Small Scale Collaborative Project

RAPSODI

PRe-clinicAl studies of a PSA-based human vaccine candidate targeting visceral, cutaneOus and mucocutaneous Leishmaniasis and Development of the associated procedures for further clinIcal trials

FP7 Contract Number: 223341

Project Final Report

Due date of deliverable:	M42
Actual Submission date:	M42

Deliverable ID:	**D0.1h**
Deliverable Title:	**Project Final report**
Responsible beneficiary:	IRD
Contributing beneficiaries:	All partners

Start Date of the Project:	January 1st, 2009	Duration	42 Months

Project co-ordinator name, title and organisation:	Mr. Gérard Papierok, VIRBAC
Tel:	+33 4 92 08 77 46
Fax:	
E-mail:	gerard-marie.papierok@virbac.com
Project website address:	www.fp7-rapsodi.eu

Revision:	V2
Dissemination Level:	CO

Section 1 - Final Publishable Summary

1.1 Executive Summary

The RAPSODI project addressed the strategic objective HEALTH-2007-2.3.4-2: "Development of a Leishmania vaccine" of European 7th Framework Program. The global goal of the RAPSODI was to develop a safe and efficient vaccine generating a broadly protecting immune response against most or all Leishmania species that cause leishmaniasis in the world. A unique vaccinal solution would thus be provided to protect against the various clinical phenotypes (namely visceral, cutaneous and mucocutaneaous leishmaniasis, VL, CL and ML respectively). Besides, RAPSODI proposed to establish all the associated assays required for the subsequent clinical trials, such as the selection of the eligible patients and assessment of vaccine efficacy.

To achieve these goals, RAPSODI gathered an international consortium of seven partnering organisation, constituted of countries from endemic areas (India, Peru, Tunisia, Spain and France). Based on successful results on canine leishmaniasis (the best VL animal model to date) that led to the first commercially available vaccine in dogs (namely Canileish®), the main achievement sought through RAPSODI was to transfer the knowledge and technology to human beings by developing a human-compatible vaccine candidate and confirming its activity in pre-clinical studies. Due to the intrinsic universality of the chosen active ingredient (PSA protein), an ambitious universal immunoprotective response to most, if not all, *Leishmania* species causing disease in humans was targeted.
RAPSODI aim was also to address the question of population selection in order to ascertain relevant and meaningful clinical trials and vaccination campaigns. Indeed, resistant individuals, when involved in either vaccinated or placebo group, represent important bias to the analysis of the results. Thus, RAPSODI objective was to investigate the parasitological, immunological and genetic features of groups of individuals with different manifestations of the infection, and subsequently apply the generated knowledge to the development of assays and field tests, which represent stand-alone results. The package (vaccine candidate + diagnostic/prognostic tools) proposed by RAPSODI would represent a global solution, and as such is believed to have a real impact on the worldwide leishmaniasis problem.

Designing and producing antigen and formulated vaccine candidates
The first step was the production of the antigen candidates under GMP standards, namely the PSA recombinant protein and 3 peptides, whose sequences are part of the PSA sequence. Rapidly, difficulties encountered in the up-scaling of the whole recombinant protein rendered this antigen option not economically viable, and it was abandoned. The efforts were then focused on the 3 peptides, which were distributed among the partners for testing on the patient samples. However, after the 1st year of the project, the preliminary results on human cells revealed that these peptide candidates would not cover both all *Leishmania* species affecting humans and the diverse HLA classes groups. The overall strategies needed thus to be rethought.
Based on the preliminary results obtained from the project, intense work was carried out to define new peptides, through *in silico* design, that would provide a good coverage of the world human population (a patent is being filled). And the whole work started again. Each partner received all the 8 peptides (5 new + 3 old) to assess their immuno protective capacity on human cells.
Then, formulated peptide-based vaccine candidates with human-compatible adjuvant and excipient were produced to determine, on dogs, their immunoprotective features.

Collecting documented samples and managing results

In order to evaluate the vaccine candidate immunogenicity and to define efficient population selection strategies, an adequate number of documented samples, representative of the different leishmaniasis scenarios (different *Leishmania* species, different status of the infection, etc) was needed. Thus, patients' recruitment plan was defined. More precisely, recruitment was concentrated on the inclusion of presumed "resistant" individuals (asymptomatic and healed, in order to identify immunological factors of resistance), symptomatic individuals, and naïve individuals (negative controls). These individuals were recruited from no or low endemic areas in France, Spain, India, Tunisia and Peru, and from highly endemic areas in India, Tunisia and Peru. The recruitment in each country was focussed on the *Leishmania* species prevalent in each area. In total, about 400 individuals according to different categories were recruited in the study. The recruitment of these human groups relied on clinical, serological, parasitological and immunological criteria agreed upon before and among the partners. Regulatory aspects (including ethical approval from the relevant authorities) were also carefully managed. Blood samples and tissues samples were collected from all these individuals processed and sent to the laboratories for analysis and assays.

A central database for gathering all obtained data was designed and setup to provide a reliable support for data storage, and a unique data warehouse to easily share consolidated data between all partners. All data for the various studies undertaken in RAPSODI was consolidated for statistical analysis. The database is composed of 341 individuals and more than 400 variables. Interesting data have been collected from these studies, and all the findings will help questioning and improving the current strategy for the treatment, control and prevention of leishmaniasis in the endemic areas.

Evaluating the vaccine candidates efficacy in vivo and ex vitro

Different formulated vaccine candidates were evaluated along three sequential dog trials, respectively aiming at i) comparing the 5 new peptides to the 3 old peptides and identify the mean concentration of use; ii) study the dose effect and identify the optimal concentration of peptides and QA21 adjuvant giving the best immune response; and iii) confirm results on a consistent group of dogs and compare the optimised vaccine candidate to the commercially availabe CaniLeish®. In the end, no particular toxic or safety concerns were raised during the study and a positive immune response was induced in more than 75% of cases at very low concentration. Such promising results are appealing further development.

Assays on human cells were also performed in order to evaluate the potential efficacy of the vaccine candidates in human. The objective was to assess the nature and the duration of the immune response engendered by the selected peptides candidates compared to control. For that purpose, specific immune responses were evaluated in individuals immune to VL, CL and ML in comparison with naïve populations. Focus were made on cell proliferation assay, cell phenotyping and cytokine production after stimulation by the antigens candidates. The results of standardized CBA carried out in the different endemic countries rendered encouraging results, even if the selected peptides candidates showed a poor antigenicity evidenced by a rather low cytokine production compared to crude antigens. Further analysis will help to define their role in the activation of a protective immune response in CL and VL patients.

Investigating intrinsic features of resistance

As mentioned above, knowing the exact immune status of an individual is a critical information necessary for implementing unbiased clinical trials. Ideally, this information would be collected by field assays; RAPSODI intended to make progress towards this goal. Studies to unravel the intrinsic features of resistance were thus necessary.

First of all, in order to be able to compare experimental results coming from the different teams, an important work of protocol standardization amongst partners was performed,

resulting in the writing of detailed procedures and implementation of training sessions in the different laboratories.

Parasitological features

The standardization task focused on a common quantitative PCR methodology aimed to both detect low parasitemia in the study population and follow-up the changes on parasitic load in those patients infected by *Leishmania*. To the best of our knowledge, this is the first time that different groups from different leishmaniasis endemic areas have attempted to estimate parasite burdens in patients affected by different clinical forms of leishmaniasis as well as in healed or asymptomatic individuals from the same areas following a common protocol. In the end, RAPSODI demonstrated that although it has been possible to estimate parasite burdens in patients affected by different clinical forms of leishmaniasis (as well as in healed or asymptomatic individuals) through a common PCR protocol, the widely variable range of results indicates that parasitemia should not be considered alone neither for the detection of asymptomatic infections nor for predicting disease outcome or relapses after treatment. However, it was possible to identify Leishmania species circulating at each study site by mean of PCR-based methods.

Immunological and cellular immune response features

A detailed analysis of the immunological status and the *Leishmania*-specific humoral and cellular immune response in the different included groups was carried out. Focus was made on the identification of asymptomatic carriers among blood donors, and also in the recruitment of symptomatic and healed patients, which gave some relevant information regarding the immune response profiles associated with both resistance and susceptibility to *Leishmania* infection. These quantitative analyses were done in serum or plasma samples through ELISA, blood and stimulated PBMCs through flow cytometry, *in vitro* cell proliferation assays and cytokine analysis in supernatant from specifically stimulated cells. Characterization of the specific cell mediated immunity to *Leishmania* infection has allowed to establish clear immunological profiles of resistance and susceptibility to the parasite.
Two kinds of tests could be designed out of these studies: i) a predictive diagnosis test able to distinguish immune from non-immune individuals based on their immunological profile; and ii) a simple and fast immunoassay to test vaccination effects in the vaccinated individuals, based on the identification of a small set of immunological markers indicative of anti-*Leishmania* immunity. Based on the identified profiles, preliminary sets of experiments were run but did not allow the drawing of reliable conclusions and further work will be needed.

Investigating genetic features of resistance

RAPSODI developed a new alternative experimental approach for evidencing inter-individual macrophage phenotypic variability as important for either the progression or the control of the infection in human leishmaniasis, leading to the identification of non specific invasive/evasive factors that limit or help the successful establishment of *Leishmania* infection in the host macrophages. Differentially regulated genes were tested on selected individuals' samples by RTQ-PCR together with the identification of informative SNPs in each of these genes. The various investigations performed allowed the identification of 223 putative relevant markers of presumed resistance/susceptibility to *Leishmania* infection. After further assays, eighteen markers were really assigned to resistance or susceptibility and 17 markers still need to be confirmed for their assignment in *Leishmania* resistance or susceptibility. A patent is being filled. These genetic markers could be used for the development of a non-invasive screening test for resistance/susceptibility to natural human leishmaniasis directly in the blood or in the PBMC-derived monocytes. Such test could be supported by a DNA chip.

This report documents briefly the main findings of the project and provides an easily accessible overview of the main achievements and their impact. The first section 1.2 briefly introduces the wider context of the project and respective scientific and technological objectives that the project set out to address. Section 1.3 briefly presents the key project achievements. Section 1.4 presents evidence of the initial impact of the project outcomes, providing details on dissemination and exploitation activities carried out by the project partners.

1.2 Description of project context and objectives

Leishmaniasis has been categorized by the World Health Organization as a “re-emerging and uncontrolled disease”, having detrimental effects on the development of endemic countries. Leishmaniasis paradox is to be considered as a neglected disease and to be the second most-dreaded parasitic disease in the modern world. Leishmaniases are widespread in 88 endemic countries on all continents except Antarctica, with 350 million people at risk, 14 million human cases permanently affected and an estimated annual incidence of 2 million cases. This results in a global morbidity of 2,090 thousands DALYs (Disability Adjusted Life Years) and a mortality rate of 59,000/year (WHO report A60/10, 2007).

Various drugs (usually quite expensive) are currently being employed, which raises the problem of the development of resistance. Besides, most of the infected people do not develop clinical symptoms and a past episode of leishmaniasis leads to lifelong immunity against re-infection with the same subspecies, once the infection is healed. This makes the development of a vaccine a realistic goal. On the other hand, vaccination is expected to be a cost-effective measure and can reach the goal of global control and/or eradication of the parasite infection. But, no such vaccine is available today.

A vaccine against *Leishmania* has been proposed as the best cost / benefit measure against leishmania. Several *Leishmania* antigen preparations have been used as vaccine candidates with different degrees of encouraging results mainly in mice models. Among the partners of RAPSODI consortium (VIRBAC and IRD), **an efficient canine vaccine** was developed. This was based upon LiESAp, a crude excreted- secreted antigen obtained from promastigote culture supernatant of *Leishmania infantum*, formulated with muramyl dipeptide (MDP). It can induce a long lasting Th1-mediated protection against experimental and natural canine VL (dogs and humans being the main reservoirs of *Leishmania)*.
In order to perform a future vaccination study, it would be appropriate to select the target population to be vaccinated and exclude naturally immunized individuals. However, tools enabling the precise categorization of the target population are also missing

The global aim of RAPSODI was to develop a human vaccine candidate against most or all *Leishmania* species that cause severe forms of leishmaniasis in the world; i.e a unique vaccinal solution able to protect the population against the various clinical phenotypes (namely visceral, cutaneous and mucocutaneaous leishmaniasis, VL, CL and ML respectively). Thus, RAPSODI’s first objective was to develop a human vaccine candidate based on a crude excreted-secreted antigen obtained from promastigote culture supernatant of *Leishmania infantum,* which gave highly promising results on dogs, a natural reservoir of visceral leishmaniasis (the more severe form). RAPSODI also proposed to investigate the possibility of extending the action of the vaccine candidate to cutaneous and mucocutaneous leishmaniasis. At the end of the project, the goal would be to have a vaccine candidate ready for human clinical trials.
RAPSODI aimed also at investigating the development of assays for population categorization, together with a marker signature for genetic susceptibility assessment.

Finally, RAPSODI objective was to provide the immunoassay tools associated to the efficiency follow-up of vaccination.

1.3 Description of the main S&T results/foregrounds

The RAPSODI project lasted 42 months starting from the 1st of January 2009. In general, the progress of the work has been roughly in line with the proposed plan; however some delays were encountered due to difficulties encountered during the project course.

The objective of the first period (M1-M18) was to define which active ingredients should be used in the vaccine for human purpose: either the recombinant protein (nsLaPSA) or a number of peptides (spPSA) from the protein. Proteins formulation and production were completed and sent to all partners in order to perform the analysis. In parallel, the formulation of the human-compatible peptide-based started, and immunological analyses were launched both in dogs and in human cells.
*Li*ESAp was produced and analysed by VIRBAC. The very low productivity of *La*PSA would result in the production of a very expensive vaccine (hundreds of euros per dose), that make unrealistic any development for human mass vaccination. As peptide production is far less expensive, the decision was finally taken to select only the peptides as active ingredients for the vaccine. Virbac subcontracted the *sp*PSA production and analysis as intended. The 3 peptides A17E, A17G and E34Pc that were found to be efficient in combination in dogs in the frame of the Vaxileish project have been produced and controlled.
However, preliminary results on human cells revealed that these 3 peptides alone may not be sufficient to cover both all species responsible for human leishmaniasis and the diverse HLA classes groups. More work, unforeseen in the initial plan, was thus needed to define additional peptides. A full investigation of peptides was performed by IRD and IMTAvH, in narrow collaboration with the other partners, with specific focus on both the genetic diversity of nsLaPSA, and the need to take into account the high polymorphism of HLA Class I and II molecules. Five peptides designed by IRD (3 HLA class I multi-epitope peptides and 2 HLA class II multi-epitope peptides) providing a good coverage of the world human population and maximizing the number of nsLaPSA epitopes have been tested by IOP, IPT, ISCIII and IMTAvH in endemic areas. In addition, 6 peptides designed by IMTAvH that evidenced reactivities in a pilot experiment may be tested by the IMTAvH in endemic areas of American tegumentary leishmaniasis.
Common protocols for clinical evaluation (inclusion/exclusion criteria), sample collection and preservation and diagnostic methods were defined for recruitment of human groups (symptomatic, asymptomatic and healed) from target populations in endemic areas for VL, CL and ML.
Common protocols of cellular biology and for immunological assays were agreed in order to establish the immunological profile (humoral and cellular) associated with resistance and susceptibility to the parasite in the different human groups.
The procedure for the evaluation of the performance of the PCR methods developed by each partner laboratory was also defined. A PCR workshop was organized in order to evaluate the PCR methods available. The goal of applying the same approach to detect and quantify *Leishmania* infection within RAPSODI project, and make this comparable among partners, was achieved.
Concerning the definition of genetic features of resistance, contrasting macrophage infection phenotypes were evidenced on limited human samples as encouraging results. It contributes to validate the proposed experimental approach to analyze the impact of human genetic diversity on the macrophage response upon infection. We evidenced the necessity to improve and standardize a micro-assay of macrophage infection applicable to limited volume of field blood samples and to large scale study. This *in vitro* cell model certainly offers a good opportunity to identify genes with differential expression between "permissive" and "less

permissive" human macrophages. SAGE libraries representative of the contrasting phenotypes (high versus low permissiveness) and of the different culture conditions (infected versus uninfected condition) were constructed and transcriptomic databases were established; it definitively validated the interest of the study.
Concerning immunological and genetic assays, different training activities and methodology transfer have been carried out during this first period.
In addition to this technical work, a project public website with a summary of the project and a booklet for presentation of the project were produced. The RAPSODI project was also presented in various congresses.

The RAPSODI activities during the second period (M19-M42) consisted in the continuation of the relevant samples collection used for the parasitological and immunological analysis. However, due to the difficulties encountered during the first period and the additional work and various changes that have been taken to overcome these issues, some additional assays on dogs turned out to be necessary to determine the efficiency and dose effect of the new peptides candidates. The production of the final human-compatible peptide-based vaccine candidates in a formulated form was also carried out. This production required several trials in conjunction with good control methods of the final products. The industrial formulation of the 8 peptides in diverse mixtures with the adjuvant (QA21) and excipient (TMS = tris, Mannitol and Saccharose) was perfectly standardized. Several batches of peptidic vaccines were manufactured under GMP standards. 3 dogs' trials were respectively performed. These dogs' experiments were respectively called LP09, LP10 and LP11. The LP09 study (comparison of the 5 new peptides to old peptides) allowed to detect the mild concentration of new peptides to use. The LP10 study (dose effect study) served to determine the concentration of peptides and QA21 adjuvant giving the best immune response in dogs, whereas the LP11 study consisted in a trial with the final peptide vaccine on a consistent group of dogs and also in a comparison between the present dog vaccine on the market CaniLeish®. As a conclusion, these trials of the peptide vaccines on a consistent group of dogs served to demonstrate that the RAPSODI peptides, at a very low concentration, induce a positive immune response for 75%. The experiments also revealed that these peptides can also be used like enhancers of vaccines for canine leishmaniasis. .

The update of the workplan also affected the recruitment campaigns and diagnostic testing had to be adapted to involve the maximum of individuals and samples with the final set of peptides. Besides, the updated plan had to take into consideration the practical issues face by some partners in the consortium:

- **In Tunisia (IPT),** the target number for CL patients, VL patients and healed VL, originally planned, was very difficult to reach, due to the instability of the political situation in Tunisia since the events in 2010. Indeed, several institutions have had no legal framework for months, making it difficult to continue the recruitment legally.
- **In Peru (IMTAvH),** the recruitment of ML (both patients with active and healed lesions) was abandoned. Besides, the budget constrains forced IMTAvH to reduce the number of individuals to be analyzed by cellular immunology methods. Because of project objectives, to obtain a vaccine candidate based on nsLaPSA, IMTAvH selected CL patients, who represent the higher proportion of tegumentary patients (over 90%) and also because ML corresponds to a second phase of the disease, i.e. almost all ML patients were formerly CL patients. A vaccine would be less necessary under the concept of preventive medicine for the ML patients group.
- **In France (IRD),** the recruitment of asymptomatics for VL infection was stopped due to the big difficulties to find these individuals in France.
- **In Spain (ISCIII),** the target number of asymtomatics was reached but some of them loss their response along the time and could not be evaluated and included in the study.

- **In India (IOP)**, VL is going down in India. As a consequence, the patients' recruitment was very challenging.

The final recruitment plan allowed the inclusion of about 400 individuals, including:

- 36 Healed CL individuals and 29 asymptomatic individuals for CL infection in Tunisia
- 22 Active CL individuals (19 Asymptomatic individuals 19 Healed CL from Peru
- 29 asymptomatic individuals for VL, 1 active VL in Tunisia
- 26 Healed VL, 16 Active VL and 26 PKDL 26 in India
- 5 asymptomatic for VL in France
- 16 asymptomatic for VL and 5 active VL in Spain
- 143 naïve individuals from the different countries.

The evaluation and assays to establish the parasitological and immunological profiles of the patients were performed with RAPSODI standardized protocols. The cell proliferation assay, cell phenotyping and cytokine production after stimulation with leishmanial crude antigens demonstrated that specific immune responses are characterized by a strong lymphoproliferative response, expansion of both CD4+ and CD8+ T cells and IFN-g production. Intracellular staining showed that specific secretion of IFN-γ is mainly produced by CD4+ T cell. Other factors like TNF-α and Granzyme B are also playing a role in the protective immune response. Therefore, all these parameters could be used as markers of immunity to leishmaniasis. Results obtained from ELISA assays to assess the humoral responses in all the groups studied have provided information about features that allow a better characterization of the immunological profiles related to resistance and susceptibility. The characterization of the humoral responses performed has pointed out the potential value of some immunoglobulin isotypes to differentiate some clinical groups in visceral and cutaneous leishmaniasis. In addition, valuable information about the differential endemicity among countries has been also provided. Finally, the different groups attempted to estimate parasite burdens in patients affected by different clinical forms of leishmaniasis as well as in healed or asymptomatic individuals from the same endemic areas. A wide range of parasite burden is observed within and among the study groups. For this reason, a specific cut-off of parasites in blood could not be related with a specific clinical or immunological status.

In parallel, further tests of the immunogenicity of the vaccine candidates was performed in the human cells collected. The sequence conservation of genes encoding soluble PSA antigens *Leishmania* isolates obtained from participating institutions of the endemic regions were designed and assessed. Tests of the immunogenicity of PSA vaccine candidates, through cellular proliferation assays (CPA) with *ex vivo* T cells were completed on all samples collected. The cellular immune protective profile elicited by the PSA vaccine candidates was analyzed. The strategy was to compare the cellular immune response of naïve individuals to infected ones (asymptomatic, healed and active Leishmaniasis patients). The objective was to identify the best PSA vaccine candidate with the best immunoprotective profile and that would be used as vaccine candidate component. The results of standardized CBA carried out in the endemic countries showed that the immune response to nsLPSA recombinant protein and their selected peptides candidates was poor in terms of antigenicity evidenced by low cytokine production. However, the synthetic peptides designed during the project rendered encouraging results. that showed evidences of their effect on immune cells although its value were obscured by the fact they also influenced immune cells from naïve individuals. Further analysis will help to define their role in the activation of a protective immune response in CL and VL patients.
Besides, experiments also assessed the nature an intensity of antibody response against nsLPSA and/or derived polypeptides. The different types of immunoglobulin classes and subclasses produced by different clinical manifestation categories, asymptomatic subjects and negative endemic controls were the subject of interest to associate them with different stages of the infection. The results of these evaluations revealed a lack of significant

reactivity against PSA and its derived E34PC peptides. However, active patients from Tunisia, where VL is the main clinical manifestation of Leishmaniasis, showed good reactivity against E34PC peptides. Thus, E34PC emerges as a good candidate to be used in ELISA diagnostic test for the identification patients with visceral leishmaniasis in Tunisia.

Combination of quantitative analyses (transcriptional profiling studies, the most contrasted differential gene expression) and qualitative analyses (cluster families associated with resistance/susceptibility to human macrophage *Leishmania* infection, top biological functions and pathways) of SAGE-libraries allowed the selection of 223 putative gene markers for their ability to distinguish the most distinct infection phenotypes and probably involved in the host-pathogen interactions associated with resistance/susceptibility towards natural *Leishmania* infection. The specificity and the efficacy (through PCR) of these markers were further tested. In the end, 35 genes were proved to act as markers for resistance or susceptibility, paving the way to a non-invasive genetic screening test (e.g. on a DNA chip), affordable and usable for the selection of population to be vaccinated in vaccine trial assays. Further work on a statistically relevant set of samples will soon be implemented to reach this goal.

A central database for gathering all data obtained has been designed. The RAPSODI database has been designed and setup to provide a reliable support for data storage, and a unique data warehouse to easily share consolidated data between all partners. The database (DB) has been created using the EpiData software (Epidata Association, Denmark http://www.epidata.dk/). All data for the various studies undertaken in RAPSODI was consolidated for statistical analysis. The data produced during the project were of several formats:

- The Case Report Forms (CRF) : papers that are filled during the recruitment of the individuals
- Biological results: data produced during biological tests, saved in Excel tables.

The database is composed of 341 individuals and more than 400 variables. Interesting data have been collected from these studies, and all the findings will help questioning and improving the current strategy for the treatment and prevention of Leishmania in the endemic areas.

1.4 Impact – Dissemination, Exploitation and Standardisation

Leishmaniasis is affecting 88 endemic countries with a potential of 360 Mo individuals at risk; its annual incidence is close to 2 Mo individuals, while its prevalence accounts for 14 Mo individuals; approximately 59 000 patients are dying each year. By developing solutions to circumvent such a “re-emerging and uncontrolled disease”, the vaccine candidate issued from RAPSODI shall have a great impact on the development of endemic countries, and improve the quality of life of their inhabitants by limiting social and health burdens. In addition, and quite independently from the vaccine development itself, the categorization tests will be very useful to enhance epidemiological data from the endemic areas, and could be useful to whatever clinical trials. Finally, the standardization and training activities will help enhancing a common view of dealing with leishmaniasis-related issues among the scientific community.

1.4.1 Impact on health and quality of life

VL is usually fatal, if not treated. CL and ML often lead to very ungracious scars, a stigma which can cause serious social prejudice, and even, in some cases, in victims being humiliated and cast out from society. As a corollary, patients keeps hidden, which contribute to the still largely under-estimation of the epidemiological data. Leishmaniasis-related disabilities impose great social and health burdens. In addition, secondary *Leishmania*-related infections can also occur in a significant number of cases, increasing the burden

caused by the disease. After 42 months of project, RAPSODI made progress toward the development of an affordable vaccine. However, due to the complexity of the task, and the urgent need for a solution, further development will probably focus on the most severe indication, namely visceral leishmaniasis.

The progress made on defining the features of resistance will also help in categorizing patients in the future. Especially, the genetic test that should be finalized directly from RAPSODI results could allow the early detection of individuals who should be carefully followed as they are susceptible to the disease. It would thus be very helpful in implementing prevention campaign in endemic areas. It should be noticed that these results are independent from the development of the RAPSODI vaccine, and could thus be useful to any programme dealing with epidemiology, prevention or therapeutic campaign.

1.4.2 Contribution to standards

A number of methods have been developed and standardised during RAPSODI's lifetime, paying special attention to the ease of access and implementation of such protocols (PCR protocols, inclusion/exclusion criteria, etc.) to ensure a very broad use around the world, and especially within the endemic area laboratories. They will be disseminated through scientific and medical communities throughout submitted and upcoming publications.

1.4.3 Impact on European and endemic area research

RAPSODI favoured a multidisciplinary approach of the leishmaniasis problem, while enabling fruitful exchanges between, on the one hand, European teams that have strong expertise in immunogenetic and animal model aspects of the disease, and on the other hand, teams from endemic areas that have a concrete and practical experience of human field research. This partnership thus increased the level of comprehension of the disease and way of thinking on both sides.
In particular, RAPSODI succeeded in standardizing various protocols among partners across the various endemic regions (Africa, Asia, South America and Southern Europe), favouring a common vision of the global leishmaniasis problem while considering regional specificities.
RAPSODI project has been helpful to increase our knowledge of asymptomatic conditions in endemic areas. In areas where VL transmission is anthroponotic, asymptomatic persons might play a role as reservoirs, and even in areas where VL is zoonotic it is speculated that these persons could also contribute to transmission. Thus, assessment of the prevalence and distribution of asymptomatic cases would contribute to a better understanding of VL transmission and help in developing control efforts. Immunological and parasitological analysis carried out in asymptomatic individuals in the framework of rapsodi has allowed to establish the best tools to characterize such condition and also to obtain a better definition of such profile.
For the most severe form of the pathology, RAPSODI has led to the better understanding of humoral and cell mediated immunity evaluated simultaneously in the four different groups' i.e.; PKDL, acute VL, healed VL and naïve group in VL endemic country (such fact is also true for the other types of leishmaniasis in the various endemic areas). The immune responses to PSA, TSLA and different peptides were investigated extensively in above groups and this set the stage for systematic evaluation of any new vaccine candidate developed for VL in future.
Interlaboratory comparison of Leishmania-Q-PCR methods performed across different countries led to standardised protocol for the detection and monitoring of parasite level in clinical sample.
The study led to the significant advancement in knowledge particularly regarding the immunopathogenesis of PKDL.

Finally, teams from the endemic countries benefited from personnal training whenever necessary. European top teams in the field (IRD and ISCIII (WHO Collaborating Centre for Leishmaniasis)) ensured training session either in their lab or on site, depending on the partner's needs.

1.4.4 Impact for endemic countries

By developing a common protocol for disease diagnostic, RAPSODI helped to an improved care of involved patients, with a better insight into their real status. This improved knowledge will in turn facilitate other leishmaniasis-related researches, increasing the corresponding level of accuracy, and allowing more in-depth studies and analyses. This impact will even be stronger once the assays will be available to field investigators, who will thus be able to simply and precisely diagnose patient status at large scale.
The teams from endemic countries will also benefit from an improved image and visibility, and could rely on the strong relationships that were built inside the consortium and together with other projects, thanks to the international meeting organised by EC and gathering all actors in the field. This will undoubtedly be an asset for further collaborations, and potentially facilitate the exchange of scientists (including young researchers) between Europe and endemic areas.

1.4.5 Economic impact

Leishmaniasis remains a severe public health problem, which is spreading to previous non-endemic areas, and the control strategies currently proposed are either ineffective, especially in the long run, or very expensive, and thus represent a high burden to the economy of the developing countries. Reservoir control by dog culling (Mediterranean basin, Latin America) has proved to be largely ineffective. Moreover, several forms of the disease are anthroponoses or have sylvatic reservoirs, rendering vectorial control through insecticide spraying unfeasible. As a consequence, all efforts (including financial) in this direction appears to have limited impact.

Although progresses have been made in the drug area to enhance patient care, the development of a vaccine still appears as an appealing and cost effective control strategy (Lee et al. Am. J. Trop. Med. Hyg., 86(3), 2012, pp. 417–425). The development of such vaccine should significantly decrease health expenses in affected developing countries. This decrease would be all the more significant, that the associated tools for diagnosis and follow-up (affordable to endemic areas) enable to concentrate efforts on the population in need. Although the RAPSODI vaccine development is still far from completion, the chosen peptide strategy still complies with the predictions of a targeted cost around 20 EUR per patient, which is 5-times less expensive than miltefosine.

Apart from the costs of control or treatment, leishmaniasis also affects productivity and welfare, and the impact is far more important than the number of cases would suggest. In particular, it causes great losses to agricultural or industrial rural development programmes by debilitating the population and depleting the labour force, and in the end constitute barriers to progress for the developing countries. On several occasions, epidemics have significantly delayed implementation of development projects (WHO report A60/10, 2007). The development of a prophylactic vaccine, like the one proposed by RAPSODI, could be useful to prevent such high impact outbreaks.

1.4.6 Impact for EU Development Co-operation Policies

In terms of the main Programme objectives, this project aimed to promote and reinforce both Community and Developing Countries scientific capacities within the context of research on vaccine development for leishmaniasis control. Leishmaniasis remains a severe

public health problem in various endemic areas (Africa, Asia, South America), which is spreading to previous non-endemic areas (including Southern Europe), with both direct and indirect economic costs.

The composition of the consortium exemplifies the broad aims of the 7th framework programme through the establishment of networks of research institutions pursuing common research goals in specific areas of interest. The four Community-based Beneficiaries involved in RAPSODI are all strong in their respective fields of research and the interactions facilitated by the project were guaranteed to have a positive impact on the European contribution to improvements in research capacity and public health in developing countries.

In general terms, outcome of this project contributed effectively to the Community's development policies in the following ways:

- Provide Developing Countries with standardized tools for patient categorization
- Strengthen Institutional development & linkages in Developing Countries
- Strengthen European-Developing Country co-operation
- Enhance European-Developing Country policy dialogue
- Enhance European Institutional collaboration

1.4.7 Contact details of the project

In order to promote the project results among the scientific community but also among the population whose life is at stake, a website has been set up (www.fp7-rapsodi.eu). A logo was also created, together with an introducing booklet and a general graphical charter, in order to provide a distinguishable identity.

Project web-site: www.fp7-rapsodi.eu

Coordinator:

VIRBAC SA
Name: Gérard PAPIEROK
Institution address: Rue 13ème LID - BP 27, 06511 Carros, France
Email: gerard-marie.papierok@virbac.com

Partners:

Institut de Recherche pour le Développement, UMR177
Name: Jean-Loup LEMESRE
Institution address: 911 Avenue Agropolis - BP 64501 - 34394 Montpellier Cedex 5, France
Email: Jean-loup.lemesre@ird.fr

Instituto de Salud Carlos III
Name: Francisco Javier MORENO NUNCIO
Institution address: 4-6 Calle Sinesio Delgado, 28029 Madrid, Spain
Email: javier.moreno@isciii.es

Indian Council of Medical Research, Institute of Pathology (IOP)
Name: Poonam SALOTRA
Institution address: Ansari Nagar, 110029 New Delhi, India
Email: salotrap@icmr.org.in

Universidad Peruana Cayetano Heredia, Instituto de Medicina Tropical Alexander von Humboldt (IMTAvH)
Name: Jorge AREVALO
Institution address: 430 Honorio Delgado, 31 Lima, Peru
Email: biomoljazz@gmail.com

Institut Pasteur de Tunis
Name: Medhi CHENIK/ Amel GARNAOUI
Institution address: 13 Place Pasteur - 1002 Tunis Belvedere, Tunisia
Email: mehdi.chenik@pasteur.rns.tn / amel.garnaoui@pasteur.rns.tn

Alma Consulting Group SAS
Name: Frédéric PEYRANE
Institution address: 55 Avenue René Cassin, 69338 Lyon, France
Email: fpeyrane@almacg.com

Syncrosome SAS
Name: Richard MITRY
Institution address: 163 Avenue de Luminy - Luminy Biotech CP 908, 13288 Marseille, France
Email: r.mitry@syncrosome.com