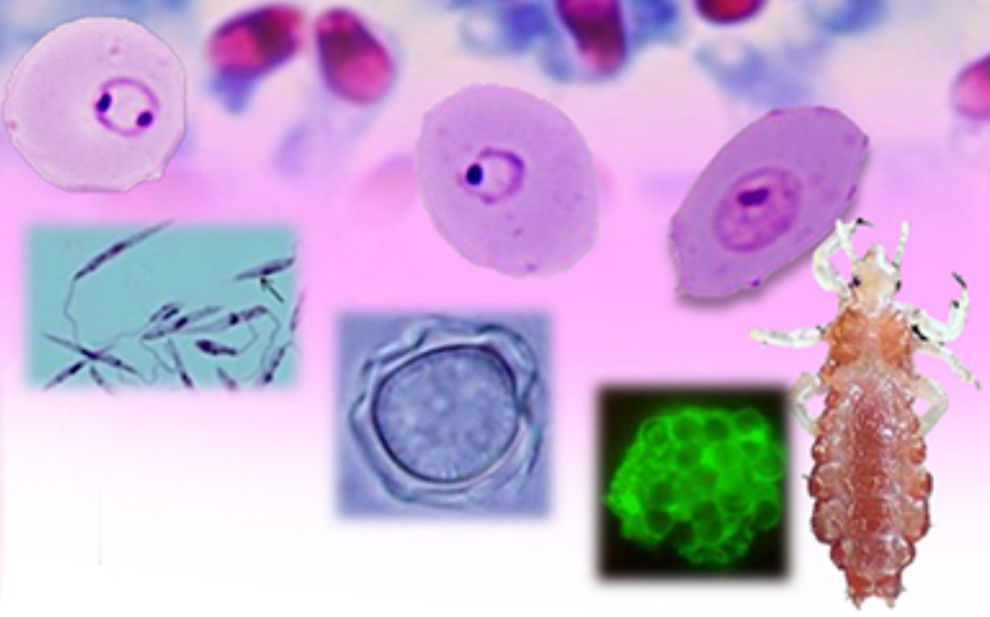

VIRTUAL MDCU CONGRESS 2022

CONCISE MESSAGES

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DEPARTMENT of PARASITOLOGY

Faculty of Medicine

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Zoonotic malaria in humans in Thailand

Chaturong Putaporntip and Somchai Jongwutiwes

Molecular Biology of Malaria and Opportunistic Parasites Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University

Since the first discovery of *Plasmodium knowlesi* infection in humans in Thailand almost two decades ago, hundreds of cases have been identified with wide geographic distribution in this country. Although the majority of *P. knowlesi*-infected patients presented with uncomplicated malaria, severe and fatal cases have been reported. Definite diagnosis requires specific molecular detection whereas microscopy merely confers presumptive identification. Like other malaria, early diagnosis and adequate treatment can prevent severe complications. Besides *P. knowlesi*, at least three other zoonotic *Plasmodium* species including *P. cynomolgi*, *P. inui* and *P. fieldi* have been diagnosed among symptomatic malaria patients in Thailand

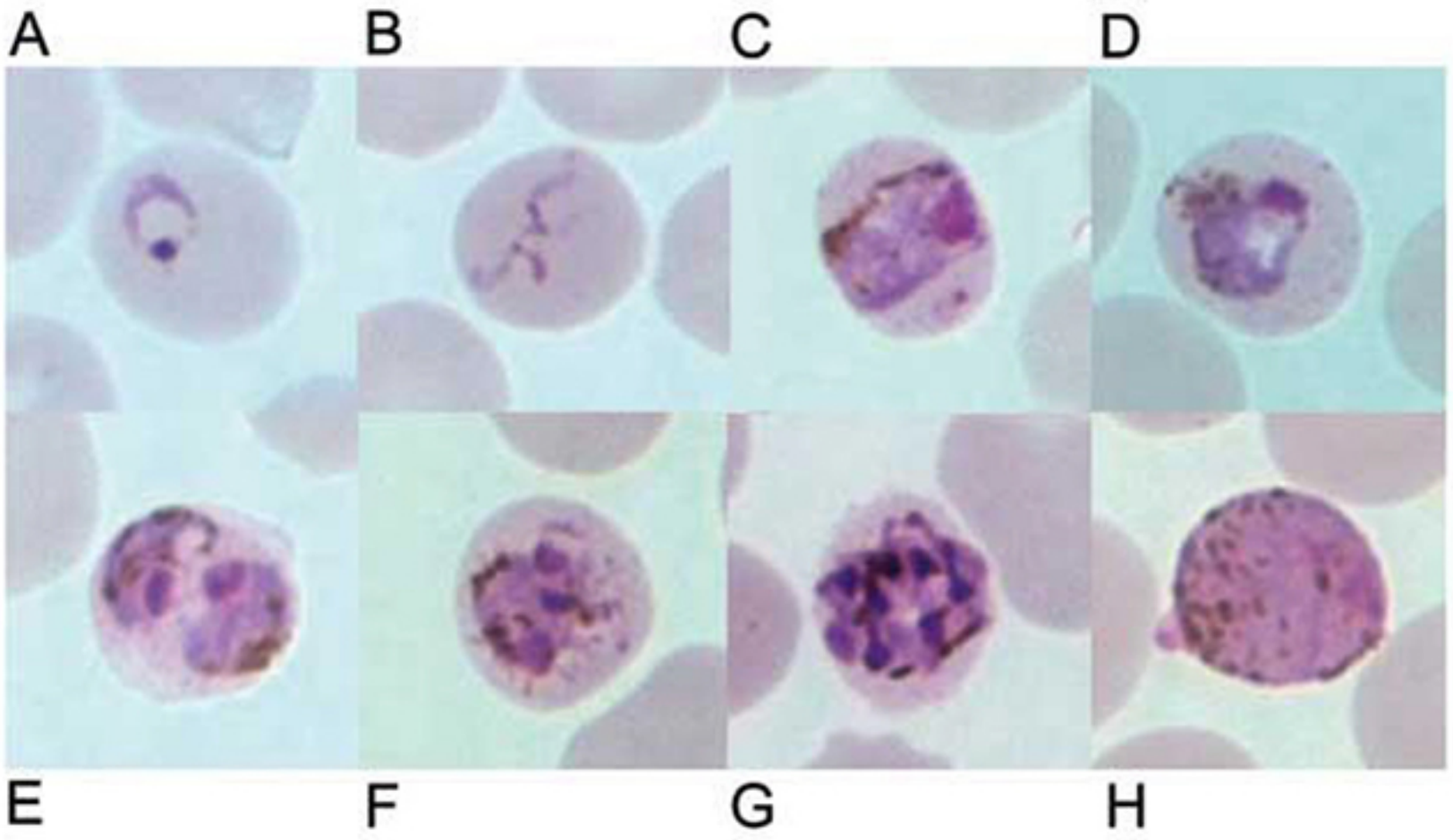

Figure. Giemsa-stained thin blood films depicting *P. knowlesi* **(A)** ring stage, **(B)** tenue form of young trophozoite, **(C)** band-shaped growing trophozoite, **(D)** growing trophozoite with little or no amoeboid activity, **(E)** double growing trophozoites, **(F)** early schizont, **(G)** late schizont in an erythrocyte with fimbriated margins, and (H) mature macrogametocyte. Discernible Sinton and Mulligan stippling is in C, D, and F. (Emerg Infect Dis 2004;10:2211-3)

Further information:

1. Jongwutiwes et al. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. ***Emerging Infectious Diseases*** 2004;10:2211-2213.
2. Putaporntip et al. Differential prevalence of *Plasmodium* infections and cryptic *Plasmodium knowlesi* malaria in humans in Thailand. ***Journal of Infectious Diseases*** 2009;199:1143-1150.
3. Jongwutiwes et al. *Plasmodium knowlesi* malaria in humans and macaques, Thailand. ***Emerging Infectious Diseases*** 2011;17:1799-1806.
4. Putaporntip et al. *Plasmodium cynomolgi* co-infections among symptomatic malaria patients, Thailand. ***Emerging Infectious Diseases*** 2021;27:590-593.
5. Putaporntip et al. Cryptic *Plasmodium inui* and *P. fieldi* infections among symptomatic malaria patients in Thailand. ***Clinical Infectious Diseases*** 2022 (in press).

Contact address for FREE diagnostic service:

p.chaturong@gmail.com (085-8103628) or jongwutiwes@gmail.com (02-2564761)

The service includes microscopy and PCR diagnosis from blood samples for all human *Plasmodium* species including *P. knowlesi*, *P. cynomolgi*, *P. inui* and *P. fieldi*.

Microsporidiosis: common, uncommon and novel species identified at King Chulalongkorn Memorial Hospital

Chaturong Putaporntip and Somchai Jongwutiwes

Molecular Biology of Malaria and Opportunistic Parasites Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University

Microsporidia are ubiquitous spore-forming obligate intracellular pathogens causing a wide spectrum of presentation including gastrointestinal, biliary, hepatic, ocular, muscular and disseminated infections. Of these, chronic diarrhea in immunocompromised patients and keratitis in immunocompetent individuals are frequently encountered. Although more than 170 genera and 1,300 species of microsporidia have been documented, less than 10 genera are known to be incriminated in human diseases. Genera *Enterocytozoon* and *Encephalitozoon* are commonly diagnosed among patients with intestinal microsporidiosis whereas *Vittaforma corneae* is frequently identified among superficial microsporidial keratoconjunctivitis patients. Diagnosis is based on identification of characteristic spores in clinical specimens stained with modified trichrome, acid fast or other stains while molecular detection may assist in identification of known genera. During the past three decades, uncommon species diagnosed at King Chulalongkorn Memorial Hospital include *Trachipleistophora anthropophthera* and *T. hominis* while a novel species closely related to *Endoreticulatus* has been identified in two patients: one with stromal keratitis and the other with disseminated infection.

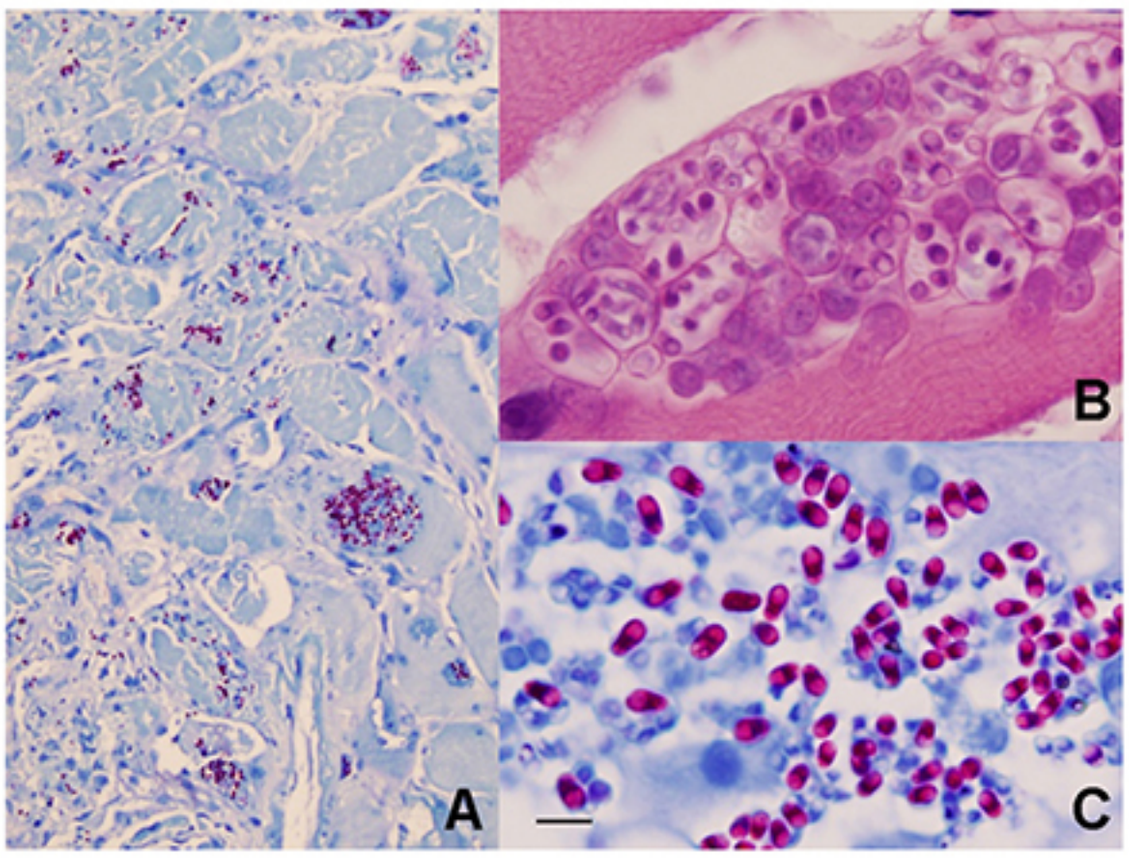

Figure. Histopathology of the patient's muscle tissues. **A**, Acid-fast stain showing inflammatory infiltration and endomysium reddish spores of *Trachipleistophora hominis*. **B**, Numerous spores inside sporophorous vesicles stained with hematoxylin and eosin. **C**, Acid-fast stain showing spores with belt-like structure and posterior vacuole. Bar = 5 µm.
(Open Forum in Infectious Diseases 2021;8:ofab494.)

Further information:

1. Pariyakanok and Jongwutiwes. Keratitis caused by *Trachipleistophora anthropophthera*. ***Journal of Infection*** 2005;51:325-328.
2. Suankratay et al. Disseminated infection caused by novel species of microsporidium, Thailand. ***Emerging InfectiousDiseases*** 2012;18:302-304.
3. Pariyakanok et al. Femtosecond laser-assisted anterior lamellar keratoplasty in stromal keratitis caused by an *Endoreticulatus*-like microsporidia. ***Cornea*** 2015;34:588-591.
4. Pariyakanok et al. Stromal keratitis with endophthalmitis caused by *Vittaforma corneae* in an immunocompetent patient: a case report. ***Ocululer Immunology and Inflammation*** 2019;27:826-828.
5. Buppajarntham et al. Myositis caused by *Trachipleistophora hominis* in a person with human immunodeficiency virus: The first case in Thailand. ***Open Forum in Infectious Diseases*** 2021;8:ofab494.

Contact address for diagnostic service:
p.chaturong@gmail.com (085-8103628) or jongwutiwes@gmail.com (02-2564761)

The service includes microscopic diagnosis from clinical samples. Species identification by DNA sequencing is available based on academic merit on a case-by-case basis.

Acanthamoebiasis: diagnosis and genotype assignment

Chaturong Putaporntip and Somchai Jongwutiwes

Molecular Biology of Malaria and Opportunistic Parasites Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University

Acanthamoeba is a pathogenic free-living protozoon existing in a wide range of environment such as soil, dust, air, freshwater, seawater, sediment, and sewage. The life cycle of *Acanthamoeba* contains a replicative trophozoite stage that feeds primarily on a variety of microbes and an environmentally resistant cystic form. Besides recalcitrant and sight-threatening keratitis which is the most common presentation of acanthamoebiasis, fatal granulomatous amoebic encephalitis and cutaneous infection in immunocompromised hosts are infrequently found. Diagnosis of acanthamoebiasis can be performed from tissue samples by microscopy, *in vitro* cultivation using non-nutrient agar plate seeded with heat-inactivated *Escherichia coli* and molecular detection. To date, 23 distinct genotypes belonging to three morphological groups have been identified in which T4 is the most common pathogenic genotype in human infections. Despite severity of illness and unfavorable treatment outcomes, early diagnosis and prompt therapeutic intervention remain to be the cornerstone for management of *Acanthamoeba* infections.

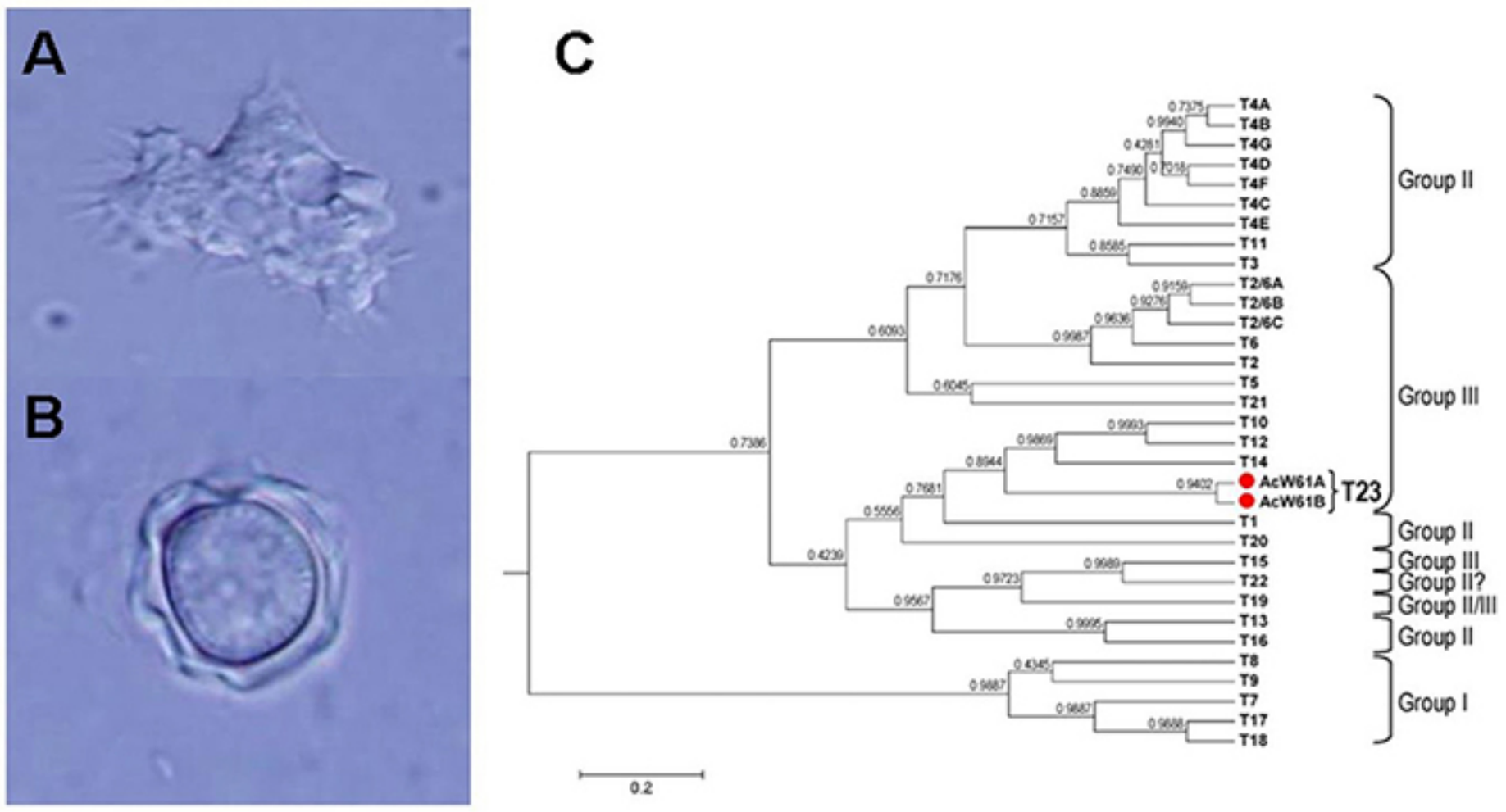

Figure. *Acanthamoeba* trophozoite **(A)**, group II cyst **(B)** and Bayesian tree inferred from the near complete *18S rRNA* sequences depicting currently known 23 genotypes **(C)**(Scientific Reports 2021;11:17290).

Further information:

1. Jongwutiwes et al. Heterogeneity in cyst morphology within isolates of *Acanthamoeba* from keratitis patients in Thailand. ***Tropical Medicine and International Health*** 2000;5:335-340.
2. Nuprasert et al. Identification of a novel T17 genotype of *Acanthamoeba* from environmental isolates and T10 genotype causing keratitis in Thailand. ***Journal of Clinical Microbiology*** 2010;48:4636-4640.
3. Satitpitakul et al. Severe keratitis caused by *Acanthamoeba* genotype T12 in Thailand: a case report. ***The American Journal of Tropical Medicine and Hygiene*** 2021;106:681-684.
3. Putaporntip et al. Analysis of *Acanthamoeba* genotypes from public freshwater sources in Thailand reveals a new genotype, T23 *Acanthamoeba bangkokensis* sp. nov. ***Scientific Reports*** 2021;11:17290.

Contact address for diagnostic service:

p.chaturong@gmail.com (085-8103628) or jongwutiwes@gmail.com (02-2564761)

The service includes microscopy, *in vitro* cultivation and PCR diagnosis from clinical specimens. Genotypic analysis will be done for Pussard and Pons' morphological groups of interest.

Laboratory diagnosis of pneumocystosis

Taweesak Tia, Chaturong Putaporntip and Somchai Jongwutiwes

Molecular Biology of Malaria and Opportunistic Parasites Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University

Pneumocystis jirovecii is an opportunistic extracellular pathogen belonging to division Ascomycota, subdivision Taphrinomycotina, comprising single-celled trophic and ascus-like cytic forms in the life cycle. *Pneumocystis jirovecii* pneumonia (PCP) is a leading cause of morbidity and mortality in immunocompromised patients. Laboratory diagnosis of *P. jirovecii* can be performed by various conventional staining methods such as Giemsa, toluidine blue and methenamine silver stains in which the diagnostic performance seems to be relied on types of clinical samples, quality of stains and experience of microscopist. Immunofluorescence staining with *Pneumocystis*-specific antibodies offers greater sensitivity than conventional staining procedures whereas the specificity depends on the competence of microscopist. Specific polymerase chain reaction targeting the ribosomal RNA genes of *P. jirovecii* confers higher sensitivity than conventional and immunofluorescence staining methods albeit cryptic colonization and true infection require clinical considerations. Co-trimoxazole remains the drug of choice for PCP while sporadic cases of infections caused by antifolate-resistant strains have been

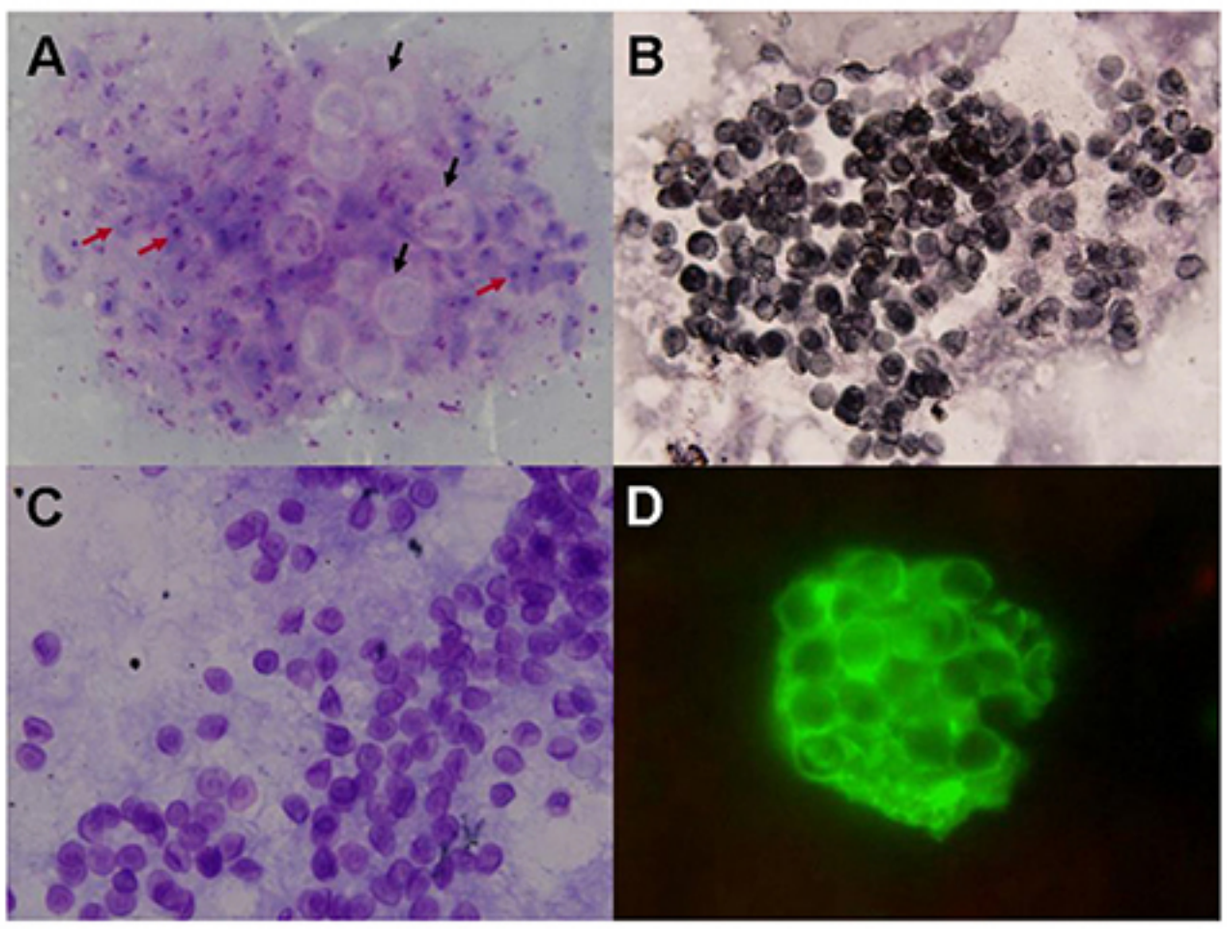

Figure. Bronchoalveloar lavage fluids from patients with pneumocystosis. **(A)** Numerous trophic (red arrows) and cystic forms (black arrows) of *P. jirovecii* stained with Giemsa stain. **(B, C** and **D)** Cysts stained with methenamine silver, toluidine blue and immunofluorescence stains, respectively. (Photos are copyrighted by the authors)

Further information:

1. Tia et al. A highly sensitive novel PCR assay for detection of *Pneumocystis jirovecii* DNA in bronchoalveloar lavage specimens from immunocompromised patients. ***Clinical Microbiology and Infection*** 2012;18:598-603.
2. Teeranaipong et al. A simple and efficient DNA extraction from respiratory samples for PCR detection of *Pneumocystis jirovecii*. ***Chulalongkorn Medical Journal*** 2018;62:725-736.

Contact address for diagnostic service:

p.chaturong@gmail.com (085-8103628) or jongwutiwes@gmail.com (02-2564761)

The official service includes microscopic examinations of Giemsa stain, immunofluorescence assay and PCR diagnosis from clinical samples. For molecular detection of anti-folate resistance in *P. jirovecii*, please contact the above addresses.

Agar plate culture for diagnosis of strongyloidiasis

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Molecular Biology of Malaria and Opportunistic Parasites Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University

Strongyloides stercoralis, a soil-transmitted nematode, is an opportunistic parasite incriminated in hyperinfection and fatal disseminated infections in immunocompromised patients, especially those who receive prolonged corticosteroid or immunosuppressive treatment. The life cycle of *S. stercoralis* comprises parasitic and free-living phases in which the third stage filariform larva is infective through skin and mucous membrane penetration. Although diagnosis of strongyloidiasis can be done by microscopy detection with direct smear and concentration methods, fluctuation of larval excretion from the intestine can compromise the efficiency of coprodiagnosis. To increase diagnostic performance, agar plate culture method has been applied on the basis of biological transformation of rhabditiform larvae to free-living adult stages and multiplication of their off-springs outside the host environment. Agar plate culture method for diagnosis of *S. stercoralis* is twice and ten-fold more sensitive than formalin-ether sedimentation and direct smear methods, respectively. Furthermore, hookworms can also be diagnosed on the basis of distinct furrows on the agar surface.

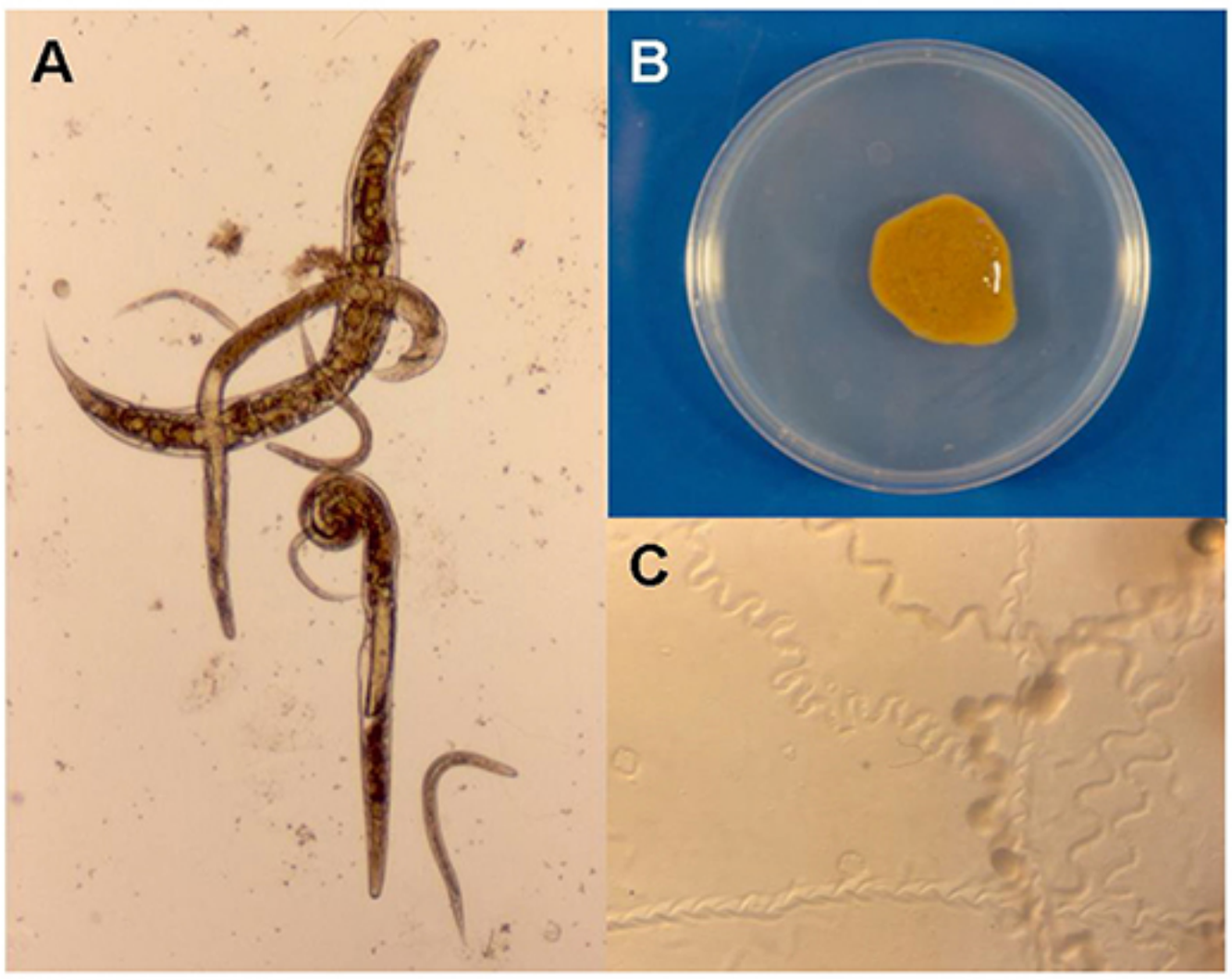


Figure. (A) Free-living stages of *Strongyloides stercoralis*, **(B)** agar plate culture and **(C)** characteristic furrows on the surface of agar. (Photos are copyrighted by Chaturong Putaporntip and Somchai Jongwutiwes)

Further information:

Jongwutiwes et al. Increased sensitivity of routine laboratory detection of *Strongyloides stercoralis* and hookworm by agar-plate culture. ***Transactions of the Royal Society of Tropical Medicine and Hygiene*** 1999;93,398-400.

Contact address for diagnostic service:
p.chaturong@gmail.com (085-8103628) or jongwutiwes@gmail.com (02-2564761)

The service includes microscopy detection and agar plate culture method from stool samples or other clinical specimens.

Leishmaniasis in Thailand

Narissara Jariyapan

Department of Parasitology, Faculty of Medicine,
Chulalongkorn University

Since 1996, several cases have been reported as autochthonous leishmaniasis in Thailand. Two major causative agents are *Leishmania* (*Mundinia*) *martiniquensis* [1] and *Leishmania* (*Mundinia*) *orientalis* [2]. Laboratory diagnosis of leishmaniasis includes microscopic examination, cultivation, and molecular methods [1,2,3]. PCR-based methods of the 3′ untranslated region of the heat shock protein 70 (type I) gene (*HSP70*-I-3'-UTR) can be used to identify and differentiate between *L. martiniquensis* and *L. orientalis*. In species with similar PCR product size, the *Bsu*RI-PCR-RFLP patterns of the 3′-UTR of *HSP70*-I fragments can be used for differentiating some species within other subgenera [3]. The PCR-based methods can be applicable to the identification of *Leishmania* DNA obtained from bone marrow, blood, saliva, and skin of human and animals and also from insect vectors.

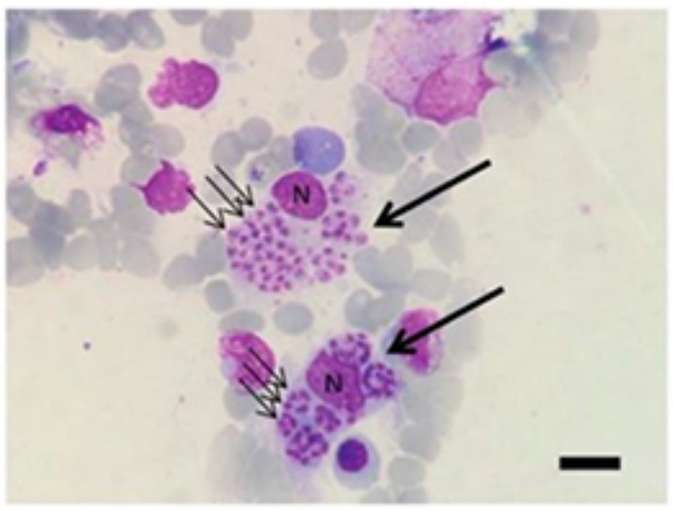
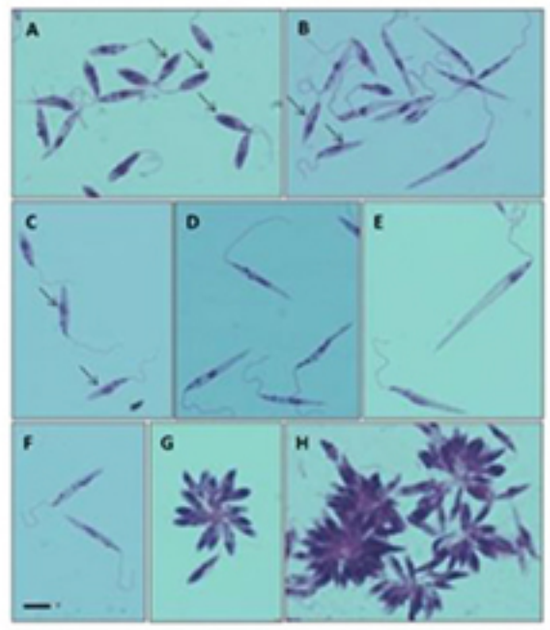


Fig. 1. Light micrograph of *Leishmania* amastigotes in bone marrow aspirate. Two infected macrophages are stained (large arrows), each with a nucleus (N) and numerous amastigotes (examples with small arrows) within the cytoplasm. The specimen was stained using Wright's stain. The bar represents 20 μm.
doi:10.1371/journal.pntd.0003339.g001



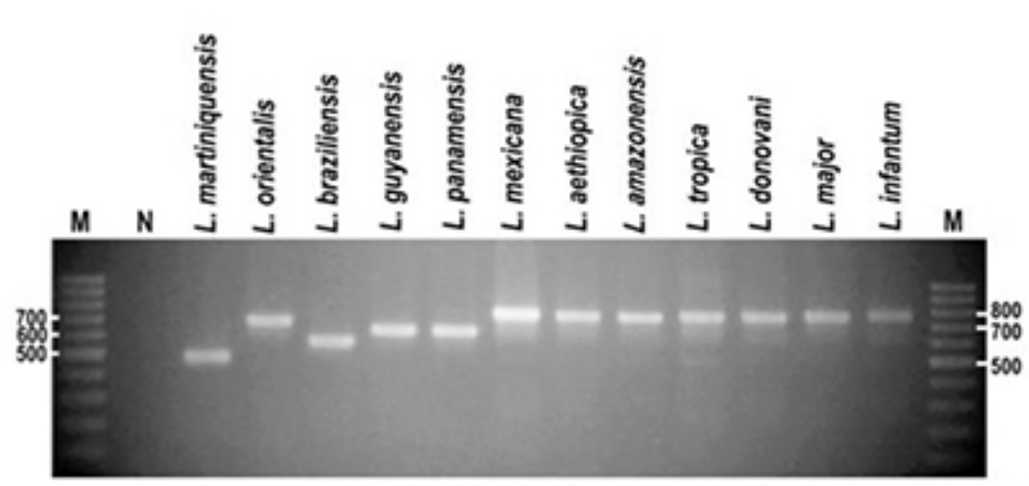


Fig 1. Agarose gel electrophoresis of *HSP70-I-3'*-UTR PCR products of 12 *Leishmania* species. M = Molecular markers and N = Negative control.

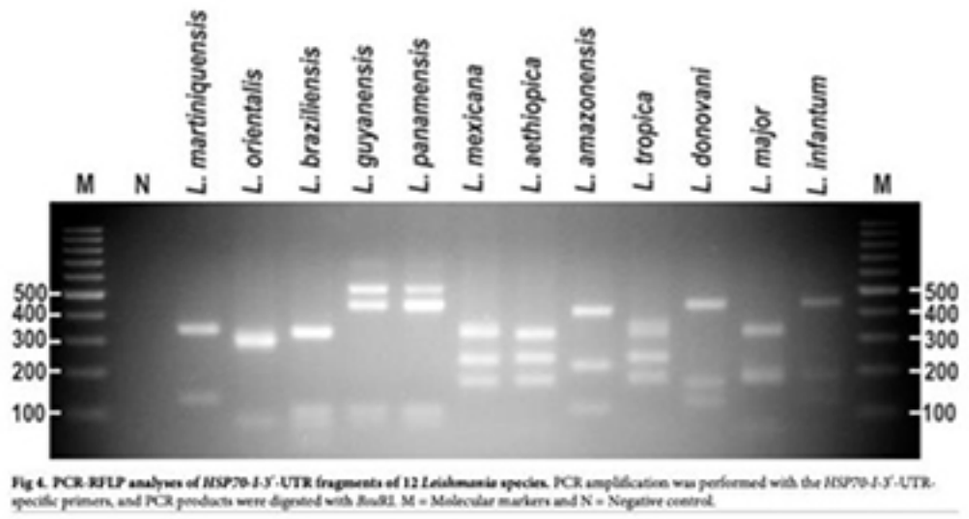


Fig 4. PCR-RFLP analyses of *HSP70-I-3'*-UTR fragments of 12 *Leishmania* species. PCR amplification was performed with the *HSP70-I-3'*-UTR-specific primers, and PCR products were digested with *Bsu*RI. M = Molecular markers and N = Negative control.

Further information:

1. Pothirat et al. First isolation of *Leishmania* from Northern Thailand: case report, identification as *Leishmania martiniquensis* and phylogenetic position within the *Leishmania enriettii* complex. PLoS Negl Trop Dis. 2014;8:e3339.
2. Jariyapan et al. *Leishmania* (*Mundinia*) *orientalis* n. sp. (Trypanosomatidae), a parasite from Thailand responsible for localised cutaneous leishmaniasis. Parasit Vectors. 2018;11:351.
3. Jariyapan et al. Molecular identification of two newly identified human pathogens causing leishmaniasis using PCR-based methods on the 3' untranslated region of the heat shock protein 70 (type I) gene. PLoS Negl Trop Dis. 2021;15:e0009982.

Contact address for FREE diagnostic service: njariyapan@gmail.com (081-9381855)

The FREE service includes microscopic examination, cultivation, and PCR-based methods for *Leishmania* parasites from saliva, blood, and tissue samples from human and animals.

Presence of the knockdown resistance (*kdr*) mutations in the head lice collected from primary school children of Thailand

Narisa Brownell and Padet Siriyasatien

Vector Biology and Vector borne Disease Research Unit, Department of Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

Recurrent cases of head lice after treatment are still problematic in Thailand. Evidence of permethrin resistance among head lice which could contribute to the increasing of treatment failure rates in Thailand has never been investigated. This is the first study in Thailand detecting permethrin resistance in human head lice using molecular method. Restriction fragment length polymorphism (RFLP) patterns and sequencing were used to identify the *kdr* T917I mutation and demonstrated three genotypic forms including homozygous susceptible (SS), heterozygous genotype (RS), and homozygous resistant (RR). Of 260 samples from this study, 156 (60%) were SS, 58 (22.31%) were RS, and 46 (17.69%) were RR. The overall frequency of the *kdr* T917I mutation was 0.31.

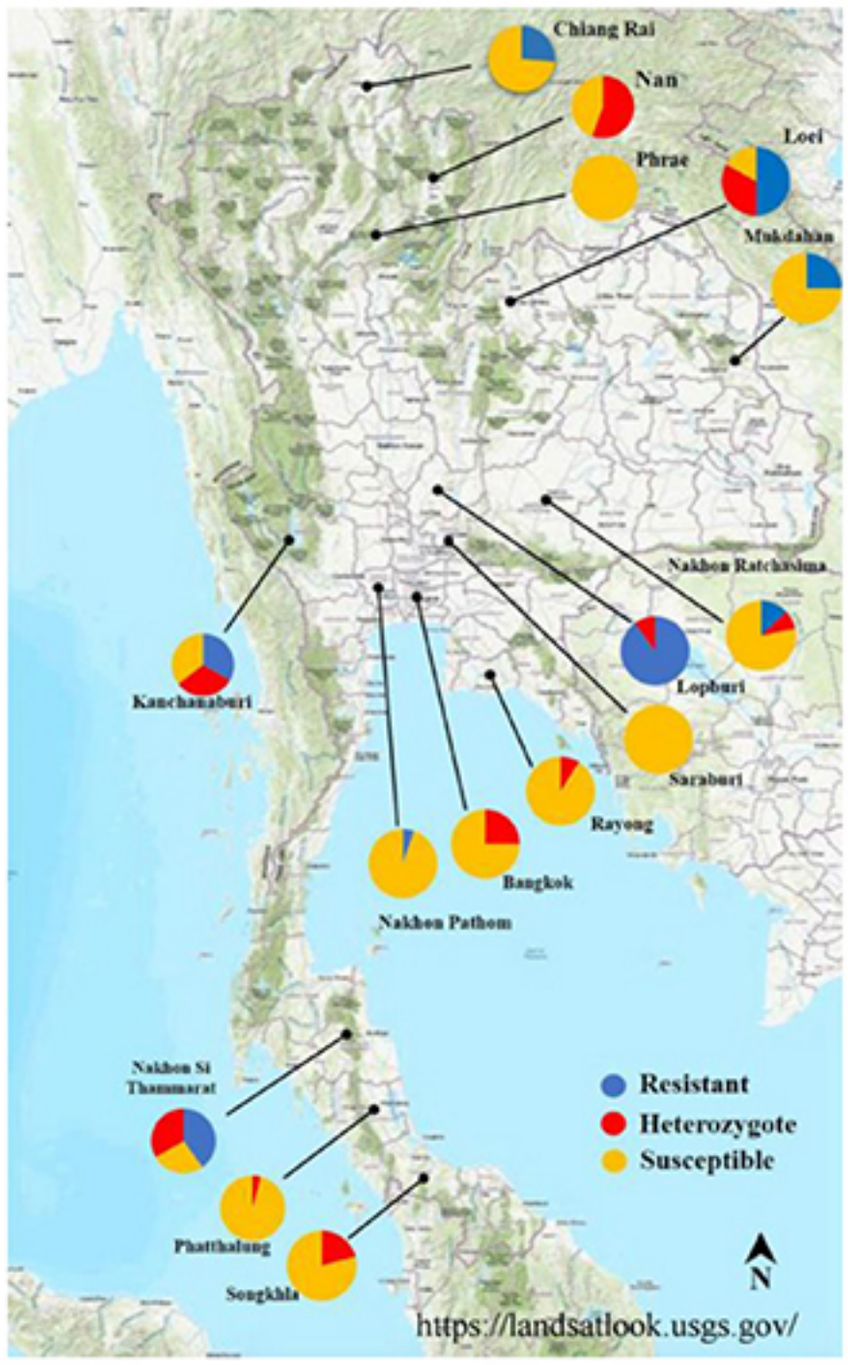


Figure Distribution of *kdr* T917I genotype in Thai human head lice collected from 6 geographical regions in the 15 provinces of Thailand. (The figure modified from the public domain https://landsatlook.udgs.gov/)

Further information:

Brownell N, Sunantaraporn S, Phadungsaksawasdi K, Seatamanoch N, Kongdachalert S, Phumee A, Siriyasatien P. Presence of the knockdown resistance (kdr) mutations in the head lice (*Pediculus humanus capitis*) collected from primary school children of Thailand. *PLoS Negl Trop Dis*. 2020 Dec 16;14(12):e0008955. doi: 10.1371/journal.pntd.0008955.

Prevalence and Risk Factors of *Opisthorchis viverrini* Infection : A Cross-Sectional Study in Sakon Nakhon Province, Thailand

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Objective :Human liver fluke infection or opisthorchiasis is a foodborne infection caused by the ingestion of raw freshwater fish containing a parasite named *Opisthorchis viverrini* .It has still been a major public health, having high infection rate, in the Northeastern region of Thailand for decades despite existing public policies with the intention to decrease the infection rate .This cross-sectional study aimed to investigate the prevalence and risk factors of opisthorchiasis in Sakon Nakhon Province, which is in the Northeastern region of Thailand, from September 2019 to October 2019.

Materials & Methods : The study was conducted in four villages from two sub-districts in Sakon Nakhon Province, Kok Pla Siew and Tong Khob .These villages have differences in their presence of fecal sludge management systems and their infection rate history .Simple random sampling was used to enroll participants .Each participant was then given a questionnaire to gather data inquiring about demographic data, surrounding environment, health behavior, and knowledge about liver fluke infection .Odds ratios, single logistic regression, and multiple logistic regression were used to analyze the data in order to identify the risk factors of the infection .In total, 238 people answered the questionnaire and subsequently provided stool for examination.

Results :The result showed that opisthorchiasis was more predominant in areas without fecal sludge management system, having 84.5 %of all infected cases diagnosed in this study .Univariate analysis also showed several other contributing factors, including age, household use of river resources, habit of eating raw fishes, and history of anti-helminthic treatment .However, using multivariate analysis, only the presence or absence of fecal sludge management system was independently associated with opisthorchiasis)adjusted OR 0.12, 95 %CI 0.03-0.53 (when adjusted for other factors.

Conclusions :In conclusion, the installation of the fecal sludge management system is a significant part in the reduction of infection rate of *Opisthorchis viverrini* and should be executed along with other public policies.