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Tackling *Plasmodium knowlesi* malaria

Landscape of current research in *Plasmodium knowlesi*

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List of abbreviations

ACT	Artemisinin-based Combination Therapies
APMEN	Asia Pacific Malaria Elimination Network
ASTMH	American Society of Tropical Medicine and Hygiene
ERG	Evidence Review Group
FISH	Fluorescent In Situ Hybridization
GMP	Global Malaria Programme
HSPH	Harvard T. H. Chan School of Public Health
IRS	Indoor Residual Spraying
LAMP	Loop-mediated amplification
LLINs	Long Lasting Insecticidal Nets
LSHTM	London School of Hygiene and Tropical Medicine
M	*Macaca*
malERA	malaria Eradication Research Agenda
MESA	Malaria Eradication Scientific Alliance
MOE	Ministry of Education
MOH	Ministry of Health
MPAC	Malaria Policy Advisory Committee
MRCUK	Medical Research Council of the United Kingdom
NHMRC	National Health and Medical Research Council Australia
NIH	National Institutes of Health
P	*Plasmodium*
PCR	Polymerase Chain Reaction
PDP	Product Development Partnership
PI	Principal Investigator
RBC	Red Blood Cells
RCUK	Research Councils United Kingdom
RDT	Rapid Diagnostic Test
UI	Uncertainty Interval
UK	United Kingdom
USA	United States of America
WHO	World Health Organization

Executive summary

Plasmodium knowlesi is the fifth species that can cause malaria in humans and was declared an emerging public health threat in 2004. 13 years later, this species still holds many unanswered questions, such as its transmission mechanism or its complete geographic distribution; threatening the effectiveness of malaria control and elimination strategies. In the last years, the number of cases reported has increased in South-East Asia and *P. knowlesi* is the first cause of malaria in Malaysia. The aim of this report was to describe the landscape of current research in *P. knowlesi,* compare it to determined needs and identify the main research gaps. Information related to ongoing research activities was collected, quality checked and validated through systematically searching online databases of grants and through direct contact with expert researchers. 20 projects have been validated and 41 experts have been contacted. The main knowledge gap is determining whether humans are always infected through the natural host or if human to human transmission is taking place in some cases. Being this the case, control and elimination strategies should be adapted in order to be effective. Current research priorities are effective vector control strategies, clinical management guidelines and new diagnostic methods. Unless more research is carried out to fulfil these gaps, we will not have the knowledge and the tools to properly fight against *P. knowlesi.*

1. Background

1.1. Malaria

The World Health Organization (WHO) in its latest World Malaria Report[1] estimated that 91 countries and territories had active malaria transmission in 2015. 212 million cases (Uncertainty Interval [UI]: 148-304 million) and about 429,000 deaths (UI: 235,000-639,000) occurred due to malaria globally and 70% of those deaths (303,000, UI: 165,000-450,000) were in children aged less than five years. Most of the cases in 2015 were in the WHO African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean Region (2%). Between 2010 and 2015, the incidence among populations at risk decreased 21% globally; the mortality rates among those populations fell by 29% among all age groups and by 35% among children under 5, one of the main population groups at high risk, together with pregnant women, patients with HIV/AIDS or non-immune immigrants[1,2]. A high proportion of this change can be attributed to the vast implementation of malaria preventive, diagnostic and treatment interventions[1].

Malaria is a life-threatening protozoan parasite infection caused by *Plasmodium* (*P.*) and transmitted to humans by *Anopheles* mosquitoes. There are 5 parasite species that can cause malaria in humans: *P. falciparum* and *P. vivax,* which account for almost all cases, *P. ovale* and *P. malariae*, which have lower incidence[1–3] and *P. knowlesi*, a malaria parasite of monkeys which is being increasingly reported in South-East Asia[4,5].

According to the World Malaria Report 2016[1], the total funding for malaria control and elimination in 2015 was estimated to be of $2,9 billion. On the other hand, the research and development funding for malaria was calculated to be of $565 million in the same year[6].

1.2. *Plasmodium knowlesi*

P. knowlesi is the fifth malaria parasite that can infect humans. However, this species of *Plasmodium* was first identified in 1927 as a natural parasite of macaques living in South-East Asia with the capacity of infecting humans only seen in laboratory conditions and therefore was overlooked by the scientific community until 2004, when a publication from Dr Singh and colleagues[5] brought this species back to the attention of the scientific community. In this paper the authors characterised blood samples from people with malaria in a district of Malaysian Borneo with Polymerase Chain Reaction (PCR) and concluded that 58% of *P. malariae* cases reported were actually caused by *P. knowlesi*, corroborating the existence of frequent natural human infections with this parasite[5,7]. The species was then identified as a public health threat, in view of the fact that it met all the criteria on the WHO definition: “initial diagnostic confusion leading to delayed detection and inappropriate control measures and high mortality in the absence of effective preventive or control measures, which becomes more difficult if there is an intermediate host difficult to control”[8].

After this study in 2004, further evidence regarding *P. knowlesi* has been collected and research has been ongoing. In 2011, WHO held an informal consultation on the public health

importance of *P. knowlesi*[9] and the creation of the Evidence Review Group (ERG) was requested in 2016[10].

Globally, there is no data of the real burden of *P. knowlesi* and the species is not mentioned in the last World Malaria Report. Most of the data available comes from the grey literature or scientific publications from Malaysia. According to the data presented by Dr Rabindra Abeyasinghe at the last Malaria Policy Advisory Committee (MPAC) meeting[11] (See 1.3. Malaria Policy Advisory Committee), in 2016 69% of the total malaria cases in Malaysia were *P. knowlesi* infections. Regarding morbidity and mortality, a comparative study in Malaysia in 2013 estimated that *P. knowlesi* was the most common cause of severe malaria and had a three-fold greater risk of severity than *P. falciparum*[12]. The majority of the information available about this species and presented in this report comes from Malaysia, the country where the first naturally acquired human case was reported in 1965, but human *P. knowlesi* cases have been confirmed across South-East Asia with cases in Cambodia, Indonesia, Myanmar, The Philippines, Singapore, Thailand or Vietnam[7,13,14].

1.2.1. Transmission

P. knowlesi is a zoonosis[11,15]. Zoonotic infections are transmitted from animals (the natural hosts) to humans directly or through an insect vector, which in this case is the mosquito. Most of the times humans act as "dead-end" hosts, meaning that there is no (or little) onwards human to human transmission[16]. Since direct transfer between humans through the mosquito has not been demonstrated to occur in nature (even though it has been proven to be possible under experimental conditions), humans are thus far considered non-competent or dead-end hosts[11,17], theory also supported by some studies in Sarawak, Malaysia, showing that parasite genotypes seem to be shared between macaques and humans[13].

The natural hosts of *P. knowlesi* are long-tailed (*Macaca fascicularis*) and pig-tailed macaques (*M. nemestrina*) and banded leaf monkeys (*Presbytis melalophus*)[15,18]. In Myanmar, the northern pig-tailed macaque (*M. leonina*) seems to be a natural host as well[10]. The insect vectors are members of the *Anopheles leucosphyrus* group[11].

The life cycle of the malaria parasites is similar for all the species. In humans and monkeys, it starts when a female mosquito injects sporozoites into the host while biting. These parasites arrive at the liver via the bloodstream and invade the hepatocytes, replicate and evolve into schizonts. Then, the hepatic schizonts break and release merozoites that will invade the red blood cells (RBCs). Inside the erythrocytes, asexual multiplication takes place and more merozoites are developed, which will invade more RBCs. Some of these merozoites may evolve into male and female gametocytes which will be absorbed by another mosquito. In the mosquito, the sporogonic cycle will take place, resulting in the production of more sporozoites that will infect another monkey or human (Fig. 1.)[19,20].

The main difference in *P. knowlesi* is that there are two levels of transmission: one among the macaques and another, less frequent, from macaques to humans (Fig 2.)[20,21]. Other key features of this species are the quotidian cycle in the RBCs, lasting approximately 24h, and the fact that the species does not relapse from residual forms in the liver[19].

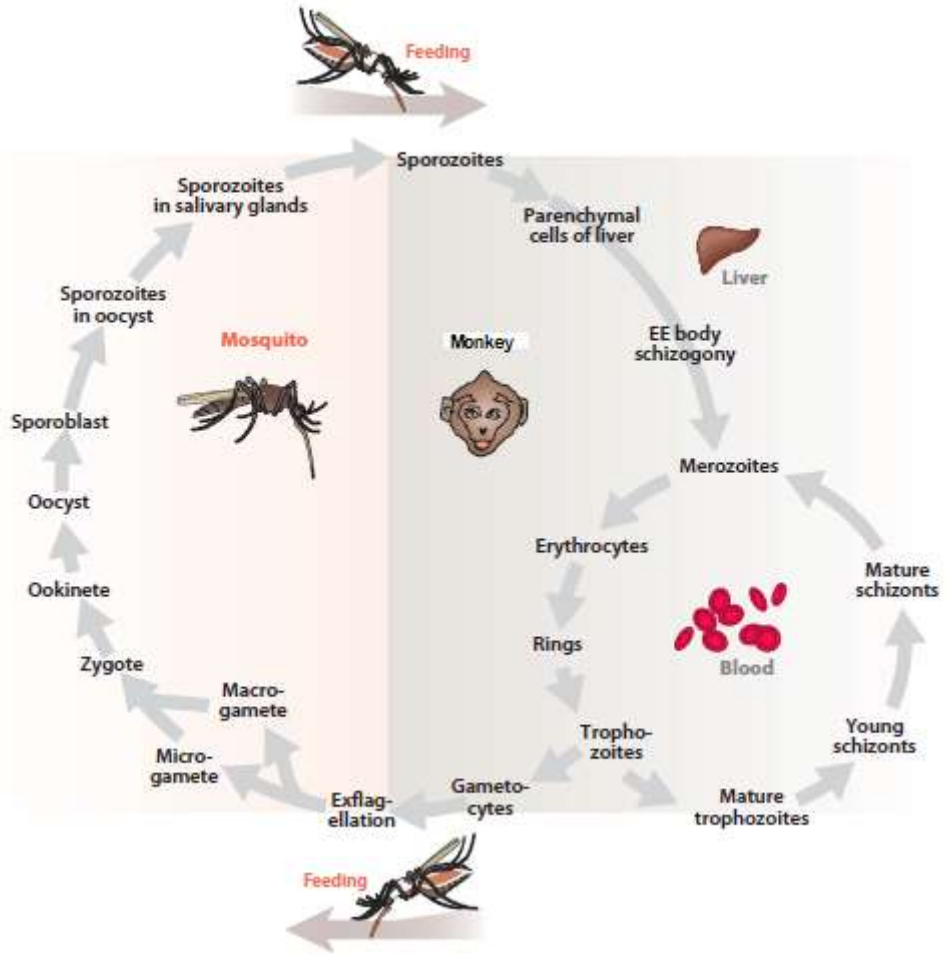

Fig. 1. Life cycle of the malaria parasite. Adapted from [19]. The cycle starts when the female mosquito injects sporozoites into the host while biting. The parasites arrive at the liver via the bloodstream and then invade RBCs, where they will develop and replicate. Afterwards, the mosquito will feed and absorb the sexual stages of the parasite, hosting the sporogonic cycle. The resulting sporozoites will stay in the salivary glands of the mosquito and will be injected again into a new host to complete the cycle.

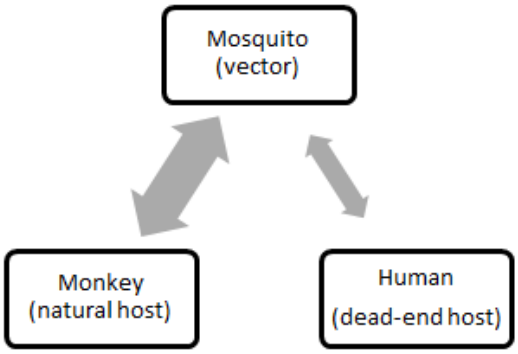

Fig. 2. Human-monkey-mosquito interaction. The main level of transmission takes place between monkeys, but there is a second level of transmission, less frequent, which involves mosquitoes biting monkeys and getting infected that will afterwards bite and infect humans.

1.2.2. Risk of infection

For any vector borne disease, the risk of human infection is highly conditioned by the distribution of the vectors. For *P. knowlesi*, since there is also a natural host involved, transmission will depend both on the insect vectors and on the monkey hosts, with the highest damage to human health when there is an equal allocation of bites between monkeys and humans[17]. The vectors of *P. knowlesi* malaria are restricted to members of the *Anopheles leucosphyrus* group, which are found in the forest and forest borders of the Philippines, Brunei, Malaysian Borneo and Indonesia[22], so people who live there or enter the ecological habitat of macaques and mosquito vectors will become at risk of getting infected[20]. According to a study conducted in Malaysia[12], almost all patients with *P. knowlesi* malaria recounted forest or plantation exposure and 50% of them reported to have seen a monkey in the past four weeks. According to the grey literature presented by Dr Rabindra Abeyasinghe in the last MPAC meeting (See 1.3. Malaria Policy Advisory Committee)[11], the data goes along these lines as well: forest workers and traditional planters accounted for the most malaria cases reported between 2014 and 2016 in Malaysia and 60% of the cases reported in 2016 were in rural areas[11]. It is thought that conversion of wild forest into wood plantations and the expansion of human activities in previous inhabited areas close to the rainforest represent considerable risk factors associated with increasing *P. knowlesi* incidence. There is an overlap of human and monkey habitat in the wood plantations, which enables the coexistence of the mosquitoes and macaques with the human population[4,23,24]. In addition, it has also been suggested that the increase in *P. knowlesi* incidence in Malaysia could be due to a loss of cross-protective immunity subsequent to the control of *P. falciparum* and *P. vivax* malaria in the region. According to the World Malaria Report 2016[1] in 2000 there were 6000 cases of *P. falciparum* reported in Malaysia, whereas in 2015, the number of cases reported was 110. A similar trend has been seen for *P. vivax,* with 5953 cases reported in 2000 and 84 in 2015. This effect could act in synergy with the influence of land use change[4].

The most common vector control strategies for malaria are Long Lasting Insecticidal Nets (LLINs) and the Indoor Residual Spraying (IRS) of houses, both focused on protecting humans. However, the characteristics of *P. knowlesi* hinder a little bit the effectiveness of these methods due to the zoonotic reservoirs in the macaques, which are a risk of disease spread even if the infection in humans is eliminated, and the outdoor biting and resting behaviour of the mosquitoes[25,26]. According to a longitudinal study carried out in an endemic area of Malaysia[25], *P. knowlesi* malaria transmission was not likely to be prevented by LLINs.

1.2.3. Diagnostics

Routine parasitological diagnostics have certain difficulties and limitations in regard to *P. knowlesi.* WHO recommends the confirmation by microscopy or Rapid Diagnostic Tests (RDTs) before treatment[1]. The former is used because the human host-adapted species have differential morphological characteristics that enable the specialists to identify them quite accurately. Nevertheless, the entry of *P. knowlesi* malaria in the picture has complicated this method of diagnosis, which has important limitations in sensitivity and specificity mainly due to the similarities that *P. knowlesi* shares with *P. falciparum* and *P. malariae*[13,20,21]. On the other hand, the RDTs for malaria are insensitive at low parasite densities and are unable to distinguish *P. knowlesi* from the other species, since cross-reactivity has been observed[7,20,21].

These may be the reasons why most *P. knowlesi* infections are misdiagnosed in routine diagnostic laboratories and due to these difficulties when identifying the species, the WHO recommended that in areas where *P. knowlesi* has been reported, microscopists should recount and report all *P. malariae* cases as *P. malariae/P. knowlesi*[9]. The use of molecular detection methods, mainly PCR, has enabled accurate identification of malaria parasites, highlighting infection in humans by *P. knowlesi*. These methods, which are more sensitive and specific than microscopy or RDTs, are thus far the only technique capable of accurately identifying *P. knowlesi*, but they are slow and expensive and it is unlikely that they will replace routine microscopy in endemic areas[20,21].

These difficulties to correctly identify *P. knowlesi* may be one of the reasons why there is little data on the real burden of the disease.

1.2.4. Clinical outcomes and pathophysiology

P. knowlesi is unusual among the primate and human malarias because the asexual part of the life cycle (the erythrocytic cycle) takes 24h to complete, rather than the 48 or 72h of the other malaria species. Given the fact that there are no clinical signs when the parasites are developing in the liver[20], if the disease is not diagnosed and treated rapidly, parasitaemia will keep increasing daily and complications will develop very fast[27,28]. If this is the case, severe *P. knowlesi* malaria is likely to occur, which has been reported in 6 to 9% of the infections, with a case fatality rate of approximately 3%[7]. In a prospective comparative study conducted in Malaysia[12], the risk of severe *P. knowlesi* malaria was reported to increase 11-fold and 28-fold with parasitaemia higher than 20,000 parasites/µL and higher than 100,000 parasites/µL, respectively; while infection with *P. knowlesi* was connected with a 3-fold higher risk of severity than with *P. falciparum.* WHO defines severe *P. knowlesi* malaria as for *P. falciparum* malaria but with 2 differences: hyperparasitaemia occurs when the parasite density is more than 100,000 parasites/µL and jaundice when the parasite density is more than 20,000 parasites/µL[29]. Clinical manifestations of severe malaria are similar for all the *Plasmodium* species and include prostration, abdominal pain, acute respiratory distress syndrome, acute renal failure, convulsions, circulatory collapse and severe anaemia, among others[12,21,30]. Most cases of the infection are uncomplicated and respond to treatment, with the variety of mild symptoms typical of the other malarias[7,21]. In this same study[12], the patients seen were older than those for *P. falciparum* or *P. vivax* malaria, association justified by the correlation between age and parasite count since a greater risk of hyperparasitaemia has been seen among older patients.

1.2.5. Drugs

As mentioned previously, infections with *P. knowlesi* can rapidly evolve to high parasitaemia. Thus, immediate diagnosis and introduction of effective treatment play a key role in avoiding morbidity and mortality[31]. Since, until recently, most of *P. knowlesi* infections were misdiagnosed as *P. malariae*, the majority of cases in Malaysia were treated with chloroquine (the recommended treatment for *P. malariae* in Malaysia) and earlier studies reported that *P. knowlesi* responded well to this drug[27,31]. However, chloroquine should be used with caution, especially when there is no species-specific diagnosis and there is risk of misdiagnosing *P. falciparum*, which is mostly chloroquine resistant[27]. According to the WHO guidelines for the treatment of malaria[12,29], different combinations of Artemisinin-based

Combination Therapies (ACTs) have been successfully used to treat uncomplicated *P. knowlesi* malaria infections, being artemether- lumefantrine the preferred combination by the Ministry of Health (MOH) of Malaysia. On the other hand, the WHO recommends intravenous artesunate for severe *P. knowlesi* malaria, as for the other malaria species[29]. Since *P. knowlesi* does not relapse from residual forms in the liver, administration of primaquine is not necessary[19]. In addition, the treatment recommendations for children are the same as for adults. Even though *P. knowlesi* malaria seems to be rare in pregnant women, the recommended treatment is intravenous artesunate for severe episodes and ACT for uncomplicated cases in the second or third trimester, whereas chloroquine should be administered in the first trimester, following the guidelines of treatment for *P. falciparum* malaria in pregnancy[29,31,32]. As far as drug resistance is concerned, mutations associated with drug resistance have not been reported so far[31].

1.2.6. Vaccines

P. knowlesi has been one of the main laboratory models in malaria vaccine development mainly because of three reasons: first, due to the possibility of growing the parasite in culture; second, in view of the fact that the parasite produces antigens similar to those of the other human malaria species and finally due to the fact that animals can be challenged in trials with predictable outcomes for the controls, being the rhesus monkeys the main hosts for trials using experimental vaccines. In addition, most of the antigens used for the development of human vaccines have been isolated from *P. knowlesi*, enabling the study in monkeys or in culture. This enabling technology, beyond testing vaccine concepts, has provided relevant data on the development of antimalarial immune responses and parasite-host interactions[19,28]. On the other hand, there is currently no vaccine in the pipeline targeting *P. knowlesi*[33].

1.2.7. Parasite genetic diversity

P. knowlesi parasites are genetically diverse, contributing to the variety of manifestations of the disease seen among patients. Recent genome sequence analysis has revealed that the *P. knowlesi* population comprises three highly divergent subpopulations; two commonly seen in human infections in Malaysia and another detected in several laboratory-maintained isolates from the 1960s. The fact that this genetic diversity is high both among humans and macaques points out to shared polymorphism and no differences between *P. knowlesi* parasites from humans and monkeys sampled in the same area, supporting the transmission pathways reviewed formerly[34,35].

1.3. Malaria Policy Advisory Committee (MPAC)

The WHO Global Malaria Programme (GMP) coordinates WHO's efforts to control and eliminate malaria. The GMP is supported and advised by the MPAC, which is a group of 15 global malaria experts that meet twice a year in order to provide independent advice to WHO to develop policy recommendations for the control and elimination of malaria[2]. On September 2016, the MPAC requested the GMP to consider forming the first ERG on *P. knowlesi*[10,36], the outcomes of which were presented in the MPAC that took place in Geneva in March 2017[37] (See Annex 1. Expert Researchers Contacted). ERGs are expert groups gathered for a limited period of time to review a specific area of work and provide evidence-based information and options for recommendations[38].

1.4. MESA Track database

The Malaria Eradication Scientific Alliance (MESA) is a coalition that works and collaborates with multiple actors in malaria elimination and eradication. With the malaria Eradication Research Agenda (malERA) initiative[39] as model and guide, its objective is to move the science of malaria eradication towards impact. One of its principal functions is creating management tools to intensify the impact of the evidence-based produced knowledge and accelerating research[40]. In order to achieve these goals, MESA collaborates with its partners and collects data that is published on an ongoing basis into the MESA Track database[41], an online, open and living database which captures research projects and institutions' research portfolios in malaria elimination and eradication.

2. Problem statement

P. knowlesi was declared an emerging public health threat in 2004[4,5]. However, major knowledge gaps still remain[27]. Lack of information regarding its transmission, misdiagnosis or incomplete geographic distribution maps are some of the issues for which we do not have clear answers. Hence, control measures and elimination strategies are not adapted to the parasite, threatening the global efforts to eliminate malaria[14]. The pressing need for further investigation is corroborated by current data suggesting an increase of *P. knowlesi* human cases in South-East Asia[21].

3. Objectives

Overall:

- Describe the landscape of current research in *P. knowlesi*, compare it to determined needs and identify the main research gaps.

Specific objectives:

1. Create a comprehensive database of the current research in *P. knowlesi*.
2. Publish the information collected in the MESA Track database.
3. Design an online mapping tool of the *P. knowlesi* research.
4. Present the landscape analysis to the WHO ERG on *P. knowlesi.*
5. Analyse the determined needs described in the literature.

4. Methods

4.1. Creation of a database of the current research in *P. knowlesi*

In order to review and describe the current landscape of research in *P. knowlesi,* I systematically collected data of funded and current research projects in *P. knowlesi*.

Following the MESA terminology[42], a project was defined as a research or development study with defined objectives which contributes to the malaria elimination and eradication

agenda. The malERA initiative[39] was used as a guide to determine whether a project was relevant and for this report all the projects related to *P. knowlesi* were considered.

4.1.1. Inclusion/ exclusion criteria

The eligibility criteria for the projects, prior to the data collection, were the following:

✓ Inclusion criteria	× Exclusion criteria
Funded research projects	Literature reviews
Active in 2012 and onwards	Other publications
Projects that included research in *P. knowlesi*	Fellowships

4.1.2. Systematic data collection

From December 2016 to April 2017 I systematically compiled information on research projects and grants active in 2012 and onwards. I pursued the information, as a first step, from public sources such as institutional websites or annual reports and through direct contact with the researchers and principal investigators. During the data collection period I contacted 41 experts (See Annex 1. Expert researchers contacted).

All the projects were collected from:

- Research grants databases:
  - National Institutes of Health (NIH)
  - Bill and Melinda Gates Foundation
  - Wellcome Trust
  - Ministries of Health of Malaysia and Australia
  - Outcomes of funding rounds of the National Health and Medical Research Council of Australia (NHMRC)
  - Medical Research Council of the United Kingdom (RCUK)
  - clinicaltrials.gov
  - grantome.com
- Research institutions*:*
  - Menzies School of Health Research (Australia)
  - London School of Hygiene and Tropical Medicine (LSHTM)
  - Harvard T. H. Chan School of Public Health (HSPH)
  - Others
- Research networks:
  - Asia Pacific Malaria Elimination Network (APMEN)
- Other sources of information:
  - American Society of Tropical Medicine and Hygiene (ASTMH) annual meeting abstracts
  - Consulted institutions on the MESA track database
- Contact via e-mail with expert researchers from the *P. knowlesi* ERG group
- Contact via e-mail with other researchers with projects related to *P. knowlesi*

The following information about the projects was collected, also according to the MESA definitions[42]:

- Title
- Objectives
- Principal institution(s): the institution that hosts the principal investigator and holds the research/development grant
- Principal investigator (PI): the person who leads the project and holds the grant
- Funding source(s): an entity which invests in research/development projects directly, or an entity which subcontracts another to execute the research/development grant
- Abstract
- Funding amount ($): in order to harmonise the funding information of the projects, the funding amount was converted into United States dollars ($), using the *Oanda* currency converter[43] and selecting as date of exchange rate the year the grant was awarded
- Funding information
- Partners on the project: partners which collaborate in the project, but do not lead the project or manage the grant
- Dates
- Country
- Theme: in order to summarize the results, the scientific theme of each project was also defined. Since the projects were assigned to the scientific theme according to its abstract and objectives, some of the projects were allocated in more than one scientific theme

4.1.3. Expert validation

As a second step, I produced a draft portfolio of the projects collected (See Annex 2. Validated and published projects) and I reached out the Principal Investigators of each project via email. I provided them with the MESA Track link to their projects and their personal portfolio, enabling them to validate the information collected, fill in the remaining gaps and provide additional projects or information.

4.2. Publication in the MESA Track database

I published the results obtained from the systematic collection of projects in the MESA track database following their guidelines for entering new data. They can be consulted here[1].

I was given a personal account and password which enabled me to enter the information in the MESA Track database and edit it. I also compared the data already in the database with the information from the systematic search, in order to carry out a quality control process and identify and correct any discrepancies.

In addition, and taking advantage of one of the features of the MESA Track database which is the creation of a personal portfolio for each Principal Investigator, I provided the link of each personal portfolio to the researchers via email, together with a short description and the aim

[1] http://www.malariaeradication.org/mesa-track/advanced-search?keywords=knowlesi

of this report; so they could check their projects, fill in any gaps or provide more information or suggestions.

4.3. Design of an online mapping tool

The third section is the design of an online mapping tool of the results obtained. This tool will be useful for interpreting and communicating the results in a more interactive and proactive way, enabling the users to present the data from different perspectives.

In order to design the online mapping tool, I brainstormed and searched online for other mapping tools so as to get ideas on how the online mapping tool should look like, trying to design a feasible and user-friendly tool. One of the models considered was the online mapping tool for malaria vectors and parasites presented by the GMP at the MPAC meeting[37].

4.4. Presentation of the landscape analysis to the WHO ERG on *P. knowlesi*

Lastly, the landscape analysis was presented to the WHO ERG on *P. knowlesi* on March 2017. (See Annex 4. Summary of ongoing research grants in *P. knowlesi*). The data was summarized and sent to the ERG Chair before the ERG meeting. In addition, I attended the MPAC in Geneva on March 2017, where the results from the ERG were publically presented.

4.5. Analysis of the determined needs as stated in the literature

To identify the *P.* knowlesi research needs as determined by the malaria scientific community, I searched the literature from the US National Library of Medicine (PubMed) using the term "*knowlesi*", including also relevant bibliographies of the resulting articles. I also searched the grey literature, including a follow-up of the new information published in the WHO World Malaria Reports from 2012 to 2016 and other WHO malaria documents launched between 2012 and 2017.

The data collected from these sources is presented in the background of this project (See 1. Background). I used this information to identify the main research needs and compare them with the database of current research.

5. Study results

5.1. Database of the current research in *P. knowlesi*

5.1.1. Inclusion/ exclusion criteria

The eligibility concerning fellowships was modified during the data collection period, and finally, I included 2 fellowships in the analysis. According to the RCUK[44], fellowships are personal awards for researchers which enable them to focus on their own investigations by giving them funding for a research project.

5.1.2. Systematic data collection

As illustrated in Fig. 3., I contacted the 15 members of the WHO ERG on *P. knowlesi* and 14 of them responded. I also contacted 13 other experts involved in *P. knowlesi* as a result of suggestions from some of the WHO ERG members, getting a response from 1 of these experts. As part of the expert validation (See 5.1.3. Expert validation), I identified and contacted 13 additional principal investigators, obtaining 7 responses.

As a result of the systematic search and consultation with experts, I have identified and assessed for eligibility 55 projects (Fig. 4.). After compiling the information, I excluded 35 projects; 4 because they were not about *P. knowlesi,* 1 because it was closed before 2012, 15 because they were duplicates from projects already found and 15 because they were lacking too much information to be added to the database (See Annex 3. Ongoing data collection). I included the other 20 projects in this analysis, validating and publishing them in the MESA track database (See Annex 2. Validated and published projects).

5.1.3. Expert validation

As a result of the information received from the 22 responses from the experts, I was able to identify 17 projects (Fig. 3.). However, from these only 1 project was included. The remaining 16 projects were excluded; 2 of them because they were duplicates and 14 because at the end of the data collection period there was too much information lacking to publish them in the database (See Annex 3. Ongoing data collection). Through the additional consultation, the experts provided valuable additional information about the projects and validated the information. In the majority of the cases, the researchers responded corroborating the information and providing further data. For one response, the researcher modified and enlarged the objectives of his project.

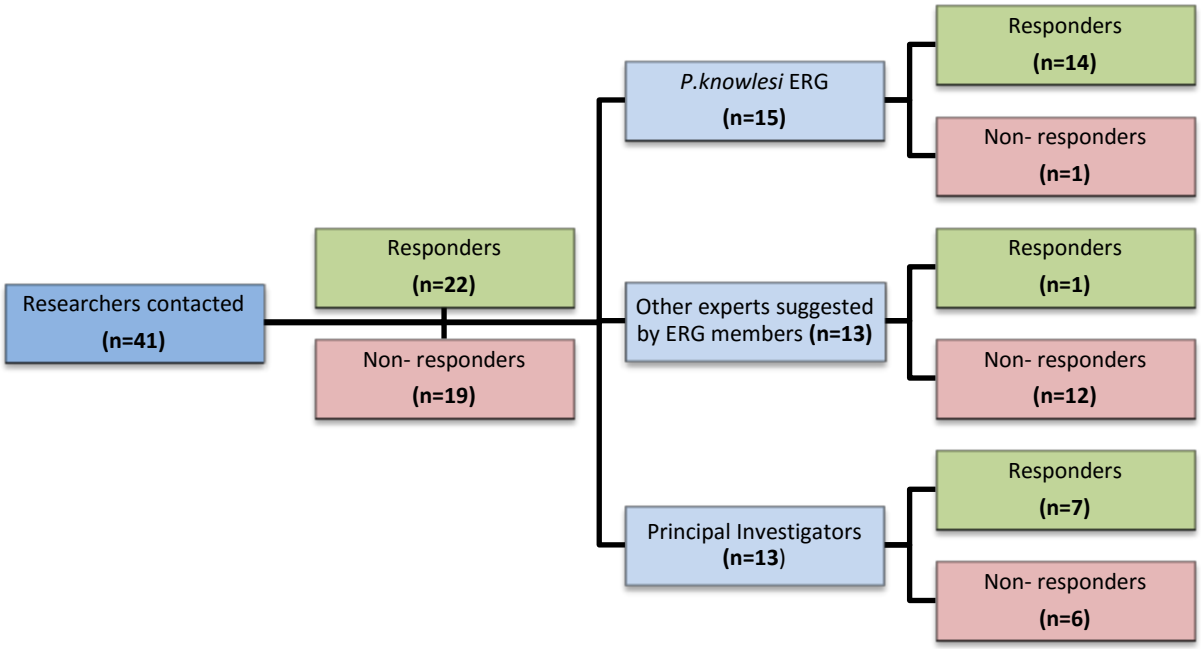

Fig. 3. Researchers contacted and number of responses obtained. 41 experts were contacted, obtaining 22 responses. Of these, 14 were from the *P. knowlesi* ERG, 1 from another expert suggested by ERG members and 7 from Principal Investigators of the projects.

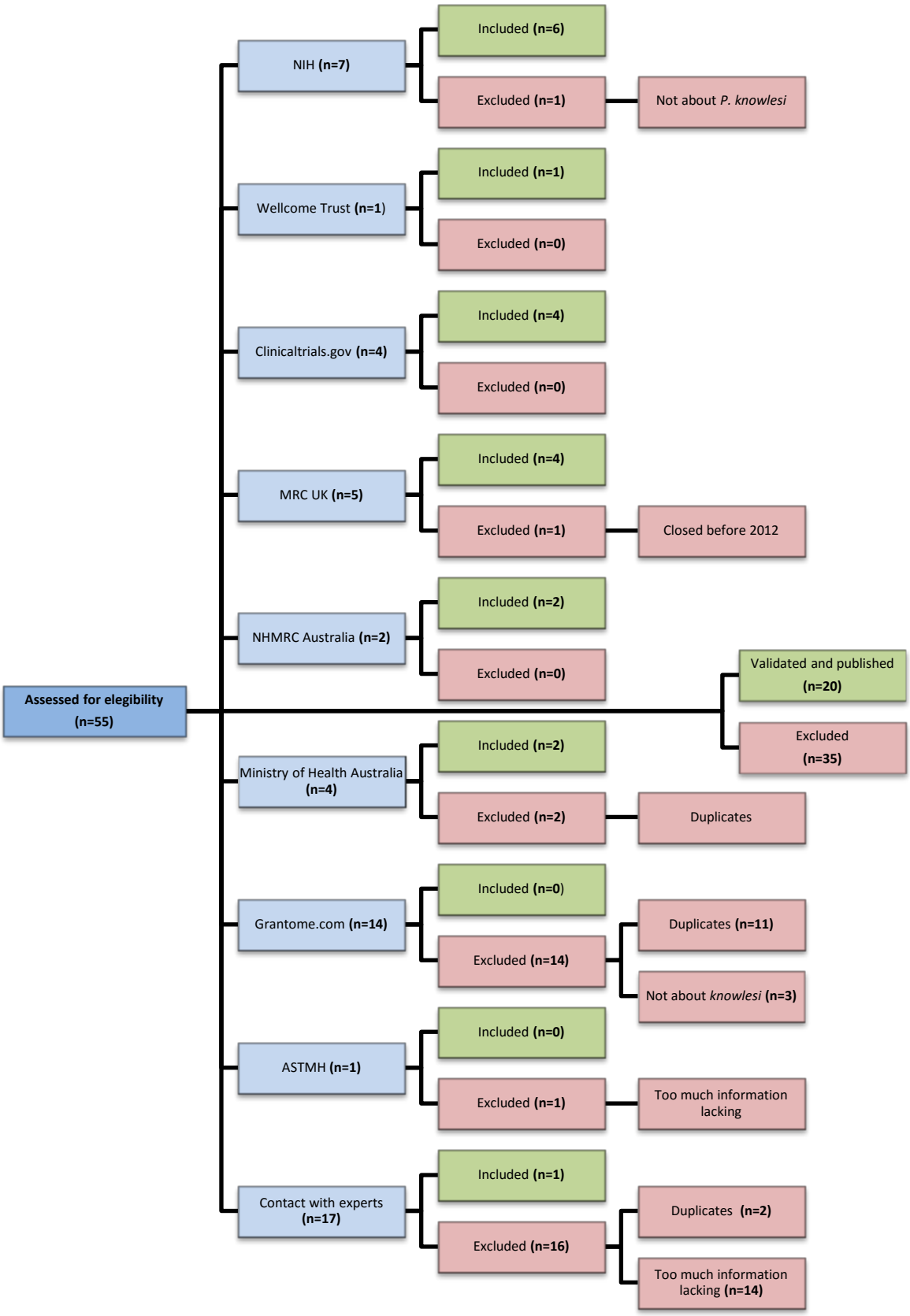

Fig. 4. Eligibility flow diagram. Data collection between December 2016 and April 2017. *n* corresponds to the number of projects identified. As a result of the systematic search and consultation with experts, 55 projects were identified and assessed for eligibility. 20 projects were validated and published.

5.1.4. Publication in the MESA Track database

As a result of the systematic collection of projects, the MESA Track database has 20 projects related to *P. knowlesi*[45]. From these 20 projects, 6 were already in the database before the start of the project and I revised and updated them with additional information, whereas I uploaded the remaining 14 projects from scratch.

At the end of the data collection, 20 out of 818 projects published in the database are related to *P. knowlesi*, 26 out of 768 institutions are involved in research projects in *P. knowlesi* and 6 out of 96 countries have projects related to *P. knowlesi* malaria[41].

A screenshot of the MESA Track database can be found in Fig. 5. The projects can be filtered by type, methodology, theme, consulted institution or region, or they can be searched by writing any word or sentence in the text search bar.

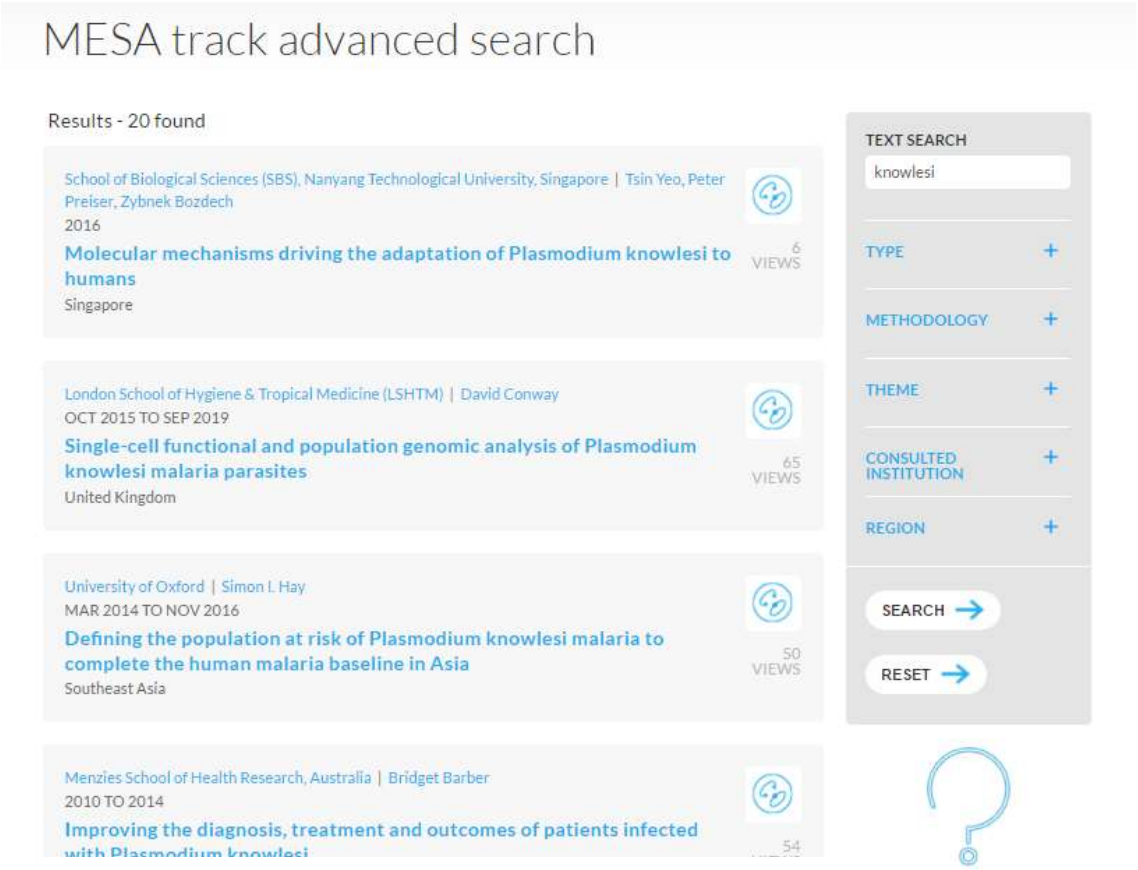

Fig. 5. Screenshot of the MESA Track database website[45]

5.2. Data analysis

The 20 projects analysed are summarized in Table 1. More detailed information on each project can be found in the Annex 2. Validated and published projects.

	Title	Objectives	Principal institution	Principal investigator	Funding source	Funding amount	Country	Partners
1	*P. knowlesi* trial of artemether-lumefantrine vs chloroquine	☑	☑	☑	☑	☒	☑	☑
2	*P. knowlesi* trial of artesunate-mefloquine versus chloroquine	☑	☑	☑	☑	☒	☑	☑
3	Artemether-lumefantrine vs chloroquine for uncomplicated *P. vivax* malaria	☑	☑	☑	☑	☒	☑	☑
4	Disease burden, risk factors and treatment of *P. knowlesi* malaria	☒	☑	☑	☑	☑	☑	☑
6	Geographic genetic profiling of human *Plasmodium* malaria	☑	☑	☑	☑	☑	☑	☑
7	Functional analysis of *P. vivax* drug resistance polymorphisms	☑	☑	☑	☑	☑	☑	☒
8	Monkeybar: defining the biomedical, environmental and social risk factors...	☑	☑	☑	☑	☑	☑	☑
9	Dissecting the red blood cell invasion pathways of the malaria parasite *P. knowlesi*	☑	☑	☑	☑	☑	☑	☑
10	Single-cell functional and population genomic analysis of *P. knowlesi* malaria parasites	☑	☑	☑	☑	☒	☑	☒
11	Structure and function of eukaryotic phosphatidylserine decarboxylase	☑	☑	☑	☑	☑	☑	☒
12	Comparative incidence and clinical spectrum of *P. knowlesi* malaria...	☑	☑	☑	☑	☑	☑	☑
13	Comparative pathophysiology and clinical epidemiology of *P. knowlesi* malaria	☒	☑	☑	☑	☑	☑	☒
14	*Plasmodium* genus and PFV fluorescent in situ hybridization (fish) assay kit	☑	☑	☑	☑	☑	☑	☒
15	An effector memory T cell-inducing subunit vaccine against malaria	☑	☑	☑	☑	☑	☑	☒
16	RBL domain malaria candidate vaccines	☑	☑	☑	☑	☑	☑	☒
17	A comparative study of the pathophysiology of severe *P. knowlesi* and *P. falciparum* malaria	☒	☑	☑	☑	☑	☑	☑
18	Improving the diagnosis, treatment and outcomes of patients infected with *P. knowlesi*	☒	☑	☑	☑	☑	☑	☒
19	Effect of paracetamol on renal function in *P. knowlesi* malaria	☑	☑	☑	☑	☒	☑	☑
20	Defining the population at risk of *P. knowlesi* malaria...	☑	☑	☑	☑	☑	☑	☒
21	Molecular mechanisms driving the adaptation of *P. knowlesi* to humans	☑	☑	☑	☑	☒	☑	☒

Table 1. Summary of validated and published projects. Among the 20 projects analysed, all 20 have data on principal institution, principal investigator, funding source and country. 16 projects have detailed objectives, 14 have known funding amount and 10 have known partners.

I categorised the projects according to 8 scientific themes and analysed them according to the principal institutions leading the projects, the geographic locations of the projects, funding disbursed and partners involved in the projects.

The scientific themes were the following:

1. Transmission
2. Risk of infection
3. Diagnostics
4. Clinical outcomes and pathophysiology
5. Drugs
6. Vaccines

7. Parasite genetic diversity
8. Basic science

When stratifying the results by theme (Fig. 6.), and taking into account that some projects can have more than one category, drugs and risk of infection account for the 18% of the projects each; followed by clinical outcomes and pathophysiology, transmission and parasite genetic diversity, which account for 14% of the projects each. Diagnostics is only addressed in 1 project, which means 4% of the total.

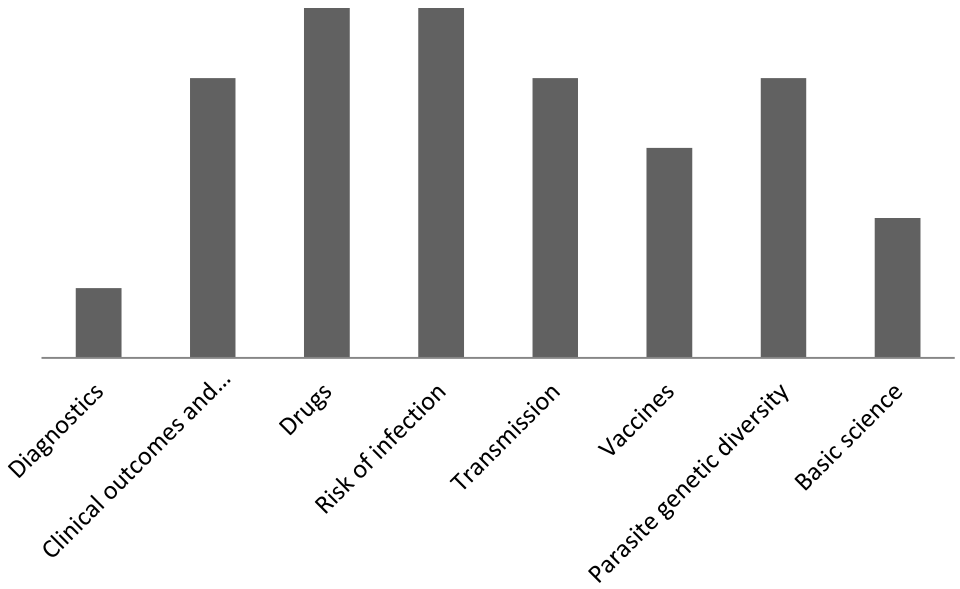

Fig. 6. Number of *P. knowlesi* projects by theme. The themes with the most projects are drugs and risk of infection, followed by clinical outcomes and pathophysiology, transmission and parasite genetic diversity.

13 projects (65%) were addressing only 1 theme in their objectives, while 6 projects (30%) were tackling 2 scientific themes and 1 project (5%) was intending to address 3 of the themes.

Regarding the principal institutions leading the projects, the results are shown in Fig. 7. Menzies School of Health Research (35% of the projects) and London School of Hygiene and Tropical Medicine (20%) are the two main institutions that have projects in *P. knowlesi*. The 9 remaining institutions have 1 project each, 5 of them are located in the USA, 2 of them in Malaysia, 1 in the UK and 1 in Singapore.

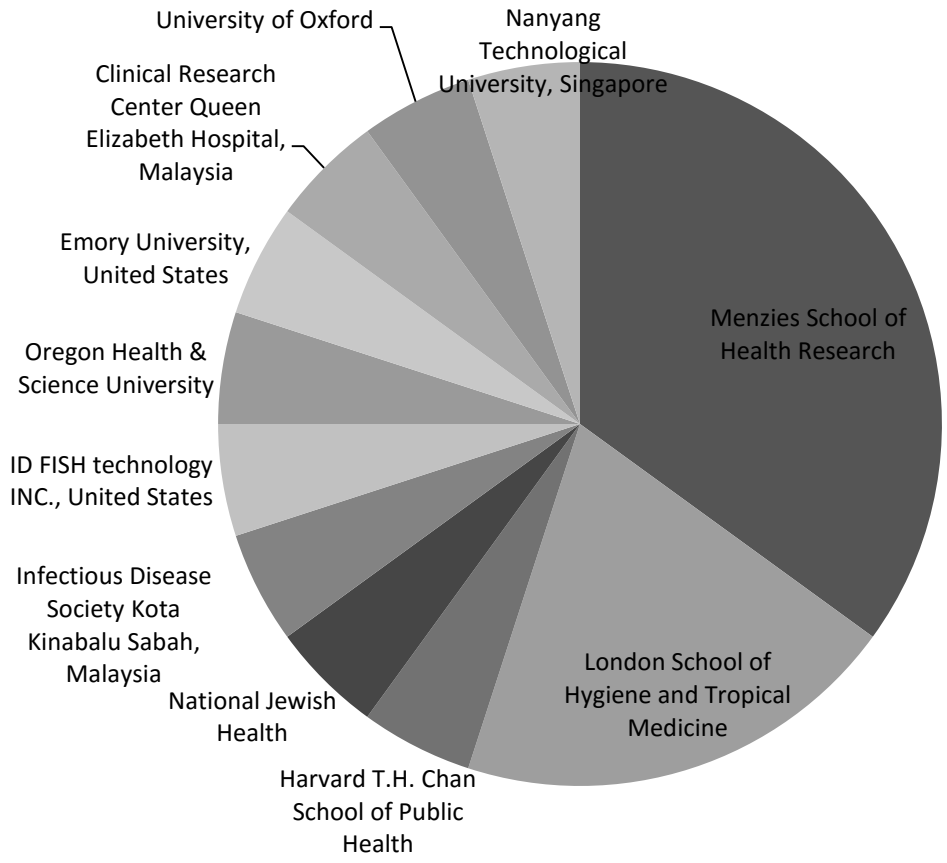

Fig. 7. Proportion of *P. knowlesi* projects by Principal Institution. Menzies School of Health Research and London School of Hygiene and Tropical Medicine are the two main institutions with projects in *P. knowlesi.*

For the geographic locations of the projects, two different analyses can be made. On the one hand, projects can be classified according to the country funding the project (Fig.8.). On the other hand, projects can be also categorized according to the country where the project is taking place (Fig. 9.).

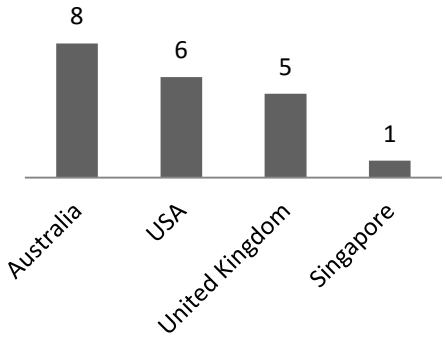

Fig. 8. Number of *P. knowlesi* projects by funding country. Australia funds 8 projects, followed by USA, UK and Singapore; with 6, 5 and 1 projects funded.

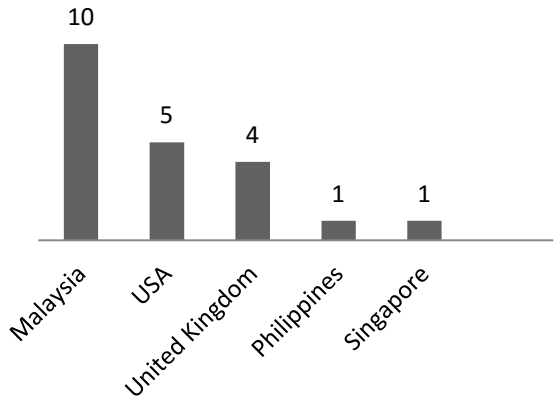

Fig. 9. Number of *P. knowlesi* projects by country where the projects are taking place. The total amount of projects is 21 instead of 20 because there is one project (Project 8) with activity in The Philippines and Malaysia.

All the 20 projects analysed have known funding source and country. According to the data on funding, the countries that act the most as funders are Australia, United States and United Kingdom (Fig. 8.). As far as countries where the projects are taking place, Malaysia is the country with the most quantity of projects (50% of the total) (Fig. 9.).

As far as the funding is concerned, from the 20 projects validated, 14 of them had known funding amount, with a total expense disbursed of $11,760,116 (Fig. 10.). For the remaining 6 projects for which we were not able to collect funding amount; it was not published for 5 projects and it was confidential for 1 project.

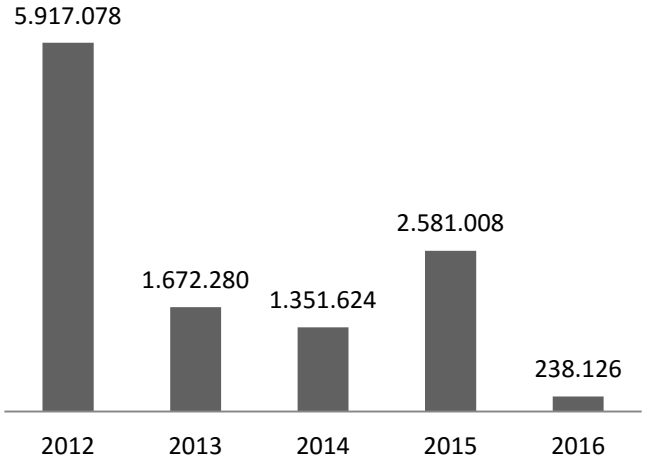

Fig. 10. Total funding amount disbursed by year, $ (2012-2016)

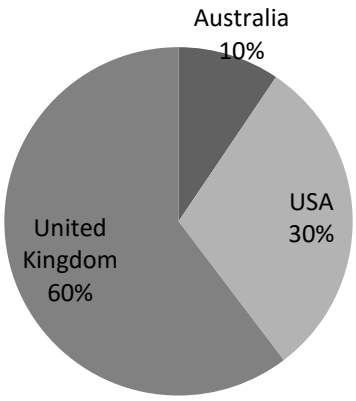

Fig. 11. Funding disbursed by country, $. The amount that Singapore is disbursing is confidential.

As it can be seen in Fig. 11., United Kingdom is funding 60% of the total amount disbursed. One of their projects, entitled *Monkeybar,* corresponds to the 39% of the funds spent by the country.

As far as the time is concerned (Fig. 12.); in 2012, 2014 and 2015, there were 5 projects tackling *P. knowlesi* that received funding. 2 projects received funds in 2013 and 3 projects received the funds in 2016.

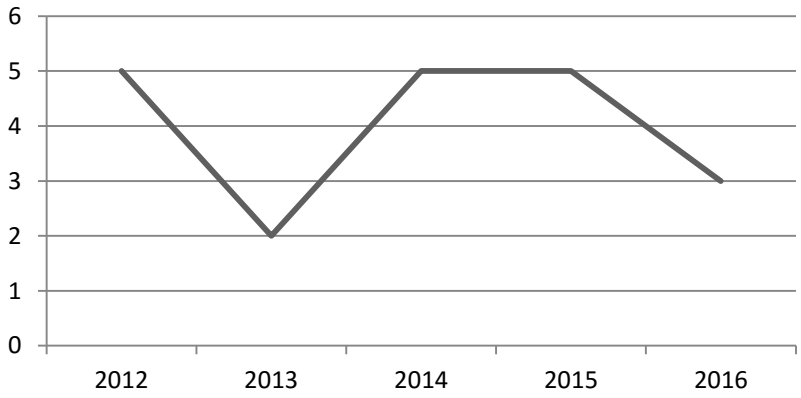

Fig. 12. Number of projects receiving funding per year.

The partners involved in the projects analysed are only known for 10 of the 20 projects. The MOH of Malaysia is involved in the majority (7 of 10) of the projects taking place in the country. Most of the other partners are universities and research centres from around the globe: Menzies School of Health Research and Charles Darwin University (Australia), Clinical Research Center Queen Elizabeth Hospital, University of Malaysia Sabah and Danau Girang Field Center (Malaysia), Oswaldo Cruz Foundation and University of Sao Paolo (Brazil), University of London, University of Glasgow and Liverpool School of Tropical Medicine (UK), Research Institute for Tropical Medicine and University of the Philippines Los Baños (The Philippines) and Mahidol Oxford Tropical Medicine Research Unit (Thailand); as well as the product development partnership (PDP) Medicines for Malaria Venture and the NIH (USA).

5.2.1. Landscape analysis

5.2.1.1. Transmission

4 projects are addressing transmission; 3 of them are hosted by the London School of Hygiene and Tropical Medicine and 1 by the Nanyang Technological University of Singapore.

Projects 8 and 9 aim to understand the conditions that allow the parasite host switch from monkey to human and determine the potential for emergence of human to human transmission, whereas projects 10 and 21 study the mechanisms of adaptation of the parasite to different hosts through genomic analyses.

These 4 projects account for 14% of the total studies and are examples of the complexity of studying the transmission of diseases with ecologies such as *P. knowlesi*[4]. *P. knowlesi* malaria is a zoonosis and direct transfer between humans by the mosquito vector has yet to be confirmed to occur in nature[11,15].

5.2.1.1. Risk of infection

The burden of *P. knowlesi*, especially in Malaysia, is studied in 5 projects. 3 of them have activity going on in Malaysia, and the other 2 are taking place in the UK.

Project 12 aims to determine the true incidence and trends of *P. knowlesi* malaria in Sabah, compared to the other malarias, whereas the risk factors for acquiring *P. knowlesi* malaria and the burden of this parasite (mainly in children) are studied in project 4. Project 8 also tackles the risk factors for infection in order to further generate risk maps for *P. knowlesi* contagion. The geographic distribution of this parasite is tackled in projects 6 and 20. Project 6 aims to track the geographical movement of malaria through genetic profiling and project 20 aims to predict spatial variation in disease risk in order to identify high-risk areas.

The above mentioned projects account for 18% of the total studies. Gaining knowledge of the geographical distribution of *P. knowlesi* and the risk status of these regions is essential for identifying areas which are a priority both for deployment of strategies and for further investigation[14,46]. Moreover, after seeing that the demographic expansion of populations may cause an increasing overlap of human and monkey habitats[23], studies examining the risk factors for acquiring the infection play an essential role in our understanding of the disease.

5.2.3.1. Diagnostics

There is only 1 among the 20 projects analysed which addresses the diagnosis of *P. knowlesi* (Project 14). It is taking place in the USA.

This project aims to develop a fluorescent in situ hybridization (FISH) assay kit able to detect all five species of *Plasmodium*, including *P. knowlesi.*

Thus far, PCR is the only definitive and reliable method to effectively diagnose *P. knowlesi*, but identification of the parasite at the species level is often done by microscopic examination or RDTs[7,13,20]. Project 14 is an example of new methods of diagnosis being developed, exploring new molecular detection methods. The objective is to move towards new tools that can change how *P. knowlesi* malaria is detected in order to provide trustworthy data about the real burden of the disease and ensure that patients receive the proper treatment in time.

5.2.3.2. Clinical outcomes and pathophysiology

4 projects are tackling clinical outcomes and pathophysiology of *P. knowlesi*. All the projects are taking place in Malaysia, and 3 of them are studying patients from hospitals in Sabah.

Project 12 aims to determine the epidemiology and clinical spectrum of *P. knowlesi* malaria at two district hospitals and provide data regarding the pathophysiology in vulnerable populations that have not been studied yet, such as pregnant women or children. Project 13 is also intending to describe the clinical features of patients with *P. knowlesi* malaria that have been hospitalized, but differently from the previous study, it aims to understand more deeply how the parasite causes severe disease. Similarly, project 18 wishes to improve the knowledge about *P. knowlesi* pathogenesis, without focusing on any special populations. Lastly, project 17 compares the pathophysiology of *P. falciparum*, *P. vivax* and *P. knowlesi* malaria of all patients admitted to the Queen Elizabeth Hospital of Sabah, Malaysia, investigating the pathophysiological correlates of severe disease. These projects are focusing not only on the disease in general terms but also on the clinical signs in vulnerable populations and in severe malaria, broadening the information available on the true spectrum of the infection.

It is only recently that the scientific community is starting to understand the whole clinical spectrum of *P. knowlesi* infection[20]. The importance of having clear protocols and guidelines to help health professionals to correctly diagnose and treat patients suffering *P. knowlesi* malaria[27] is often highlighted in the literature. Along these lines, 14% of the projects analysed in this report aim to describe the clinical outcomes and pathophysiology of the disease.

5.2.3.3. Drugs

5 projects are tackling the treatment of *P. knowlesi* malaria and 4 of them are clinical trials conducted in Sabah, Malaysia.

Projects 1 and 2 examine whether the combinations of artemether–lumefantrine and artesunate–mefloquine, respectively, are superior to chloroquine in the treatment of uncomplicated *P. knowlesi* malaria. Project 3, on the other hand, aims to evaluate the combination of artemether–lumefantrine for *P. vivax* malaria and extrapolate the results to all malaria species prevalent in Malaysia. The overall goal of these 3 clinical trials is to help the authorities develop a unified and aligned treatment guideline. Project 19 is also a clinical trial,

but instead of studying the treatment of malaria, it aims to address whether paracetamol can provide renal protection in patients with severe *P. knowlesi* malaria. Finally, project 4 evaluates the optimal treatment for non-severe *P. knowlesi* and *P. vivax* malaria by comparing two currently antimalarial medications in Malaysia.

Considering that the current WHO Malaria Treatment Guidelines (2015) provide the same official treatment recommendations for *P. vivax, P. ovale, P. malariae* and P. *knowlesi* infections[29], the hospital guidelines in the endemic areas recommend oral ACT and intravenous artesunate for the treatment of non-severe and severe *P. knowlesi* malaria respectively[32]. Different combinations of ACTs for uncomplicated infections are being tested in some clinical trials and there is also interest on testing the effectiveness of artemisinin-based combinations for *P. vivax* infections, with the intention of extrapolating the results to the other species.

5.2.3.4. Vaccines

3 of the 20 projects analysed are tackling vaccine development. However, the connection between *P. knowlesi* and vaccines in these 3 projects is more directed to use *P. knowlesi* parasites as a laboratory model for vaccine trials addressed to *P. falciparum* and *P. vivax* rather than developing vaccines aimed at fighting against *P. knowlesi* malaria.

Project 9 is aimed at developing transgenic *P. knowlesi* models with modified adhesive proteins to test vaccine candidates for *P. vivax*, project 15 is trying to determine if a vaccine is protective by challenging with *P. knowlesi* sporozoites and project 16 is intended to test some hypothesis of immunization with *P. knowlesi* malaria parasites.

Malaria vaccines are an area of intensive research and even though most vaccine research is focused on *P. falciparum*[33,47], *P. knowlesi* has its own space in the area as well. For instance, there are several examples in the literature where rhesus monkeys have been the prime host for trials using experimental vaccines for *P. knowlesi* malaria[19] and studies using this model parasite have largely contributed to advancing positions in vaccine progress, providing relevant information about the antimalarial immune responses and the parasite-host interactions after infection[28].

5.2.3.5. Parasite genetic diversity

The genetic diversity of *P. knowlesi* is analysed in 4 projects: 6, 7, 10 and 21.

By using genetic profiling and barcoding methods researchers are able to differentiate between species, discriminate amongst infections originated from different populations of the same species or examine the mechanisms of adaptation of the parasite. Moreover, in project 7, the study of the parasite's polymorphisms will be used for the study of critical genes involved in many processes relevant to other malaria species, such as *P. vivax.*

Even though, as stated in the literature, little is known about the degree of parasite genetic diversity in naturally acquired *P. knowlesi* infections[35], this area can provide further and valuable evidence to understand host-switching events and possible adaptation to humans, together with providing further information about the mechanisms of natural selection[34].

5.2.3.6. Basic science

2 projects are related to the basic biological mechanisms of the *P. knowlesi* parasite.

The process of RBC invasion by malaria parasites is likely to be an important target in the development of new tools to fight against the parasite. Project 9 tries to investigate the role of the adhesive proteins when invading RBCs by identifying which parasite proteins and host proteins are required for *P. knowlesi* to invade human RBCs. Another possible target in the development of vaccines and drugs is the lipid composition of the parasite cells' membrane, as studied in project 11, which uses modified *P. knowlesi* parasites to study the consequences of interventions in the phospholipid metabolism of pathogens.

P. knowlesi has a historical knowledge as an experimental malaria model[28]. The successful adaptation of *P. knowlesi* to grow and multiply *in vitro* in human RBCs has created a novel and promising model for malaria research[48], shedding light on several aspects of the parasite's biology[49].

5.3. Design of an online mapping tool

The results of the brainstorming process for the design of an online mapping tool are presented as follows.

The home page of this tool will be a world map. The user will be able to filter the results and see only what he or she is interested in. There will be a bar which will allow the user to move over the years, from 2012 to 2016 and see the changes and the trends over time.

The different filters will be:

- Theme: each of the 8 themes will have a colour and a symbol. When checking for themes, each project will be displayed as the theme it belongs to in the map, pinned in the geographic region the project is being carried out. The user will be able to change years and check which project started each year or display all the data at the same time.
- Institutions: the institutions of the projects will be also displayed on the map. It will be possible to see only principal institutions, only partners, or both, with different shape of the logos to be able to differentiate between them. In this case, each institution will be pinned in the region where their facilities are, and the pin will have the logo of the institution to make it easier to recognise the centres. Moreover, the size of the logo will be proportional to the number of projects each institution has in the MESA Track database (Fig. 13.).
- Funding: Funding will have different options of visualisation. Countries will be coloured according to the amount they are disbursing (when the *funding country* option is selected) or they will be coloured according to the amount of money that is being spent in the country (when the *country receiving funds* option is selected). As well as for the other filters, it will be possible to check each year or all the years at the same time.

- New projects: there will be the option of seeing which new projects started each year. By moving across the years' bar, the projects will appear in the country they are being carried out.

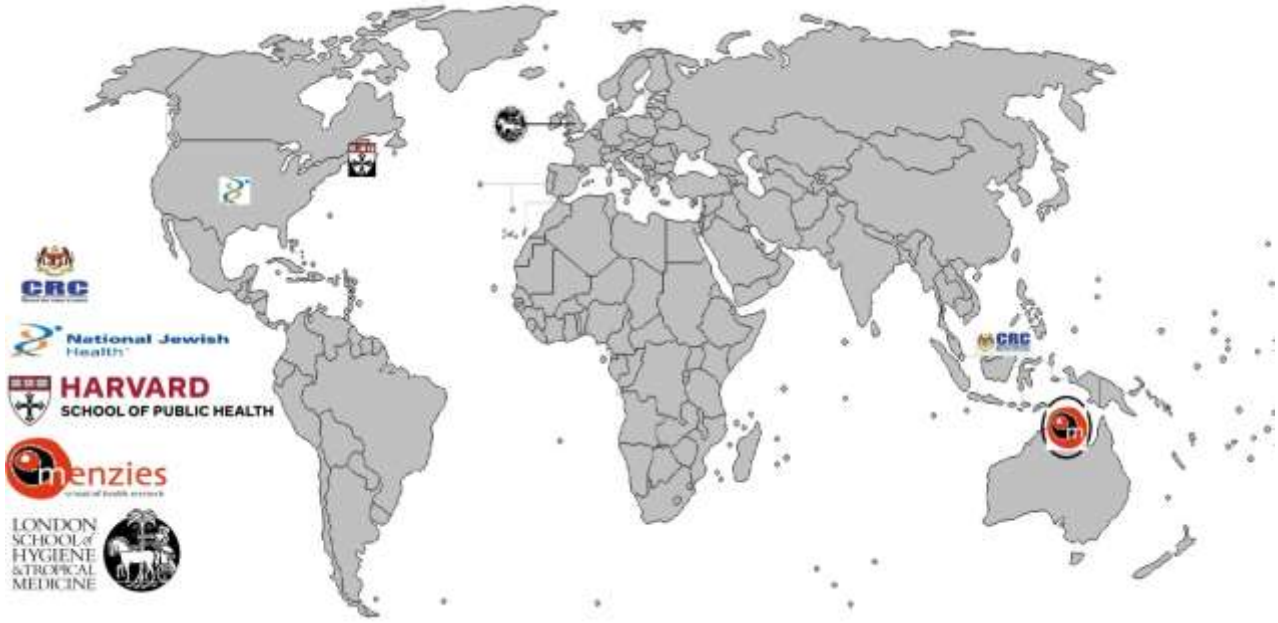

Fig. 13. Draft of the possible appearance of the online tool, when filtering by principal institutions and all years.

In addition, the tool will have extra features, such as the possibility of getting directed to the project in the MESA Track database when clicking on a certain project or getting directed to the websites of the institutions by clicking on them. Moreover, by moving the cursor through the different pins, a pop-up bubble with key information about the project will appear and will disappear when moving the cursor away.

5.4. Research gaps

Some of the gaps presented in the literature are being covered by the current research landscape. Significant achievements have been made, but there are still other challenges and gaps that persist even after examining the ongoing research.

A major step towards increased acknowledgement of *P. knowlesi* was done when the MPAC supported the creation of the ERG, whose task was to point out research gaps and provide options for recommendations.

5.4.1. Transmission

We are only beginning to understand *P. knowlesi* malaria transmission and there are major challenges on the way. Seeking for clear evidence of human to human transmission is one of the main research priorities at this moment, acknowledged in the literature and extensively highlighted by the ERG and the MPAC[11,37]. 4 of the projects analysed are tackling this issue, trying to gain evidence-based knowledge on the potential for emergence of human to human transmission and trying to fill this knowledge gap. This evidence would be useful to

successfully implement malaria control programmes and interventions to limit the transmission of *P. knowlesi* to humans[20,50]. Another problem stated in the literature is the quality of the data available, which is so geographically specific that generalizations cannot be made[4]. The ERG also highlighted that the study of the vector host preferences and feeding habits is another key priority area which needs further investigation[11]. Likewise, studies detecting asymptomatic infections which would throw some light on the likelihood of human reservoirs of *P. knowlesi* infection would also be highly recommended[5].

5.4.2. Risk of infection

Understanding the geographical distribution of *P. knowlesi* is another key point for identifying high-risk infection areas and designing proper elimination strategies and surveillance systems. It is also vital for detecting areas where malaria transmission is likely to occur even after human malarias have been controlled and eliminated. The MPAC of September 2016 also recognized the research gaps in the epidemiological distribution of *P. knowlesi* infection in humans and the risks of infection[10,11]; and the need of maps illustrating vector distribution, human *P. knowlesi* cases and environmental risk factors was also expressed by the ERG[11]. 5 of the projects analysed are addressing these gaps, aiming to provide this extremely needed evidence. However, further research is still needed, since there are still large parts of South-East Asia for which the risk status is unknown[14,46]. Along these lines, longitudinal studies of communities that live in the forest would be extremely helpful in order to determine if these exposed populations possess a substantial burden of infection and whether it resolves without treatment or if it is asymptomatic, together with studies analysing the relationship between forest cover and the distribution of the host and vector species[24]. Moreover, further and more extensive entomological studies are needed, such as getting more data on the behaviour patterns of the vectors[4,11,20]. As stated in the literature, more research is needed to design proper malaria control strategies, since the currently used methods such as LLINs and IRS seem not to be effective against *P. knowlesi* vectors, which have been seen to mainly feed outdoors[25].

5.4.3. Diagnostics

The lack of feasible and effective diagnostic methods and tools with the capacity to clearly differentiate between *Plasmodium* species and correctly identify *P. knowlesi* has widely been acknowledged by the ERG and also in the literature. Only one among the twenty projects compiled is focused on developing new laboratory diagnostic methods, highlighting the need of the development of new tools for its detection. RDTs for *P. knowlesi* are needed, affordable and convenient for the endemic regions and with the capacity to detect the parasite even when parasitaemia levels are low. Moreover, *P. knowlesi*-specific serological assays and markers need to be generated, as well as new technologies such as loop-mediated amplification (LAMP) kits or cytogenetic techniques like FISH assay kits. The development of a quantitative PCR would also be extremely convenient to determine, for instance, the real proportion of the population infected with *P. knowlesi*[11,20].

5.4.4. Clinical outcomes and pathophysiology

Even though much is known regarding the clinical manifestations of *P. knowlesi* malaria, the clinical management of the disease was also defined as a research priority by the ERG[11]. The majority of the data available thus far comes from a limited number of case reports, small

retrospective and prospective studies and relatively small field studies[20] and therefore further research is needed. The 4 projects analysed that are tackling clinical outcomes are trying to provide evidence-based data that can be useful to create medical texts tackling the distinctive clinical outcomes of the disease and facilitate its clinical management, as well as providing detailed information on severe malaria[11,27] and further evaluate the relevance and thresholds of the laboratory findings that define it. However, it is still unclear why some patients develop severe disease while others just present uncomplicated infections[13]. More extensive studies are also needed so as to determine the case fatality rates, as well as further reports on the clinical aspects of *P. knowlesi* malaria in children and pregnant women[20].

5.4.5. Drugs

In the countries where efforts are being done to increase identification of *P. knowlesi* malaria, the delivery of more timely and optimal treatments has contributed to a considerable reduction in *P. knowlesi* case-fatality rates[50,51]. This is, in part, due to the high sensitivity of the parasite to artemisinins and the susceptibility to chloroquine and mefloquine, which enable an effective treatment of the disease if diagnosed in time[11]. Nevertheless, according to the literature, more treatment studies are needed concerning the ideal management of *P. knowlesi* infections, such as studies on the optimal treatment for clearance of parasites, studies on the efficacy of the different treatments in severe infection or the obtaining of complete efficacy and safety data on ACT use in this concrete species of *Plasmodium*[7,20,23,32]. Some of these gaps are being covered by the current research, as seen in the 5 projects analysed, which aim to compare different ACTs versus chloroquine or define the optimal treatment for non-severe disease.

On the other hand, none of the projects analysed addresses the possibility of *P. knowlesi* resistance to antimalarial drugs and even though resistance of *P. knowlesi* to antimalarial drugs has not been documented yet, it is another field of study that the scientific community should not fail to remember[32].

5.4.6. Parasite genetic diversity

The ERG also emphasized the need for supplementary studies in the area of parasite genetic diversity research[11] and 4 of the projects analysed are responding to this need. Examination of the parasite's gene expression and analysis of the role of host and parasite genetic diversity, as well as detailed genetic analysis of sequences of the parasite, both from human and monkey infection, would unveil further understanding of how *P. knowlesi* pathogenesis works, determining whether regional variation is taking place[20].

5.4.7. Outcomes from the MPAC

The ERG identified four main research priorities (Box 1), which were presented at the MPAC[11].

- Evidence for human to human transmission
- New laboratory diagnostic methods
- Entomology
- Clinical management

Box 1. Research priorities identified by the ERG[11]

After analysing these research priorities and the additional evidence presented by the ERG, as well as acknowledging the increase of *P. knowlesi* malaria in Malaysia, the MPAC concluded the following[37]:

- If *P. knowlesi* cases continue to be reported in Malaysia, the country will need guidance on whether the target of eliminating malaria by 2020 can be taken into consideration.
- If human to human transmission is confirmed to occur in Malaysia, *P. knowlesi* will need to be considered a human malaria infection and elimination will be needed for certification of malaria-free status.
- The work done regarding the classification of transmission of the Avian Flu or the Middle East Respiratory Syndrome could be useful for further understanding how to classify *P. knowlesi* transmission.

Box 2. MPAC conclusions about the outcomes of the ERG on *P. knowlesi*[37]

6. Discussion

As the MPAC concluded[37] and considering that the efforts of countries to eliminate malaria are proving to be successful (since 2007 seven countries have been certified by the WHO as having achieved at least three consecutive years with zero local cases of the disease[2]), determining whether human to human *P. knowlesi* transmission is taking place is indispensable. If proved, *P. knowlesi* malaria would need to be considered a "fully" human malaria infection and it would need to be eliminated as well to get the certification. The global elimination ("interruption of local transmission of a specified malaria parasite in a defined geographical area as a result of deliberate activities"[52]) and eradication ("permanent reduction to zero of the worldwide incidence of infection caused by human malaria parasites as a result of deliberate activities"[52]) agenda does not have, as yet, *P. knowlesi* malaria in the spotlight, but it may need to adapt before long.

Another main gap concerning *P. knowlesi* is the acknowledgement of its importance. A major step towards increased distinction was done when the MPAC recognized the importance of this species in several areas of South-East Asia by supporting the creation of the ERG. Nevertheless, whereas *P. knowlesi* was present, although lightly mentioned, in previous editions of the World Malaria Report, the parasite is not acknowledged in the last report, from 2016[1]. In order to speed up the efforts to raise awareness and recognition of *P. knowlesi* malaria and improve its surveillance, regular inclusion of this parasite in the World Malaria Report would be extremely beneficial[50].

Diagnostics is clearly an issue that is being neglected in the *P. knowlesi* research agenda. The fact that the diagnostic methods are neither sensitive nor specific enough to correctly identify these species poses a great threat to the population, delaying the administration of effective treatment and worsening the possible outcomes of the disease. The recommendation of the WHO to record all *P. malariae* cases in regions were *P. knowlesi* has been reported as *P. malariae/ P. knowlesi* is a good strategy to improve case management and have a better estimate of the real burden of this parasite while new methods are being developed.

As far as the geographic locations of the projects are concerned, the countries funding the most number of research studies are Australia, USA and UK (Fig. 8.); whereas the countries where the most projects are taking place are Malaysia, USA and UK (Fig. 9.). In such a way, it makes sense that Malaysia accounts for the majority of projects, since the burden of *P. knowlesi* malaria in the region is huge. In addition, we should bear in mind that the majority of the projects from the ongoing data collection (See Annex 3. Ongoing data collection) are taking place in Malaysia, meaning that there is research work in Malaysia that we have not acknowledged in the analysis. Having the data for these projects would considerably increase the total number of research projects in the country. It is also worth mentioning that the MOH of Malaysia is highly involved in the research taking place in the country, being partner in 7 out of the 10 projects. There is not a relation between the number of projects and the funding that countries get, considering that the funding amounts vary a lot depending on the objectives, duration, etc. of each project.

Back to the funding amounts, UK accounts for the 60% of the total funding disbursed (Fig. 11.). This has a lot to do with the *Monkeybar* project (Project 8), which accounts for 65% of the total assets disbursed by the country in its 5 projects. This project is the main reason why the year 2012 stands out in Fig.10. 78% of the assets spent this year correspond to this project, being 39% of the total amount disbursed between 2012 and 2016. This project is funded by the MRC, the main funding source of the country. Complementary, USA budgets 30% of the total funding disbursed, through the NIH. Regarding Australia, the two main funding sources are Menzies School of Health Research and the NHMRC.

The 2 fellowships included in the analysis (Projects 9 and 13) account for $2,037,061, which means the 17% of the total funding amount disbursed between 2012 and 2016.

During the data collection process, some questions arose. First, information about funded projects was not easy to access, especially data regarding funding sources and funding amounts. In the countries were the funds for the grants come from the taxpayers, such as USA (NIH) or UK (MRC), the data is publically accessible, being uploaded into the databases and frequently updated. On the other hand, when the funding source is a private donor or a university, any (or little) of this information is published, making it difficult to track the grants back to their funding amounts or even get the objectives or details of the projects. This poses a threat on the visibility of the studies carried out and makes it more difficult to disseminate the research landscape. Nevertheless, after collecting and validating the information on the 20 projects analysed in this report, the results are now publically available in the MESA Track database and have been shared with the ERG, aiming to increase the visibility of the research landscape. The fact that funding amount is missing for 6 projects may be distorting the data concerning funding, as well as the projects that were not included in the analysis due to lacking data (See Annex 3. Ongoing data collection). The unbalances seen between number of projects and funding amount in the different years may be due to this missing data and with all the information the analysis could possibly be more equal.

Regarding the design and implementation of vector control strategies, there is a need to differentiate between malaria control and malaria elimination. The tools and strategies

implemented will be different if we aim to reduce incidence of the disease to a locally acceptable level or if we want to actually interrupt the local transmission.

Neither the ERG nor the MPAC mentioned the role of community health workers when tackling *P. knowlesi.* It is important to highlight that they should be familiar with this species of *Plasmodium*, especially in high-risk areas such as Malaysia, and that they could help to raise awareness of the risks posed by this species.

There are other research needs that the ERG did not mention, such as the impact of this species of *Plasmodium* on the health systems of the areas most affected or the social perception of *P. knowlesi* malaria among the population from South-East Asia. These are also research areas that would need to be considered, among others.

This report had several limitations. First of all, the inclusion/exclusion criterion regarding fellowships was modified during the data collection period and the fellowships found were included in the analysis (Projects 9 and 13). This modification was owing to the fact that the objectives and funding information of the projects were considered significant to obtain results as much robust as possible. In the second place, the access to potential projects depended on the availability of the researchers, their willingness to collaborate and the efficiency of email exchange. In general terms, the responsiveness from the researchers was high, they showed interest in the project and they provided essential information, being essential for the data collection process and validation of the information. However, given the fact that the real total number of projects is unknown and that the search for projects is an ongoing process, at some point we needed to stop the data collection period and move forward to the data analysis. For this reason, for some of the projects we could not get all the information necessary to be added to the database (See Annex 3. Ongoing data collection). However, the projects will be eligible to be added to the MESA Track database in the future. Moreover, the fact that due to time constraints we were not able to build a prototype or get feedback on the design of the online mapping tool is another limitation that we acknowledge.

7. Conclusions and recommendations

The main conclusion that can be drawn from this report is that we lack the evidence-based data needed to confirm whether human to human transmission is taking place. However, taking into account the results of this report, it can be also concluded that there are projects going on that tackle this research gap. We should also be aware of the existing and expected future changes in the levels of exposure to zoonotic malaria, which will need the development of suitable preventive and control strategies, bearing in mind that the animal reservoir makes *P. knowlesi* malaria impossible to eradicate.

Secondly, even though research tackling *P. knowlesi* accounts for only a small part of all the research and development efforts in the field of malaria, there is actually research in *P. knowlesi* going on, especially in Malaysia. Along these lines, certain countries are leading the efforts to understand *P. knowlesi*, as seen both in the literature and in the projects analysed.

Powerful partnerships among institutions are taking place. The amount of collaborations between different countries, research institutions and MOH shows the joint efforts and

sharing rationality shaping the research landscape. In addition, the MOH of Malaysia stands as one of the main institutions tackling *P. knowlesi*.

In order to improve the way *P. knowlesi* is being addressed, I recommend the following:

- A surveillance and monitoring system reporting all the entries of *P. knowlesi* into the human population, together with confirmation with PCR, would be useful to determine the real burden of the disease and detect possible changes in transmission[13].
- Design and implementation of suitable vector control strategies tackling the *P. knowlesi* vectors would also help to control the disease.
- Better RDTs able to correctly identify *P. knowlesi* should be developed.
- Guidelines and standardized procedures when diagnosing *P. knowlesi* with PCR would be extremely useful for the health professionals, as well as determining thresholds of the laboratory findings that define severe disease[20,27].
- Community health workers should be aware of the existence of *P. knowlesi,* able to rapidly identify the clinical symptoms and well-trained to give a correct diagnosis and treatment of the disease. Creation of specific medical texts which describe the clinical features of *P. knowlesi* malaria would also be practical for the management of the disease.
- Specific treatment guidelines for the management of *P. knowlesi* in areas where cases have been reported would be extremely useful[27].

P. knowlesi is one of the main causes of malaria in South-East Asia, especially in Malaysia. While the scientific community collects evidence on whether human to human transmission is taking place, one of the main gaps at the moment, efforts must also be placed in vector control strategies and in a better clinical management of the disease, as well as in developing new and suitable diagnostic methods. Much research is taking place in order to fill all the remaining gaps related to this species of *Plasmodium*, but further evidence is still needed.

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Last but not least, I place a deep sense of gratitude to my friends and family, for their patience and for being a source of inspiration during the development of this report.

10. Annexes

10.1. Annex 1. Expert researchers contacted

ERG Members

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10.2. Annex 2. Validated and published projects

Identification number	1
Title	***P. knowlesi* trial of artemether-lumefantrine vs chloroquine (CAN KNOW)** **Official title: Artemether-lumefantrine vs chloroquine in patients with acute uncomplicated *P. knowlesi* Malaria: a randomized open label trial in Sabah, Malaysia (CAN KNOW trial)**
Objectives	The investigators aim to test whether the fixed combination of artemether-lumefantrine is superior to chloroquine in order to define the optimal treatment for uncomplicated *P. knowlesi* infection in both adults and children in this region.
Principal institution(s)	Menzies School of Health Research, Australia
Principal Investigator (PI)	Nicholas Anstey, Timothy William
Funding source(s)	Menzies School of Health Research, Australia

Abstract	Preliminary studies have supported the background efficacy of local standard anti-malarial medications in the treatment of uncomplicated *knowlesi* malaria; however there are no current WHO treatment guidelines for this infection. There are both health cost benefits to a more rapidly acting agent, and due to difficulties with microscopic identification there may be more effective treatments for all malaria species if an aligned treatment guideline could be supported. Artemether-lumefantrine should be compared against chloroquine due to the fact that it is a first line anti-malarial recommended in Malaysia.
Funding amount ($)	-
Funding information	-
Partners on the project	Ministry of Health Malaysia
Dates	January 2014 to March 2015
Country	Malaysia
Region	South-East Asia
URL	https://clinicaltrials.gov/ct2/show/study/NCT02001012
Link on MESA Track	http://www.malariaeradication.org/mesa-track/p-knowlesi-trial-artemether-lumefantrine-vs-chloroquine
Theme(s)	Drugs

Identification number	2
Title	**P. *Knowlesi* trial of artesunate-mefloquine versus chloroquine (ACT KNOW)** **Official title: Artesunate-mefloquine vs chloroquine in patients with acute uncomplicated *P. knowlesi* and *P. vivax* malaria: a randomized open label trial in Sabah, Malaysia (ACT KNOW trial)**
Objectives	The investigators aim to test whether the fixed combination of artesunate-mefloquine is superior to chloroquine in order to define the optimal treatment for both uncomplicated *P. knowlesi* and *P. vivax* infection in both adults and children in this region.
Principal institution(s)	Menzies School of Health Research, Australia
Principal Investigator (PI)	Nicholas Anstey, Timothy William
Funding source(s)	Menzies School of Health Research, Australia
Abstract	Preliminary studies have supported the background efficacy of local standard anti-malarial medications in the treatment of uncomplicated *knowlesi* malaria, however, this has not been tested systematically and there are no current WHO treatment guidelines for this infection. There are both health cost benefits to a more rapidly acting agent, and due to difficulties with microscopic identification, there may be more effective treatments for all malaria species if an aligned treatment guideline could be supported. In addition, no therapeutic efficacy monitoring of current first-line antimalarials used for the treatment of *P. vivax* malaria has been conducted in Malaysia.
Funding amount ($)	-
Funding information	-
Partners on the project	Ministry of Health Malaysia
Dates	October 2012 to December 2014
Country	Malaysia
Region	South-East Asia
URL	https://clinicaltrials.gov/ct2/show/NCT01708876

Link on MESA Track	http://www.malariaeradication.org/mesa-track/artesunate-mefloquine-vs-chloroquine-patients-acute-uncomplicated-p-knowlesi-and-p-vivax
Theme(s)	Drugs

Identification number	3
Title	**Artemether-lumefantrine vs chloroquine for uncomplicated *P. Vivax* malaria in Malaysia (PRIMAL)** **Official title: Artemether-lumefantrine vs chloroquine in patients with acute non-severe *P. vivax* malaria in Sabah, Malaysia (PRIMAL trial)**
Objectives	The key objective of this study is to evaluate artemether-lumefantrine for *P. vivax* malaria in order to facilitate potential policy change to a unified ACT guideline for all malaria species in Sabah (mainly *P.vivax* and *P.knowlesi*).
Principal institution(s)	Menzies School of Health Research, Australia
Principal Investigator (PI)	Timothy William
Funding source(s)	Menzies School of Health Research, Australia
Abstract	Both artemether-lumefantrine and chloroquine are currently used and recommended by Malaysian Ministry of Health as blood stage treatments for non-severe *P. vivax* and *P. knowlesi* malaria. Microscopic misdiagnosis between *Plasmodium* species remains a large issue in Sabah, Malaysia and elsewhere. Preliminary data in a recently completed RCT evaluating artesunate-mefloquine vs chloroquine for *P. vivax* showed up to 36% *P. vivax* recurrence with chloroquine monotherapy by day 28 post treatment without primaquine. Based on these data, blood stage chloroquine treatment failure rates should also be evaluated in the context of standard concurrent (rather than delayed) liver stage primaquine dosing, due to both its potential blood stage synergistic effect in addition to known decreased recurrence rates. As artemether-lumefantrine is one of the current first-line Ministry of Health ACTs used in Sabah with a lower adverse event profile compared to artesunate-mefloquine, this was recommended as the more appropriate ACT to evaluate against chloroquine.
Funding amount ($)	-
Funding information	-
Partners on the project	Ministry of Health Malaysia
Dates	January 2015 to December 2016
Country	Malaysia
Region	South-East Asia
URL	https://clinicaltrials.gov/ct2/show/record/NCT02348788
Link on MESA Track	http://www.malariaeradication.org/mesa-track/artemether-lumefantrine-vs-chloroquine-uncomplicated-p-vivax-malaria-malaysia-primal
Theme(s)	Drugs

Identification number	4
Title	**Disease burden, risk factors and treatment of *knowlesi* malaria**
Objectives	To estimate the burden from *P. vivax* and *P. knowlesi* malaria in children in Sabah, Malaysia
Principal institution(s)	Menzies School of Health Research, Australia
Principal Investigator (PI)	Matthew Grigg

Funding source(s)	National Health and Medical Research Council (NHMRC), Australia
Abstract	*Plasmodium knowlesi* is a form of monkey malaria recently found to also cause increasing numbers of natural infections in humans in South-East Asia. This research will describe the burden of *P. knowlesi* malaria in an area of Malaysian Borneo. The risk factors for acquiring *P. knowlesi* malaria will be assessed. Finally the optimal treatment for non-severe cases of *P. knowlesi* and *P. vivax* malaria will also be evaluated by comparing the 2 currently recommended anti-malarial medications in Malaysia.
Funding amount ($)	70,878.2
Funding information	AUD 78,412
Partners on the project	Ministry of Health Malaysia
Dates	January 2014 to December 2015
Country	Malaysia
Region	South-East Asia
URL	https://www.nhmrc.gov.au/grants-funding/outcomes-funding-rounds (Results of the 2013 NHMRC Grant Application Round)
Link on MESA Track	http://www.malariaeradication.org/mesa-track/disease-burden-risk-factors-and-treatment-knowlesi-malaria
Theme(s)	Risk of infection Drugs

*Project 5 was excluded, it can be found in the Ongoing Data Collection Annex (identification number XV)

Identification number	6
Title	**Geographic genetic profiling of human *Plasmodium* malaria**
Objectives	- To generate a library of apicoplast and mitochondrial genomic sequence variants across multiple human *Plasmodium* species: *P. falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae*, and *P. knowlesi* using existing raw genomic sequence data generated by collaborating investigators with external funding. - Development of new analytical approaches which can discriminate the different species even in mixed infections by creating a statistical algorithm to infer informative SNP haplotypes within and between species from complex mixed infections. The perfect linkage disequilibrium or "perfect phylogeny" across the co-inherited organelle SNPs leads to an opportunity to construct phylogenetic trees that represent the relationship between haplotypes. Crucially this allows modelling approaches to disaggregate complex mixed infections. - To refine the existing barcoding methodology for discriminating between infections originating from geographically distinct populations of the same species. - To produce a proof of principle in collaboration with overseas research colleagues who have raw genomic sequence data suspected to contain mixed species co-infection. - To develop analytical software which can infer barcodes from complex mixed infections which are commonly found in malaria patients in many parts of the world.
Principal institution(s)	London School of Hygiene and Tropical Medicine (LSHTM)
Principal Investigator (PI)	Taane Gregory Clark
Funding source(s)	Medical Research Council (MRC), United Kingdom
Abstract	Malaria caused by *Plasmodium falciparum* kills about 600,000 people per year, and increased population mobility through international air travel carries further risks of re-introducing parasites to elimination areas and dispersing drug resistant parasites to new regions. A simple genetic marker that quickly and accurately

	identifies the geographic origin of infections would be a valuable tool for locating the source of outbreaks, and spotting the spread of drug resistant parasites from Asia into Africa. Genetic markers have proved extremely valuable in tracking and eradicating diseases, such as Polio. However, the previous candidates for malaria genetic barcodes have relied on identifying DNA markers found in the parasite nucleus, which shows too much genetic variation between individual parasites to be used accurately. Now, DNA sequences found outside the nucleus in organelles called the mitochondria and the apicoplast have been analysed. These are only inherited through maternal lines and therefore much more stable over generations than nuclear DNA sequences. The research outlined in this methodology proposal will create computational tools which will help to exploit use of mitochondria and the apicoplast sequences to create reliable genetic barcodes for tracking the geographical movement of malaria in an operational context. We will create a publically available online resource to facilitate the widespread use of barcoding. It will be of practical use to malaria control agencies and research groups worldwide.
Funding amount ($)	501,586
Funding information	£338,843
Partners on the project	National Institutes of Health (NIH), United States Oswaldo Cruz Foundation (Fiocruz), Brazil Charles Darwin University, Australia University of Sao Paolo, Brazil
Dates	April 2015 to March 2018
Country	United Kingdom
Region	Europe
URL	http://gtr.rcuk.ac.uk/projects?ref=MR%2FM01360X%2F1
Link on MESA Track	http://www.malariaeradication.org/mesa-track/geographic-genetic-profiling-human-plasmodium-malaria
Theme(s)	Parasite genetic diversity Risk of infection

Identification number	7
Title	**Functional analysis of *Plasmodium Vivax* drug resistance polymorphisms**
Objectives	To understand the impact of polymorphisms in putative drug resistance transporters which have been identified in molecular epidemiological studies of *P. vivax*. The macaque parasite *Plasmodium knowlesi* will be used for the analysis of naturally occurring *P. vivax* polymorphisms, due to its close phylogenetic relatedness and its versatility as a system for reverse genetics and drug susceptibility assays. CRISPR/Cas9-based approaches directly in *P. knowlesi* for the functional analysis of the *P. vivax* drug transporter genes and their polymorphisms will be used.
Principal institution(s)	Harvard T.H. Chan School of Public Health (HSPH)
Principal Investigator (PI)	Manoj T. Duraisingh
Funding source(s)	National Institute of Allergy and Infectious Diseases (NIAID), NIH
Abstract	Drug resistance in malaria is one of the most formidable barriers to treatment, control and elimination. Putative polymorphisms in drug resistance transporters have been identified in *Plasmodium vivax*, the most widespread of all of the species causing human malaria. However, the ability of these polymorphisms to confer chloroquine resistance or the magnitude of the resistance conferred by

	these mutations remains unknown. This gap in our knowledge is largely due to the absence of an in vitro culture system, robust drug assays, and reverse genetics for the study of *P. vivax*. Reverse genetic analyses of specific drug transporter polymorphisms of *P. vivax* will be critical to our understanding of their relevance to antimalarial resistance, the development of strategies for limiting the spread or reversal of drug resistance, and their validation as drug resistance markers in population level studies and surveillance for control and elimination measures. Our work will also establish *P. knowlesi* as a powerful heterologous platform for the study of critical genes involved in many processes relevant to *P. vivax*.
Funding amount ($)	238,126
Funding information	-
Partners on the project	-
Dates	June 2016 to May 2018
Country	United States
Region	North America
URL	https://goo.gl/ZadmVD
Link on MESA Track	http://www.malariaeradication.org/mesa-track/functional-analysis-plasmodium-vivax-drug-resistance-polymorphisms
Theme(s)	Parasite genetic diversity

Identification number	8
Title	**MONKEYBAR: Defining the biomedical, environmental and social risk factors for human infection with *Plasmodium knowlesi*; opportunities for prevention and control of an emerging zoonotic infection**
Objectives	We aim to identify the scale of public health threat posed by *P. knowlesi*, through characterisation of the biological, environmental and social factors responsible for triggering its emergence within human populations. By understanding these factors we will generate risk maps to define and focus appropriate control strategies. This will allow an understanding of the conditions that permit the parasite host switch from macaque to human, and predictions of the risk of further species crossover events may be possible.
Principal institution(s)	London School of Hygiene and Tropical Medicine (LSHTM), Infectious and Tropical Diseases department
Principal Investigator (PI)	Chris Drakeley
Funding source(s)	Medical Research Council (MRC), United Kingdom (Environmental and Social Ecology of Human Infectious Diseases Initiative (ESEI) (Grant ref. G1100796))
Abstract	Malaria is caused by a single celled parasite found predominantly in the blood of its host, and is transmitted between hosts by the bite of a mosquito. Greater than 100 species of malaria exist, infecting many different animals. Until recently, humans were thought to be the natural hosts for 4 malaria species. However, since 2004 reports appeared of malaria infections in humans that are caused by a parasite species found previously in certain types of macaque in South-East Asia. This species is *Plasmodium knowlesi* and, although benign in its natural monkey host, it has caused severe and even fatal disease in a proportion of human sufferers. Currently, we know little about the true burden of disease caused by this macaque parasite, or why it has emerged as a human pathogen. Initial descriptions of humans with *Plasmodium knowlesi* malaria suggested that they had been bitten and infected while working in forested areas, common in many countries of South-East Asia. However, a more recent study in Malaysian Borneo

	shows that communities there are also being infected, even though there is little forest in existence. There may be a number of factors related to the environment, the types of mosquito in the area, people's behaviour or movement and their proximity to troops of macaques that will have an effect on how at risk they are to being infected with *Plasmodium knowlesi*. We have built a network of researchers with different skills and expertise from the UK, the Philippines, Malaysia and Australia to tackle the various knowledge gaps by working together in a concerted approach. We plan to conduct studies in both the Philippines (on Palawan island) and in Sabah, a region of Malaysian Borneo. A larger number of cases of human *Plasmodium knowlesi* infection have been found in Sabah, compared to Palawan, and we will attempt to find out why communities here seem at higher risk. In treating their infections we can produce guidelines to help other clinicians faced with this disease. We also want to study the macaques, the mosquitoes that transmit the parasite, and the environment in which the infections occur to give us the whole picture and to produce maps that describe the risk factors existing in the different areas. We hope this will provide important information to ministries of health trying to control malaria disease in the affected regions and prevent further outbreaks of malaria originating from primates. Methods: We propose an interdisciplinary approach to examine the extent of infection with *P. knowlesi* at 2 study sites and to identify risk factors for infection that ultimately contribute to its control. A case control study, the first of its kind for *P. knowlesi*, will allow analysis of broad scale spatial and socio-behavioural factors associated with symptomatic infections. Alongside this we will conduct detailed entomological trapping experiments and primatology studies within habitats of potential epidemiological importance (closed canopy primary forest, secondary forest, etc.). These data together with mosquito and macaque infection rates will be incorporated into spatial and mathematical models to generate risk maps for *P. knowlesi* infection. We will use these maps to define areas in which to conduct cross sectional surveys to identify asymptomatic infections and exposure patterns with *P. knowlesi*.
Funding amount ($)	4,622,644
Funding information	£2.896.960 (MRC) + £5,000 (London International Development Centre)
Partners on the project	Danau Girang Field Centre, Malaysia Clinical Research Center Queen Elizabeth Hospital, Malaysia University of Malaysia Sabah (UMS), Malaysia University of Glasgow, United Kingdom University of London (Royal Veterinary College), United Kingdom Research Institute for Tropical Medicine (RITM), Philippines Liverpool School of Tropical Medicine, United Kingdom University of the Philippines Los Baños (UPLB), Philippines Menzies School of Health Research, Australia
Dates	March 2012 to September 2017
Country	The Philippines Malaysia
Region	South-East Asia
URL	http://gtr.rcuk.ac.uk/projects?ref=G1100796 http://malaria.lshtm.ac.uk/MONKEYBAR
Link on MESA Track	http://www.malariaeradication.org/mesa-track/monkeybar-defining-biomedical-environmental-and-social-risk-factors-human-infection
Theme(s)	Risk of infection Transmission

Identification number	9
Title	**Dissecting the Red Blood Cell Invasion Pathways of the Malaria Parasite *Plasmodium knowlesi***
Objectives	- To unravel the process of red blood cell (RBC) invasion by malaria parasites, an important target for novel drugs and vaccines - To identify parasite factors which may be causing the rise in human infections - To identify novel vaccine candidates - To develop a transgenic *P. knowlesi* model to test existing vaccine candidates for *P. vivax*
Principal institution(s)	London School of Hygiene and Tropical Medicine (LSHTM), Infectious and tropical diseases department
Principal Investigator (PI)	Robert William Moon
Funding source(s)	Medical Research Council (MRC), United Kingdom
Abstract	The parasites produce a range of adhesive proteins enabling them to bind to specific proteins on the surface of red blood cells and establish the process of red blood cell invasion. Because of their crucial role in the invasion process these parasite proteins are important vaccine targets. They also determine how effectively parasites can replicate and so can affect disease severity as well as determining which hosts are susceptible to malaria. In this project I will investigate the role of these proteins during the invasion process using a malaria parasite known as *Plasmodium knowlesi*. I will use highly efficient techniques to genetically modify the parasite and generate parasites in which I have deleted genes encoding the adhesive proteins. This will enable me to determine which are essential for invasion and which can be deleted without any effect on the invasion process. Using similar techniques I will also add fluorescent "tags" to each of the proteins coded by the target genes, so that I can determine where the adhesive proteins are in the cell and where they move during the invasion process. The "tags" will also allow me to identify parasite proteins that interact with the adhesive proteins as well as what they specifically bind to on the host red blood cell surface. This will provide critical insight into the mechanism of invasion of all malaria parasites, as well as identifying precisely which parasite proteins and host proteins are required for *P. knowlesi* to invade human red blood cells. The latter is of particular importance as it may explain how a macaque malaria parasite is able to spread to infect humans and determine the potential for emergence of human-to-human transmission of the parasite.
Funding amount ($)	1,805,966
Funding information	£1,085,780 (MRC UK) + £62,600 (Bloomsbury Colleges). Fellowship
Partners on the project	Medicines for Malaria Venture (MMV)
Dates	July 2015 to June 2020
Country	United Kingdom
Region	Europe
URL	http://gtr.rcuk.ac.uk/projects?ref=MR%2FM021157%2F1
Link on MESA Track	http://www.malariaeradication.org/mesa-track/dissecting-red-blood-cell-invasion-pathways-malaria-parasite-plasmodium-knowlesi
Theme(s)	Vaccines Transmission Basic science

Identification number	10

Title	**Single-cell functional and population genomic analysis of *Plasmodium knowlesi* malaria parasites**
Objectives	- Analysis of *P. knowlesi* by flow cytometric sorting from blood of macaque and human fresh isolates, and sequencing from single cells and pools of individual sorted cells. - Test a hypothesis of vector-specific susceptibility to the divergent *P. knowlesi* types - Design of genetic manipulation experiments to test for loci that are involved in host adaptation and potentially enhancing reproductive isolation
Principal institution(s)	London School of Hygiene and Tropical Medicine (LSHTM), Infectious and tropical diseases department
Principal Investigator (PI)	David Conway
Funding source(s)	Biotechnology and Biological Sciences Research Council (BBSRC), United Kingdom
Abstract	Genomic analyses of parasites can give insights into mechanisms of adaptation to different hosts. There are divergent genetic types of *Plasmodium knowlesi* infecting humans, now discovered to be associated with different macaque monkey reservoir host species. Sequence analysis reveals substantial genome-wide divergence between these types, although this is particularly high in some chromosomal regions.
Funding amount ($)	-
Funding information	Studentship
Partners on the project	-
Dates	October 2015 to September 2019
Country	United Kingdom
Region	Europe
URL	http://gtr.rcuk.ac.uk/projects?ref=studentship-1618502
Link on MESA Track	http://www.malariaeradication.org/mesa-track/single-cell-functional-and-population-genomic-analysis-plasmodium-knowlesi-malaria
Theme(s)	Parasite genetic diversity Transmission

Identification number	11
Title	**Structure and function of eukaryotic phosphatidylserine decarboxylase**
Objectives	- Examine the regulation of the enzyme phosphatidylserine decarboxylase (PSD), which produces the essential membrane component phosphatidylethanolamine - Obtain specific biochemical information about new ways to selectively block the activity of the enzyme in pathogens including bacteria, fungi and malarial parasites. - Test the hypothesis that the *Plasmodium knowlesi* PSD (PkPSD) proenzyme is initially a serine protease that undergoes a molecular metamorphosis to become a decarboxylase - Examine the lipid regulation of the PkPSD by testing for the presence of specific phospholipid binding sites - Investigate the hypothesis that lipid regulation of PkPSD is mechanistically coupled to proenzyme processing by inducing conformational changes to the enzyme that either activate or inhibit the protease function.
Principal institution(s)	National Jewish Health
Principal Investigator (PI)	Dennis R. Voelker
Funding source(s)	National institute of general medical sciences (NIGMS), NIH

Abstract	Phosphatidylethanolamine (PE) is an essential lipid in organisms ranging from bacteria to humans and a pivotal enzyme in the production of this phospholipid is phosphatidylserine decarboxylase (PSD). Although the deduced primary structure of eukaryotic PSD has been known for more than a decade, the details about how the activity of this enzyme is regulated have been elusive. In addition, PSD belongs to an unusual family of enzymes that contain a pyruvoyl prosthetic group. Progress in understanding eukaryotic PSD enzymes has been hampered by its integral membrane structure and relative lability in the presence of detergents. Recently, we cloned a cDNA encoding PSD from the parasite *Plasmodium knowlesi* (PkPSD). The PkPSD exists in both soluble and membrane bound forms. The availability of soluble forms of PkPSD has now enabled new lines of inquiry into the structure and function of this enzyme. Using coupled in vitro transcription/ translation systems we have begun to dissect the early events that regulate the conversion of nascent proenzyme to the mature enzyme, consisting of a small subunit containing the pyruvoyl prosthetic group, and a large subunit. We have now devised a system for examining the in vitro processing of the proenzyme to the mature enzyme, and have succeeded in expressing high levels of the proenzyme in bacteria. Utilizing these systems we now plan to conduct experimentation to elucidate the mechanisms of proenzyme processing and post translational regulation of catalytic activity. From these studies we will obtain a comprehensive view of how the lipid composition of cell membranes allosterically influences the activation of an essential enzyme in phospholipid synthesis. Understanding this aspect of PSD regulation coupled to membrane lipid composition will have important consequences for intervening in phospholipid metabolism of pathogens and mammalian cells with unregulated growth.
Funding amount ($)	1,217,280
Funding information	-
Partners on the project	-
Dates	August 2013 to July 2017
Country	United States
Region	North America
URL	https://goo.gl/PywZQK
Link on MESA Track	http://www.malariaeradication.org/mesa-track/structure-and-function-eukaryotic-phosphatidylserine-decarboxylase
Theme(s)	Basic science

Identification number	12
Title	**Comparative incidence and clinical spectrum of *Plasmodium knowlesi* malaria, a longitudinal study in Sabah, Malaysia**
Objectives	- Determine the true incidence and trend of *knowlesi* malaria in Sabah, in comparison to the other human malaria species. This will be achieved through collaboration with the Sabah Public Health Laboratory (Ministry of Health Malaysia), and will involve the implementation of state-wide molecular testing of every case of microscopy-diagnosed malaria in Sabah during the 5-year study period. - Determine the epidemiology and clinical spectrum of *knowlesi* malaria at two district hospitals, including changes over time. - Provide for the first time accurate information regarding the true incidence of *knowlesi* malaria in Sabah, and the trend of this species over time in comparison to the other human malaria species. - Provide crucial information regarding the clinical features of *knowlesi* malaria in

	populations that have not been previously well-studied, including children and pregnant women.
Principal institution(s)	Infectious Disease Society Kota Kinabalu Sabah, Malaysia
Principal Investigator (PI)	Timothy William
Funding source(s)	National institute of allergy and infectious diseases (NIAID), NIH
Abstract	Malaysia has one of the most successful malaria control programs in South-East Asia, with marked reductions in the incidence of *Plasmodium falciparum* and *P. vivax* over recent decades and an aim to eliminate these species by 2020. However, the simian parasite *P. knowlesi* has emerged as a major cause of human malaria, and we recently reported that in Sabah, east Malaysia, combined notifications of P. *knowlesi* and the microscopically near-identical *P. malariae* increased >10-fold between 2004 - 2011. However, microscopic diagnosis of *P. knowlesi* is known to be problematic, and definitive diagnosis requires molecular methods. Hence, whether the apparent increase in *P. knowlesi* is due to increased recognition of the species remains uncertain, and the true incidence and trend of *knowlesi* malaria in Sabah, in comparison to the other human malaria species, has not been determined. *P. knowlesi* has a 24-hour replication cycle and can result in high parasitaemia with consequent complications. Risk of severe disease appears higher than that of *falciparum* malaria, and fatal cases have been reported. However, many knowledge gaps remain with regards to the epidemiology and clinical features of *knowlesi* malaria. Large prospective longitudinal studies have not been conducted, and no prospective study has evaluated the clinical features of *knowlesi* malaria in children or the occurrence and consequences of *knowlesi* malaria in pregnancy.
Funding amount ($)	273,456
Funding information	-
Partners on the project	Menzies School of Health Research, Australia Ministry of Health Malaysia
Dates	April 2015 to March 2020
Country	Malaysia
Region	South-East Asia
URL	https://goo.gl/zBrcO5
Link on MESA Track	http://www.malariaeradication.org/mesa-track/comparative-incidence-and-clinical-spectrum-plasmodium-knowlesi-malaria-longitudinal
Theme(s)	Risk of infection Clinical outcomes and pathophysiology

Identification number	13
Title	**Comparative pathophysiology and clinical epidemiology of *knowlesi* malaria**
Objectives	- Describe the epidemiological and clinical features of the hospitalised patients, including changes over time. - Understand how *P. knowlesi* causes sever disease.
Principal institution(s)	Menzies School of Health Research, Australia
Principal Investigator (PI)	Bridget Barber
Funding source(s)	National Health and Medical Research Council (NHMRC), Australia. Results of the 2014 NHMRC Grant Application Round
Abstract	The simian parasite P. *knowlesi* is the most common cause of malaria in Malaysia and can cause severe and fatal disease. We are currently conducting a study of all malaria patients admitted to a tertiary referral hospital in Sabah, Malaysia.

Funding amount ($)	231,095
Funding information	$AUD 264,598.19. Fellowship
Partners on the project	-
Dates	October 2014 to 2019
Country	Malaysia
Region	South-East Asia
URL	https://goo.gl/RR4whn
Link on MESA Track	http://www.malariaeradication.org/mesa-track/comparative-pathophysiology-and-clinical-epidemiology-knowlesi-malaria
Theme(s)	Clinical outcomes and pathophysiology

Identification number	14
Title	***Plasmodium* genus and PFV fluorescent in situ hybridization (fish) assay kit**
Objectives	- To manufacture 3 lots of kits and completion the stability study of the kits and reagents - Analytical Sensitivity Study using patient samples positive for *P. falciparum* and *P. vivax* - Reproducibility Study near limit of detection using patient samples - Completion of Specificity Study (including Analytical Specificity and Interference Substances) - Clinical studies at 3 sites; PCR on all clinical study samples and sequencing on all PCR positive samples and analysis of data - Develop *P. ovale*, *P. malariae* and *P. knowlesi* specific FISH assays.
Principal institution(s)	ID FISH technology INC., United States
Principal Investigator (PI)	Jyotsna S. Shah
Funding source(s)	National Institute of Allergy and Infectious Diseases (NIAID), NIH
Abstract	Even though malaria is a frequently encountered disease in many developing countries, it is difficult to make the right diagnosis relying on clinical signs only. Drug selection for the treatment of malaria depends on the species of malaria present. Delayed or missed diagnosis of *falciparum* malaria increases the risk of complicated or severe disease, which may be fatal vulnerable populations. To prevent unnecessary anti-malarial treatment and future drug-resistance strains of malaria parasites, it is important to confirm clinical suspicious with a good laboratory test. In light of the changing drug policies of many African countries, including Tanzania and Kenya, where the expensive artemisinin combination therapy (ACT) drugs are prescribed as first-line treatment, a good laboratory confirmation will also have an impact on the economics. The Giemsa stain is helpful. However, when parasite levels are very low, or in mixed infections, the information obtained by examination of Giemsa stained smear by microscopy is limited, and in some cases, biased, by the inability to devote the necessary amount of time to the examination of blood smears. PCR would help. Unfortunately it is time-consuming and expensive. Thus, FISH Tests for detection of *Plasmodium* and for differentiation of *P. falciparum* and *P. vivax* in air-dried blood smears has potential in the rapid diagnosis of this disease. P- Genus FISH Assay detects all five species of *Plasmodium, P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi.* Therefore the Genus FISH assay can be used for screening.
Funding amount ($)	885,321
Funding information	NIAID Funding for fiscal year 2014

Partners on the project	-
Dates	July 2003 to October 2015
Country	United States
Region	North America
URL	https://goo.gl/TtGK5Q
Link on MESA Track	http://www.malariaeradication.org/mesa-track/plasmodium-genus-and-pfv-fluorescent-situ-hybridization-fish-assay-kit
Theme(s)	Diagnostics

Identification number	15
Title	**An effector memory T cell-inducing subunit vaccine against malaria**
Objectives	- To use cytomegalovirus as a novel tool to immediately intersect incoming parasites at the liver stage, thus preventing blood infection and disease. - To determine whether the RhCMV/Pk4 vaccine is protective by challenging with *P. knowlesi* sporozoites - To know whether a TEM-inducing vaccine can improve the level and duration of sterilising immunity induced by subunit vaccines against malaria parasites.
Principal institution(s)	Oregon Health and Science University (OHSU), United States
Principal Investigator (PI)	Klaus J. Früh
Funding source(s)	National Institute Of Allergy And Infectious Diseases (NIAID), NIH
Abstract	The ultimate goal of this project is to develop a sterilising vaccine against malaria. The specific hypothesis tested in this proposal is that sterilising immunity against malaria can be achieved by induction of a lasting effector memory T cell (TEM) response targeting the liver stage of *Plasmodium* parasites. Repeated immunisations with live or irradiated sporozoites are known to protect vaccinated individuals against malaria challenge. Recent evidence suggests that this protection correlated with the presence of frequent pluripotent TEM, suggesting that permanent sterilising immunity against malaria requires the induction of high levels of long-lived TEM by vaccination. To test this hypothesis, we propose to use recombinant cytomegalovirus (CMV) as a vaccine vector because CMV is the prototypical virus inducing long-lived TEM that do not show signs of T cell exhaustion. This unique capability of CMV-vectors was recently applied to induce long-lived TEM against simian immunodeficiency virus, resulting in protection against SIV-challenge that was by far superior to conventional heterologous prime/boost vaccines with respect to efficacy and duration. Since sterilising protection against *Plasmodium knowlesi* parasites was only partial and short-lived when heterologous prime/boost vaccines were used, we will examine whether CMV-derived vaccine vectors will similarly confer lasting and efficacious immunity against challenge with *Plasmodium knowlesi* (Pk) sporozoites. We propose to generate recombinant RhCMV expressing four *Pk* antigens previously used for heterologous prime-boost vaccination: the circumsporozoite protein (CSP), the sporozoite surface protein 2 or thrombospondin-related adhesion protein (SSP2 or TRAP), the apical merozoite antigen-1 (AMA1) and the C-terminus of the merozoite surface protein 1 (MSP1c). We will inoculate animals with this panel of four recombinant RhCMV/Pk vectors and monitor the development of TEM in blood, lung and liver.
Funding amount ($)	455,000
Funding information	-
Partners on the project	-

Dates	February 2013 to January 2016
Country	United States
Region	North America
URL	https://goo.gl/al1ccv
Link on MESA Track	http://www.malariaeradication.org/mesa-track/effector-memory-t-cell-inducing-subunit-vaccine-against-malaria
Theme(s)	Vaccines

Identification number	16
Title	**RBL Binding Domain Malaria Candidate Vaccines**
Objectives	- Test the hypothesis that immunization with an RBL red blood cell binding domain can limit a lethal infection, and that the combination of multiple RBL binding domain regions will provide greater protection by testing the potential of *P. knowlesi* RBL binding domains to function as immunogens that will attenuate the usual lethality of this parasite - Test the hypothesis that antisera to homologous binding domain regions from *P. vivax* RBL proteins will inhibit invasion of merozoites in vitro, as a prelude to possible pre-clinical vaccine trials using these proteins in NHPs.
Principal institution(s)	Emory University, Atlanta, United States
Principal Investigator (PI)	Mary R. Galinski
Funding source(s)	National Institute Of Allergy And Infectious Diseases (NIAID), NIH
Abstract	A malaria vaccine would be extremely valuable tool to help control malaria. Novel approaches and new paradigms are needed that can expedite the testing and provide some form of validation for theoretically sound malaria vaccine candidates. This proposal is straightforward, while also being innovative, risky and exploratory. We will learn whether RBL-based malaria vaccines can provide protection against erythrocytic challenge and also develop valuable tools for advancing investigations relating to merozoite invasion of red blood cells. This research will also gain valuable information on the immune response to the RBLs and pave the way for potential pre- clinical malaria vaccine trials based on *P. vivax* RBL immunogens.
Funding amount ($)	484,000
Funding information	-
Partners on the project	-
Dates	March 2011 to February 2015
Country	United States
Region	North America
URL	https://goo.gl/JmralW
Link on MESA Track	http://www.malariaeradication.org/mesa-track/rbl-binding-domain-malaria-candidate-vaccines
Theme(s)	Vaccines

Identification number	17
Title	**A comparative study of the pathophysiology of severe *knowlesi* and *falciparum* malaria**

Objectives	To understand and compare the pathophysiology of *knowlesi* malaria and *falciparum* malaria.
Principal institution(s)	Menzies School of Health Research, Australia
Principal Investigator (PI)	Tsin Yeo Nicholas Anstey
Funding source(s)	National Health and Medical Research Council (NHMRC), Australia
Abstract	As part of the prospective enrolments of all patients admitted to Queen Elizabeth Hospital, Kota Kinabalu, Sabah, this project investigated pathophysiological correlates of severe disease, and compared *falciparum, vivax* and *knowlesi* malaria.
Funding amount ($)	685,576
Funding information	$AUD 660,293.75
Partners on the project	Mahidol Oxford Tropical Medicine Research Unit (MORU) Clinical Research Center Queen Elizabeth Hospital, Malaysia Ministry of Health Malaysia
Dates	December 2012 to 2015
Country	Malaysia
Region	South-East Asia
URL	https://goo.gl/mpMWXO
Link on MESA Track	http://www.malariaeradication.org/mesa-track/comparative-study-pathophysiology-severe-knowlesi-and-falciparum-malaria
Theme(s)	Clinical outcomes and pathophysiology

Identification number	18
Title	**Improving the diagnosis, treatment and outcomes of patients infected with *Plasmodium knowlesi***
Objectives	To improve the knowledge about *Plasmodium knowlesi* pathogenesis
Principal institution(s)	Menzies School of Health Research
Principal Investigator (PI)	Bridget Barber
Funding source(s)	National Health and Medical Research Council (NHMRC), Australia
Abstract	The simian parasite *Plasmodium knowlesi* is increasingly recognized as a cause of uncomplicated and severe human malaria in South-East Asia and is now the most common cause of malaria in Malaysian Borneo. Although *P. knowlesi* is at least as likely to cause severe disease in adults as *Plasmodium falciparum*, little is known about its pathogenesis.
Funding amount ($)	124,858
Funding information	$AUD 139,141.39. NHMRC Postgraduate Scholarships: Medical Postgraduate Scholarship
Partners on the project	-
Dates	2010 to 2014
Country	Malaysia
Region	South-East Asia
URL	https://goo.gl/Uaoahd
Link on MESA Track	http://www.malariaeradication.org/mesa-track/improving-diagnosis-treatment-and-outcomes-patients-infected-plasmodium-knowlesi
Theme(s)	Clinical outcomes and pathophysiology

Identification number	19
Title	**Effect of Paracetamol on Renal Function in *Plasmodium Knowlesi* Malaria (PACKNOW)** **Official title: Effect of Paracetamol on Renal Function in *Plasmodium knowlesi* Malaria: A Randomised Controlled Clinical Trial (PACKNOW Trial)**
Objectives	To assess the effect of paracetamol on renal function and oxidative stress in patients with *knowlesi* malaria
Principal institution(s)	Clinical Research Center Queen Elizabeth Hospital, Malaysia
Principal Investigator (PI)	Giri M. Rajahram
Funding source(s)	Menzies School of Health Research, Australia
Abstract	*Plasmodium knowlesi* is the most common cause of malaria, and malaria deaths, in Sabah, Malaysia. Acute kidney injury (AKI) is a common feature of severe *knowlesi* malaria; however the mechanisms of AKI in *knowlesi* malaria are unknown. In *falciparum* malaria, recent evidence suggests that oxidative stress from haemolysis-related cell-free haemoglobin (CFHb) may contribute to pathogenesis of AKI. The investigators hypothesize that paracetamol may provide renal protection in patients with severe *knowlesi* malaria by reducing the hemoprotein-induced lipid peroxidation that occurs in haemolytic conditions. As there is currently no consensus that exists concerning adequate medical treatment for severe malaria complicated by intravascular haemolysis and AKI, the potential application of paracetamol would be of great benefit, especially as it is safe and widely available. The main activity proposed is a randomised, open label, controlled trial of regularly-dosed paracetamol, versus no paracetamol, in patients with *knowlesi* malaria, to assess the effect of paracetamol on renal function and oxidative stress.
Funding amount ($)	-
Funding information	-
Partners on the project	Menzies School of Health Research, Australia Ministry of Health Malaysia
Dates	October 2016 to August 2018
Country	Malaysia
Region	South-East Asia
URL	https://clinicaltrials.gov/ct2/show/study/NCT03056391
Link on MESA Track	http://www.malariaeradication.org/mesa-track/effect-paracetamol-renal-function-plasmodium-knowlesi-malaria-packnow
Theme(s)	Drugs

Identification number	20
Title	**Defining the population at risk of *Plasmodium knowlesi* malaria to complete the human malaria baseline in Asia**
Objectives	To predict spatial variation in *Plasmodium knowlesi* disease risk in order to identify priority areas for surveillance based on regions with sparse data and high estimated risk.
Principal institution(s)	University of Oxford, United Kingdom
Principal Investigator (PI)	Simon I. Hay
Funding source(s)	Wellcome Trust
Abstract	Infection by the simian malaria parasite*, Plasmodium knowlesi*, can lead to severe and fatal disease in humans, and is the most common cause of malaria in parts of

	Malaysia. Despite being a serious public health concern, the geographical distribution of *P. knowlesi* malaria risk is poorly understood because the parasite is often misidentified as one of the human malarias. Human cases have been confirmed in at least nine South-East Asian countries, many of which are making progress towards eliminating the human malarias. Understanding the geographical distribution of *P. knowlesi* is important for identifying areas where malaria transmission will continue after the human malarias have been eliminated.
Funding amount ($)	164,330
Funding information	£100,000 – Enhancement Grant 095066/Z/10/A
Partners on the project	-
Dates	March 2014 to November 2016
Country	United Kingdom
Region	Europe
URL	Wellcome Trust Grants Awarded 2000/01 to 2014/15 database
Link on MESA Track	http://www.malariaeradication.org/mesa-track/defining-population-risk-plasmodium-knowlesi-malaria-complete-human-malaria-baseline-asia
Theme(s)	Risk of infection

Identification number	21
Title	**Molecular mechanisms driving the adaptation of *Plasmodium knowlesi* to humans**
Objectives	-To compare the genome of *Plasmodium knowlesi* isolates from patients with severe and uncomplicated malaria and a reference strain. -To in vitro culture adapt *P. knowlesi* isolates obtained from patients with severe and uncomplicated malaria. -To compare the intrerythrocitic transcriptome of *Plasmodium knowlesi* isolates from patients with severe and uncomplicated malaria and a reference strain. -To identify and correlate changes in the parasite transcriptome and genome with host adaptation as well as virulence.
Principal institution(s)	School of Biological Sciences (SBS), Nanyang Technological University, Singapore
Principal Investigator (PI)	Peter Preiser Zybnek Bozdech Tsin Yeo
Funding source(s)	Ministry of Education (MOE) Singapore
Abstract	A new challenge has arisen in South East Asia due to *P. knowlesi*, a simian parasite normally found to infect the macaque monkeys causing a significant number of human malaria infections. Today, *P. knowlesi* infections are the major cause of human malaria in certain regions. It is not known whether *P. knowlesi* is solely transmitted from the monkey to the human or whether human to human transmission is possible. The discovery of *P. knowlesi* as a significant threat to humans makes it of critical importance to understand the changes that drive adaptation to humans as well as the molecular factors leading to severe disease in human patients. The hypotheses here are: 1) Genetic or transcriptional changes will provide insights into the molecular changes that underpin adaptation of *P. knowlesi* to humans 2) Parasites that cause severe disease vs uncomplicated disease will have either genomic or transcriptomic differences. Taken together this study will provide us with a comprehensive understanding of parasite factors that contribute to virulence.

Funding amount ($)	-
Funding information	Singapore Ministry of Education Academic Research Fund Tier 1. Researchers were not able to share funding amount.
Partners on the project	-
Dates	2016
Country	Singapore
Region	South-East Asia
URL	Information from emailing with the PIs
Link on MESA Track	http://www.malariaeradication.org/mesa-track/molecular-mechanisms-driving-adaptation-plasmodium-knowlesi-humans
Theme(s)	Transmission Parasite genetic diversity

10.3. Annex 3. Ongoing data collection

Identification number	I
Title	**Natural selection and genetic diversity of leading *Plasmodium knowlesi* vaccine antigens**
Objectives	-
Principal institution(s)	University of Malaysia
Principal Investigator (PI)	Lau YL Fong MY
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Parasite genetic diversity Vaccines

Identification number	II
Title	**Characterization of *Plasmodium knowlesi* circumsporozoite protein and production of sporozoites for potential vaccine development**
Objectives	-
Principal institution(s)	University of Malaysia
Principal Investigator (PI)	Lau YF Fong MY Indra Vythilingam
Funding source(s)	-

Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Vaccines

Identification number	III
Title	**Host-vector population genetics in *Plasmodium knowlesi* malaria from Malaysia**
Objectives	-
Principal institution(s)	University of Malaysia
Principal Investigator (PI)	Lau YF Fong MY
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Parasite genetic diversity

Identification number	IV
Title	**Rapid point-of-care (PoC) molecular tests for the detection of malaria and newly developed anti-*Plasmodium* and anti-*knowlesi* ELISAs for the diagnosis of human Malaria**
Objectives	-
Principal institution(s)	University of Malaysia
Principal Investigator (PI)	Lau YL, Indra Vythilingam Klemens JS
Funding source(s)	-
Abstract	-

Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Diagnostics

Identification number	V
Title	**The phylogeography and ecology of human and non-human primate malarias and their vectors in Malaysia**
Objectives	-
Principal institution(s)	University of Malaysia
Principal Investigator (PI)	Indra Vythilingam Yvonne YAL Lau YL
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Risk of infection

Identification number	VI
Title	**An integrated community based approach for malaria control**
Objectives	-
Principal institution(s)	University of Malaysia
Principal Investigator (PI)	Indra Vythilingam Lau YL Fong MY
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-

Partners on the project	-
Dates	-
Country	-
Region	-
URL	-
Link on MESA Track	-
Theme(s)	Risk of infection

Identification number	VII
Title	**Genetic diversity and temporal variations in composition of subpopulations of *Plasmodium knowlesi***
Objectives	-
Principal institution(s)	University of Malaysia Sarawak (UNIMAS)
Principal Investigator (PI)	Balbir Singh
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	Kapit Hospital, Malaysia London School of Hygiene and Tropical Medicine (LSHTM)
Dates	2016 to 2018
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Parasite genetic diversity

Identification number	VIII
Title	**Genomics and population genetics of *Plasmodium knowlesi* in South-East Asia**
Objectives	-
Principal institution(s)	University of Malaysia Sarawak (UNIMAS)
Principal Investigator (PI)	Balbir Singh
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	London School of Hygiene and Tropical Medicine (LSHTM) King Abdullah University of Science and Technology, Saudi Arabia
Dates	2013 to 2017
Country	-

Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Parasite genetic diversity

Identification number	IX
Title	**Identification of vectors of *Plasmodium knowlesi* and other malaria parasites in Sarawak**
Objectives	-
Principal institution(s)	University of Malaysia Sarawak (UNIMAS)
Principal Investigator (PI)	Balbir Singh
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	Sarawak Health Department, Ministry of Health (MOH) Malaysia
Dates	2014 to 2017
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Transmission

Identification number	X
Title	**Epidemiology of *knowlesi* and other simian malarias in humans in the Betong Division of Sarawak**
Objectives	-
Principal institution(s)	University of Malaysia Sarawak (UNIMAS)
Principal Investigator (PI)	Balbir Singh
Funding source(s)	-
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	Sarawak Health Department, Ministry of Health (MOH) Malaysia
Dates	2013 to 2017
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-

Theme(s)	Risk of infection Clinical outcomes and pathophysiology

Identification number	XI
Title	**Mapping of *knowlesi* malaria vectors in Peninsular Malaysia**
Objectives	To identify and characterize *anopheline* mosquito immature habitats To examine the spatial distribution of *knowlesi* malaria vectors To map *knowlesi* malaria vector distributions using geographic information systems
Principal institution(s)	-
Principal Investigator (PI)	Rohani Ahmad
Funding source(s)	National Institute of Health (NIH) Ministry of Health (MOH) Malaysia
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Risk of infection

Identification number	XII
Title	**Insecticide susceptibility status in *Anopheles crescens*: vector of *Plasmodium knowlesi* in peninsular Malaysia**
Objectives	To attain better understanding of the resistance situation in *knowlesi* malaria areas To determine the level and type of insecticide resistance in *A. crescens* mosquitoes To evaluate the metabolic resistance in *A. crescens* mosquitoes to assess the implications of the current vector control strategies against vectors of *P. knowlesi* To characterize the spatial distribution of resistance in *A. crescens* mosquitoes in a variety of ecological settings and then to correlate this resistance with pesticide usage
Principal institution(s)	-
Principal Investigator (PI)	Rohani Ahmad
Funding source(s)	National Institute of Health (NIH) Ministry of Health (MOH) Malaysia
Abstract	-
Funding amount ($)	-
Funding information	-

Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Transmission

Identification number	XIII
Title	**Bionomics of *knowlesi* malaria vectors in Sabah**
Objectives	To determine the entomological risk-factors associated with *P. knowlesi* infection within an endemic areas in Sabah To characterize the ecology and behaviour of *knowlesi*
Principal institution(s)	-
Principal Investigator (PI)	Rohani Ahmad
Funding source(s)	National Institute of Health (NIH) Ministry of Health (MOH) Malaysia
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Risk of infection

Identification number	XIV
Title	**Field evaluation of outdoor residual spraying for the control of simian malaria in Sabah**
Objectives	To evaluate the effectiveness of outdoor residual spraying of a new formulation of deltamethrin for malaria control To compare the effectiveness of this formulation with current deltamethrin WG in malaria control To determine the residual efficacy of the new formulation of insecticide and deltamethrin WG against malaria vectors To determine and compare the residual lifespan and effectiveness of these insecticides on different wall surface materials To determine a long-lasting insecticide for outdoor residual spray to control malaria vectors
Principal institution(s)	-
Principal Investigator (PI)	Rohani Ahmad

Funding source(s)	National Institute of Health (NIH) Ministry of Health (MOH) Malaysia
Abstract	-
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	Malaysia
Region	South-East Asia
URL	-
Link on MESA Track	-
Theme(s)	Transmission Risk of infection

Identification number	XV
Title	**Experimental design of *Plasmodium knowlesi* infection in susceptible versus refractory non-human primate model hosts**
Objectives	To identify host features that confer protection against malaria disease and relate these observations to developments intended to reduce host susceptibility to *Plasmodium* infections.
Principal institution(s)	Emory University, United States
Principal Investigator (PI)	Allison Hankus
Funding source(s)	-
Abstract	Longitudinal studies with *P. Knowlesi* in *Macaca mulatta* (Rhesus monkey) and *M. fascicularis* (Kra monkey) were designed to identify features at the host-pathogen interface conferring varying degrees of host susceptibility to parasite infection. To further our understanding of pathological significance during the course of infection, necropsies were performed at various points of infection allowing deeper analysis of affected tissues and organ systems uniquely influenced between these NHP cohorts.
Funding amount ($)	-
Funding information	-
Partners on the project	-
Dates	-
Country	-
Region	-
URL	http://www.abstractsonline.com/pp8/#!/4114/presentation/4441
Link on MESA Track	-
Theme(s)	Transmission

10.4. Annex 4. Summary of ongoing research grants in *P. knowlesi*

Summary of ongoing research grants in *Plasmodium knowlesi*

Maria Tusell Rabassa

March 2017

ISGlobal Barcelona Institute for Global Health

MESA MALARIA ERADICATION SCIENTIFIC ALLIANCE

Background

We have compiled a dataset of **current funded research projects** and **grants** on *Plasmodium knowlesi*, which aims to

- ✓ **support** the work of the WHO Evidence Review Group (ERG) on *Plasmodium knowlesi*
- ✓ and **describe** the landscape of current research activities,

utilizing the MESA Track database:
www.malariaeradication.org/mesa-track

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What is MESA track?

MESA track is an **online**, **open** and **living database** which captures **research projects** and institutions' research portfolios in malaria elimination and eradication.

Project: Research or **development study** with **defined objectives** which contributes to the malaria elimination and eradication agenda.

Inclusion criteria

- **Funded** research projects
- Active in **2012** and onwards
- *P. Knowlesi* related research objectives

Exclusion criteria

- Literature reviews
- Other publications

http://www.malariaeradication.org/mesa-track/advanced-search?keywords=knowlesi

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Methodology

The projects have been collected from:

- Direct correspondence with:
  - ERG members
  - Principal investigators of the MESA track projects
  - Other collaborators
- Ministry of Health of Malaysia
- Ministry of Health of Australia
- Research grants databases:
  - NIH
  - Bill & Melinda Gates Foundation
  - Wellcome Trust
  - NHMRC Australia
  - MRC UK
  - clinicaltrials.gov
  - grantome.com
- Research institutions
  - Menzies School of Health Research
  - LSHTM
  - Harvard School of Public Health

Information collected:

- Title
- Objectives
- Principal institution(s)
- Principal investigator
- Funding source(s)
- Abstract
- Funding amount ($)
- Funding information
- Partners
- Dates
- Country

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MALARIA ERADICATION SCIENTIFIC ALLIANCE

Initially, MESA track had **6** projects on *P. knowlesi*.
After the additional searching and information collected from experts, there are **19** projects to date.

1. Inputs

2.Validation through contact with PIs.

19 projects collected thus far.

Fluent and **ongoing** process.
Researchers keep **sharing** information.

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MALARIA ERADICATION SCIENTIFIC ALLIANCE

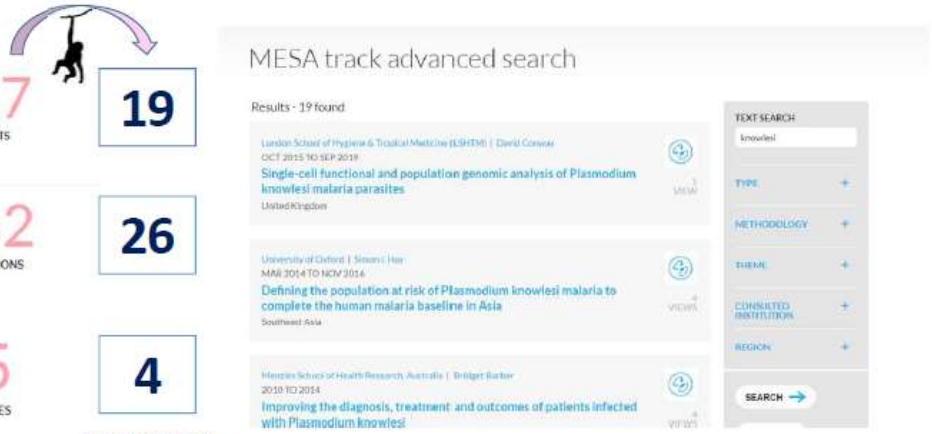
What is IN MESA track right now?

817 PROJECTS → **19**

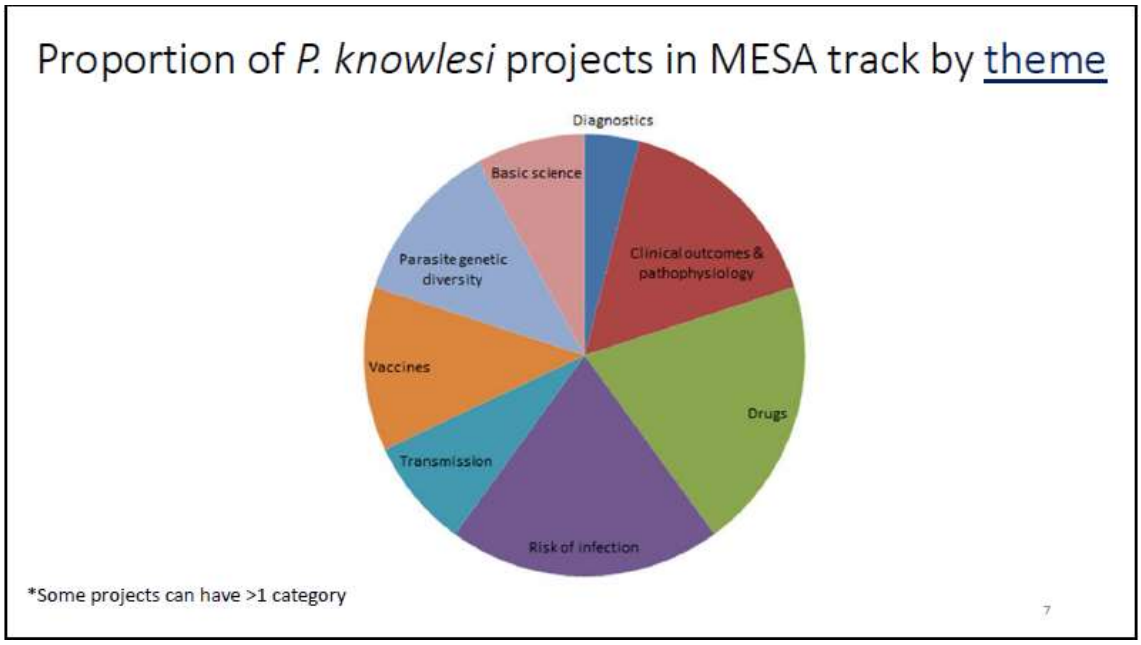
762 INSTITUTIONS → **26**

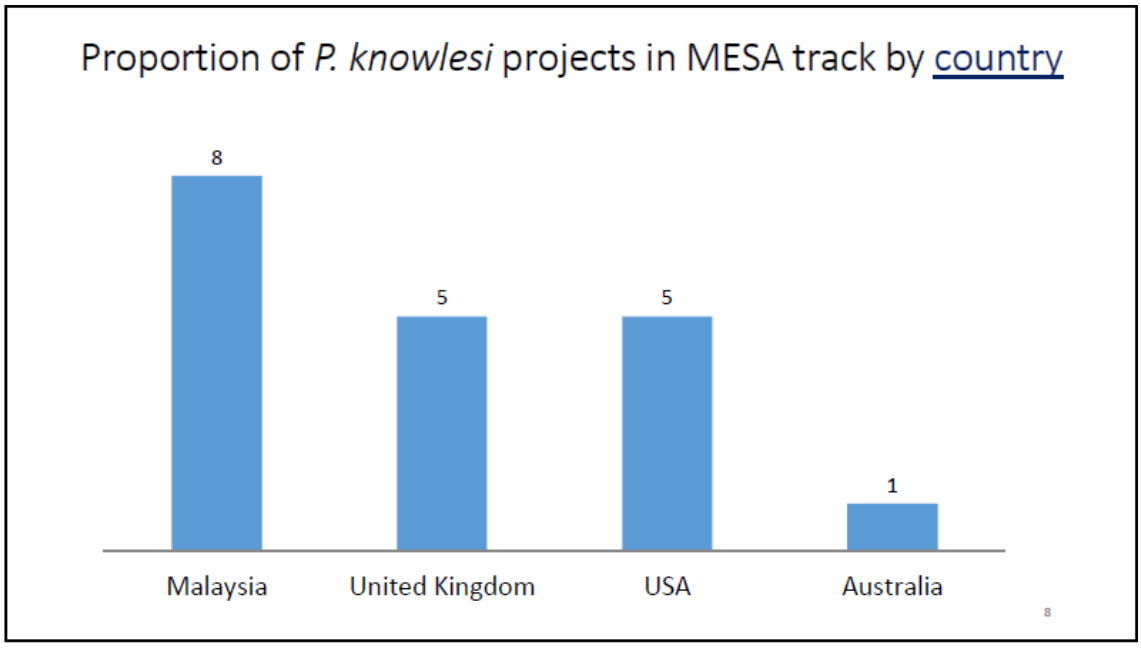
95 COUNTRIES → **4**

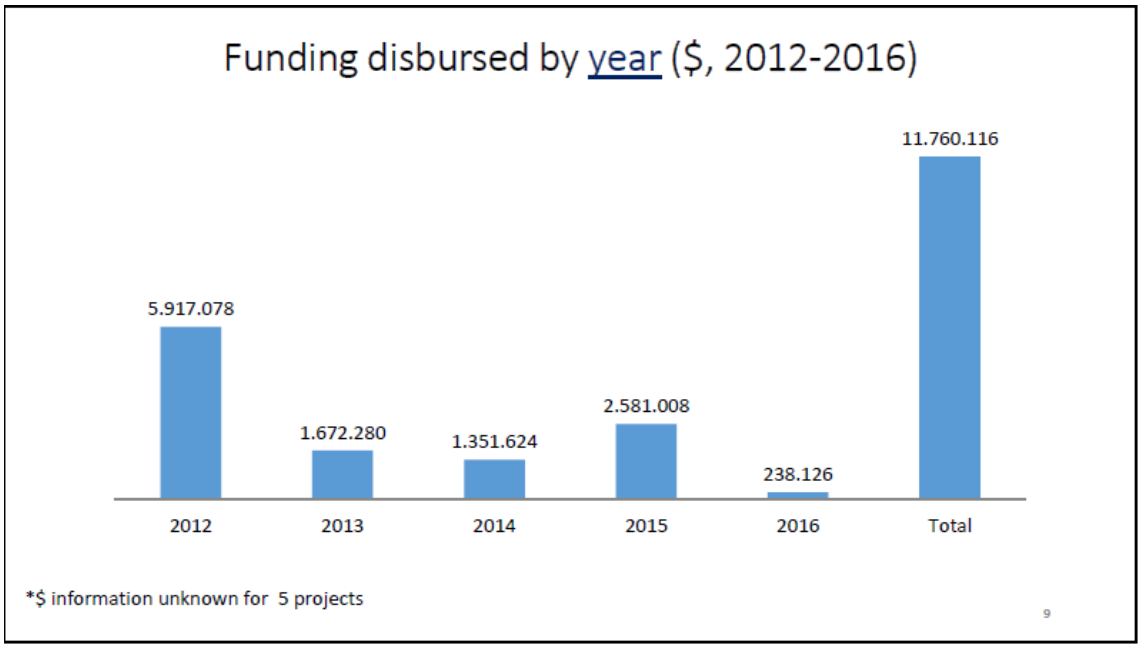
on *P. knowlesi*

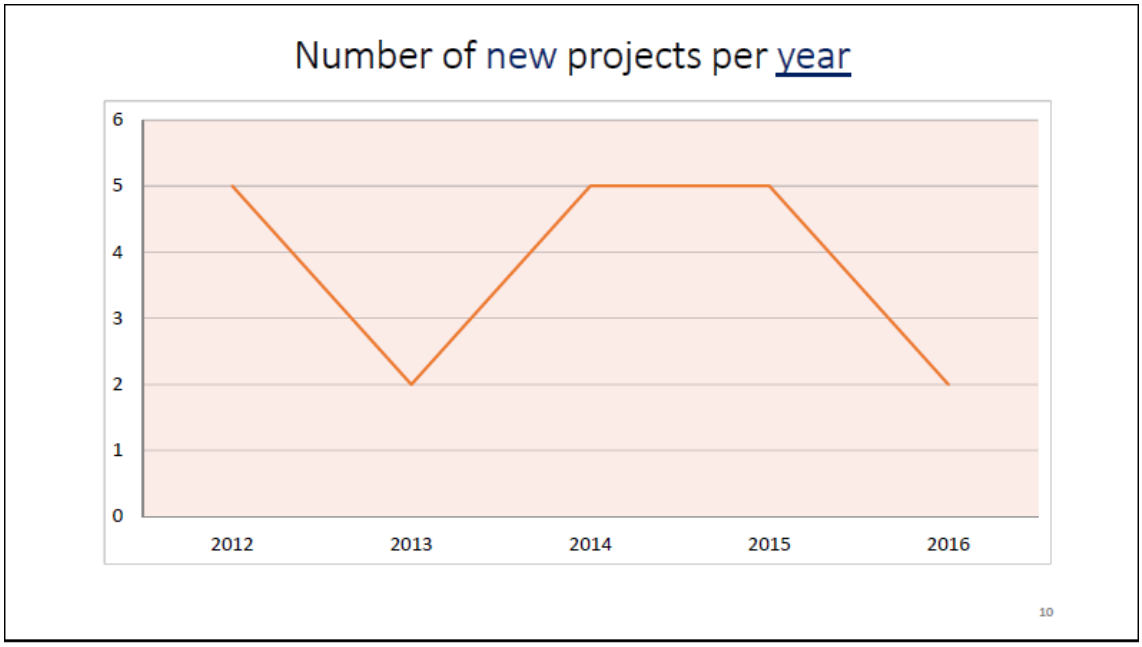


6


Proportion of P. knowlesi projects in MESA track by theme
Diagnostics
Basic science
Clinical outcomes & pathophysiology
Parasite genetic diversity
Vaccines
Drugs
Transmission
Risk of infection
*Some projects can have >1 category
7


Proportion of P. knowlesi projects in MESA track by country
8
5
5
1
Malaysia
United Kingdom
USA
Australia
8


Funding disbursed by year ($, 2012-2016)
11.760.116
5.917.078
1.672.280
1.351.624
2.581.008
238.126
2012
2013
2014
2015
2016
Total
*$ information unknown for 5 projects
9


Number of new projects per year
6
5
4
3
2
1
0
2012
2013
2014
2015
2016
10

Summary of projects published in MESA Track

Title	Objectives	Principal institution	Principal investigator	Funding source	Funding amount	Country	Partners
P. Knowlesi Trial of Artemether-lumefantrine vs Chloroquine	☑	☑	☑	☒	☒	☑	☑
P. Knowlesi Trial of Artesunate-mefloquine Versus Chloroquine	☑	☑	☑	☒	☒	☑	☑
Artemether-lumefantrine vs Chloroquine for Uncomplicated P. *Vivax* Malaria	☑	☑	☑	☒	☒	☑	☑
Effect of Paracetamol on Renal Function in *Plasmodium knowlesi* Malaria	☑	☑	☑	☒	☒	☑	☑
Functional analysis of *Plasmodium vivax* drug resistance polymorphisms	☑	☑	☑	☑	☑	☑	☒
Structure and function of eukaryotic phosphatidylserine decarboxylase	☑	☑	☑	☑	☑	☑	☒
Comparative incidence and clinical spectrum of *Plasmodium knowlesi*	☑	☑	☑	☑	☑	☑	☒
Plasmodium genus and PFV fluorescent in situ hybridization (fish) assay kit	☑	☑	☑	☑	☑	☑	☒
An effector memory T cell-inducing subunit vaccine against malaria	☑	☑	☑	☑	☑	☑	☒
RBL Binding Domain Malaria Candidate Vaccines	☑	☑	☑	☑	☑	☑	☒

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Title	Objectives	Principal institution	Principal investigator	Funding source	Funding amount	Country	Partners
Geographic genetic profiling of human *Plasmodium* malaria	☑	☑	☑	☑	☑	☑	☑
Monkeybar: Defining the biomedical, environmental and social risk factors...	☑	☑	☑	☑	☑	☑	☑
Dissecting the Red Blood Cell Invasion Pathways of the Malaria Parasite *Pk.*	☑	☑	☑	☑	☑	☑	☑
Single-cell functional and population genomic analysis of *Pk.* malaria parasites	☑	☑	☑	☑	☒	☑	☒
Comparative pathophysiology and clinical epidemiology of *knowlesi* malaria	☒	☑	☑	☑	☑	☑	☒
Disease burden, risk factors and treatment of *knowlesi* malaria	☒	☑	☑	☑	☑	☑	☑
Defining the population at risk of *Plasmodium knowlesi* malaria	☑	☑	☑	☑	☑	☑	☒
A comparative study of the pathophysiology of severe *knowlesi* and *falciparum* malaria	☒	☑	☑	☑	☑	☑	☑
Improving the diagnosis, treatment and outcomes of patients infected with *Plasmodium knowlesi*	☒	☑	☑	☑	☑	☑	☒

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Ongoing data collection

Title	Objectives	Principal institution	Principal investigator	Funding source	Funding amount	Country	Partners
Natural selection and genetic diversity of leading *Plasmodium knowlesi* vaccine antigens	☒	☑	☒	☒	☒	☒	☒
Characterization of *Pk* circumsporozoite protein and production of sporozoites for potential vaccine development	☒	☑	☒	☒	☒	☒	☒
Host-vector population genetics in *Pk* malaria from Malaysia	☒	☑	☒	☒	☒	☑	☒
Rapid PoC molecular tests for the and newly developed *anti-Plasmodium* and *anti-knowlesi* ELISAs	☒	☑	☒	☒	☒	☒	☒
The phylogeography and ecology of human and non human primate malarias and their vectors in Malaysia	☒	☑	☒	☒	☒	☑	☒
An integrated community-based approach for malaria control	☒	☑	☒	☒	☒	☒	☒
Genetic diversity and temporal variations in composition of subpopulations of *Pk.*	☒	☑	☒	☒	☒	☒	☑
Genomics and population genetics of *Plasmodium knowlesi* in Southeast Asia	☒	☑	☒	☒	☒	☑	☑

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Title	Objectives	Principal institution	Principal investigator	Funding source	Funding amount	Country	Partners
Identification of vectors of *Plasmodium knowlesi* and other malaria parasites in Sarawak	☒	☑	☒	☒	☒	☑	☑
Epidemiology of *knowlesi* and other simian malarias in humans in the Betong Division of Sarawak	☒	☑	☒	☒	☒	☑	☑
Mapping of *knowlesi* malaria vectors in Peninsular Malaysia	☑	☒	☑	☑	☒	☑	☒
Insecticide susceptibility status in *Anopheles crescens*: vector of *Plasmodium knowlesi* in peninsular Malaysia	☑	☒	☑	☑	☒	☑	☒
Bionomics of *knowlesi* malaria vectors in Sabah	☑	☒	☑	☑	☒	☑	☒
Field evaluation of outdoor residual spraying for the control of simian malaria in Sabah	☑	☒	☑	☑	☒	☑	☒
Molecular mechanisms driving the adaptation of *Plasmodium knowlesi* to humans	☒	☑	☑	☑	☒	☒	☒

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Final remarks

- To date, we have identified **19 projects** addressing ***Plasmodium knowlesi*** in the MESA Track database, with
  - ✓ **15** projects with identified **funding source**
  - ✓ **14 projects** with known **funding amount**
  - ✓ **15** with detailed **objectives**
  - ✓ **9** with known **partners** on the project
- We have identified a **further 15** projects, for which we are still collecting information (e.g. funding information & project objectives).

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Final remarks

- By theme, **risk of infection** and **drugs** account for **20%** of the projects each. **Clinical outcomes and pathophysiology** accounts for **16%** of the projects.
- **Diagnostics** only accounts for **4%** of the projects.
- Most of the work is taking place in **Malaysia**.
- In the 14 projects where we were able to collect funding amount, the **total amount disbursed** has been **$11,760,116** .

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