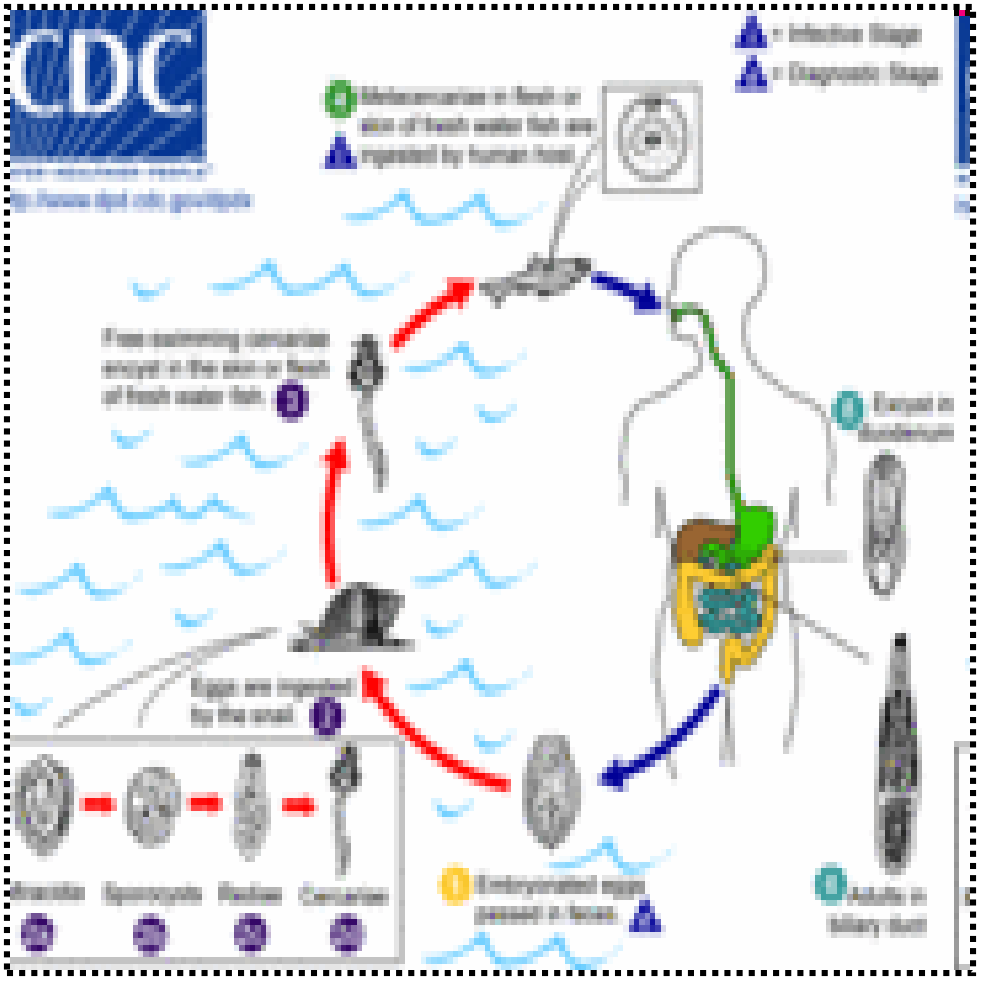
Opisthorchiasis in Laos: studies in central and southern Lao PDR

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Life cycle



Opisthorchis viverrini

- Tropism:
  - ✓ Bile duct (liver)
- Eggs via stool
  - ✓ Shouldered, operculate eggs
- 2 intermediate hosts (IH):
  - ✓ 1st IH: Fresh water snails
  - ✓ 2nd IH: specific species (cyprinoid, freshwater fish:
    → Human infection: consumption raw or undercooked 2nd IH

History in Laos

- ***O. viverrini*:**
  - First discovery in Laos in 1929 with prevalence of:
    - Vietiane: 15%
    - Khammouane: 23%
- **After discovery:**
  - Almost 100 year later, *O. viverrini* remains highly prevalent in the country
  - However, little data on the disease is available in the country
  - Most of information related to *O. viverrini* obtained from a neighboring country of Thailand

Partnership Project

- Research partnership, since 2005
  - National Institute of Public Health, Vientiane (Mahosot, CMPE, …),
  - & Swiss Tropical and Public Health Institute

- Focus: food-borne and water-borne trematodiasis (e.g., opisthorchiasis):

  Parasitology ➔ epidemiology ➔ morbidity

  ➔ treatment ➔ control

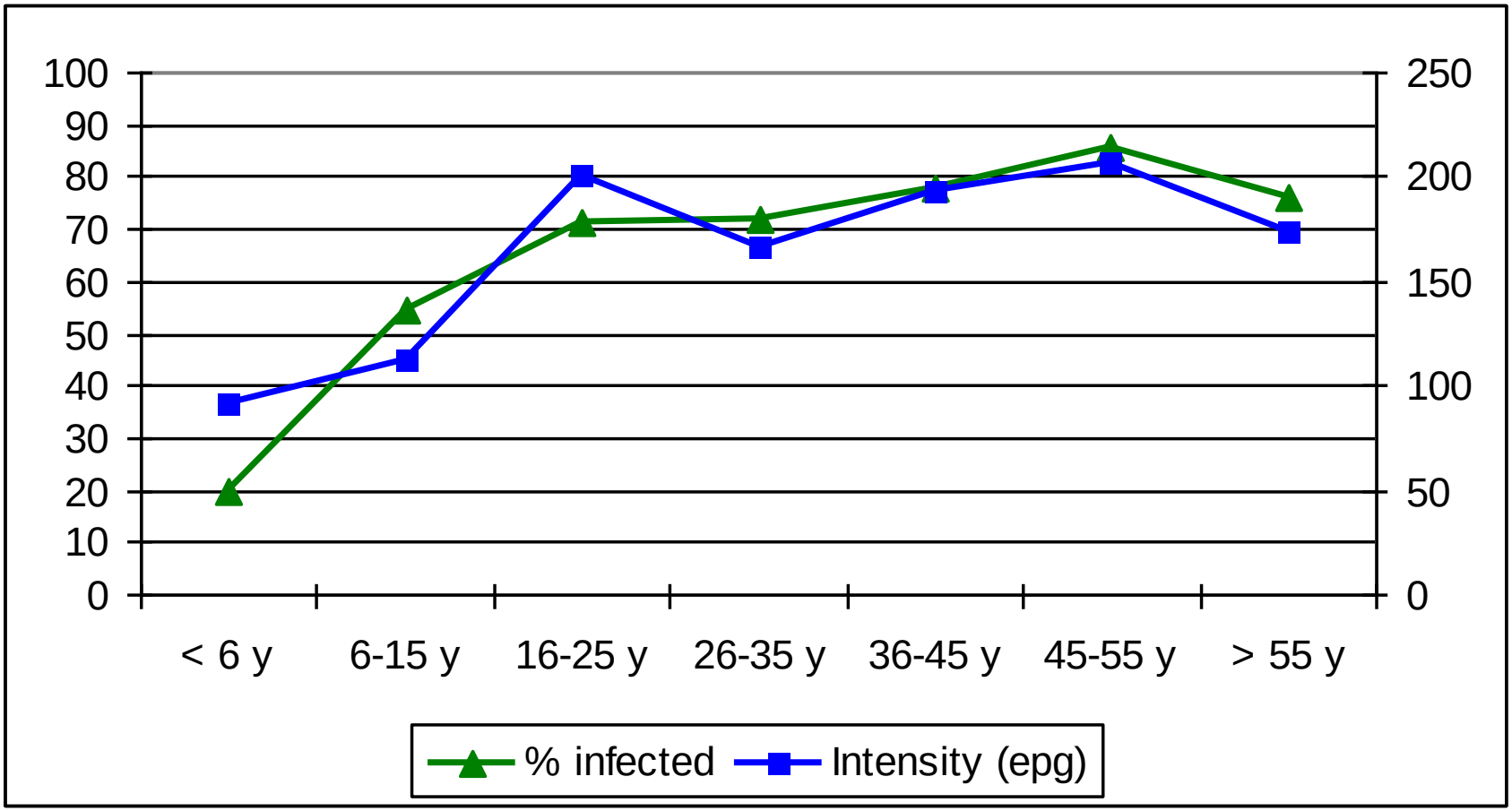
(1) Epidemiology

Prevalence of intestinal parasitic infections in 2006

(2 techniques: Kato-Katz, FECT, n = 669).

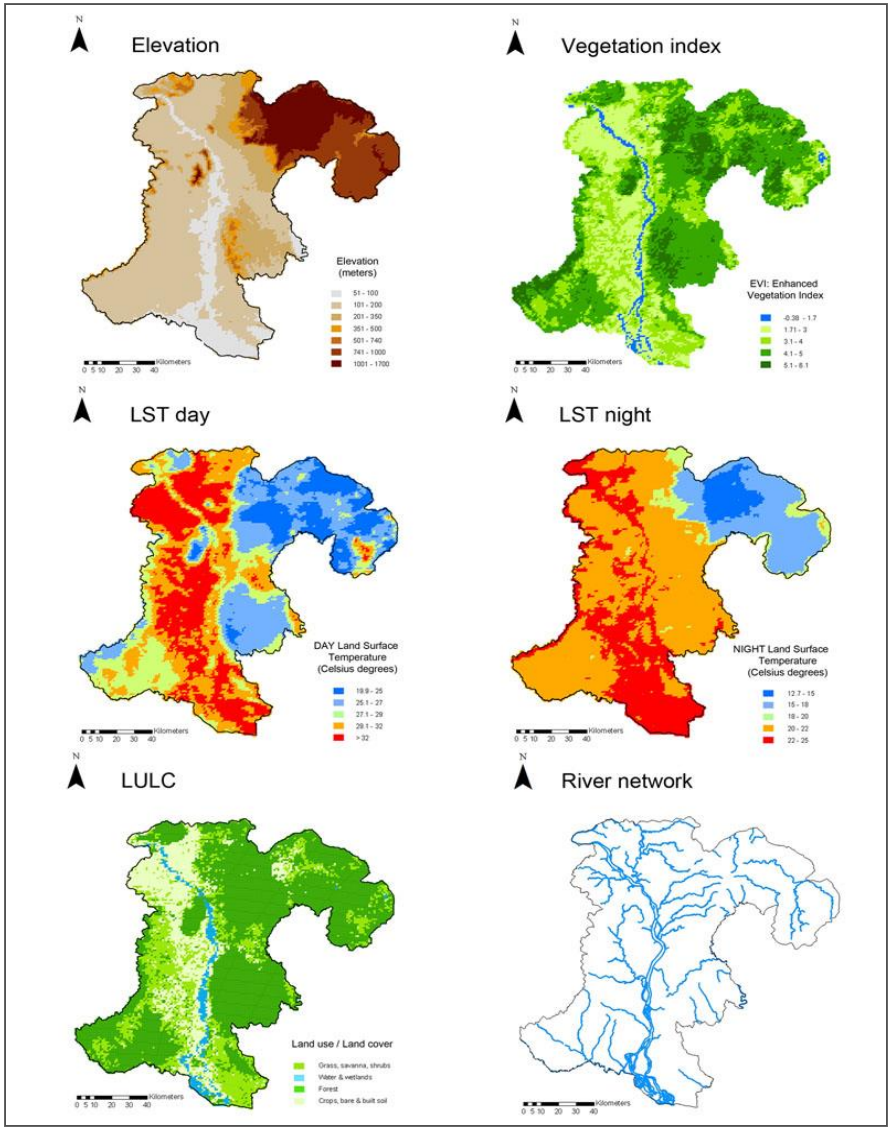
Parasites	Prevalence (95% CI)	Study settings		
	(n = 669)	Khong (n = 225)	Mounlapa-mok (n = 232)	Paksong (n = 212)
Trematodes				
O. viverrini	64.3 (60.6–67.9)	92.0	90.9	5.7**
S. mekongi	24.2 (21.0–27.5)	68.0	3.9	0.0**
Echinostoma spp.	6.0 (4.2–7.8)	12.9	4.7	0.0**
Nematodes				
Hookworm	76.8 (73.6–80.0)	71.1	66.0	94.8**
A. lumbricoides	31.7 (28.2–35.2)	7.1	6.0	85.9**
T. trichiura	25.0 (21.7–28.3)	13.3	8.2	55.7**
S. stercoralis	4.6 (3.0–6.0)	9.8	3.9	0.0**
E. vermicularis	3.6 (2.2–5.0)	3.1	4.4	3.3
Cestodes				
Taenia spp.	3.7 (2.3–5.2)	1.8	4.3	5.2
H. diminuta	2.7 (1.5–3.9)	0.0	0.0	8.5**
D. latum	0.5 (<0.1–0.9)	0.5	0.9	0.0
Intestinal protozoa				
B. hominis	13.6 (11.0–16.2)	19.6	6.5	15.1**
E. coli	7.2 (5.2–9.1)	3.0	8.4	10.4**
G. intestinalis	4.9 (3.3–6.6)	3.0	5.9	6.1
E. nana	0.6 (<0.1–1.2)	0.6	0.4	0.5

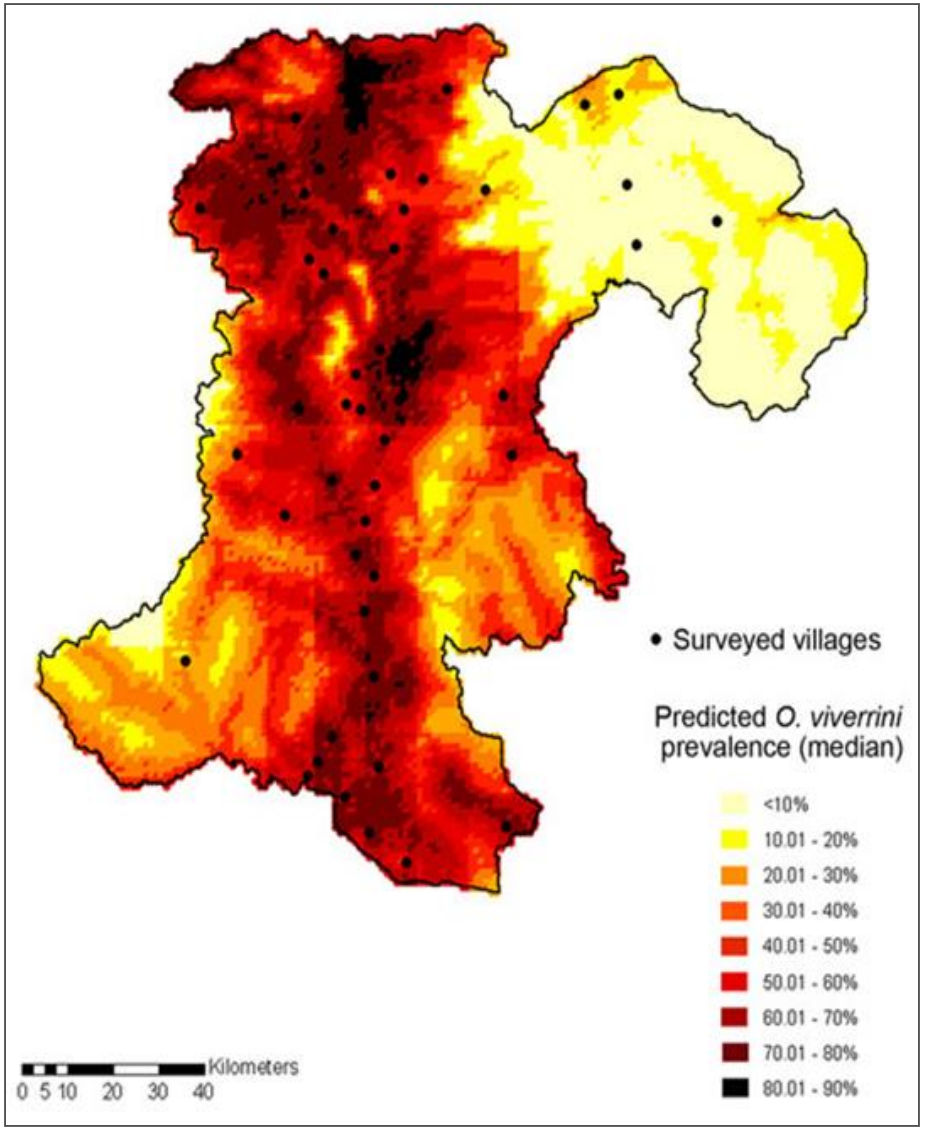
(1) Epidemiology (cont')



(Sayasone et al 2007)

(1) Epidemiology (cont')





(Forrer et al 2012)

(2) DIAGNOSIS

Helminth species	Sensitivity of different Kato-Katz thick smear sampling efforts							
	Baseline survey ($n=90$)				Days 28–30 posttreatment follow-up ($n=66$)			
	1 stool 1 smear	1 stool 3 smears	3 stools 1 smear	3 stools 3 smears	1 stool 1 smear	1 stool 3 smears	3 stools 1 smear	3 stools 3 smears
	n (%)	n (%)	n (%)	n ('gold' standard)	n (%)	n (%)	n (%)	n ('gold' standard)
S. mekongi	36 (45.6)	54 (68.4)	65 (82.3)	79 (100)	2 (14.3)	3 (21.4)	8 (57.1)	14 (100)
O. viverrini	74 (83.1)	84 (94.4)	84 (94.4)	89 (100)	2 (18.2)	4 (36.4)	5 (45.4)	11 (100)

Study was carried out among 93 children in primary and secondary schools on Don Long Island, Khong district, Champasack province, Lao PDR in February and Mar 2007. Sensitivity is compared before ($n=90$) and after PZQ administration ($n=66$), using the maximum sampling effort as the diagnostic 'gold' standard for the following sampling efforts: 1×1 sampling effort examines the first Kato-Katz thick smear only; 1×3 examines the first stool specimen by triplicate Kato-Katz thick smea 3×1 examines 3 stool specimens by a single Kato-Katz thick smear for each specimen.

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(3) Morbidity

Indicator measurements	Intensity of infection		
	Adult worms discovered from purging stool sample		
	Mean	IRR (95%, CI)	*P*-value
Self-report morbidity			
Right Upper Quadrant pain			
No	87	1.0	
Yes	225	2.6 (1.1 – 6.3)	0.022
Abdominal discomforts			
No	50	1.0	
Yes	135	2.7 (1.4 – 5.2)	0.004
Physical examination			
Jaundice			
No	103	1.0	
Yes	235	2.3 (1.1 – 5.9)	0.011
Ultrasound examination			
Intra-hepatic bile duct dilation			
No	92	1.0	
Yes	1188	12.9 (5.2 – 20.2)	0.003
Common bile duct dilatation			
No	94	1.0	
Yes	383	4.1 (1.1 – 19.1)	0.026
Any liver fibrosis			
No	87		
Yes	265	3.1 (1.1 – 8.2)	0.012
Size of left liver lobe			
≤ 2 SD	140	1.0	
> 2 SD	67	0.5 (0.3 – 0.9)	0.023

(Sayasone et al 2012)

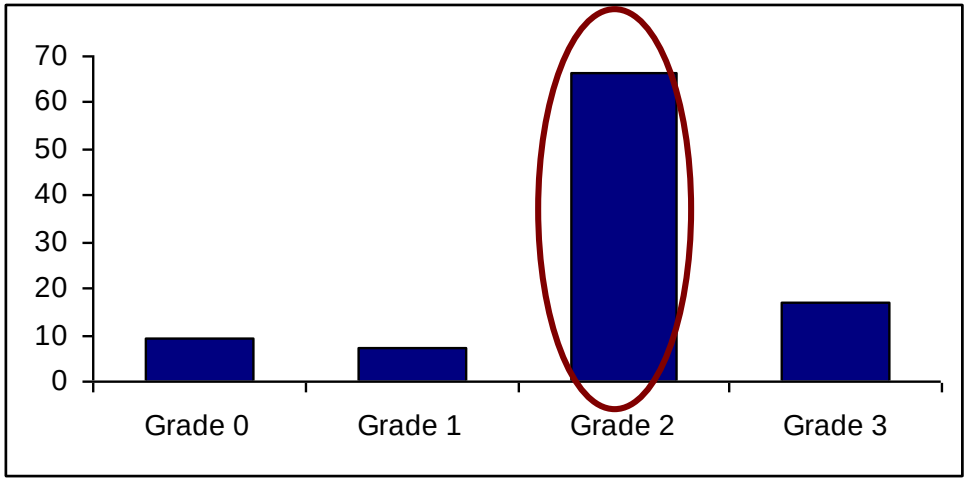
(3) Morbidity cont`



- **Patient with *Opisthorchis* infection**
- **Age > 20 years**
- **n = 431**

(Soukhathammavong Ayé et al 2011)

(3) Morbidity (cont`)

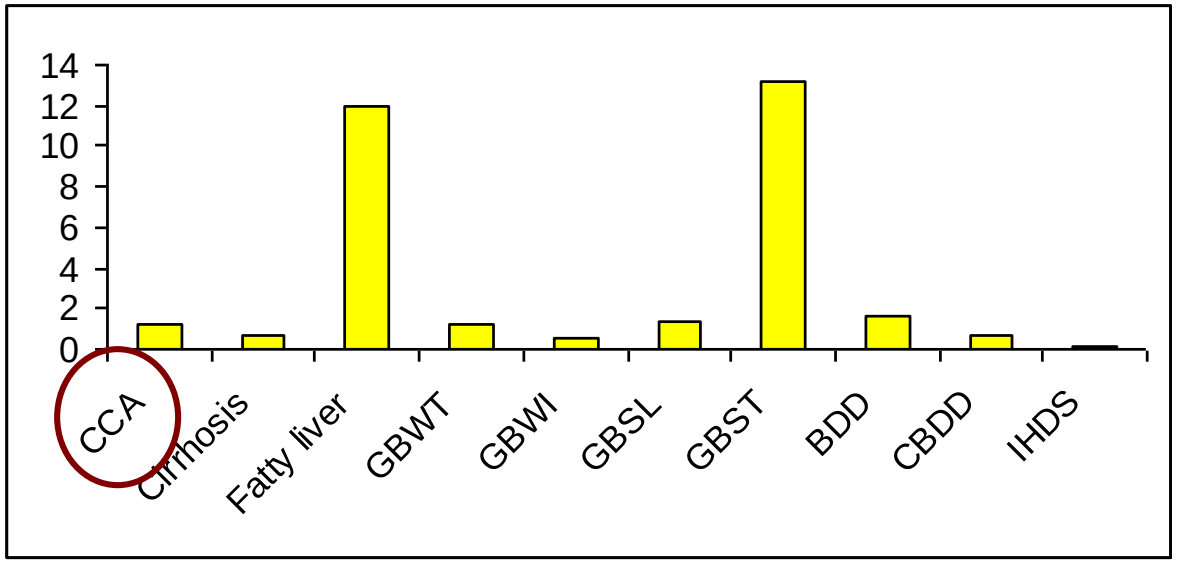


Grade 0- normal parenchymal liver

Grade 1- mild peridutal fibrosis

Grade 2- moderate periductal fibrosis

Grade 3- Advanced periductal fibrosis



CCA- suspected cholangiocarcinoma

GBWT- Gallbladder wall thickening

GBWI- Gallbladder wall irregular

GBSL- Gallbladder sludge

GBST- Gallbladder stone(s)

BDD- Bile duct dilated

CBDD- Common bile duct dilated

IHDS- Intrahepatic duct stone(s)

(Soukhathammavong Ayé et al 2011)

(4) Treatment-Efficacy of PZQ against *Ov*

Table 3.0. viverrini infection intensity before (D0) and posttreatment (D28) and egg reduction rate for maximum and minimum diagnostic effort.

Treatment		Maximum diagnostic effort (363 datasets, *n* = 64)						Minimum diagnostic effort (161 datasets, *n* = 85)					
	Infection intensity	n	# cured	% cured	D0 GM (EPG)	D28 GM (EPG)	ERR (%)	n	# cured	% cured	D0 GM (EPG)	D28 GM (EPG)	ERR (%)
40 mg/kg	All infections	35	25	71.4	307.4	0.99	99.7	35	33	94.3	411.8	0.26	99.94
	1–999 EPG	24	18	75.0	96.4	0.48	99.5	24	24	100	142.3	0	100
	1,000–9,999 EPG	9	6	66.7	2,460	2.1	99.92	9	7	77.8	2,817	1.4	99.95
	≥ 10,000 EPG	2	1	50.0	27,344	9.0	99.97	2	2	100	23,778	0	100
75 mg/kg	All infections	29	28	96.6	308.1	0.05	99.98	35	35	100	383.7	0	100
	1–999 EPG	20	20	100	103.7	0.00	100	24	24	100	181.1	0	100
	1,000–9,999 EPG	9	8	88.9	3,424.2	0.16	100	10	10	100	1,568	0	100
	≥ 10,000 EPG	0	0	na	na	na	na	1	1	100	18,816	0	100

EPG, eggs per gram of stool; ERR, egg reduction rate; GM, geometric mean; na, not applicable.

doi:10.1371/journal.pntd.0001726.t003

Levis L *et al*. 2012

(4) Tribendimidine trial (proof of concept 2009)

- Why do we need to conduct this trial?
  - Today, treatment of opisthorchiasis based on a single drug (PZQ)
  - As shown in our study, PZQ does not have a curate of 100% for treatment of *O. viverrini*
  - Long run of MDA program might encourage the resistance
  - Side effects of PZQ → low coverage of MDA

→ ***Need an alternative drug*** to secure the treatment

(4) Proof of concept trial (cont')

	Mefloquine	Artesunate	Mefloquine–artesunate	Tribendimidine	Praziquantel
Kato-Katz thick smear technique					
Opisthorchis viverrini					
Patients cured/patients infected	0/19 (0)	1/24 (4%)	1/17 (6%)	19/24 (79%)	14/22 (64%)
GM eggs per g of stool (range)	1114·1 (498·9–2488·1)	1229·4 (625·1–2417·7)	669·1 (320·8–1395·7)	44·7 (11·6–171·7)	43·1 (16·6–111·7)
Egg reduction rate	28·7%	31·5%	36·6%	99·3%	98·4%
Co-infection with hookworm					
Patients cured/patients infected	3/15 (20%)	4/20 (20%)	2/12 (17%)	11/17 (65%)	2/17 (13%)
Formalin-ether concentration technique					
O viverrini					
Patients cured/patients infected	4/19 (21%)	8/24 (33%)	8/17 (47%)	23/24 (96%)	21/22 (95%)
GM egg per g of stool (range)	182·3 (77·0–433·5)	156·2 (82·2–297·0)	114·0 (69·2–187·3)	NA	NA
Egg reduction rate	71%	60%	75%	99%	99%

Data are number (%) of patients, unless otherwise indicated. GM=geometric mean. NA=not applicable.

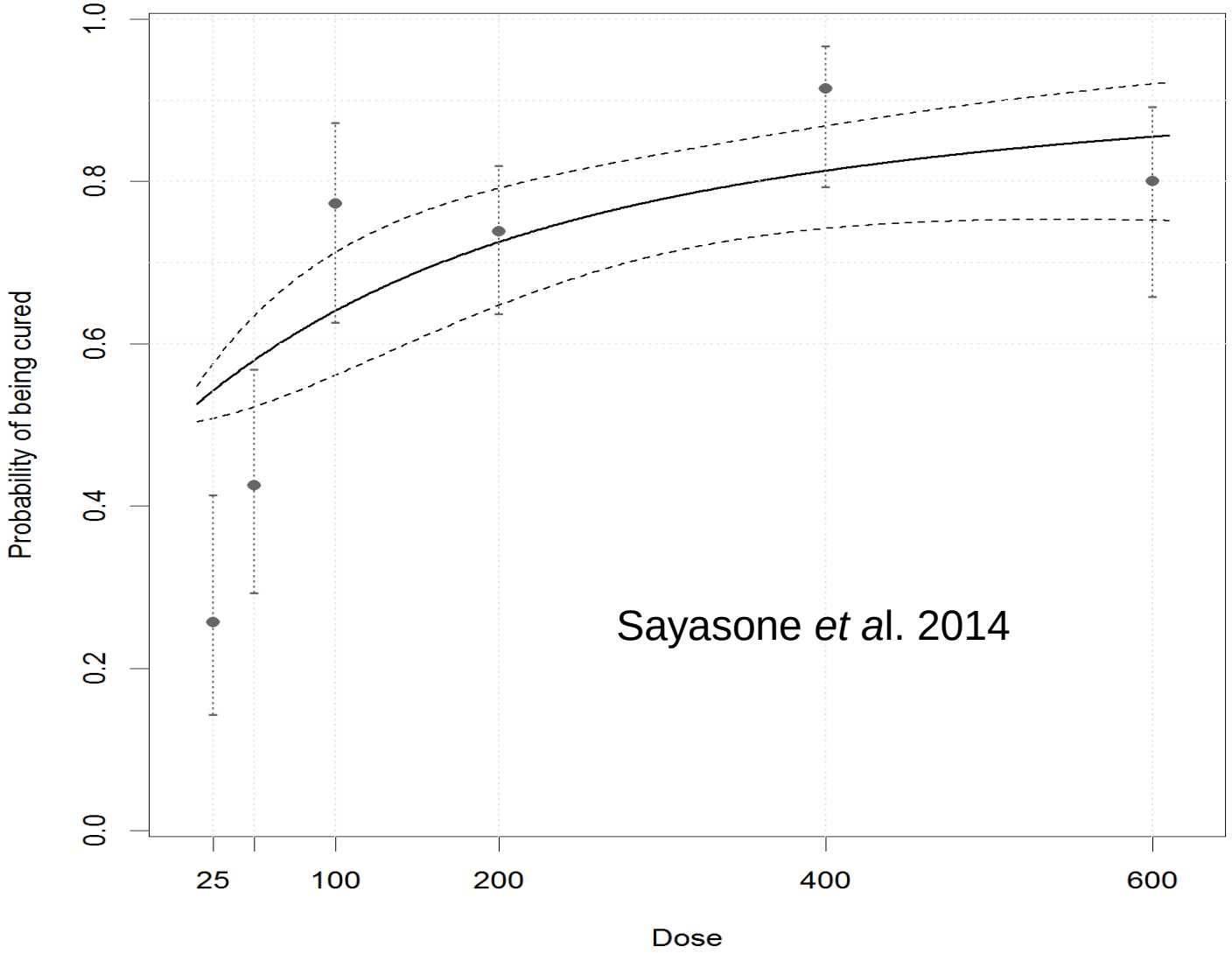
Table 4: **Per-protocol analysis of prevalence and cure rate of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel treatment in school children infected with *Opisthorchis viverrini* at follow-up**

Soukhathammavong *et al*. 2011

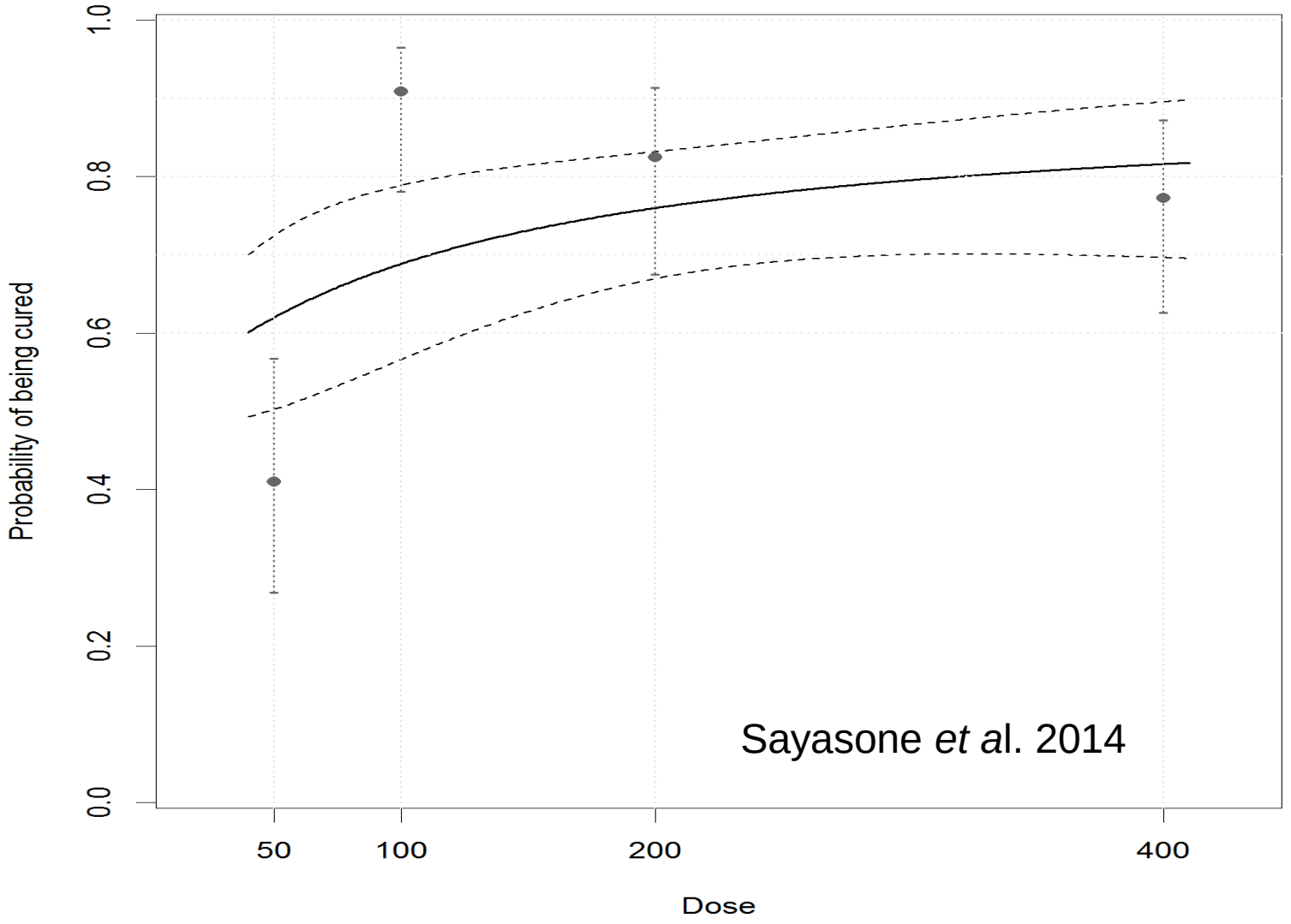
(4) Further TBM trial

- 2012-2014, we conducted a phase 2 tribendimidine trial for treatment of liverfluke in Southeast Asia:
  - Assessing the most effective dose and Pharmacokinetic of TBM
  - Assessing the efficacy and safety of TBM

(4) Trial Results in adults (Phase 2)



(4) Trial Results in Children (Phase 2)



Conclusions

- ***Opisthorchis viverrini* infection in Laos:**
  - ❑ Infection is associated with hepatobiliary morbidity ➔ **treatment required**
  - ❑ CCA not documented in Lao communities, but suspected cases seen
  - ❑ Repeated/long time of PZQ mass-treatments bear risk for resistance➔ **alternative drug needed**
  - ❑ High prevalence and infection intensity→ need a concerted action for disease control → **reduced community burden**

- **What next?**
  - ❑ Develop alternative, sustainable interventions (i.e., ecoHealth approaches) → testing (IDRC)
  - ❑ Trial to assess the efficacy of different doses of PZQ

Thank you for your attention

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