Outcomes from the Evidence Review Group on *Plasmodium knowlesi*

Malaria Policy Advisory Committee
Geneva, Switzerland

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Outline

• Why an ERG on *Plasmodium knowlesi*
• Members of the ERG
• WHO Consultation on *P. knowlesi* (2011)
• Brief history and current situation
• Transmission, hosts and vectors
• Diagnosis, clinical and treatment
• Human-vector-human transmission, is it taking place?
• Research priorities
Why an ERG on *Plasmodium knowlesi*?

The MPAC meeting of September 2015 recommended the constitution of an ERG to address the following knowledge gaps;

- The epidemiological distribution of *P. knowlesi* infection in humans including common clinical outcomes, the range and distribution of the primary hosts and vectors.
- The most effective methods of control and prevention including diagnostics and treatment and the potential impact on the success of malaria elimination programmes.
- The plausibility of human-vector-human transmission and potential future changes that may influence the levels of exposure to *P. knowlesi*.
- Operational research priorities to limit *P. knowlesi* transmission to humans
- Scope to be expanded to include other primate malarias
Evidence Review Group

Members

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• Dr Nicholas Mark Anstey
• Dr John Kevin Baird
• Dr Christopher Drakeley
• Dr Jenarun Bin Jelip
• Dr Yee Ling Lau
• Dr Asmad Matusop
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WHO Informal Consultation on the Public Health Importance of *P. knowlesi*

- Held in 2011 to review the *P. knowlesi* situation
- The Consultation provided 17 recommendations, many of which have contributed to our current understanding
- These included recommendations on diagnostics, determining vector and host distribution, protocols on diagnostic procedures and management among other areas
Transmission and factors for zoonotic infections

**HOST**
- Long-tailed macaque (*M. fascicularis*)
- Pig-tailed macaque (*M. nemestrina*)
- Banded leaf monkey (*P. melalophus*)

**VECTOR**
- An. leucosphyrus mosquitoes:
  - *An. latens* (Sarawak)
  - *An. balabacensis* (Sabah)
  - *An. cracens* (Peninsular Malaysia)
  - *An. dirus* (Viet Nam)

**ENVIRONMENT**
- Dense jungle and forest fringe areas

**SOCIAl**
- Employment
- Migration
- Others

**MALARIA**
- *Plasmodium knowlesi*
Natural hosts in Sarawak, Malaysian Borneo

*Macaca fascicularis*
Long-tailed macaque

*Macaca nemestrina*
Pig-tailed macaque

Natural hosts in Peninsular Malaysia and Myanmar

*Presbytis melalophus*
Banded leaf monkey
Peninsular Malaysia

*Macaca leonina*
Northern pig-tailed macaque
Myanmar

Source: koushik/naturism.co.in
Factors contributing to increase of reported *P. knowlesi* infections

- Improved diagnostic capacity
- Reduction in human malaria cases and awareness of Pk
- Loss of relative immunity due to low rates of malaria
- Change in land use patterns creating increased opportunity for spill over of infections to humans – through closer associations with natural reservoir hosts or access to infected vectors
Host-parasite interactions

• Two distinct *P. knowlesi* populations identified in human patients from Malaysia have been linked to *M. nemestrina* and *M. fascicularis*, respectively
  
  – The strain associated with *M. fascicularis* is thought to be circulating and infecting humans in areas of continental Asia, where *M. nemestrina* is absent
  
  – This *M. fascicularis*-associated strain may have a distinct relationship with environmental and socioeconomic variables compared to the mixture of parasite infections in patients from Malaysia

• The presence of Leucosphyrurus Complex vectors in Malaysia including Dirus Complex vectors in continental Asia further adds to the possibility of different relationships between disease risk and the environment in these two regions
Vectors

- *P. knowlesi* vectors are members of the An. leucosiphyrus group
  - found throughout the region
  - associated with dense jungle and forest fringe
  - rest and feed outdoors (exophagic) typically after dusk

- In Sarawak the forest breeding *An. latens* was found to be the primary vector
  - *An. latens* has been found to harbor other simian malaria parasites: *P. inui*, *P. coatneyi*, and *P. fieldi*

- *An. balabacensis* implicated as vector in Sabah and it prefers to breed in ground pools formed in fruit orchard, rubber and palm oil plantations

- *An. cracens* is considered a major knowlesi malaria vector in peninsular Malaysia

- *An. dirus* appears to be the primary vector in Viet Nam and continental Asia
Vector habitat

Slow running streams

Animal foot paths

Source: Dr. Rohani Ahmad, Institute of Medical Research (IMR), Malaysia, 2016
Vector habitat

Stagnant water

Ground pools

Sources: Dr. Rohani Ahmad, Institute of Medical Research (IMR), Malaysia, and EntoPest Unit of Sabah Health Department, Malaysia, 2016
Larval sampling

Source: EntoPest Unit of Sabah Health Department, Malaysia, 2016
In 2016:

P. knowlesi cases contributed 69% of total reported cases.

9 mixed cases (43%) were involved Pk infection
- 7 cases (Pk + Pf)
- 2 cases (Pk + Pv)
DISTRIBUTION OF HUMAN MALARIA AND P.KNOWLESI CASES BY GENDER in MALAYSIA 2014-2016

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<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>196</td>
<td>494</td>
<td>81</td>
<td>268</td>
<td>126</td>
<td>259</td>
</tr>
<tr>
<td>Men</td>
<td>1138</td>
<td>2090</td>
<td>589</td>
<td>1372</td>
<td>557</td>
<td>1264</td>
</tr>
</tbody>
</table>
DISTRIBUTION OF HUMAN MALARIA AND P. KNOWLESI CASES BY AGE GROUP IN MALAYSIA (2014-2016)

Source: Vector Borne Disease Sector, Disease Control Division, MOH
DISTRIBUTION OF HUMAN MALARIA CASES BY INFECTION STATUS (SPORADIC/CLUSTER) IN MALAYSIA 2016

DISTRIBUTION OF ZOONOTIC MALARIA CASES BY INFECTION STATUS
SPATIAL DISTRIBUTION OF HUMAN MALARIA CASES IN MALAYSIA (2016)
SPATIAL DISTRIBUTION OF ZOONOTIC MALARIA CASES IN MALAYSIA (2016)
SPATIAL DISTRIBUTION OF HUMAN MALARIA & ZOONOTIC MALARIA CASES IN MALAYSIA (2016)
SPATIAL DISTRIBUTION OF VECTOR SPECIES FOR ZOONOTIC MALARIA IN MALAYSIA (2016)
DISTRIBUTION OF HUMAN MALARIA CASES BY OCCUPATION IN MALAYSIA, 2014-2016
DISTRIBUTION OF ZOONOTIC MALARIA CASES BY OCCUPATION IN MALAYSIA, 2014-2016

Source: Vector Borne Disease Sector, Disease Control Division, MOH
DISTRIBUTION OF ZOONOTIC MALARIA CASES BY LOCALITY STATUS IN 2016

- Traditional Village: 60%
- Estate / Farm: 20%
- Logging Site: 13%
- Construction Site (Jungle): 3%
- Jungle: 3%
- OA Village: 1%

Source: Vector Borne Disease Sector, Disease Control Division, MOH
First successful survey in Indonesia

Contribution of *Plasmodium knowlesi* to multi-species human malaria infections in North Sumatera, Indonesia

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**Sumatra results**

<table>
<thead>
<tr>
<th></th>
<th>18 ssu rRNA assay</th>
<th>SICAvar assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total P. knowlesi cases</strong></td>
<td>76</td>
<td>377</td>
</tr>
<tr>
<td><strong>P. knowlesi mono infection</strong></td>
<td>42 (55.3%)</td>
<td>215 (57.0%)</td>
</tr>
<tr>
<td><strong>P. knowlesi + P. vivax</strong></td>
<td>16 (21.1%)</td>
<td>65 (17.2%)</td>
</tr>
<tr>
<td><strong>P. knowlesi + other Plasmodium spp. infections</strong></td>
<td>18 (23.7%)</td>
<td>97 (25.7%)</td>
</tr>
<tr>
<td>Cases positive by both assays</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>Total P. knowlesi cases detected with any assay</strong></td>
<td>443</td>
<td></td>
</tr>
<tr>
<td><strong>P. knowlesi mono infection</strong></td>
<td>254/443 (57.34%)</td>
<td></td>
</tr>
</tbody>
</table>

Relative frequencies (percentages) read vertically.
Evidence from Aceh, Indonesia

**Fig. 2** Study recruitment and laboratory testing results 38 index cases were enrolled through passive surveillance and triggered 36 RACD events. One RACD event covered three contemporaneous indexes cases from the same household. In passive surveillance, 37 cases were confirmed by PCR and by RACD, there were six PCR-confirmed cases, resulting in a total of 43 cases. Pan-LAMP Pan-loop-mediated isothermal amplification
Plasmodium knowlesi infected about 25% of confirmed malaria cases, and nearly half of those were gametocytemic.

Data from Vietnam

Plasmodium knowlesi occurred in 33 of 70 An. dirus carrying malaria.

Table 2. Number of salivary glands of Anopheles dirus mosquitoes infected with parasites collected in different sites in the forest near Khanh Phu, Vietnam

<table>
<thead>
<tr>
<th></th>
<th>No. of PCR positive salivary glands</th>
<th>No. of examined for gametocyte DNA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Forest fringe</td>
<td>In the forest</td>
</tr>
<tr>
<td>Pf</td>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>Pv</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Pm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pk</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Pf + Pv + Pk</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Pf + Pk</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pv + Pk</td>
<td>1</td>
<td>14</td>
</tr>
<tr>
<td>Pf + Pv</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Pf + Pm</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>59</td>
</tr>
</tbody>
</table>
Diagnosis

- *P. malariae* and *P. knowlesi* may not be reliably distinguished by microscopy
  - PCR is the definitive diagnostic method
- pan-Plasmodium RDTs can be used for screening but not confirmation of *P. knowlesi*
- *P. knowlesi*-specific RDTs have demonstrated low sensitivity
  - Products are in the pipeline but performance to date is not yet optimal
RDTs for detection of knowlesi malaria

<table>
<thead>
<tr>
<th>RDT</th>
<th>Sensitivity</th>
<th>Sensitivity (&lt;1,000 p/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OptiMal-IT</td>
<td>72%</td>
<td>45%</td>
</tr>
<tr>
<td></td>
<td>32%</td>
<td>-</td>
</tr>
<tr>
<td>FirstResponse</td>
<td>74%</td>
<td>25%</td>
</tr>
<tr>
<td>CareStart</td>
<td>42%</td>
<td>-</td>
</tr>
<tr>
<td>Paramax-3</td>
<td>35%</td>
<td>-</td>
</tr>
<tr>
<td>Binax NOW</td>
<td>26%</td>
<td>0%</td>
</tr>
<tr>
<td>ParaHIT</td>
<td>23%</td>
<td>-</td>
</tr>
</tbody>
</table>

Clinical symptoms and parasitemia

• Most human *P. knowlesi* cases are chronic and symptomatic but some can be severe leading to death
  – Clinical studies in Sarawak, Malaysian Borneo, indicated > 10% of patients with *P. knowlesi* malaria developed severe disease as classified by the WHO with approximately 1% CFR

• *P. knowlesi* has the shortest asexual replication cycle of all Plasmodium species leading to rapidly increased parasitemia levels
  – Relatively high parasitemia (lower than for falciparum) is associated with severe *P. knowlesi* malaria
  – Patients having parasitemia >15,000 parasites/ul should be treated urgently and closely monitored until parasitemia is controlled, especially if > 45 years.
Age distribution and gender: Sabah MOH *P. knowlesi* notification data 2007 - 2014

Source: Menzies School of Health Research

- Patients with Pk older than those with Pf and Pv (median 32, 23, 24 yrs)
- 80% male
  (in children, 64% male)
- Females with Pk older than males: 38 vs. 31 yrs (45 yrs vs. 34 yrs in adults)
- Bimodal age distribution among females with peaks at 12 and 52 years
Who is at risk of severe disease and death from *P. knowlesi*?

*Source: Menzies School of Health Research*

- Parasitaemia and disease severity increase with age (Daneshvar *CID*, 2009, Barber, *CID*, 2013).
- Severe Pk not yet reported in children <12 yrs.
- Sabah MOH notifications 2010-2014: 0% mortality in 373 children (Rajahram, *EID*, 2016)
- Youngest reported death 31 years (range 31-84)
- Gender: females 19% of 4217 Sabah *P. knowlesi* notifications, but 46% of fatal knowlesi cases. Not significant after controlling for age. (Rajahram *et al*, *EID*, 2016)
- Larger cohorts: **Age** and **parasitaemia** are independently associated with severe disease (Sabah District data (Grigg *et al*, n=481; under review) and Sabah Tertiary data (Barber *et al*, n=146; under review)
Parasites outside Malaysia benign?

• “What we see in Vietnam, at least the work I’ve been involved in, seems very different to the picture in Malaysia - all infections are pretty much asymptomatic, sub-microscopic a lot of the time, and very often in mixed species infections... I think other studies from Mekong have showed the same thing now too. I wonder if the Pk strains in this region my not be particularly well adapted to humans for some reason, and only cause very low level, and transient infection.”

- Richard Culleton, 24 Feb 2017

It may be that *P. knowlesi* outside of Malaysian Borneo is different, more often causing low-grade asymptomatic carriage rather than aggressive and symptomatic infections.
Knowlesi malaria – is human-vector-human transmission happening?

**Key Axiom:** *Absence of evidence is not evidence of absence*

- No outbreak of *P. knowlesi* was reported in areas without presence of macaques.
- Genetic analysis of strains in macaques and human infections of *P. knowlesi* showed
  - same lineage – but it would take a very long time – several decades to centuries for a change to occur
  - absence of dhfr mutations in spite high SP pressure in humans
- Two largescale case control studies in Malaysia failed to show the presence of submicroscopic infections of *P. knowlesi* in humans. The indonesian study did detect asymptomatic infections
- One published study from Vietnam reported the presence of sporozoites of both *P. vivax* and *P. knowlesi* in the same mosquitoes. One conducted in Malaysia too, but results of this were not presented, nor details on the PCR methodology used.
Conclusion - is human-vector-human transmission happening

• Human *P. knowlesi* is still largely a zoonosis

• But all indications are that human to human transmission can take place, and probably is taking place in some situations, although not very efficiently yet. But this could change with time and with parasite adaptation.
Treatment

• *P. knowlesi* is highly sensitive to artemisinins; and variably and moderately sensitive to chloroquine and mefloquine

• ACT KNOW open-label, random controlled trial (2016) compared artemesunate-mefloquine (A-M) and chloroquine (CQ) for the treatment of uncomplicated *P. knowlesi* malaria
  – A-M treated patients showed improved outcomes, demonstrating:
    • faster parasite clearance than CQ treated patients
    • lower risk of anaemia within 28 days
    • faster fever clearance
    • shorter duration of hospital bed occupancy
Treatment of uncomplicated knowlesi malaria

Artemether-lumefantrine vs chloroquine RCT (CAN KNOW study) (Grigg et al. unpublished)

Rationale: no efficacy data for A-L despite being used in Malaysia, likely better safety profile compared to ASMQ

- A-L (n=58); CQ (n=65)
- No treatment failure in either arm by day 42
- Better early therapeutic response with A-L
  - PCT median 18 vs. 24 h; p=0.021
  - PCT$_{50}$ 7.2 h vs. 8.2 h
  - PCT$_{90}$ 13.7 h vs. 15.6 h
  - Microscopy negative at 24 h: 76% vs. 60%
  - Microscopy negative at 48 h: both 100%
- ↓ risk of anaemia at day 28 with A-L: 66% vs. 81%
- No difference in adverse events or SAEs between groups

**Recommendation:** ACT is preferred over CQ for treatment of uncomplicated *P. knowlesi* (irrespective of the presence of chloroquine-susceptible *P. vivax* in co-endemic areas)
Research Priorities

- **Evidence for human-to-human transmission**
  - Presence of mixed infections of *P. knowlesi* with human malaria species (*P. falciparum, P. vivax, P. malariae*) in the mosquito vectors
  - Vector host preferences and feeding habits – High human blood index in human *P. knowlesi* vectors
  - Laboratory studies and parasite genomics

- **Laboratory diagnosis**
  - Development of new rapid diagnostic tests for *P. knowlesi*
  - Development of high throughput tests (LAMP) for *P. knowlesi*
  - Selection of serological markers to assess human *P. knowlesi* transmission intensities
  - Development of a quantitative PCR (e.g., to determine what proportion of the population is infected with *P. knowlesi*).

- **Entomology**
  - Mapping vectors of *P. knowlesi* and overlay on human *P. knowlesi* incidence/prevalence maps, and those of environmental risk factors

- **Clinical management**
Thank you.

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Dr Andrea Bosman
Dr Kamini Mendis
Ms Glenda Gonzales
References


References


Dr.Rohani Ahmad, Institute of Medical Research (IMR), Malaysia
EntoPest Unit of Sabah Health Department, Malaysia, 2016
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