Executive summary (5-10 pages)

The project aimed to assess how widespread is increased susceptibility of insecticide-resistant *Anopheles* to Plasmodium infection, and the risk associated with this phenomenon in relationship to human behaviour regarding the use of bed nets and insecticides. In this multidisciplinary study, social sciences play a critical role. Sociological and anthropological data were important to understand human behaviour related to prevention of mosquito bites.

The social science studies focused on the evaluation of the human perception of the risk associated with malaria in different localities, and evaluation of how human behavior (regarding the use of malaria control tools) vary in space and time, in order to evaluate its impact on the risk of malaria transmission at the local level and to generalize to broader contexts.

The study focussed on three different groups of factors potentially contributing a risk of a reversal in malaria control, linked to evolution of insecticide resistance and related increase Anopheles sensitivity to Plasmodium. These were the mosquito infection status and its determinants (in particular, the genotype at loci involved in resistance evolution: *kdr*, *Ace1*), mosquito behaviour (propensity to bite indoor and outdoor, time of biting) and its change over time, and the human behaviour (propensity to be indoors or outdoors during the night, and mosquito avoidance behaviour) and its change over time. The studies were carried out in Senegal, Côte d'Ivoire, and Benin.

The studies in Dielmo, Senegal, and Agboville, Côte d'Ivoire, of the biology of infection of mosquitoes, found that resistant mosquitoes (bearing the L1014F allele) tend to have higher infection rates than sensitive mosquitoes, both when the infections were acquired in wild populations and when introduced experimentally. There were not enough sensitive mosquitoes available from Benin for a comparison with resistant mosquitoes. These results support previous studies on lab strains [3, 4, 6]. In Dielmo, we observed that the trend in infection rates was not steady: the infection rates of *An. gambiae* first increases after the implementation of impregnated mosquito nets, from 2006 to 2014, but then decreased to a rate never seen before during the longitudinal survey from 2014 to 2016. Conversely in *An. funestus*, the rate of infection decreased from 2006 to 2014, and then increased again. Various factors could account for these changes in rates of infections. Specifically, the decreases could be related to chemotherapy given to humans to treat malaria, and to the dramatic drop in the number of bites (linked to a likely drop in the population size of mosquitoes). The increases could be due to a lowered vigilance in malaria control (e.g. populations making less use of mosquito nets) or also potentially to the increase in the frequency of the *kdr-R* mosquitoes in the population (as seen, from ~8 to ~12% in e.g. *An. arabiensis*). The latter explanation would account more easily for the interspecific differences in trends of infection (resistant alleles being rarer in *An. funestus*), but the change of the frequency was very slow (only a 4 point increase). It would be of great concern if this is the only explanation (as future evolution of resistance alleles would then have a very high impact), but hopefully, the global drop in the number of bites will largely compensate for any increase in the rate of infection of mosquitoes in Dielmo (**Error! Reference source not**

found.). In other locations (Côte d'Ivoire and Benin), the proportion of resistant mosquitoes was already very high, so the frequency of resistance and therefore the rate of infection is unlikely to worsen, unless there is also a link with the frequency of *Ace 1*. This locus remains to be investigated (its frequency being too low for now to get enough *Ace1-S* mosquitoes to be compared with those who are *Ace1-R* regarding their susceptibility to infection by *Plasmodium*).

Mosquitoes still bite mostly in night-time in all our three sites, not early in the evening or after people awaken in the morning. This pattern is quite reassuring. There is a switch to an higher proportion of outdoor bites in Dielmo, but this is because the number of outdoor bites has not decreased as fast as the rate of indoor biting, not because mosquitoes that were biting indoors are now biting outdoors. This is also reassuring. In other sites the proportion of bites occurring indoor rather than indoor is similar to that before the implementation of nets in Dielmo, suggesting that the same pattern might hold regarding this behaviour of mosquitoes.

Regarding human behaviour, a variety of issues were identified that still prevent some people from using mosquitoes nets properly, and several factors were identified that might lead, in the future, either to an increased coverage or to a lowered coverage, depending on which factors predominate. These factors can be classed into economic (poverty), social (education, etc.), cultural (beliefs and traditions), political (frustration regarding how the malaria issue is handled), environmental (heat) and technological (net quality).

Economic factors play a role that could evolve positively as poverty is reduced in the future: populations use nets for other purpose than what they are supposed to be used for, e.g. agriculture (to protect crops from pests), cassava transformation, or wedding dresses. This allows people to earn a small amount of money which can be very helpful in cases of extreme poverty. The affected people also indicate that it is difficult for them to renew nets when they are damaged, in particular in Benin. This could be addressed by distributing new nets more often. On the other hand the proportion of families owning and using at least one net remains very high in all three locations, despite these issues.

Social factors could play a major role as well. Many different understandings of malaria and of how it is transmitted persist. Not everyone makes the link with mosquito bites, which is very important for motivating the use of nets. Hence, there is room for progress regarding the coverage with education. We can be optimistic in this regard since the populations consider information campaigns as important, indicating they are rather receptive. However, when informing them, it is very important to help them to distinguish mosquitoes that transmit malaria from those that don't, and to understand they are different kind of mosquitoes with different behaviours, as mosquitoes biting in the daytime (which do not transmit malaria) have convinced some people that the mosquitoes have adapted to nets by changing their behaviour and therefore that nets are not useful anymore. Given the inconvenience of using nets (heat, discomfort due to the texture), this is a factor that could lead to a lowered coverage. The fact that the entomological data do not indicate that changes in mosquito behaviour have occurred that would make nets inefficient (resistance to the insecticide lowers

the efficiency but the mechanical barrier of the net remains), makes it easier to identify the appropriate message: net use continues to be highly desirable. It may also be important to take into account the perceptions of malaria as either dangerous or benign. It is not always considered the most problematic disease in the study sites, and it might be important to underline that the decline in malaria is tightly tied to control measures and that vigilance has to be maintained for the control efficiency to persist.

Cultural aspects might also play a role, and are linked to education as well. Populations have "alternative" methods that have traditionally been implemented. The use of these alternative methods is linked to economic aspects (they are less expensive) and political aspects (the populations concerned perceive the methods implemented by the state as serving obscure goals). Approaches that answer the populations' most emergent needs (such as sanitation) could help regarding the political aspects of this, as the people would then see their ideas being taken into account (the implementation of control tools would then be less perceived as a top-down approach).

As regards the environmental aspects, it is difficult for the concerned countries to really weight in the balance to fight climate change, although the effect of high temperatures on net use could be put on the list of arguments. Locally however, there could be policies and support to populations for constructions to be fresher, so that populations are more motivated to use nets. This would also help populations to adhere to national policies as this would show state entitlement to improve their comfort.

Regarding technological issues, it should be possible to take into account the preferences of potential uses for different shapes and textures of nets, in order to improve coverage. It would also be useful to find a mechanism to deliver nets for free, while informing the population of their actual price (e.g. by a label on the package), so nets are not perceived as low quality because of their low (or zero) cost.

In summary, we are not in the worst scenario possible either regarding entomological aspects or sociological aspects, but the risks are not null. The risk is therefore medium, and efforts have to be maintained to improve net coverage and ensure that there is no reduction in the intensity of malaria control.

Publications (*publications of the project in italics*)

[1] Francine Mariette Ahodekon. Analyse prospective de la perception du risque de paludisme par la communauté de tori-bossito (bénin). Master's thesis, Master en Sociologie - Anthropologie, spécialité "Études Prospectives et Développement". Département de Sociologie-Anthropologie (DS-A) de la Faculté des Sciences Humaines et Sociales (FASHS), Université d'Abomey-Calavi, Bénin., February 2017. Soutenue le 17/02/2017.

[2] Haoues Alout, Roch K Dabiré, Luc S Djogbénou, Luc Abate, Vincent Corbel, Fabrice Chandre, and Anna Cohuet. Interactive cost of plasmodium infection and insecticide resistance in the malaria vector anopheles gambiae. *Scientific Reports*, 6:29755, 2016.

[3] Haoues Alout, Nicaise Tuikue Ndam, Marcel Maurice Sandeu, Innocent Djégbe, Fabrice Chandre, Roch Kounbobr Dabiré, Luc Salako Djogbénou, Vincent Corbel, and Anna Cohuet. Insecticide resistance alleles affect vector competence of *Anopheles gambiae s.s.* for *Plasmodium falciparum* field isolates. *PLoS ONE*, 8(5):e63849, May 2013.

[4] Haoues Alout, Bienvenue Yameogo, Luc Salako Djogbénou, Fabrice Chandre, Roch Kounbobr Dabiré, Vincent Corbel, and Anna Cohuet. Interplay between malaria infection and resistance to insecticides in vector mosquitoes. *Journal of Infectious Diseases*, page jiu276, 2014.

[5] Ténin Aminatou Coulibaly. Impacts de l'utilisation des pesticides chimiques dans le périmètre maraîcher de houeyiho sur le niveau de résistance des vecteurs du paludisme aux insecticides au sud du bénin. Master's thesis, Université Abobo-Adjamé, July 2017. Soutenue le 17/07/2017.

[6] S. Doucouré, O. Thiaw, A. Souhoufi, C. Bouganali, Diagne N., and C. Sokhna. An. gambiae s.l. susceptibility to insecticides and pattern of kdr mutation in Ndiop, Senegal, a village under bed nets coverage. Oral communication - MIM Conference - 15-20 April 2018, April 2018.

[7] Bruno Houdjo Noutangni. Analyse prospective des déterminants de la non utilisation de la moustiquaire imprégnée en milieu aizo de tori bossito. Master's thesis, Master en Sociologie -Anthropologie, spécialité "Études Prospectives et Développement". Département de Sociologie-Anthropologie (DS-A) de la Faculté des Sciences Humaines et Sociales (FASHS), Université d'Abomey-Calavi, Bénin., March 2017. Soutenue le 02/03/2017.

[8] Christian Mitri, Kyriacos Markianos, Wamdaogo M Guelbeogo, Emmanuel Bischoff, Awa Gneme, Karin Eiglmeier, Inge Holm, N'Fale Sagnon, Kenneth D Vernick, and Michelle M Riehle. The kdr-bearing haplotype and susceptibility to *Plasmodium falciparum* in *Anopheles gambiae*: genetic correlation and functional testing. *Malaria Journal*, 14(1):391, 2015.

[9] Christabelle Sadia. Etude de l'influence du gène kdr, responsable de la résistance aux insecticides sur la susceptibilité des moustiques au Plasmodium. Master's thesis, University Nangui Abrogoua, Abidjan, January 2017. 21/01/2017.

[10] Fatoumata Seck. Evalution comparative de l'efficacité de techniques artificielles pour gorger et infecter les vecteurs du paludisme au laboratoire. Master's thesis, Université Cheikh Anta Diop de Dakar (UCAD) , Senegal Faculté des Sciences et Technique, Département de Biologie Animale, Master en Biologie Animale option Entomologie Médicale, November 2017. Soutenue le 13/11/ 2017.

[11] Seynabou Sougoufara, Omar Thiaw, Aurélie Cailleau, Nafissatou Diagne, Myriam Harry, Charles Bouganali, Pape M Sembène, Souleymane Doucoure, and Cheikh Sokhna. The impact of periodic distribution campaigns of long-lasting insecticidal-treated bed nets on malaria vector dynamics and human exposure in Dielmo, Senegal. 2018.

[12] O. Thiaw, S. Doucouré, S. Sougoufara, C. Bouganali, L. Konaté, Diagne N., O. Faye, and C. Sokhna. Dynamics of insecticide resistance in field population of Anopheles arabiensis in Dielmo, a Senegalese village under universal coverage of insecticide-treated nets. Oral communication - MIM Conference - 15-20 April 2018, April 2018.

[13] Roland Tossou. Mécanismes de défense des moustiques face aux insecticides : activités enzymatiques et mutations génétiques au sein des populations de Anopheles gambiae dans la commune de Tori-Bossito, sud du Benin. Master's thesis, Université Abobo-Adjamé, October 2017. Soutenue le 12/10/2017.

[14] A. W. Yadouleton, A. Cailleau, M. Chouaibou, R. Tossou, O. Briët, T. Smith, and M Akogbeto. Dynamic density, sporozoite rates and entomological inoculation rates of Anopheles gambiae at Tori-Bossito, Benin. Poster communication - MIM Conference - 15-20 April 2018, April 2018.