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Case Series of Three Malaria Patients From Thailand Infected With the Simian Parasite, *Plasmodium Cynomolgi*

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Abstract

Background

While human cases of simian malaria, *Plasmodium knowlesi*, are now regularly recognized in Southeast Asia, infections with other species such as *P. cynomolgi* are still rare. There has been a handful of clinical cases described, all from Malaysia, and retrospective studies of archived blood samples in Thailand and Cambodia have discovered the presence *P. cynomolgi* in isolates using polymerase chain reaction (PCR) assays.

Case presentation

In Thailand, an ongoing malaria surveillance study enrolled two patients from Yala Province diagnosed with *P. vivax* by blood smear, but who were subsequently found to be negative by PCR. Expanded PCR testing of these isolates detected mono-infection with *P. cynomolgi*, the first time this has been reported in Thailand. Upon re-testing of 60 isolates collected from Yala, one other case was identified, a co-infection of *P. cynomolgi* and *P. vivax*. The clinical course for all three was relatively mild, with symptoms commonly seen in malaria: fever, chills and headaches. All infections were cured with a course of chloroquine and primaquine.

Conclusion

In malaria-endemic areas with macaque populations, cases of simian malaria in humans are being reported at an increasing rate, although still comprise a very small percentage of total cases. *P. cynomolgi* and *P. vivax* are challenging to distinguish by blood smear; therefore, PCR can be employed when infections are suspected or as part of systematic malaria surveillance. As Thai MoPH policy schedules regular follow-up visits after each malaria infection, identifying those with *P. cynomolgi* will allow for monitoring of treatment efficacy, although at this time *P. cynomolgi* appears to have an uncomplicated clinical course and good response to commonly used antimalarials.

Background

The first naturally-acquired human infection of the simian malaria parasite, *Plasmodium cynomolgi*, was reported from Malaysia in 2014 [1]. Cases have continued to be reported from Malaysia [2–5, 7] and retrospectively detected in isolates from Cambodia and Thailand [6, 8]. An ongoing malaria surveillance study of Thailand has enrolled malaria patients to monitor infections in border provinces and determine resistance patterns. In 2021, three *P. cynomolgi* infections were identified in study participants from Yala Province, of which two were mono-infections.

Malaria Case Presentations

The malaria surveillance study, ongoing since March 2019 in Yala, Sisaket, Ubon Ratchathani, and Ratchaburi Provinces in Thailand, looks to consent and enroll individuals diagnosed with malaria, defined as evidence of any species of parasites in the blood using malaria rapid diagnostic test (RDT) and or microscopy. First, a single venous blood sample is drawn, with a complete blood count (CBC), glucose 6-phosphate dehydrogenase (G6PD) CareStart™ RDT (Access Bio, Inc., USA) and fluorescent spot testing (R&D Diagnostics Ltd., Greece) performed by local Ministry of Public Health (MoPH) or Royal Thai Army (RTA) staff. The remaining blood sample shipped to US Armed Forces Research Institute of Medical Sciences (AFRIMS) in Bangkok, Thailand for additional testing: repeat blood smears, speciation verification using polymerase chain reaction (PCR), quantitative G6PD testing (Pointe Scientific, USA), PCR for molecular markers of resistance and submicroscopic gametocytemia as well as *ex-vivo* and *in-vitro* drug susceptibility assays. At the time of writing, 149 malaria patients have been enrolled: 128 *P. vivax* cases, 14 *P. falciparum* and four *P. knowlesi* cases. A short description of three *P. cynomolgi* cases and the locations within Thailand (Figure 1) follows.

Case A

A 53-year-old woman presented at a malaria clinic in Ban Nang Sata District, Yala Province, in March, 2021 with 38°C fever, headache, and chills for five days. The hematological assessment showed white blood count (WBC) at 4,200/mm^3, hemoglobin at 10.9 g/dL, and platelets at 191,000/mm^3. Further history revealed she worked a rubber plantation and that her husband had recently been diagnosed and treated for *P. vivax* infection.

Case B

A 55-year-old woman was part of a malaria active case detection investigation by malaria clinic staff from Ka Bang District, Yala Province, in February, 2021. The patient reported a history of headache and fever for eight days, although on the day of examination, the subject's tympanic temperature was 37°C. Laboratory examination revealed WBC at 4,800/mm^3, hemoglobin at 11.7 g/dL, and platelet count at 330,000/mm^3. The patient reported to be living and working on a rubber plantation.

Case C

In June 2021, a 25-year-old male on active duty in the Royal Thai Army presented at a malaria clinic in Yala District, Yala Province, with a complaint of five days of fever and nighttime chills. His temperature was 37.8°C. Hematology findings showed slight thrombocytopenia at 123,000/mm^3, WBC at 6,900/mm^3, and hemoglobin at 12.5 g/dL. The patient stated he had been stationed in Yala District for at least 20 months, going out on daily patrols and sleeping overnight in the forest. He reported using mosquito repellent and mosquito coils for personal protection.

Using microscopy, all three subjects were diagnosed with *P. vivax*; all presented with uncomplicated illness, had normal G6PD activity and reported no prior history of malaria. Each patient was treated by local health care staff with chloroquine and a two-week radical cure course of primaquine following Thai

national treatment guidelines. All were found to be clinically well within five days of initiating the antimalarials, with no recurrences at subsequent follow-up visits required by the Thai MoPH scheduled at 14-, 28-, 60- and 90-days post-treatment.

Laboratory Investigations

Blood smears were prepared and read by two World Health Organization (WHO)-certified microscopists at the AFRIMS labs in Bangkok, Thailand. In brief, thick and thin smears were prepared on the same glass slide and air-dried and fixed in methanol, stained for 45 minutes (min) in 3% diluted Giemsa stain, and examined at an oil immersion magnification of ×100. Parasite counting was done per 500 white blood cells (WBC) in thick films, and percent parasitemia was calculated based on the actual WBC count. Parasites resembling *P. vivax* were detected, with densities of 25, 10, and 2,718 parasites/μL blood for Case A, B, and C, respectively. Only Case C had gametocytemia at four gametocytes per 200 WBCs. Malaria parasite morphologies (Figure 2) showed growing trophozoite stages with amoeboid-shaped cytoplasm and prominent Schüffner's stippling. Yellowish-brown pigments dispersed within the cytoplasm were also found in some infected cells. Trophozoites with either single, double, or triple chromatin dots were detected in the blood films. No ring forms were detected in any slide. Only Case C had rare parasites visible on thin film, although erythrocytes were not clearly enlarged or distorted. This study conducts 5-species (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*) real-time PCR testing at AFRIMS on all malaria isolates received. Briefly, genomic DNA is extracted from ethylenediaminetetraacetic acid (EDTA) whole blood using EZ1 DNA blood kit with automated EZ1 Advanced XL purification system (QIAGEN, Valencia, CA, USA) and confirmed for *Plasmodium* speciation by multiplex-real time PCR, using species-specific primers and probes [9, 10]. Two of the study patients (A and B) were found to be negative by multiplex real-time PCR, with *P. vivax* reported for Case C.

Since asexual parasites had been observed on blood smear for Cases A and B, speciation singleplex real-time PCR testing for *P. cynomolgi* was then performed on these isolates as well as all other samples collected from Yala (n=60). Primers and probes specific to small subunit rRNA, sexual (sporozoite) stage (Genbank accession number L08242.1) were selected, with sequences as follows: Forward: 5'-ATTGCGGTCGCAAATAATGAAG-3', Reverse: 5'-GGTATGATAAGCCAGGGAAGTG-3'and Probe: 5'-FAM-TACTCGCTCCTTCTGTTCCCTGGA-BHQ1'). The reaction was carried out in a 25 μl reaction using Rotor-Gene Multiplex PCR kit (QIAGEN, Hilden, Germany) with cycling conditions consisting of an initial activation step at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 15 seconds and annealing /extension at 60°C for 15 seconds. Blood from a macaque infected with *P. cynomolgi* was used as a positive control. Mono-infection with *P. cynomolgi* was confirmed by PCR in Cases A and B, with Case C having co-infection with *P. vivax*. All other Yala samples were negative for *P. cynomolgi*.

Discussion

P. cynomolgi, a malaria species with Southeast Asian macaques as a natural host, can infect humans through the bites of *Anopheles spp.* mosquitoes [1–3, 11]. This report describes three individuals enrolled

in a malaria surveillance study in Thailand who were found to have *P. cynomolgi* infection, although after an initial microscopic diagnosis of and treatment for *P. vivax*. Diagnosis by blood smear requires qualified, experienced microscopists, to distinguish the two species, which can be especially difficult at very low parasitemias, and since *P. cynomolgi* cannot be identified by malaria RDTs, diagnostic testing by PCR is usually required. The only other publication on *P. cynomolgi* prevalence in Thailand conducted PCR assays on 1,152 archived samples from malaria patients in Tak, Ubon Ratchathani, Chanthaburi, Yala, and Narathiwat Provinces during the period of 2007 to 2017 [8]. There were nine *P. cynomolgi* infections detected, all co-infections: *P. cynomolgi* with *P. vivax* (n=7), with *P. falciparum* (n=1), or with both *P. vivax* and *P. knowlesi* (n=1). Cases were distributed across various years, diagnosed between April and December (rainy season is May-October), and found in all provinces, although Yala had five of the nine cases (55%). In these *P. cynomolgi* clinical cases from 2021, two of the three were mono-infections, which is the first time this has been reported in Thailand. There is one case report of *P. cynomolgi* mono-infection from a European tourist traveling through Thailand (Surat Thani Province) and Malaysia [3]. However, the origin of infection could not be confirmed.

With an initial microscopic diagnosis of *P. vivax*, the patients were not questioned for a history of contact with macaques. At the follow-up visits by the Yala study team, Case A and B did report the presence of macaques near their homes. In Thailand, the main hosts of *P. cynomolgi*, *P. knowlesi*, *P. inui*, and *P. coatneyi* are *Macaca fasicularis* and *M. nemestrina*, with recent reports in stump-tailed macaques, *M. arctoides* [12]. Co-infections of simian malaria are not uncommon in macaques, with the presence of two or three species simultaneously detected in 18–40% of monkeys [12, 13], which may explain why some human studies report co-infections more than mono-infections [2, 5]. *P. cynomolgi* was first reported as a mono-infection in a Malaysian woman in 2014 [1], and up to now, cases have been shown to exist in both peninsular Malaysia and Borneo Malaysia, the latter where *P. knowlesi*, another simian malaria is endemic [5, 7]. There have been six other studies reporting the prevalence of *P. cynomolgi* in humans in Southeast Asia, shown in Table 1.

Table 1
Summary of literature on *P. cynomolgi* cases in Southeast Asia

Location	Sample set	N	Diagnosis by PCR
Borneo Malaysia (Sarawak)[2]	Malaria patients	332	All mixed infections: *P. cynomolgi* & *P. knowlesi* (n=5)
Borneo Malaysia (Sabah)[4]	Survey for asymptomatic, low-density malaria cases	876	*P. cynomolgi* (n=2)
Borneo Malaysia (Kapit)[5]	Malaria patients	1,047	All mixed infections: *P. cynomolgi* & *P. knowlesi* (n=6)
Peninsular Malaysia[7]	Survey of communities living at forest fringe	645	*P. cynomolgi* (n=9)
Cambodia (Pailin/Battambang)[6]	Survey of asymptomatic submicroscopic malaria cases	1,361	*P. cynomolgi* (n=11) Mixed infection of *P. cynomolgi* & *P. vivax* (n=2)

Legend

Data on prevalence of *P. cynomolgi* taken from references 2, 4-7 is summarized. Columns from left to right: Location names country and province/state of the study, samples set describes from which population blood samples were collected, and N is number of samples tested by PCR. Diagnosis by PCR presents number of isolates found to have *P. cynomolgi* mono-infections or *P. cynomolgi* mixed infections.

To date, most of the publications reporting on human *P. cynomolgi* infections are retrospective testing of blood samples. In the two clinical case reports of mono-infection, and past experimental infections in humans [1, 2, 11], undifferentiated flu-like symptoms have been present, with symptoms occurring at very low parasitemias and not progressing in severity. In humans, the antimalarial treatment required for *P. cynomolgi* is not well studied, but macaques in *P. cynomolgi* drug and vaccine studies respond well to chloroquine and primaquine, the regimen for *P. vivax* in Thailand [14]. All the patients from Yala recovered rapidly, and there were no recurrences over three months of active follow-up. The low prevalence of simian malarias infecting humans means the parasites are not under frequent antimalarial drug selection pressure and should remain susceptible to treatment [6]. In Imwong et al., two Cambodian individuals were found to have *P. cynomolgi* again three months after the initial diagnosis, but it was not possible to conclude whether it was a relapse, new infection, or persistent blood-stage infection.

The Thai P. *cynomolgi* survey study by Putaporntip et al. demonstrated that *P. cynomolgi* has been infecting humans in Thailand for the last 15 years and is likely underdiagnosed. However among the published studies reviewed here, the prevalence of *P. cynomolgi* has been less than 1.5% in samples tested [8]. In Thailand, the first clinical case *P. knowlesi* was reported in 2004, and by 2017, cases began to be regularly reported by the Thailand MoPH, peaking at 53 cases at the time if this writing (2021) [15,

16]. It is not yet understood if the increases in human simian malaria infections are due to better detection methods, the result of human encroachment into macaque habitats, or both. The three Yala patients were diagnosed separately in time and space, although Yala province borders with Perak and Kelantan States in Malaysia where *P. cynomolgi* has been documented [7, Figure 1]. Whole-genome sequencing of the isolates is planned, which will allow lineage comparisons among these three cases as well as with data available from cases in the neighboring Malaysian states [7]. To mitigate the potential spread of *P. cynomolgi* and *P. knowlesi* and remain on track for malaria elimination, increased vigilance will be required for any signs of increased transmission in Yala and other areas in Thailand where exposure to macaques is possible.

Conclusions

This cases series is the second time human *P. cynomolgi* infections have been documented in Thailand and the first report of mono-infections, along with a description of the clinical course of each. *P. cynomolgi* is quite challenging to distinguish from *P. vivax* microscopically, and while this may lead to underdiagnosis, the disease course is usually mild and should be adequately and rapidly treated using antimalarial regimens for *P. vivax*. Molecular characterization is the most accurate way to detect these rare infections, but the capabilities may not reach the areas which need it most. Going forward, for all samples collected during this malaria surveillance study, primers for *P. cynomolgi* will be included for 6-species real time PCR verification. Although the diagnoses may not be available before treatment is administered, the results will allow for a more accurate estimation of infection prevalence in Thailand and evaluation of treatment efficacy during the 90-day Thai MoPH follow-up period.

Abbreviations

AFRIMS: Armed Forces Research Institute of Medical Sciences

DNA: deoxyribonucleic acid

EDTA: ethylenediaminetetraacetic acid

G6PD: Glucose 6-phosphate dehydrogenase

MoPH: Ministry of Public Health

PCR: polymerase chain reaction

rRNA: ribosomal ribonucleic acid

RTA: Royal Thai Army

WBC: white blood cell

WHO: World Health Organization

Declarations

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Disclaimer

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense. The investigators have adhered to the policies for protection of human subjects as prescribed in AR 70–25.

Ethics approval and consent to participate

Ethical approval for the conduct of this malaria study was obtained from the Walter Reed Army Institute of Research Institutional Review Board (WRAIR IRB) #00000794 in Silver Spring, Maryland, US on 12 September 2018 and Institute for Development of Human Research Protection (IHRP) IRB #00006539 in Bangkok, Thailand on 22 October 2018. Signed informed consent was obtained from all individuals prior to participation.

Consent for publication

Not applicable

Competing Interests

The authors declare that they have no competing interests.

Availability of data and materials

The majority of the data generated is presented in this article, but requests may be made to the corresponding author. Permission from Thai MoPH and Royal Thai Army will also be required.

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Contributions

Study concept, design and support: MS, JSG, MW, NW, BV, SD, WK, PL, BV, ST, AS Study execution and collection of samples/data: PS, KP, KP, MS, WK, PL, SS, PS, CK, SS, CC, MA, PB, CM, CT, ST Performed assays and interpreted data: PS, KP, KP, WK, PL, SS, PS, SS, CC, MA, PB, CM, CT, ST Drafting of the manuscript: PS, KP, KP, MS, PL, WK, CC, MW. All authors read and approved the final manuscript.

References

1. Ta TH, Hisam S, Lanza M, Jiram AI, Ismail N, Rubio JM. First case of a naturally acquired human infection with *Plasmodium cynomolgi*. Malar J. 2014;13:68.
2. Singh B, Kadir KA, Hu TH, Raja TN, Mohamad DS, Lin LW, Hii KC. Naturally acquired human infections with the simian malaria parasite, *Plasmodium cynomolgi*, in Sarawak, Malaysian Borneo. Internat J Infect Dis. 2018;73Suppl:68.
3. Hartmeyer G, Stensvold CR, Fabricius T, Marmolin E, Høgh S, Nielsen H, Kemp M, Vestergaard L. *Plasmodium cynomolgi* as Cause of Malaria in Tourist to Southeast Asia, 2018. Emerg Infect Dis. 2019;25:1936-1939.
4. Grignard L, Shah S, Chua TH, William T, Drakeley CJ, Fornace KM. Natural Human Infections With *Plasmodium cynomolgi* and Other Malaria Species in an Elimination Setting in Sabah, Malaysia. J Infect Dis. 2019;220:1946-1949.
5. Raja TN, Hu TH, Kadir KA, Mohamad DSA, Rosli N, Wong LL, et al. Naturally Acquired Human *Plasmodium cynomolgi* and *P. knowlesi* Infections, Malaysian Borneo. Emerg Infect Dis. 2020; 26:1801-1809.
6. Imwong M, Madmanee W, Suwannasin K, Kunasol C, Peto TJ, Tripura R, et al. Asymptomatic Natural Human Infections With the Simian Malaria Parasites *Plasmodium cynomolgi* and *Plasmodium knowlesi*. J Infect Dis. 2019;219:695-702.
7. Yap NJ, Hossain H, Nada-Raja T, Ngui R, Muslim A, Hoh BP, et al. Natural Human Infections with Plasmodium cynomolgi, P. inui, and 4 other Simian Malaria Parasites, Malaysia. Emerg Infect Dis. 2021;27:2187-2191.
8. Putaporntip C, Kuamsab N, Pattanawong U, Yanmanee S, Seethamchai S, Jongwutiwes S. *Plasmodium cynomolgi* Co-infections among Symptomatic Malaria Patients, Thailand. Emerg Infect Dis. 2021;27:590-593.
9. Kamau E, Alemayehu S, Feghali KC, Saunders D, Ockenhouse CF. Multiplex qPCR for detection and absolute quantification of malaria. PLoS One. 2013;8:e71539.

10. Reller ME, Chen WH, Dalton J, Lichay MA, Dumler JS. Multiplex 5' nuclease quantitative real-time PCR for clinical diagnosis of malaria and species-level identification and epidemiologic evaluation of malaria-causing parasites, including *Plasmodium knowlesi*. J Clin Microbiol. 2013;51:2931-2938.
11. Coatney GR, Elder HA, Contacos PG, Getz ME, Greenland R, Rossan RN, Schmidt LH. Transmission of the M strain of *Plasmodium cynomolgi* to man. Am J Trop Med Hyg. 1961;10:673-678.
12. Fungfuang W, Udom C, Tongthainan D, Abdul Kadir K, Singh B. Malaria parasites in macaques in Thailand: stump-tailed macaques (*Macaca arctoides*) are new natural hosts for *Plasmodium knowlesi*, *Plasmoidum inui*, *Plasmodium coatneyi* and *Plasmodium fieldi*. Mal J. 2020;19:350.
13. Zhang X, Kadir KA, Quintanilla-Zariñan LF, Villano J, Houghton P, Du H, et al. Distribution and prevalence of malaria parasites among long-tailed macaques (*Macaca fascicularis*) in regional populations across Southeast Asia. Malar J. 2016;15:450.
14. Dow GS, Gettayacamin M, Hansukjariya P, Imerbsin R, Komcharoen S, Sattabongkot J, et al. Radical curative efficacy of tafenoquine combination regimens in *Plasmodium cynomolgi*-infected Rhesus monkeys (*Macaca mulatta*). Malar J. 2011;10: 1-10.
15. Ngernna S, Rachaphaew N, Thammapalo S, Prikchoo P, Kaewnah O, Manopwisedjaroen K, et al. Case report: Case series of human *Plasmodium knowlesi* infection on the Southern Border of Thailand. Am J Trop Med Hyg. 2019;101:1397–401.
16. Thailand Malaria Elimination Program, Ministry of Public Health, Thailand. http://malaria.ddc/ma.moph.go.th/malariar10/index_newversion.php. Accessed 17 December 2021.

Figures

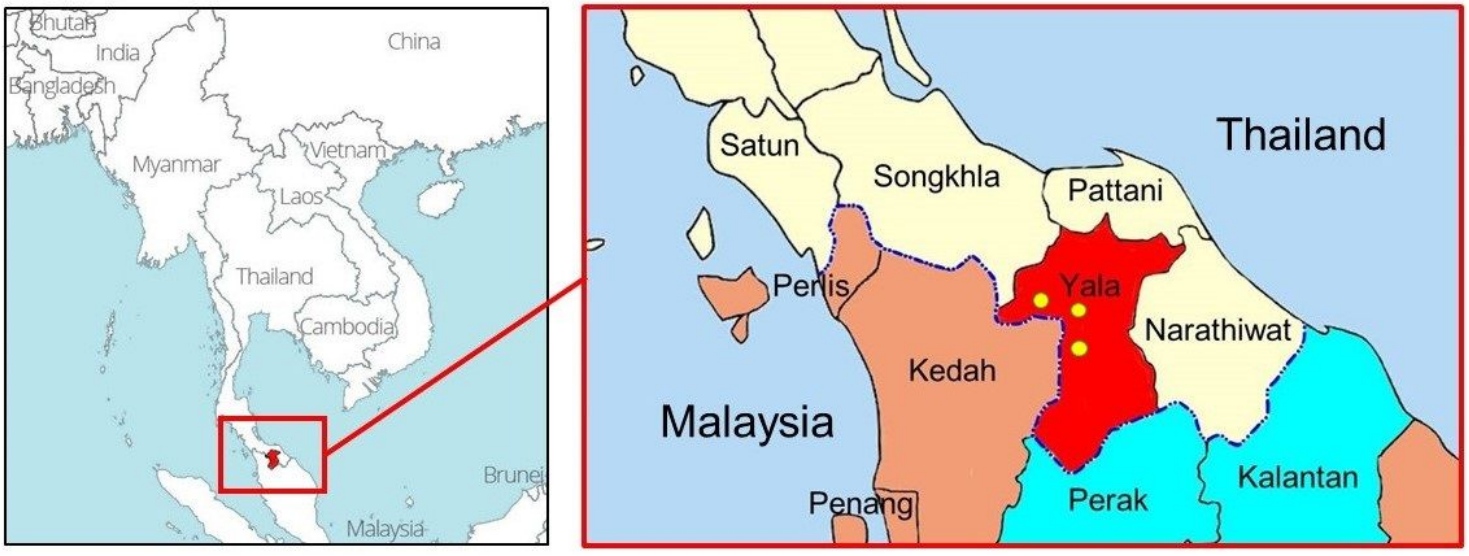

Figure 1

Location of human *P. cynomolgi* cases in Thailand

Legend Map of Yala Province, Thailand with location of detected human *P. cynomolgi* cases (yellow dots). The royal blue dotted line indicates the border between Thailand and Malaysia. Provinces in light yellow and red are located in Thailand, and those that are brown and light blue are in Malaysia, with the two states of Perak and Kelantan being two areas with previously reported human *P. cynomolgi* cases [7].

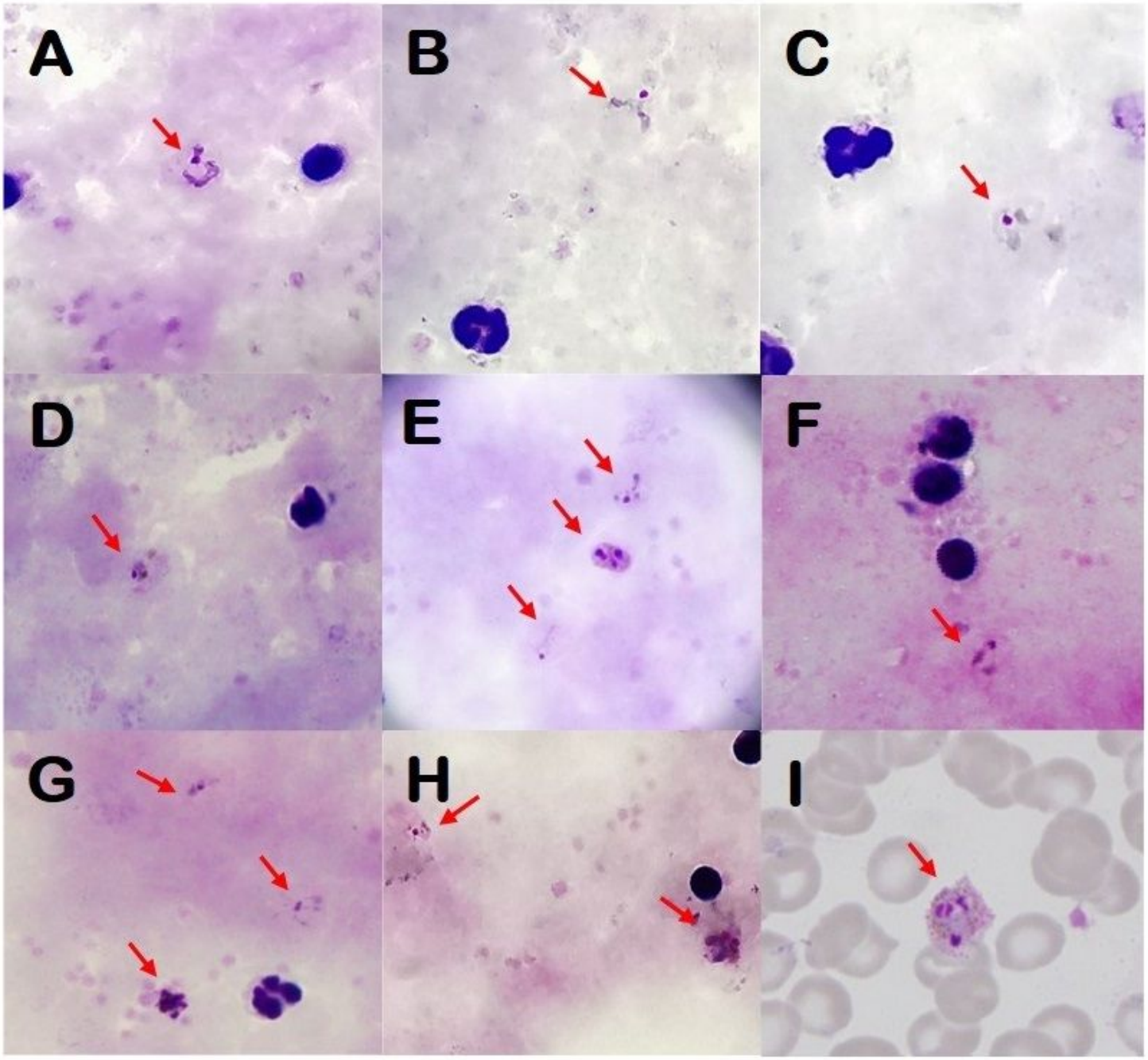

Figure 2

Morphology of *P. cynomolgi* parasites in Giemsa-stained blood smears

Legend Shown are malaria parasites detected in Giemsa stained films at a magnification of 100x. Panels A-E are from Case A (thick film), showing growing trophozoite stages with amoeboid-shaped cytoplasm.

Yellowish-brown pigments were visible (D and E) with multiple chromatin dots in some parasites (E). F: Case B (thick film) with growing trophozoite stages. G and H: Case C (thick film) with parasites resembling *P. vivax* were found in each field of view. Early schizont with merozoites also seen in (G). I: Case C (thin film). Dominant Schüffner's stippling (pink, scattered dots) and yellowish-brown pigments in a trophozoite. Erythrocytes were slightly enlarged.