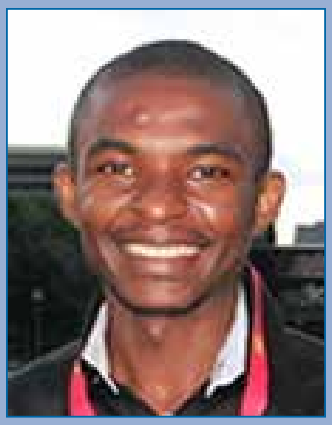

Clovis Seumen Tiogang

Seuman Tiogang obtained a M.Sc in Biochemistry from the University of Yaounde I in 2012. His Master dissertation focuses on the coinfection of malaria and intestinal nematodes in school-aged children. Since 2014, his main research focus has been on the interactions between Ascaris lumbricoides secreted proteins and the host immune response to malaria.

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Evaluation of the effect of Ascaris suum secreted proteins on host immune response to Plasmodium berghei crude extract in BALB/c mice

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Introduction

Malaria and geohelminthiasis are challenging public health problems in developing countries, particularly in Sub-Saharan Africa, where they coexist. Although the rapid scale up of malaria interventions in recent years have significantly reduced the global burden and case fatalities, this reduction is however heterogenous as the disease remain highly endemic in most afro-tropical countries including Cameroon. These difficulties are mainly due to poverty, inadequate allocation of resources for control and the emergence and spread of drug resistance in the parasite and insecticide resistance in the *Anopheles* vectors. This is further worsened by the absence of a plausible vaccine. Until date, studies geared towards understanding the immune interactions and its implication on malaria and geohelminthiasis co-infections have remained poorly understood. While some schools of thought suggest a protective effect of helminths(Lyke *et al.*, 2006; Brutus *et al.*, 2007; Waknine-Grinberg *et al.*, 2010), others claim helminths infections worsen malaria through enhancing the incidence and gametocyte carriage; and may also down-regulate the development of protective immunity against malaria while exacerbating malaria induced liver pathology(Nacher *et al.*, 2000; Nacher *et al.*, 2001; Helmby, 2009)

Among helminthes parasites, ascaris have gained interest due to their observed modulatory effect on the host immune response to allergies and autoimmune diseases. Indeed, it has been observed that the highest density of helminthic infections coincides with the lowest incidence of allergic and autoimmune diseases due to the immunomodulatory mechanism elicited by worms to prevent their elimination(Cooper, 2009). In malaria and ascaris co-infection, whether ascaris has a protective effect against malaria or against malaria induced immunopathology with the aim to protect the host from destruction thereby preventing their elimination remain unknown. Therefore, the present study aimed at assessing the effect of *A. suum* secreted proteins on immune response to *Plasmodium berghei* crude extract in BALB/c mice model.

Materials and Methods

Adult worm's secreted proteins were isolated, quantified and analyzed. Age- and sex-matched BALB/c mice, 6 weeks old, were used in all experiments as well as *Plasmodium berghei* NK65 (*PbNK65*). Mice were distributed into 7 groups (4 controls and 3 interventions) of 6 mice each, immunized with *PbNK65* crude antigen and the parasitaemia was monitored for 21 days. Total IgG level were determined in the plasma. All data were analyzed and p-value<5% were considered statistically significant.

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Results and Discussions

ASP precludes body weight loss in malaria immunized BALB/c mice

Body weight loss was more pronounced in the control groups. The difference was even more pronounced day 14 post-inoculation (pi), where body weights among ASP exposed mice were higher than in the control group. Interestingly, this difference was statistically significant for ASP2 (p=0.0008) mice group but not for ASP1 and ASP3 groups. ASP3 mice showed a rapid decrease of body weight after inoculation up to day 7pi compared to ASP1 and ASP2. The decrease in body weight in pre-immunized malarial mice is presumably due in part to the decrease in food and water intake, a disturbed metabolic function and hypoglycemia that have been shown to be associated with malaria. ASP may decrease body weight loss, a malaria complication, associated with malaria infection in a dose dependent manner.

Reduced parasitemia in malaria immunized BALB/c mice with two doses of ASP

All mice groups presented a peak of parasitemia on day 7pi, except ASP2 mice. On the other hand, another peak of parasitemia slower than the previous was observed on day 14pi. A reduced parasitemia was observed for ASP2 but this difference was not statistically significant.

ASP decreases severity of malaria in immunized BALB/c mice in a dose-dependent manner

ASP2 mice survived more their counterpart of other groups. The Median survival time was higher for ASP2 mice. However, ASP1 and ASP3 mice showed a significantly lower survival time when compared to iRBC mice (see table 1). Our data indicate an overall decrease severity of malaria in mice exposed to ASP. This decrease was more pronounced in mice receiving two doses of ASP. In fact, the former survived longer than the others as indicated by an increase in post-inoculation survival time in those mice. These results corroborate with that carried out by Rocha and colleagues to understand protective effect of *A. suum* extract on experimental arthritis. Their result suggests that *A. suum* extract giving per os protects against Arthritis severity, an inflammatory disease. (Rocha et al 2008)

Table 1: Kaplan Mayer survival time comparison

	Median survival Time (days)	IQR[Q1-Q3]	p-value
iRBC	15	[14-18]	-
ASP1*	7	[6.75-15.8]	0.044
ASP2	20	[10-20]	0.45
ASP3*	12.5	[7-17.5]	0.033

*Mean Survival time significantly lower than the control group. P-value<0.05. IQR: InterQuartile Range

ASP stimulates the production of antimalarial total IgG malaria immunized BALB/c mice in a dose-dependent manner

Total IgG specific to *PbNK65* change analysis showed an increase in total IgG at day 7pi in all mice groups and a decrease was observed in ASP2 and ASP3 mice on day 14pi (Fig.1). ASP2 antibody titer was higher than those of ASP1 and ASP3 on day 7 pi (Fig.2). These changes could be indicative of a better immune memory resulting in a rapid and sufficient production of total IgG to counteract the infection. However, when combined with the survival rate of mice in each group, no dead was recorded in ASP2 as well as iRBC mice at this time whereas four and three dead were already recorded in ASP1 and ASP3 respectively. Hence, even though total IgG production may have been stimulated by ASP, this increase production was not enough to confer a protection against malaria infection.

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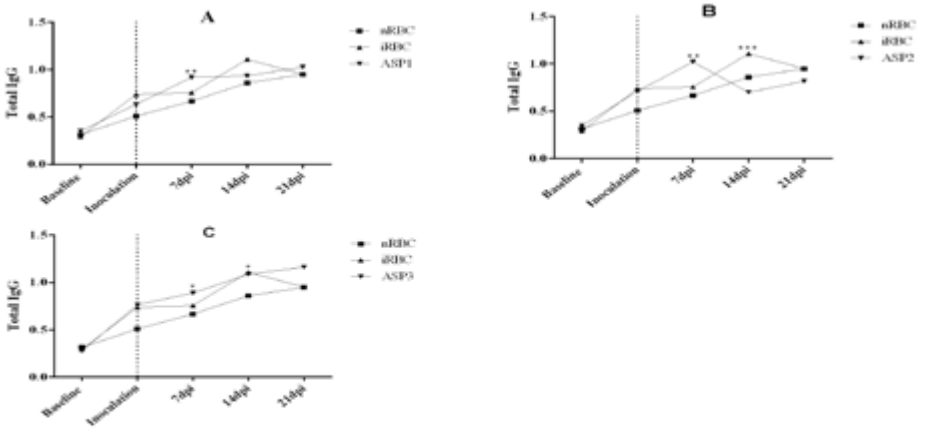

Figure 1: The plasma levels of anti-*PbNK65* total IgG in ASP1 mice (A), ASP2 mice (B) and ASP3 (C). Plasma was collected before immunization, inoculation and on day 7, 14 and 21 post-inoculation. Specific IgG titer against *PbNK65* crude antigens was determined in groups of 6 mice by ELISA and absorbance reading at 405nm. Results are presented as the mean of absorbance. *p-value <0.05, **p-value<0.01 and *** P-value <0.001.

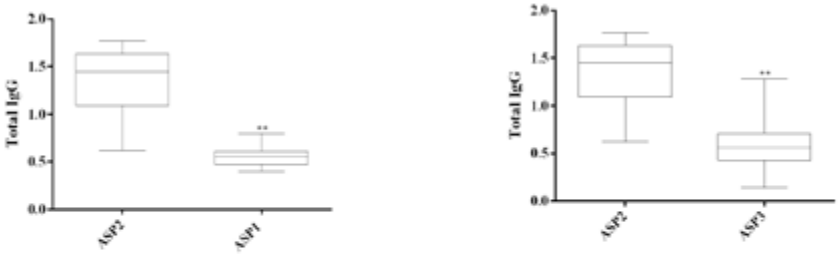

Figure 2: The plasma levels of anti-*PbNK65* total IgG at day 7 p.i. in ASP1 mice versus ASP2 mice (A) and ASP2 versus ASP3 (B). Plasma was collected day 7 post-inoculation. Specific IgG titer against *PbNK65* crude antigens was determined in groups of 6 mice by ELISA and absorbance reading at 405nm. Results are presented as the mean of absorbance. Two asterisks (**) mean a p-value<0.01.

Conclusions

Malaria remains a challenging public health concern in poor countries worldwide and shares the same endemic area with ascaris infection. Interaction between both diseases is a reality but mechanisms and pathways are unknown and uncertain. This interaction constitutes a gap the way to discover an effective vaccine against malaria and further eradicate the disease. The present study contributes to the better understanding of ascaris effect on malaria especially immune response to malaria and open avenue to new research to decipher the parasite effector and its mechanism of modulation of host immune response.

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