Molecular Surveillance for Drug Resistant *Plasmodium falciparum* Imported to Ontario

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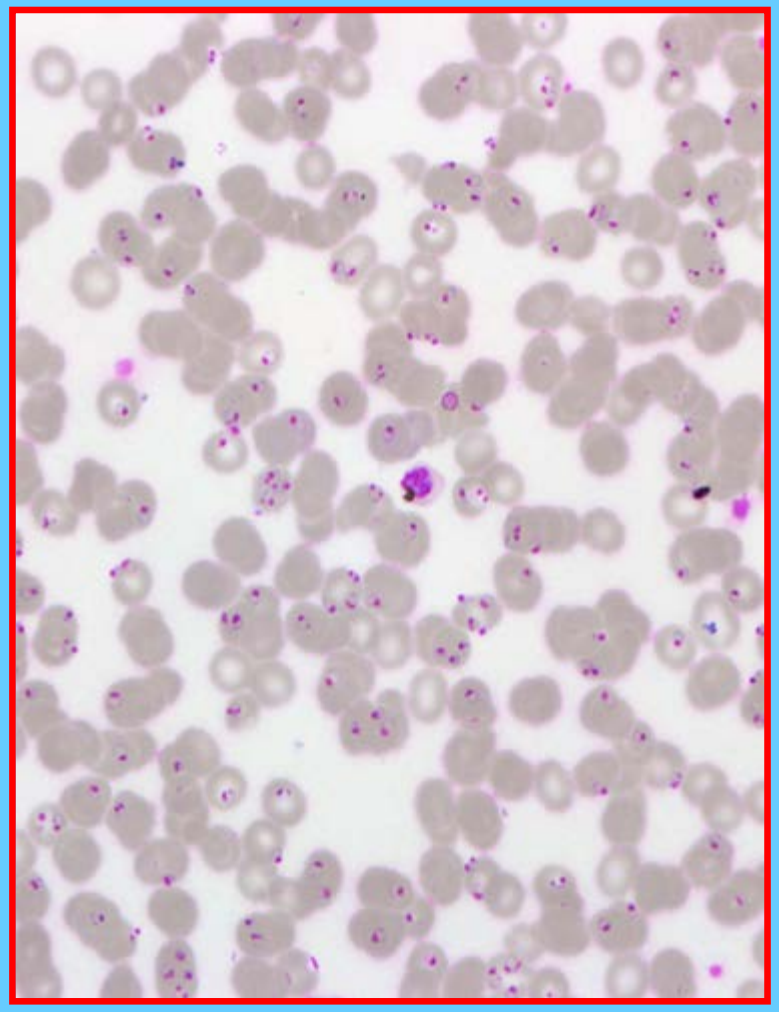
Medicine
UNIVERSITY OF TORONTO

Disclosures

- Committee to Advise on Tropical Medicine and Travel (CATMAT)
  - External advisory body to the Public Health Agency of Canada

What do we know about malaria in Canada?



CanTravNet

GEOSENTINEL
The Global Surveillance Network of the ISTM and CDC

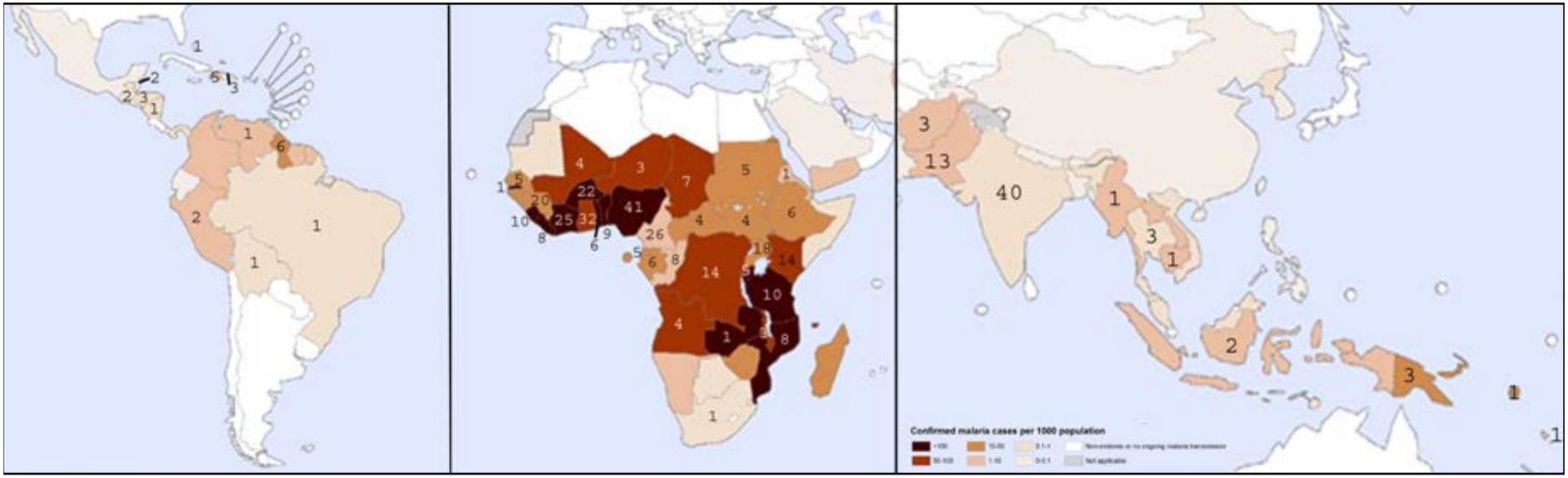
CMAJ OPEN

Research

Malaria in travellers returning or migrating to Canada: surveillance report from CanTravNet surveillance data, 2004–2014

Andrea K. Boggild MSc MD, Jennifer Geduld MSc, Michael Libman MDCM, Cedric P. Yansouni MD, Anne E. McCarthy MD, Jan Hajek MD, Wayne Ghesquiere MD, Jean Vincelette MD, Susan Kuhn MD, David O. Freedman MD, Kevin C. Kain MD

- **400-500 cases / year**
- **62% due to *Plasmodium falciparum***
- **40% of cases occur in VFRs**
- **Severe malaria in ~5% of cases**
- **1-2 deaths / year**



Malaria Epidemiology in Canada

Table 3: Most common source countries by year of import for 456 cases of malaria among ill returned travellers and new immigrants evaluated at CanTravNet sites, 2004–2014

	No. of diagnoses of malaria (no.	Total no. of cases of malaria reported to the Public	Cases reported to the Public Health Agency of Canada each	Most common source countries (no. of cases)*		
				First	Second	Third†
2				hanistan (1)	Guatemala (1)	Venezuela (1)
2				ana (2)	Guinea (2)	–
2				eria (6)	Ivory Coast (4)	Mozambique (4)
2				a (5)	Ivory Coast (3)	Cameroon (2), Nigeria (2)
2				ya (5)	Nigeria (5)	–
2				a (5)	Ghana (4)	–
2				neroon (3)	Ghana (3)	Honduras (3), Nigeria (3)
2				a (10)	Ghana (7)	Nigeria (6)
2				a (7)	Pakistan (6)	Sierra Leone (5)
2				neroon (12)	Guinea (7)	Nigeria (7)
2014‖	49 (48)	Unavailable	Unavailable	Benin (4)	Ghana (4)	–
Total	456 (437)	4190	11.3	Nigeria (41)	India (40)	Ghana (32)

Most imports from West Africa

Nigeria, Cameroon, and Ghana top source countries from Africa

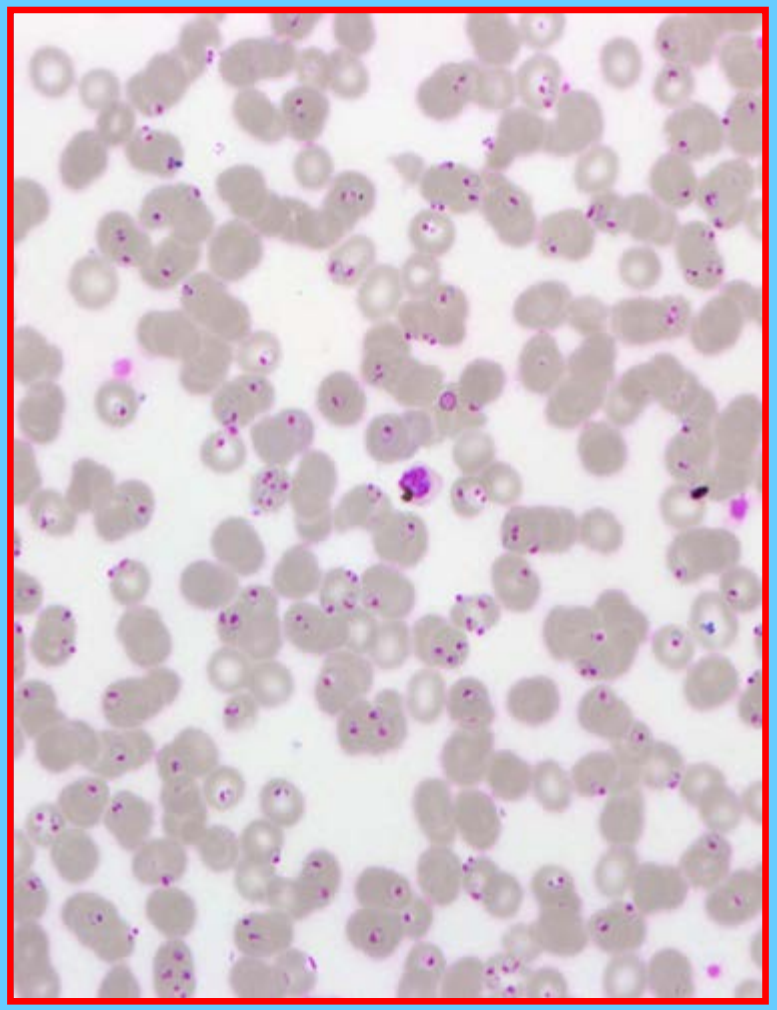
Very few cases from southeast Asia

***P. vivax* imports predominantly from Indian sub-continent**

*Country of exposure may be unknown or unattributable with multicountry itineraries.
†Not noted if more than 5-way tie for third place.
‡Data for Sept. 1, 2004, to Dec. 31, 2004.
§Extrapolated to 1 year.
¶Data for Jan. 1, 2014, to Aug. 31, 2014.

***CMAJ Open* 2016. DOI:10.9778/cmajo.20150115**

Why is Epidemiology of Imported Malaria Important?



Malaria Treatment

- **Is the infection caused by *P. falciparum*?**
  - Leads to severe disease in non-immunes and requires prompt initiation of treatment
  - Chloroquine is DOC for non-falciparum malaria
- **Does the patient fulfill severity criteria?**
  - Severe malaria, regardless of species, is *always* treated with parenteral anti-malarials
- **Is the infecting organism likely to be drug resistant?**
  - Most falciparum malaria is chloroquine resistant

Quick Reference to Global Distribution of Drug Resistant *Plasmodium* spp.		
Chloroquine-resistant *P. falciparum*	**Multi-drug resistant *P. falciparum***	**Chloroquine-resistant *P. vivax***
ALL malarious areas EXCEPT in Americas North of the Panama canal (Mexico, DR/Haiti, Central American countries) and parts of the Middle East	Southeast Asia along the Thai borders of Myanmar (Burma) and Cambodia, Burma, Vietnam, and in some parts of the Amazon basin	PNG, Guinea (Irian Jaya), Indonesia, Myanmar, Solomon Islands, focally in South America (Colombia, Brazil, Guyana, Peru)

The NEW ENGLAND JOURNAL of MEDICINE

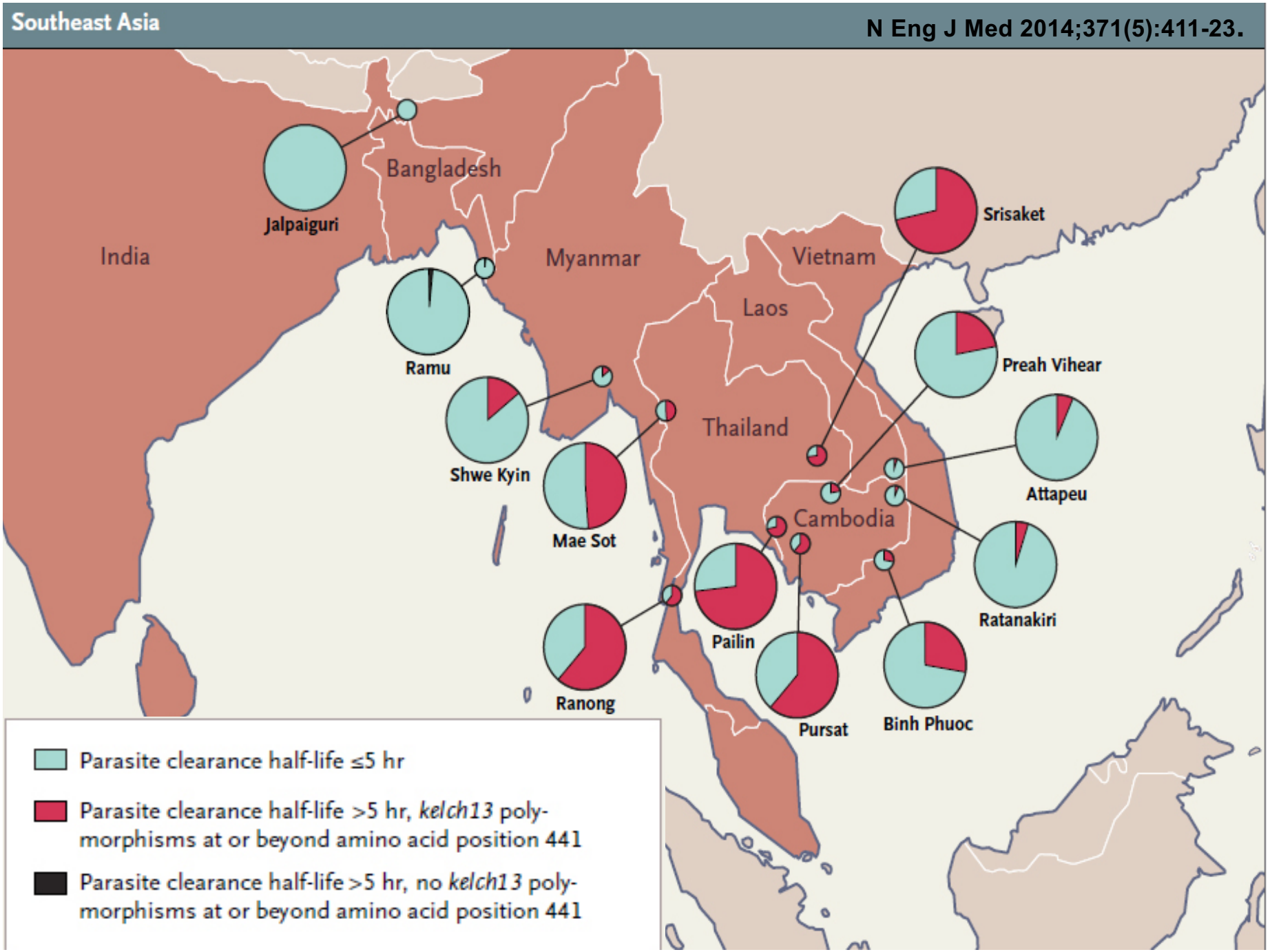
N Eng J Med 2014;371(5):411-23.

ORIGINAL ARTICLE

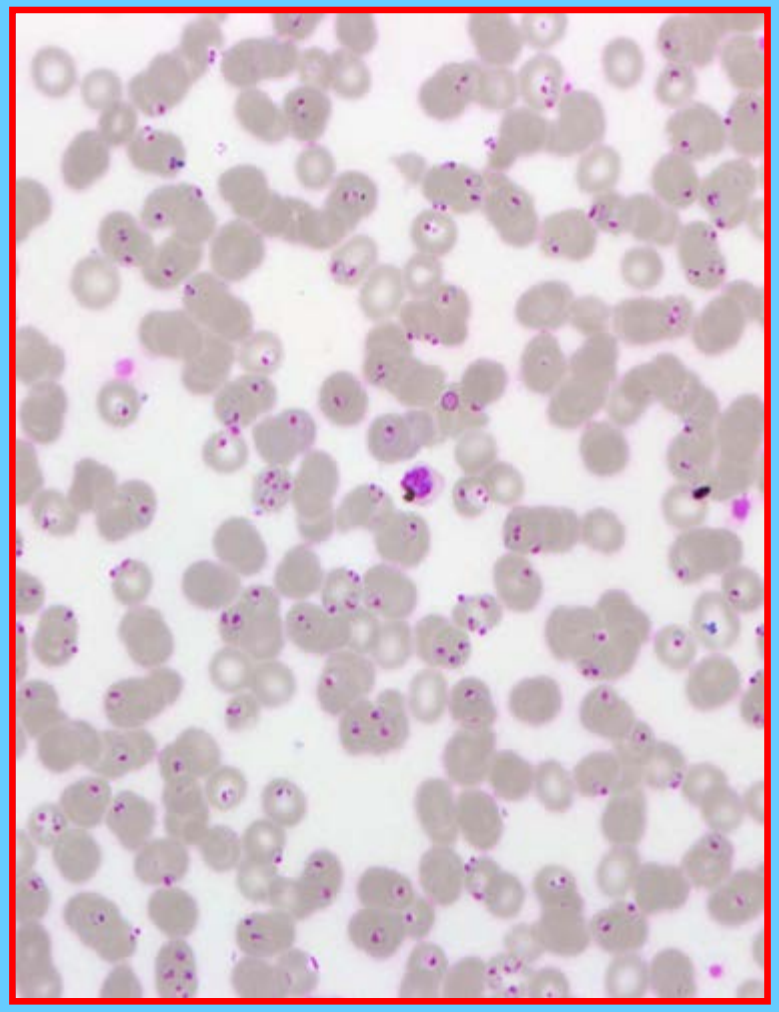
Spread of Artemisinin Resistance in *Plasmodium falciparum* Malaria

E.A. Ashley, M. Dhorda, R.M. Fairhurst, C. Amaratunga, P. Lim, S. Suon, S. Sreng, J.M. Anderson, S. Mao, B. Sam, C. Sopha, C.M. Chuor, C. Nguon, S. Sovannaroth, S. Pukrittayakamee, P. Jittamala, K. Chotivanich, K. Chutasmit, C. Suchatsoonthorn, R. Runcharoen, T.T. Hien, N.T. Thuy-Nhien, N.V. Thanh, N.H. Phu, Y. Htut, K-T. Han, K.H. Aye, O.A. Mokuolu, R.R. Olaosebikan, O.O. Folaranmi, M. Mayxay, M. Khanthavong, B. Hongvanthong, P.N. Newton, M.A. Onyamboko, C.I. Fanello, A.K. Tshefu, N. Mishra, N. Valecha, A.P. Phyo, F. Nosten, P. Yi, R. Tripura, S. Borrmann, M. Bashraheil, J. Peshu, M.A. Faiz, A. Ghose, M.A. Hossain, R. Samad, M.R. Rahman, M.M. Hasan, A. Islam, O. Miotto, R. Amato, B. MacInnis, J. Stalker, D.P. Kwiatkowski, Z. Bozdech, A. Jeeyapant, P.Y. Cheah, T. Sakulthaew, J. Chalk, B. Intharabut, K. Silamut, S.J. Lee, B. Vihokhern, C. Kunasol, M. Imwong, J. Tarning, W.J. Taylor, S. Yeung, C.J. Woodrow, J.A. Flegg, D. Das, J. Smith, M. Venkatesan, C.V. Plowe, K. Stepniewska, P.J. Guerin, A.M. Dondorp, N.P. Day, and N.J. White, for the Tracking Resistance to Artemisinin Collaboration (TRAC)

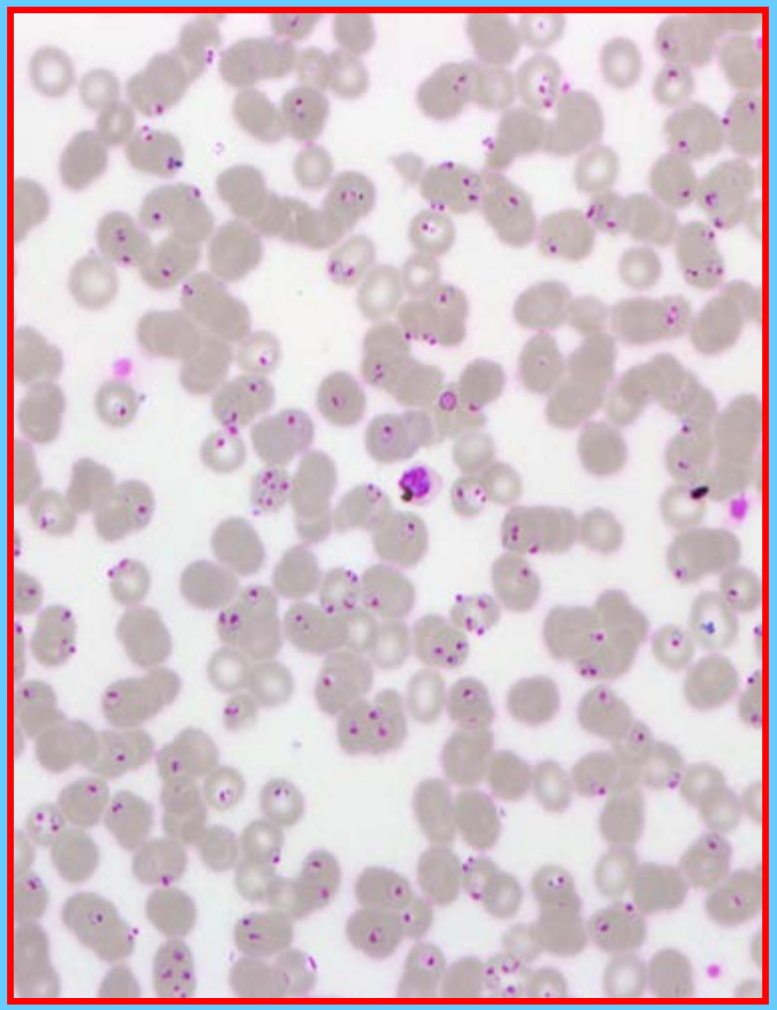
ABSTRACT


Southeast Asia
N Eng J Med 2014;371(5):411-23.
India
Bangladesh
Myanmar
Laos
Vietnam
Thailand
Cambodia
Jalpaiguri
Ramu
Shwe Kyin
Mae Sot
Ranong
Pailin
Pursat
Srisaket
Preah Vihear
Attapeu
Ratanakiri
Binh Phuoc
Parasite clearance half-life ≤5 hr
Parasite clearance half-life >5 hr, kelch13 polymorphisms at or beyond amino acid position 441
Parasite clearance half-life >5 hr, no kelch13 polymorphisms at or beyond amino acid position 441

If Malaria is Treated Empirically, then Why is Surveillance for Resistance Important?



First: The Back Story



Access to Drugs for NTDs in Canada

HEALTH CARE

Why world-beating tropical drugs are so hard to get in Canada

Doctors say they're needed badly, but drug makers say the market is too small for them to turn a profit

WHO Malaria Treatment Guidelines

Treating uncomplicated *P. falciparum* malaria

Treatment of uncomplicated P. falciparum malaria

Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended artemisinin-based combination therapies (ACT):

- artemether + lumefantrine
- artesunate + amodiaquine
- artesunate + mefloquine
- dihydroartemisinin + piperaquine
- artesunate + sulfadoxine–pyrimethamine (SP)

Strong recommendation, high-quality evidence

Non-immune travellers

Treat travellers with uncomplicated *P. falciparum* malaria returning to non-endemic settings with ACT.

Strong recommendation, high-quality evidence

abc NEWS

LOG IN

Coartem Approved to Treat Malaria

By SCOTT ROBERTS

April 9

Government of Canada | Gouvernement du Canada

Because we don't have reasonable access to WHO recommended first-line therapy, we must be vigilant for threats to our continued use of drugs such as atovaquone-proguanil (Malarone)

Medical Access to Artesunate or Quinine for Malaria Treatment Streamlined in Canada through the Canadian Malaria Network (CMN)

Medicine
UNIVERSITY OF TORONTO

Am. J. Trop. Med. Hyg., 76(2), 2007, pp. 208–223

AJTMH 2007;76(2):208-223.

ATOVAQUONE-PROGUANIL: REPORT FROM THE CDC EXPERT MEETING ON MALARIA CHEMOPROPHYLAXIS (II)

ANDREA K. BOGGILD,* MONICA E. PARISE, LINDA S. LEWIS, AND KEVIN C. KAIN

Atovaquone is highly lipophilic with low aqueous solubility and is therefore poorly absorbed unless consumed with a fatty meal

Co-administration of atovaquone and a fatty meal leads to a 5-fold increase in maximum plasma concentration (*C*max) over fasting

Indicat
in adults
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Current
for proph
it is not
Treatm
adults and children weighing ≥ 5 kg (AI).

Dosing. *Adult.* Adult tablets contain 250 mg atovaquone

cure rates of uncomplicated *P. falciparum* malaria of 87–100%, with eight of nine trials showing radical cure rates of

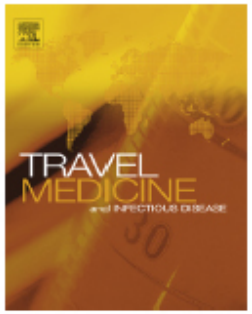
Atovaquone-Proguanil Failure

Travel Medicine and Infectious Disease (2015) xx, 1–5

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevierhealth.com/journals/tmid



Failure of atovaquone-proguanil malaria chemoprophylaxis in a traveler to Ghana

Andrea K. Boggild [a,b,c,*], Rachel Lau [c], Denis Reynaud [d], Kevin C. Kain [a,b,e], Marvin Gerson [f]

Medicine
UNIVERSITY OF TORONTO

TMAID 2015;13(1):89-93.

Serum Drug Concentrations

Taking AP on an <u>empty stomach</u> can lead to sub-therapeutic serum concentrations of the drug by 1000-fold

Table 1 Clin … atovaquone-proguanil chem…

Specimen	Parasitemia (by thin film microscopy)	Expected plasma drug concentration	Plasma drug concentration[a], atovaquone	Plasma drug concentration[a], proguanil
Day 1 of illness	3%	Atovaquone: 11.5 μg/mL; Proguanil: 0.509 μg/mL	2 ng/mL (0.002 μg/mL)	1.3 ng/mL (0.0013 μg/mL)
Day 3 of illness	<0.1%	Atovaquone[b]: 9.43 μg/mL; Proguanil[b]: 0.102 μg/mL (102 ng/mL)	1.3 ng/mL (0.0013 μg/mL)	0.7 ng/mL (0.0007 μg/mL)

[a] By LC-MS/MS; limit of detection for UV-HPLC is 100 ng/mL.

[b] Half-life of atovaquone is 59 h, and that of proguanil is 14.5 h [11].

TMAID 2015;13(1):89-93.

Am. J. Trop. Med. Hyg., 76(2), 2007, pp. 208–223

AJTMH 2007;76(2):208-223.

ATOVAQUONE-PROGUANIL: REPORT FROM THE CDC EXPERT MEETING ON MALARIA CHEMOPROPHYLAXIS (II)

ANDREA K. BOGGILD,* MONICA E. PARISE, LINDA S. LEWIS, AND KEVIN C. KAIN

Resistance to atovaquone results from a single point mutation in parasite *cytochrome b*, which leads to reduced binding affinity for atovaquone

Resistance to proguanil involves the stepwise development of point mutations in the *dhfr* gene

Indicat
in adults
ing in are
Current
for proph
it is not currently FDA-approved for this indication.

Treatment of uncomplicated *P. falciparum* malaria in adults and children weighing ≥ 5 kg (AI).

Dosing. *Adult.* Adult tablets contain 250 mg atovaquone

pediatric populations.

Treatment: Randomized comparator clinical trials indicate cure rates of uncomplicated *P. falciparum* malaria of 87–100%, with eight of nine trials showing radical cure rates of

Atovaquone-Proguanil Treatment Failures

TABLE 3
Reported cases of AP failure for treatment of *P. falciparum*

Patient age, sex	Immune status	Dose, duration	Country of acquisition	Molecular marker of resistance
45, M[34]	Semi-immune	Four adult tabs daily, 3 days	Nigeria	Cyt b Tyr268Asn
24, F[35]	Non-immune traveler	Four adult tabs daily, 3 days	Kenya	Cyt b Tyr268Ser
28, M[36]	Non-immune traveler	Four adult tabs daily, 3 days	Mali	Cyt b Tyr268Ser
28, M[37]	Non-immune traveler	Four adult tabs daily, 3 days	Cameroon	Cyt b Tyr268Ser DHFR triple-codon mutation 51,59,108
1.5, M[38]	Non-immune traveler	One adult tab daily, 3 days	Ivory Coast	Wt cyt b and DHFR
4, M[38]	Non-immune traveler	One adult tab daily, 3 days	Ivory Coast	Cyt b Tyr268Ser DHFR triple-codon mutation 51,59,108
Adult, F[38]	Semi-immune	Four adult tabs daily, 3 days	Ivory Coast	Cyt b Tyr268Ser
38, F[39]	Semi-immune	Four adult tabs daily, 3 days	Democratic Republic of Congo	Wt cyt b
30, M[40]	Semi-immune	Four adult tabs daily, 3 days	Gambia	Wt cyt b
33, M[40]	Non-immune traveler	Four adult tabs daily, 3 days	Kenya, Tanzania	Wt cyt b
56, M[40]	Semi-immune	Four adult tabs daily, 3 days	Nigeria	Wt cyt b
25, F[41]	Non-immune traveler	Two adult tabs twice a day, 3 days	Sierra Leone	Cyt b Tyr268Ser DHFR C59R, S108N

AJTMH 2007;76(2):208-223.

P. falciparum isolate genotypes

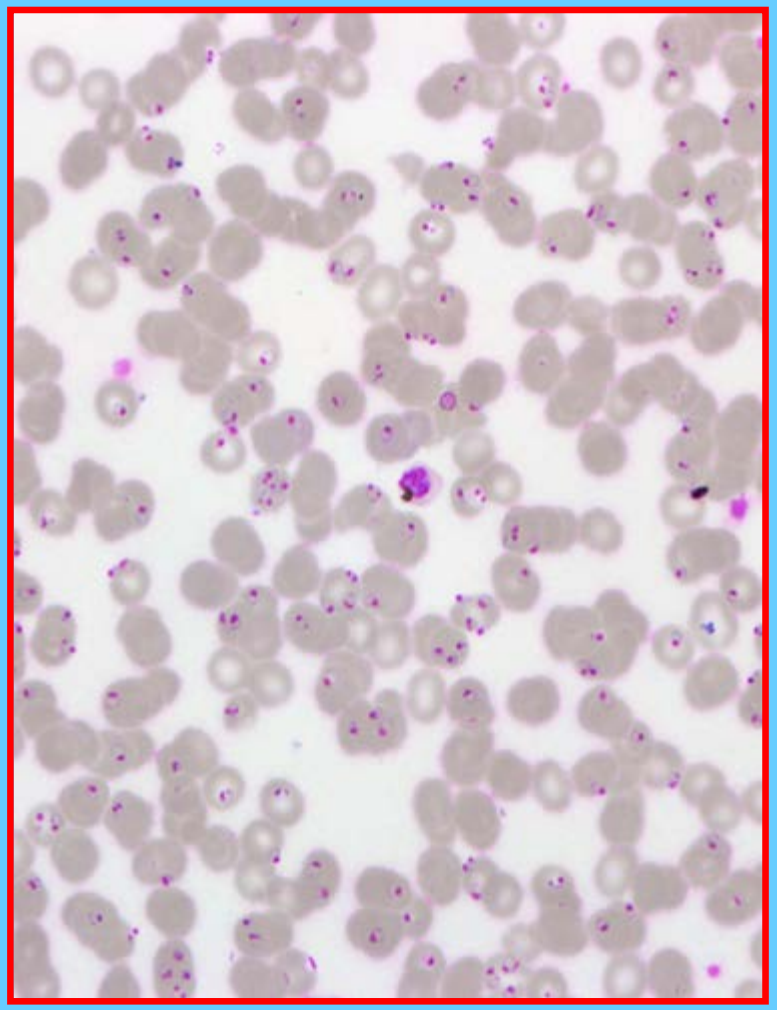
Pf Isolate	Cyt B Y268C/S/N	DHFR N51I	DHFR C59R	DHFR S108N
Initial isolate	Y		R	N
1-month failure isolate	S			
2-month failure isolate	S			

Low levels of drug pressure due to inadequate absorption selects for minor mutant populations leading to a fully resistant infection over time

Clinical Cases

- Initial treatment doses of Malarone not taken with a sufficiently fatty meal
- Sub-therapeutic serum levels of atovaquone during initial treatment enable selection of mutant clones
- Isolates fully resistant to proguanil and then become resistant to atovaquone
- **Take home message: Administer atovaquone-proguanil with fat!!!**

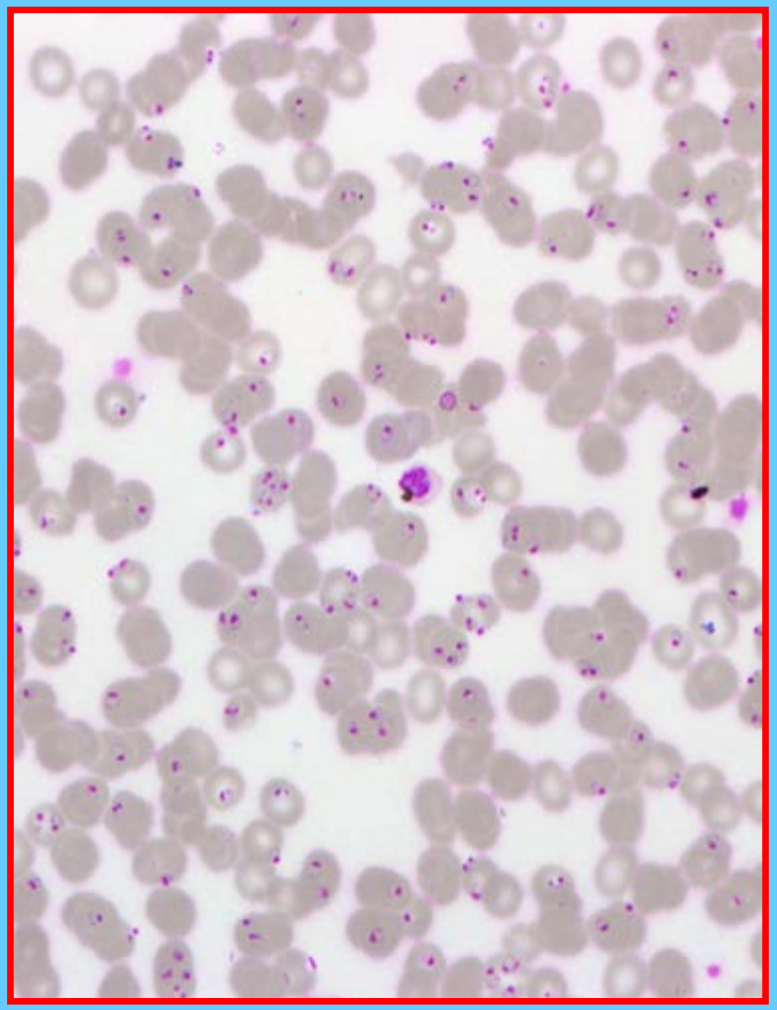
OBJECTIVE AND RATIONALE

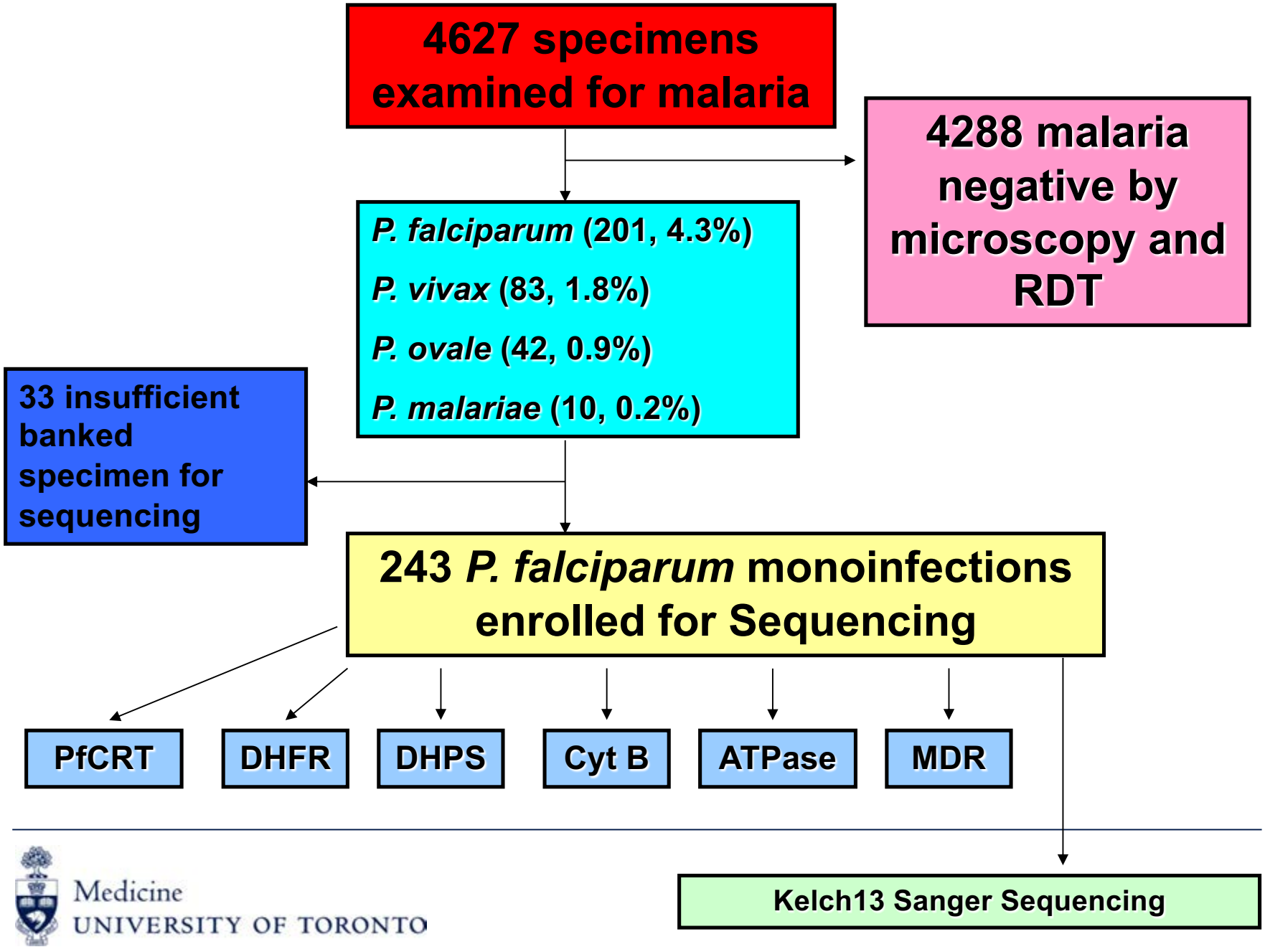


Objective and Rationale

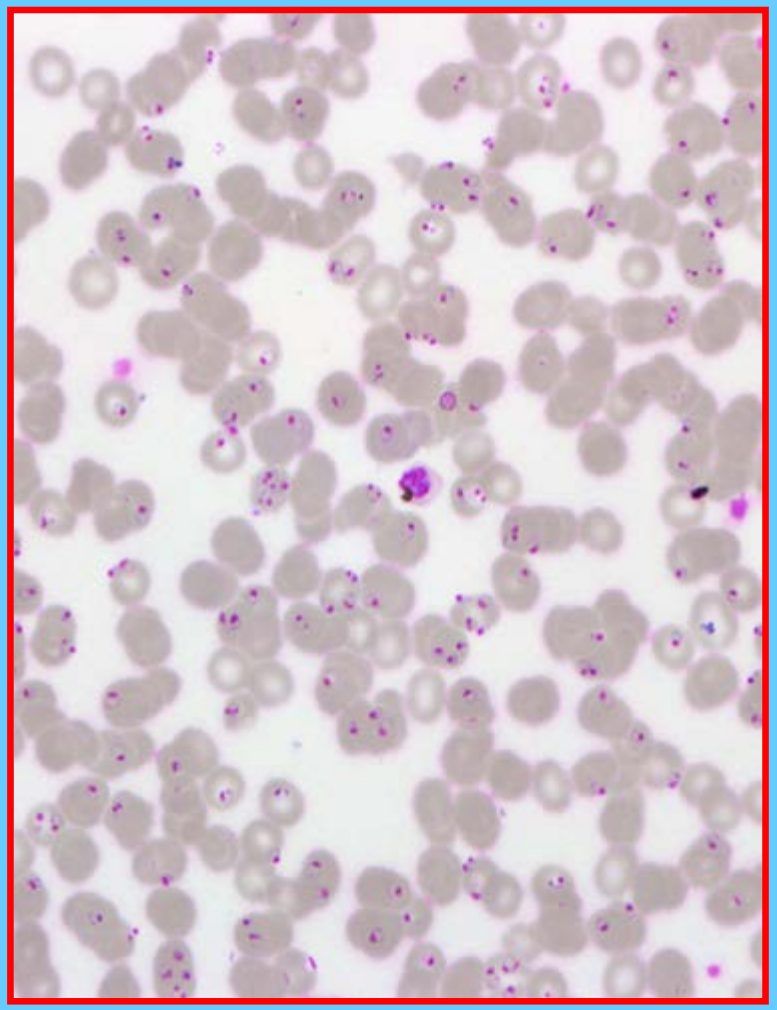
- *Plasmodium falciparum*:
  - Potentially fatal
  - Imported with regularity to Ontario
  - Almost always treated in Canada with second-line agents that may be difficult to absorb and to which isolates elaborate resistance
- We aimed to understand the frequency and pattern of SNPs conferring resistance to common antimalarials in isolates of *P. falciparum*

METHODS




4627 specimens examined for malaria
4288 malaria negative by microscopy and RDT
P. falciparum (201, 4.3%)
P. vivax (83, 1.8%)
P. ovale (42, 0.9%)
P. malariae (10, 0.2%)
33 insufficient banked specimen for sequencing
243 P. falciparum monoinfections enrolled for Sequencing
PfCRT
DHFR
DHPS
Cyt B
ATPase
MDR
Kelch13 Sanger Sequencing

RESULTS



RESULTS

- *P. falciparum* isolates confirmed by PCR as monoinfections by enrolment period
  - Jul 2008 - Jun 2009: 75 cases
  - Jul 2013 - Jun 2014: 79 cases
  - Jul 2017 - Jun 2018: 89 cases

RESULTS

Demographic and Parasitologic Characteristics	Enrolment Period				P-value
	Total (N=243)	2008 - 2009 (N=75)	2013 - 2014 (N=79)	2017 - 2018 (N=89)	
Mean Age, years (SD)	39.2 (18.3)	40.9 (17.1)	38.0 (16.6)	38.9 (20.6)	0.61
Female sex, No (%)	66 (28.1)	18 (25.4)	19 (25.0)	29 (33.0)	0.47
Parasitemia, percent (median, range)	0.3 (0.01-24.0)	0.3 (0.01-17.8)	0.3 (0.01-12.0)	0.7 (0.01-24.0)	0.1

RESULTS

Region of Acquisition Number (%)	Total (N=243)	2008-2009 (N=75)	2013-2014 (N=79)	2017-2018 (N=89)	P-value
West Africa	81 (33.3)	20 (26.7)	17 (21.5)	38 (42.7)	0.14
East Africa	18 (7.4)	4 (5.3)	8 (10.1)	6 (6.7)	
Africa, other	33 (13.5)	12 (16)	13 (16.5)	8 (9)	
Caribbean	1 (0.4)	1 (1.3)	0 (0)	0 (0)	
Southeast Asia	5 (2.1)	0 (0)	4 (5.1)	1 (1.1)	
South America	1 (0.4)	0 (0)	0 (0)	1 (1.1)	
Unknown	104 (42.8)	33 (44)	36 (45.6)	35 (39.3)	

RESULTS - PfCRT

Gene	Frequency of MT Genotype (%)			p-Value
	2008 - 2009	2013 - 2014	2017 - 2018	
CRT K76T	56.8	38.4	PND	**0.03**
CRT N75E	52	37.5	PND	0.10
CRT M74I	52	37.5	PND	0.10
CRT C72S	1.4	1.3	PND	1.00

- Genetic markers of resistance to **Chloroquine** appear to be diminishing over time

RESULTS - DHFR

Gene	Frequency of MT Genotype (%)			p-Value
	2008 - 2009	2013 - 2014	2017 - 2018	
DHFR C50R	0	0	1.3	1.0
DHFR N51I	88	92.3	95.7	0.55
DHFR C59R	90.7	94.9	93.3	0.58
DHFR S108N	89.3	97.3	100	**<0.001**
DHFR I164L	1.4	0	PND	0.49

- Genetic markers of resistance to Proguanil appear to be **increasing** over time

RESULTS – Cyt b

Gene	Frequency of MT Genotype (%)			p-Value
	2008 - 2009	2013 - 2014	2017 - 2018	
CytB Y268S	0	0	0	1.00
CytB Y268C	0	0	0	1.00
CytB Y268N	0	0	0	1.00

- Genetic mutants that would be resistant to **atovaquone** were NOT detected over time

RESULTS - ATPase

Gene	Frequency of MT Genotype (%)			p-Value
	2008 - 2009	2013 - 2014	2017 - 2018	
ATPase A623E	1.3	1.3	0	0.55
ATPase S769N	0	0	0	1.00

- ATPase mutations conferring resistance to **artemether** were very rare

RESULTS - MDR

Gene	Frequency of MT Genotype (%)			p-Value
	2008 - 2009	2013 - 2014	2017 -2018	
MDR1 N86Y	42.7	14.3	7.4	**<0.001**
MDR1 Y184F	49.3	60.3	53	0.40
MDR1 S1034TR	0	0	PND	1.00
MDR1 N1042D	0	0	0	1.00
MDR1 D1246Y	17.6	3.8	3.5	**<0.003**
MDR1 copy #	1.1 (0.83 – 1.4)	1.1 (0.31 – 2.3)	1.9 (0.73 – 5.4)	**<0.001**

- Mixed results: reduced frequency of some multidrug mutations, but increased MDR copy # over time

RESULTS – Kelch 13

Gene	Frequency of MT Genotype (%)			p-Value
	2008 - 2009	2013 - 2014	2017 - 2018	
K13 above position 440	0	0	0	1.0

- 20 SNPs in the Kelch13 gene that are associated with artemisinin resistance were **NOT** detected

5 Key Points – *Pf* Resistance

- Withdrawal of chloroquine from country-level formularies may have translated into reduced CRT and MDR mutations over time
- DHFR mutations are increasing over time, as is MDR copy number
- Genetic markers of resistance to atovaquone and the artemisinins are rare
- Findings biased towards West African isolates and not generalizeable to all Pf imports to Canada
- Prevalence of resistance to common antimalarials among *P. falciparum* isolates imported to Ontario necessitates ongoing surveillance

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- Ruwandi Kariyawasam, PhD Candidate, University of Toronto
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- Public Health Ontario Laboratory

Public Health Ontario
PARTNERS FOR HEALTH

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THANK YOU!

Pyrosequencing Readout = Pyrogram