic plasticity and they are easily disseminated by human activities.

Aedes albopictus is an excellent example. This species has spread from tropical areas to areas with temperate climates which do not allow a constant followup of generations, e.g. during winter periods. As a consequence, the species goes through a winter diapause during which the larvae in the eggs are not able to hatch and remain in the egg-shell until the living conditions allow a further development.

Rapid transportation systems connect the world's biota more than any time in earth's history. Within a couple of hours or days organisms are transported from one continent to another. Beside economic activities, human migration and tourism is increasing the risk for spreading both disease vectors and diseases. Especially the international trade, mainly of used tires and occasionally of lucky bamboo (*Dracaena* spp.) cuttings, is the vehicle for the spread of most of the invasive mosquitoes.

Between 1995 and 2017 the scientists of the KABS could record in the frame of the routine mosquito monitoring programme five exotic mosquito species for Germany namely *Uranotaenia unguiculata* (1995), *Aedes albopictus* (2007), *Aedes japonicus* (2009), *Culiseta longiareolata* (2011) as well as *Ae. koreicus* (2015). All species are considered established in the Upper Rhine valley after having produced at least 3 generations in the new territory and have successfully overwintered.

Whereas the intercontinental spread is mainly facilitated by the global merchandise especially with used tires, the spread within and between neighboring countries is most likely that females of *Aedes albopictus* are introduced as "blind passengers" in vehicles.

In Europe, *Ae. albopictus* has probably been present in Albania since at least 1979, but didn't spread due to the political isolation. However, in the early 1990s *Ae. albopictus* was passively introduced into Italy, due to the international trade of used tires followed by a rapid spread into other areas in

Italy. Having become established in Italy, *Ae. albopictus* was spreading by vehicles and boats along the Mediterranean coast including France, Spain, Croacia, other Balkan countries, as well as Greece and Turkey. The species is, today, principally present in the whole northern and some parts of the southern mediterranean basin with an increasing tendency of spreading northwards across the Alps into central European countries. Taking into account that *Aedes albopictus* is a vector of at least 22 arboviruses, including Dengue, Chikungunya, Zika and Yellow Fever viruses and that Italy is a favourite country for German tourists the risk of the introduction of *Ae. albopictus* into Germany via returning tourists in vehicles from Italy and therefore an increasing public health risk is evident. This risk has to be considered as serious when we take into account that the Asian tiger mosquito is already involved in the autochthonous transmission of dengue and Chikungunya viruses in Europe, namely dengue in Southern France and Croatia and Chikungunya in Italy 2007.

As a consequence, the German Mosquito Control Association (KABS) started in 2005 a monitoring program from Basel to Heidelberg along motorway A5 (E35) coming from Italy as suspected port of entry for *Ae. albopictus* adults. In the frame of the first monitoring program in the time period 2005 to 2009, *Ae. albopictus* eggs were found the first time in an ovitrap at a resting station north of the city Weil am Rhein. In order to assess the risk for the introduction of *Ae. albopictus* a collaboration of scientific, traffic and governmental institutions in close cooperation with the public was initiated. Furthermore, public awareness has been increased by press releases and thorough information via internet, radio and TV to be able to recognize tiger mosquitoes. This was done for the KABS-area in Southwest-Germany and nation-wide by the "Mückenatlas". The highway monitoring revealed that about 40% of all service and resting stations and some camp grounds along the highway A5 between Basel and Hesse were infested by *Ae. albopictus* what indicate the permanent introduction of the Asian tiger mosquito into Germany.

The increased public awareness resulted in numerous records of adult *Ae. alboipictus* females by alert people who have send females to the KABS or the Mückenatlas. Nowadays, we have in Southwest Germany established populatons of the Asian tiger mosquito in cities like Freiburg, Lörrach, Karlsruhe

and Heidelberg. All populatons are controlled by the application of Bti and additional with the so-called Sterile Insect Technique (SIT). Males of the Asian tiger mosquito are sterilized by gamma-radiation and are released in infested areas. The sterile males mate with the "wild females" and the offspring is not viable.

The ultimate goal is to assess newly developing *Ae. albopictus* populations as early as possible and to initiate control activities by the KABS and the Institute for Dipterology a sister organization of KABS.

The successful control programme of KABS can serve as a model also for tropical countries which was proven already in African countries like Kenia, Ghana or Burkina Faso. The transfer of techniques and the close cooperation of organisations between mosquito infested areas can help in the frame of partnerships to control mosquito-borne diseases such as Malaria.

Which is the most dangerous animal for humans?







Mosquito SpeciesWorld-wide>3500About 1000 species are vectors of dangerous diseases(60 species are most important)Europe102Germany52

Each minute a person is dieing on a mosquito bite in the tropics

Importance of mosquitoes for the evolution and socioeconomic development of the human population!



Major mosquito-borne diseases - World-wide - 2018

MALARIA People at risk People infected Deaths:

>3 billion 216 million/year ~500.000/year

 ARBOVIRUSES
 – Dengue, West Nile, Chikungunya,

 Zika and Japanese Encephalitis virus

 People at risk:
 > 2.5 billion

 People infected:
 > 390 million/year

 Fatal cases:
 > 30.000/y

LYMPHATIC FILARIASIS People at risk: People infected

1,1 billion 120 million

Evolution of mosquitoes









Homo sapiens about 200.000 years

Evolution of Mosquitoes Occur at least since the Mesozoic period (oldest fossils in amber from cretaceous period (~100 mill. years)



Mosquitoes adapted to a great variety of aquatic habitats



Evolution of mosquito-borne diseases

Co-evolution between humans and mosquitoborne diseases;

Zoonoses develop into human diseases – prozess started 6 mill years ago and is still going on e.g. *Pl. knowlesi*.

It is a important part of human evolution!

Vectorial Capacity vs. Vector Competence

• Vectorial capacity is a measurement of the efficiency of vector-borne disease Transmission

• Vector competence is an evaluation of the vector's capability (mechanical or biological) to transmit a pathogen

• Therefore, vector competence is actually an additional component of vectorial capacity



The most important factors for the development of mosquitoes



Water



Temperatur

Adaptation of their life cycles to various conditions



MALARIA

Human Malaria-Pathogens

- 🌶 Plasmodium falciparum (Malaria tropica)
- Plasmodium vivax (Malaria tertiana)
- Plasmodium ovale (Malaria tertina)
- > Plasmodium malariae (Malaria quartana)
- » Plasmodium knowlesi ….

Disease Burden

- > 40 % of the world population is exposed at risk of infection
- ~~216 millions clinical cases/year (80% cases in Africa)
- ~ 0,5 million deaths/year (>90% in Africa)

Each min, a child is dieing in Africa on malana Annual costs formalaria in Africa alone almost 2 billion USS





Yellow fever/Dengue/Zika/West-Nile Virus



Flavivirus: The viron (50 nm) consist of a nucleocapsid and a lipoprotein envelope; Genom is a single-stranded RNA with approximately 11.000 base pairs and composed of 10 genes namely 3 structural protein genes (core protein©, membrane associated Protein (M) and the envelope protein (E) – neutralization activities.



West-Nil-Fieber in Europa 2018 (ECDC)

In 2018, as of 15 November 2018, EU Member States have reported 1 491 human cases in Italy (569), Greece (309), Romania (277), Hungary (214), Croatia (53), France (25), Austria (20), Bulgaria (15), the Czech Republic (5), Slovenia (3) and Cyprus (1). EU neighbouring countries reported 557 human cases in Serbia (415), Israel (128) and Kosovo* (14). To date, 171 deaths due to West Nile virus infection have been reported by Greece (45), Italy (42), Romania (42), Serbia (35), Kosovo* (3), Bulgaria (2), the Czech Republic (1) and Hungary (1).



Peoples Sciences (www.kabsev.de) thorough information and involvement of the public



Breakthrough in biological control of mosquitoes



Discovery of Bti in the Negev Desert by Dr. Yoel Margalith in August 1976

Soil bacteria can be found in almost each habitat, part of the nature;



Cry4A (125 kDa), Cry4B (135 kDa),
 Cry10A (58 kDa), Cry11A (68 kDa)
 Texia: CyrA (17 kDa)

Toxins are as effective as chemicals

Bacillus thuringiensis israelensis and Bacillus sphaericus



4 major protein toxins: Cry4A (125 kDa), Cry4B (135 kDa), Cry10A (58 kDa), Cry11A (68 kDa) 5th toxin: CytA (27 kDa)

Targets: mosquitoes and blackflies



Binary protein toxin: 51.4 kDa and 41.9 kDa Additionally: 100 kDa

Targets: Anophelines and Culex spp.



Ultimate Goal of our mosquito control operations





Members: Communities/Cities

- Number of members: 93
- Inhabitants: 2,7 Million People
- Control Area: 6.000 km2 60.000 Hectares
- Breeding area:
- Number of sites
- Permanent Staff:
- Temporary staff
- Budget: ٠
- appr. 4 Million Euro

1,25 €/pers/year

6.000

37

300

Average costs: ٠



The four most dangerous mosquito species

- 1. Anopheles gambiae s.l. (African fever mosquito)
- 2. Aedes aegypti (Yellow fever mosquito)
- 3. Aedes albopictus (Asian tiger mosquito)
- 4. Culex pipiens s.l. (House mosquito)









Lessons we have to learn:

- To fight the mosquitoes we need to understand our enemy and we need all bullets to our disposal to fight them
- We need close cooperation globally wide and new ideas to overcome all constraints – helping hands

PAMCA - AMCA -- EMCA can be the platform for this activities.



Dr. Florence Fouqué, WHO Geneva: Malaria Situation and Challenges



MALARIA SITUATION IN 2017

In 2017, an estimated 219 million cases of malaria occurred worldwide Most malaria cases were in the African Region (200 million or 92%) There were an estimated 435 000 deaths from malaria globally.

Children aged under 5 years are the most vulnerable group affected by malaria, they accounted for **61% (266 000) of all malaria deaths**.

The African Region accounted for 93% of all malaria deaths.



MALARIA TRENDS



EVOLUTION OF DEPLOYMENT OF DIAGNOSTIC (2010-2017)

The number of Rapid Diagnostic Tests (RDT) deployed in African Region has been multiplied by 5, allowing the testing of about 80% of suspected cases



EVOLUTION OF DEPLOYMENT OF TREATMENTS (2017-2017)



MALARIA TRENDS BY REGION AND COUNTRIES FOR 2017



FUNDING FOR MALARIA CONTROL



MALARIA PARASITE CYCLE



MALARIA CYCLE INTO THE HUMAN HOST



MALARIA PARASITES



MALARIA CLINICAL SYMPTOMS



MALARIA CYCLE IN THE MOSQUITO



DIAGNOSTIC METHODS

Broadly 3 methods available:

- Microscopy
- RDT (Rapid Diagnostic Tests
- PCR

MALARIA TREATMENT



MALARIA VECTORS = ANOPHELES



VECTOR LIFE-CYCLE



CHALLENGES IN MALARIA CONTROL BECAUSE OF CLIMATIC CHANGES



Figure 1. A, Mean village elevations divided in quartiles, highlighting the generally higher terrain to the west of the subcounty and the lower terrain to the southeast between the Mubuku and Sabo rivers. B, Plasmodium falciparum test positivity rate (PIPR) in each village before the flood, with the highest rates in villages at lower elevation and along the major rivers. C, PIPR in each village after the flood, with relatively high positivity rates in the majority of villages. D, Absolute difference in the PIPR from the preflood to postflood periods. The PIPR for villages with <30 tests performed in the respective period is not shown.

CHALLENGES IN MALARIA CONTROL BECAUSE OF ENVIRONMENTAL CHANGES



FIGURE 1. Locations of the International Centers of Excellence for Malaria Research (ICEMR) sites, including the seven sites reporting studies on urban malaria.

CHALLENGES IN MALARIA CONTROL



Key challenges identified in Global Technical Strategy for Malaria 2016 - 2030

Matthias Feil: Workshop approach and results

Design Thinking as the appropriate method for the 2,5 days' workshop was quickly selected for several purposes: it is an approach to solve problems and to develop new ideas. The goal is to find solutions that are compelling from the perspective of the person concerned. It assumes that problems can be solved better if people of from different disciplines work together in an environment encouraging creativity, iointly investigate the specific needs and motivations of concerned people and finally develop concepts which are



validated and tested several times. The procedure is based on the work of designers, which is understood as a combination of understanding, observation, ideation, refinement, design and learning.



Source: SAP SE

On the first day (Discover) we defined 6 teams with about 6 persons each by accident and with the objective to have team members of different origin country to ensure diversity and intercultural exchange between all participants. The overarching challenge was called 'How might we help preventing illness by

diseases?'. mosquito-borne The subject matter and world's experts Professor Norbert Becker and Madame Florence FOUQUÉ had their key notes on mosquito borne diseases (like Malaria) including prevention through vector control, diagnosis and of treatment. Some the team



members have heard first time about these facts. During synthesis phase each team consolidated the learnings, collected the most surprising facts and created the team individual fictive persona which could be a real person concerned by the existing challenges as a potential victim or as a professional fighting against mosquitos. The teams made all use of the possibility to dedicatedly interview both experts with the specific persona in mind. At the end of the first day, each team finally defined a clear problem statement they would continue to explicitly solve during the next day.

The second day (Design) each team started the creative work through ideation applying empathy with an attitude of deferring judgements on wild ideas. Following, all teams started with a low fidelity prototype of their prioritized ideas to allow validation by experts and other teams in the subsequent step. Then, all



teams presented their rough ideas to the plenum and in front of the experts (Mrs. FOUQUE and Mr. Prof. Becker) for validation of the individual concepts. Each team received critical but constructive feedback about what could or couldn't work and what else would have to be taken under consideration. Interesting was that the experts were already deeply impressed by the quick solution proposals after their presentation at that time.

On the morning of the third day all teams had three hours to integrate the received feedback into a next iteration prototype and final presentation. This presentation took place again in the plenum with additional presence of the press, the organizing Rotary members, and the rector of the University

Prof. Dr. Marjan DEMA. All teams did a great job! They came with focused solution on dedicated aspects as the following results demonstrate:

First team created an 'Automatic Epidemic Forecasting and Response System' collecting different sources of data to help health workers by registration capabilities and delivering information through smart phones.



Second team proposed an educating and prevention support providing gamification application for smartphones called MOSQUIT which provides information and prevention behaviors and measures to kids, youth and adults accordingly.



The 3rd team provided solutions on the current problem of missing or lacking infrastructure by satellite and drones supported mapping of any region, by restoring most important existing bad roads and creating new roads based on the investigated information taking also alternative vehicles like boats, drones into account. Those vehicles not only provide drugs to hospitals but already represent mobile hospitals for local treatments.



4th team created 'Rotar Iron against Malaria' solution and focused on economic aspect to support population of concerned countries struggling with corruption and the consequence of effectivity loss: tax reduction for NGO funding. It would allow local government, WHO and industry working together in a triple win situation resulting in effective and efficient help to population.



5th team invented 'The Zapper' which is an electric and autarchic trap to attract and kill mosquitos in a CO2 neutral way by using solar energy. Additionally, the killed and collected mosquitos can be further used as fertilizer and animal food.



6th team created 'Malaria Diagnostic Scanner & Micro App'. It consists of the combination of little box, the scanner, and connected smartphone: a little and easy to use scanner for peripheral usage diagnosing a person's blood in short time and at the place of living; on a connected smartphone a specific application runs which allows to quickly send diagnosis data for fast validation and treatment by a doctor working remotely; based on this information the doctor can initiate the delivery of the necessary drugs to the patient 2-3 days earlier



Dr. Heiner Grönewald: Best experience of my life

40 young people, most of them students of medicine, pharmacology, economy, biology, and psychology, came together to learn about Malaria and to create innovative ideas to fight against it. We were lucky to have set up such a perfect blend of different people with different experiences, skills, and characters. Everybody was very much engaged in the subject, so that the common creativity produced fine solutions without even researching in the World-Wide-Web. And the collaboration worked just smoothly - regardless of nationality,



academic background, gender, age, religion, and language. Of course the event was conducted in the English language which everybody managed perfectly.

This was due to the personality of the individual persons and to the methodology that Matthis Feil, our well proven professional moderator, used to tease out the brilliant ideas even of the more reserved participants. This "Design Thinking" is excellent to bring together a good approach, with the right people, in the right environment - with fun and cultural exchange.

The best ideas are worth nothing, if they cannot be conveyed to others. The working groups presented their outcome by posters that they presented and explained orally in a very professional manner. Even when the local press was around, a local presenter jumped in without any discontinuity.

The attending scientists from WHO and the World Mosquito Control Association, Florence Fouqué and Norbert Becker, enjoyed the workshop atmosphere and were impressed by the depth of the ideas produced and the "fresh blood" they experienced. So they were convinced that the group did not produce ideas just for "the lowest shelve in a large cabinet", no, they even proposed to continue the collaboration by helping to make some results fly in reality.

During a creative workshop phase I walked from table to table and studied the way of working together and the outcome. All of a sudden, I overheard a comment given to a Greek by a Kosovar that compensated all the effort by the organisers: "this is the best experience of my life".

Heiner Grönewald



Cultural excursion to Prizren





Panta Rhei

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"The fight against Malaria and other related mosquito-born diseases"

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