Incidence and Risk Factors of Hookworm Infection in Population at Baan Tungsorhongsar, Thakadan, Sanamchaikhet, Chachaoengsao

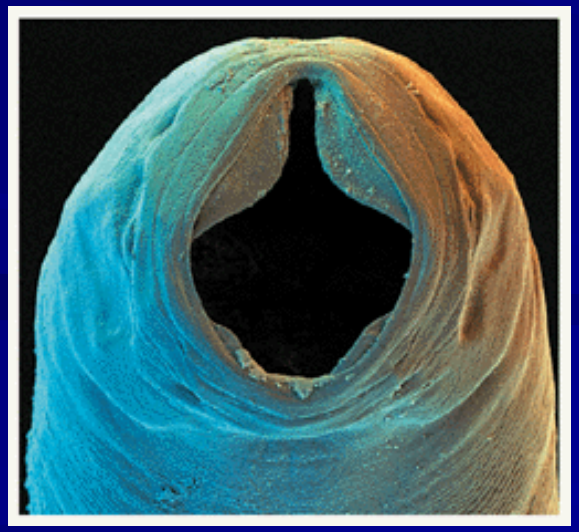
Introduction

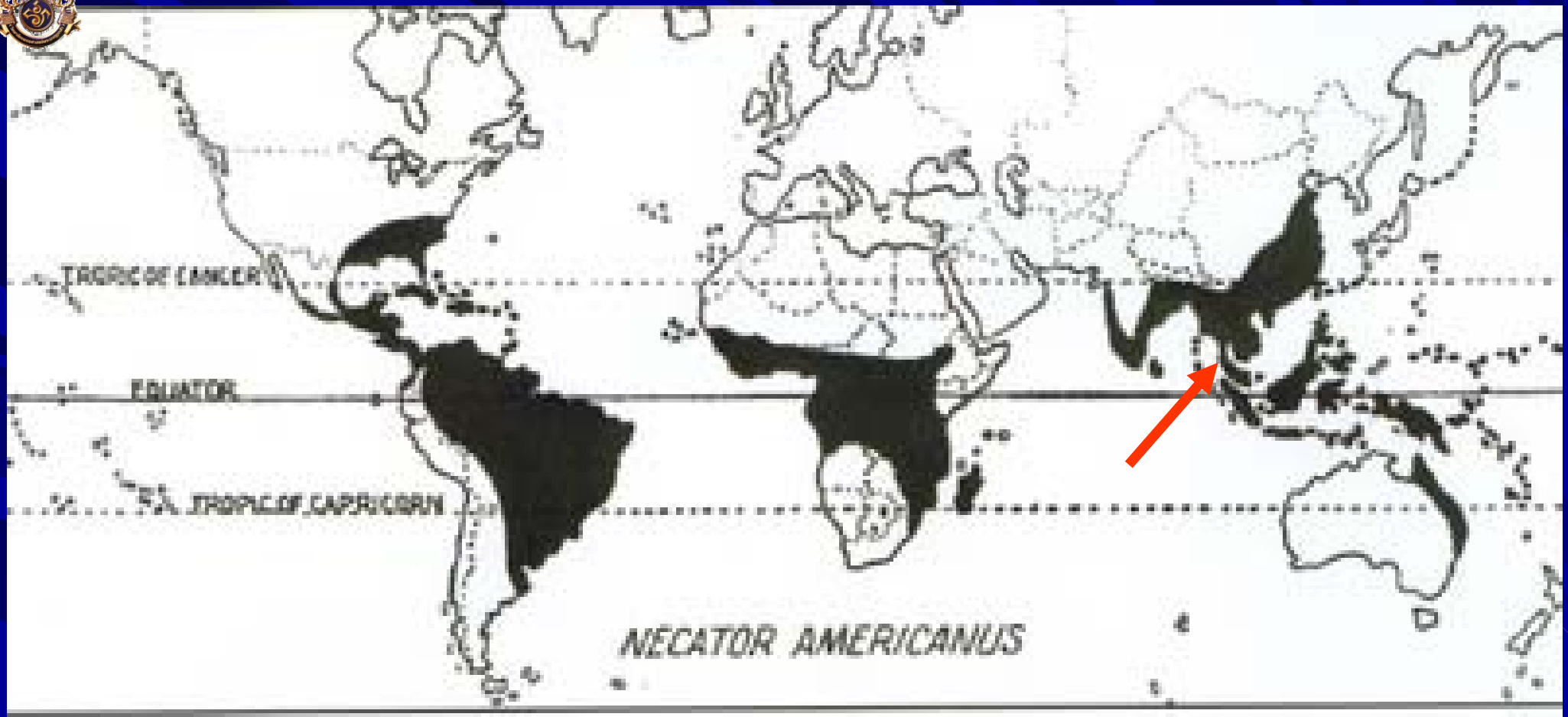
- Hookworm infection is one of the most common chronic parasitic infection.
  - 740 million cases worldwide
  - Most are impoverished people
- The greatest number of hookworm cases occurred in Asia, followed by sub-Saharan Africa.

(de Silva et al. , Trends Parasitol 2003; 19:547-51)

Introduction

- There are 2 species of human hookworms
  - *Necator americanus*
  - *Ancylostoma duodenale*





World-wide distribution of endemic Hookworm (Necator americanus) .
(Mississippi Historical Society)

Introduction

- 3 species of animal hookworms
  - *Ancylostoma ceylanicum*
  - *Ancylostoma caninum*
  - *Ancylostoma braziliense*
- Cause zoonotic disease in human
  - Cutaneous larva migrans

(Prociv et al., Acta Trop 1996; 62:23-44)

Introduction

- Hookworm infection is still a major public health problem in Thailand.

Introduction

- Hookworm infection rates in rural areas of Sakaew, Nan and Kanchanaburi were 5.0%, 25.5% and 46.2%, respectively.

(Maipanich et al., J Trop Med Parasitol 2004; 27:51-8)

Introduction

- Patients with chronic hookworm infection have greater risk of
  - Iron-deficiency anemia
  - Growth retardation in children
  - Low birth weight
  - Impaired lactation
  - Increased maternal mortality

Introduction

- A cross-sectional study in 2005 at Tungsorhongsar, Chacheongsao province
- Prevalence of hookworm infection was 12%.

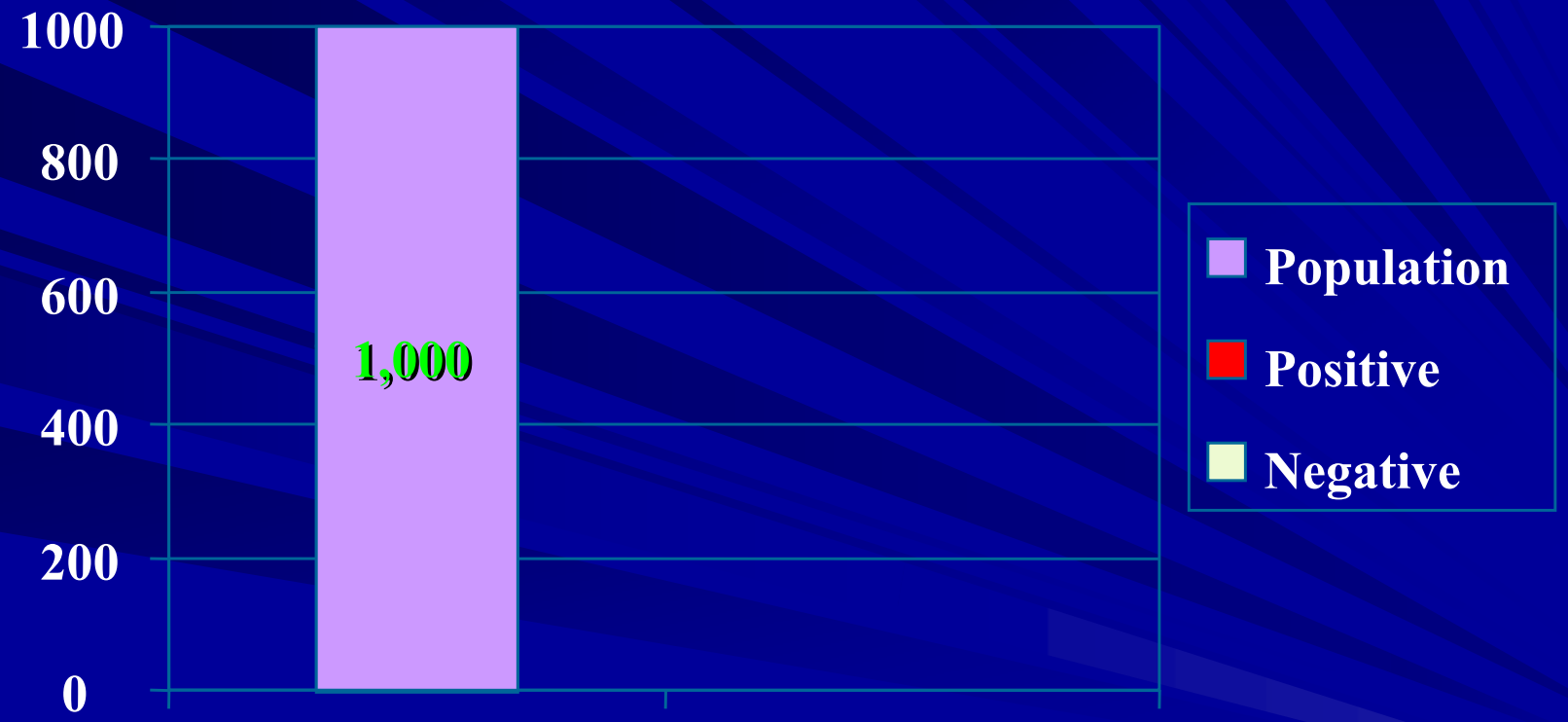
Introduction

- Information regarding epidemiology is required to design the more effective public health intervention.
- True risks can not be obtained from cross-sectional studies.

Objectives

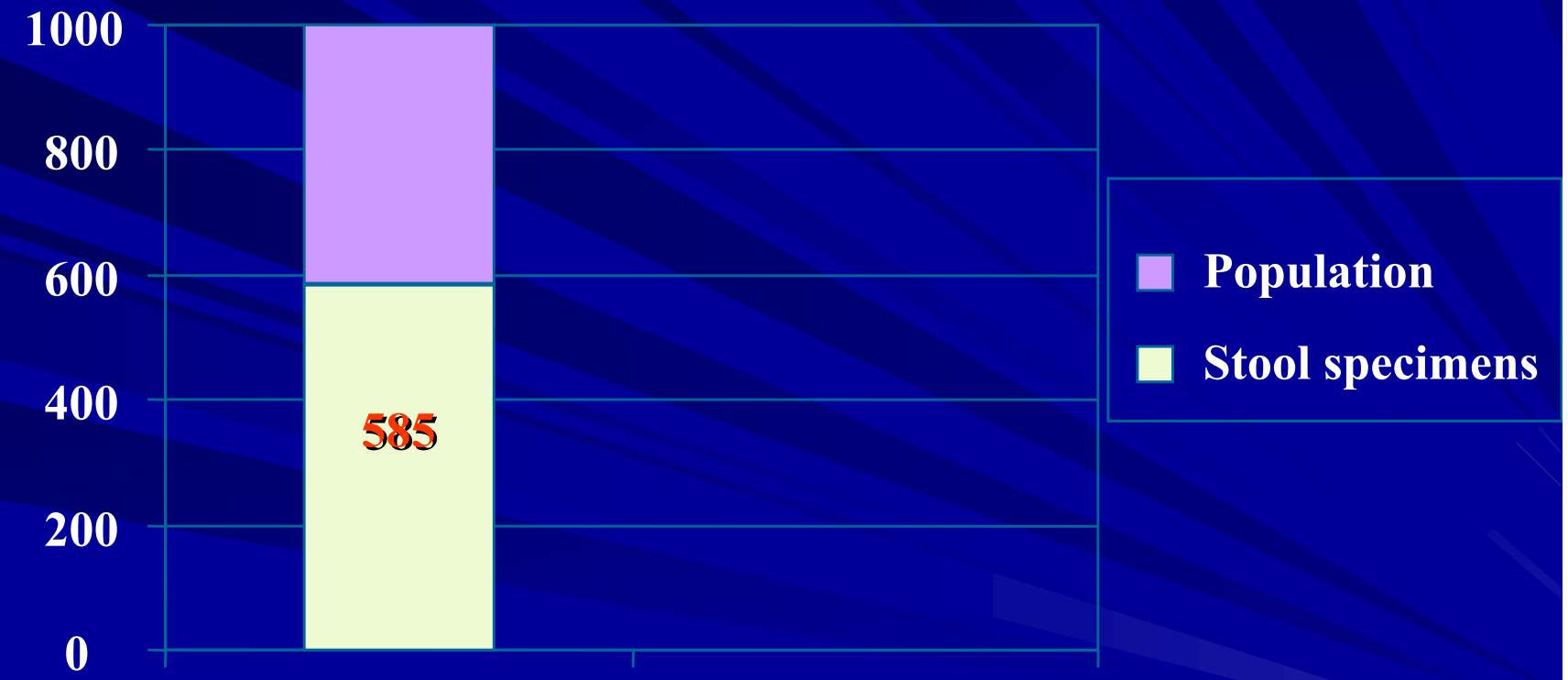
- To identify the incidence and risk factors of hookworm infection.
- To identify species of hookworm using PCR technique.

Materials and Methods



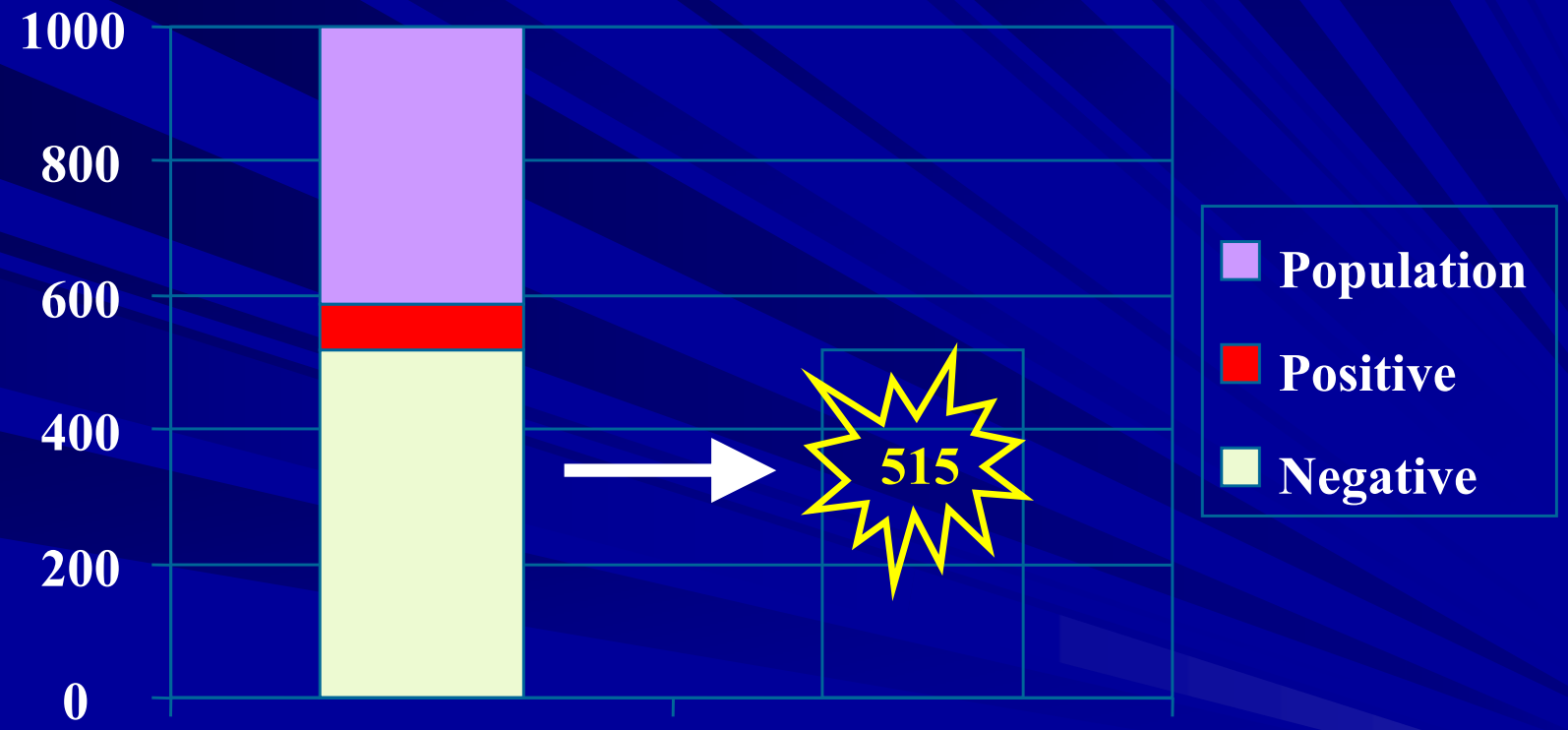
Population

Materials and Methods



Population

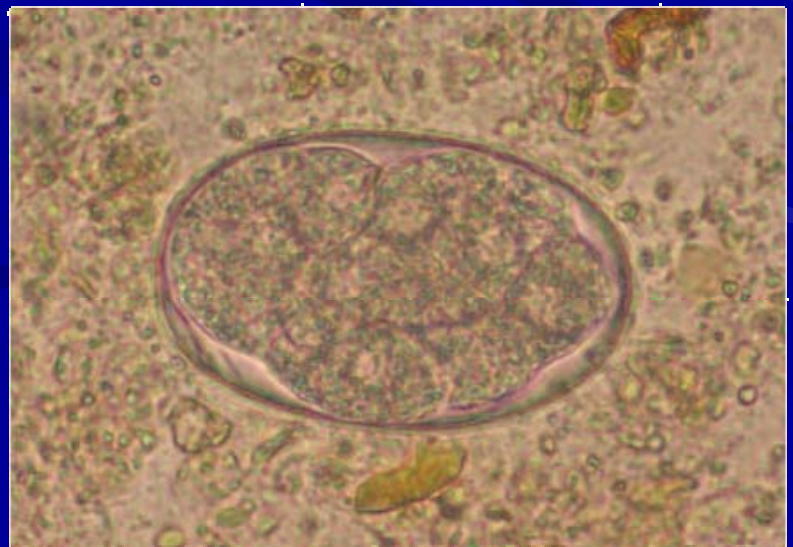
Materials and Methods



Population

Materials and Methods

- Stool examination
  - Simple smear
  - Kato thick technique



Species identification
PCR Amplification

- **RTHHW1F** GAT GAG CAT TGC WTG AAT GCC G
- **RTHHW1R** GCA AGT RCC GTT CGA CAA ACA G

- **Conditions:**

Denature	94˚C	2 min	1 cycle
Annealing	64˚C	1 min	
Extension	72˚C	2 min	
Denature	94 ˚C	30 s	45 cycles
Annealing	64 ˚C	30 s	
Extension	72 ˚C	30 s	
Final extension	72 ˚C	7 min	

(Traub RJ et al., unpublished method)

PCR amplification of rRNA gene

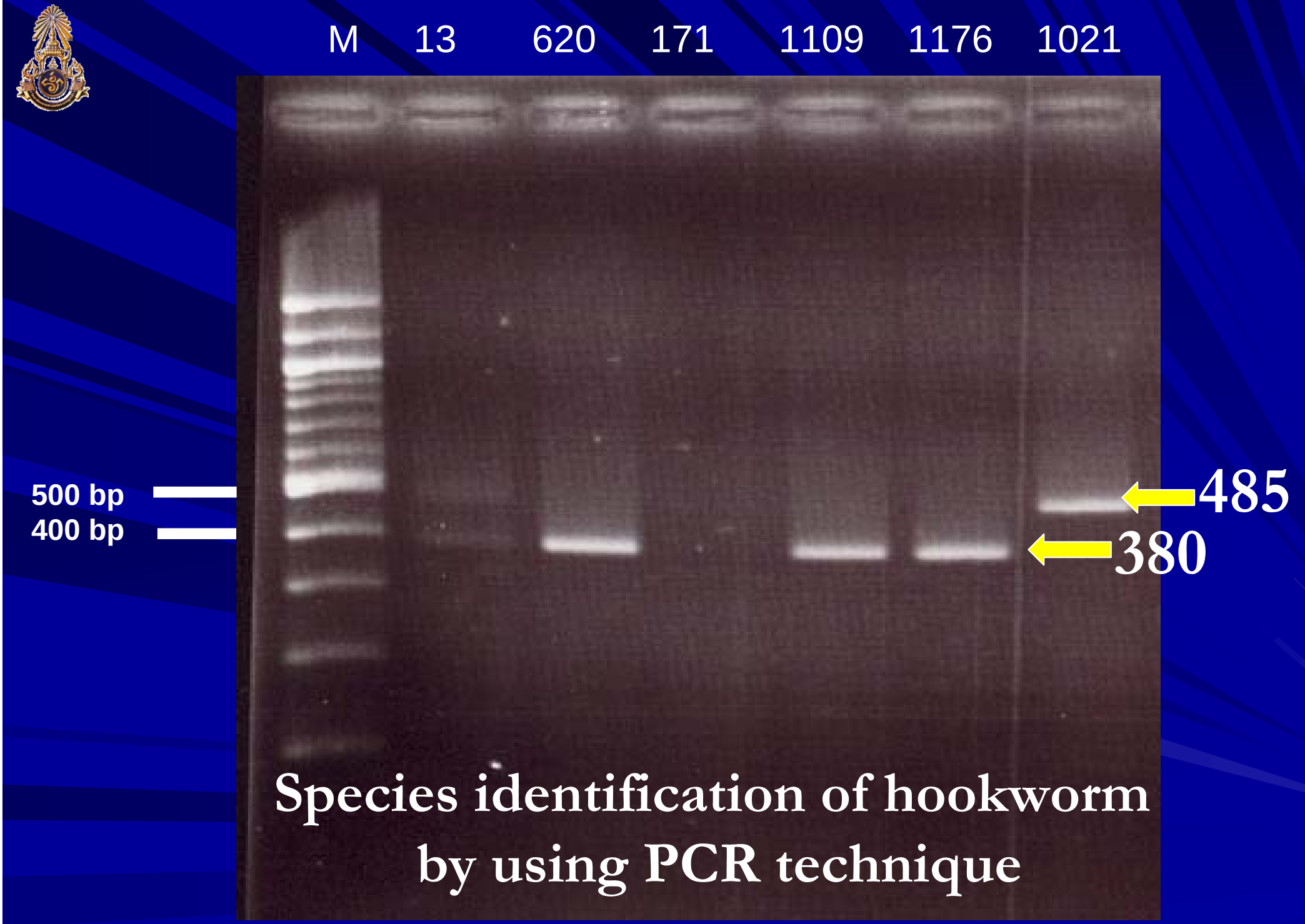
485 bp

= *Necator americanus*

380 bp

= *Ancylostoma* spp.

DNA sequencing


M
13
620
171
1109
1176
1021
500 bp
400 bp
485
380
Species identification of hookworm
by using PCR technique

Materials and Methods

- Standardized Questionnaire
  - Demographic data
  - Risk behavior
  - Health status

Materials and Methods

- To study the distribution of hookworm infection in the village, Geographic Information System (GIS) Technology was performed.

Data analysis

- Statistical analysis of risk factors was performed using univariate and Poisson-multivariate analysis by STATA program.

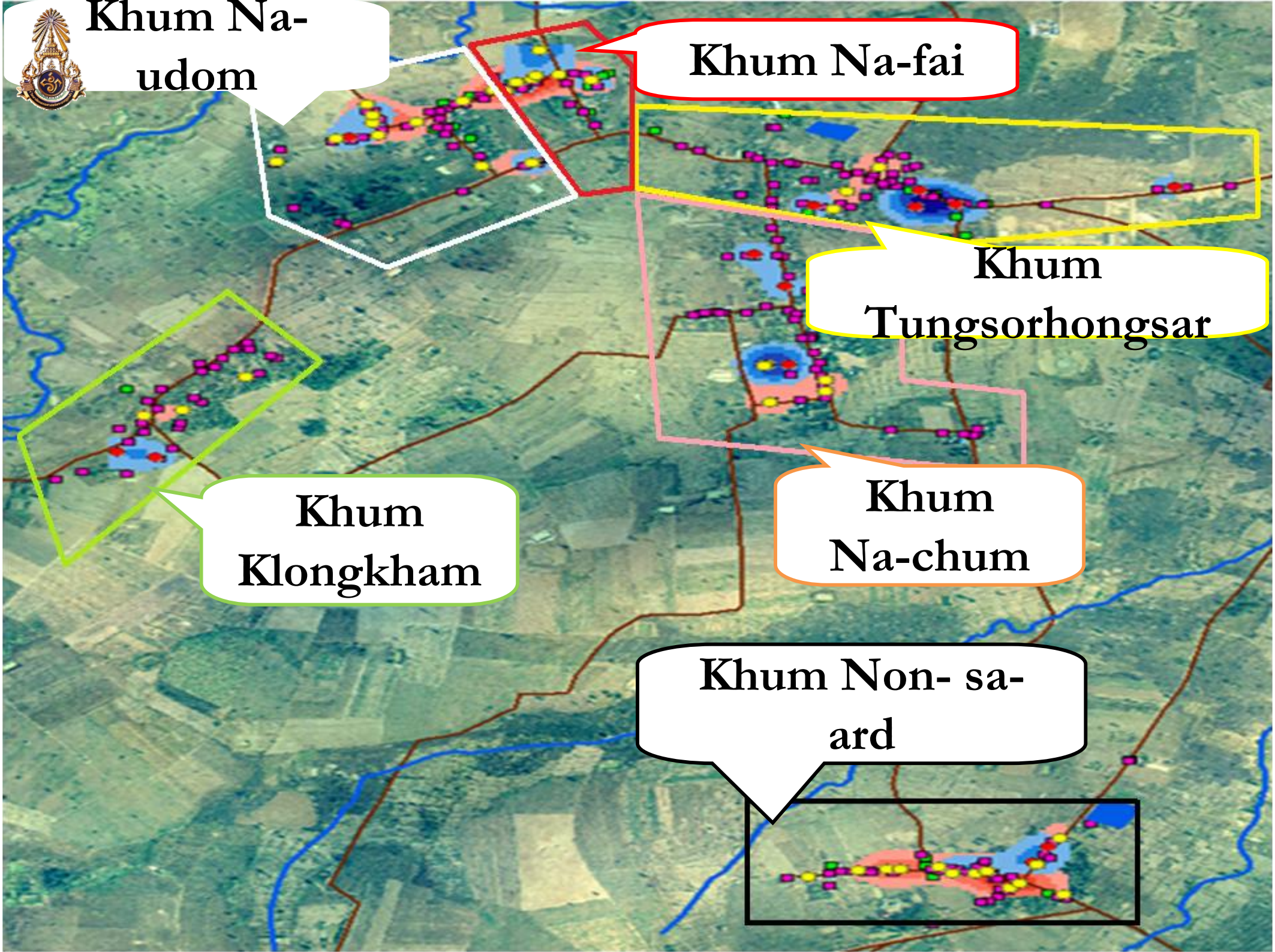
Results

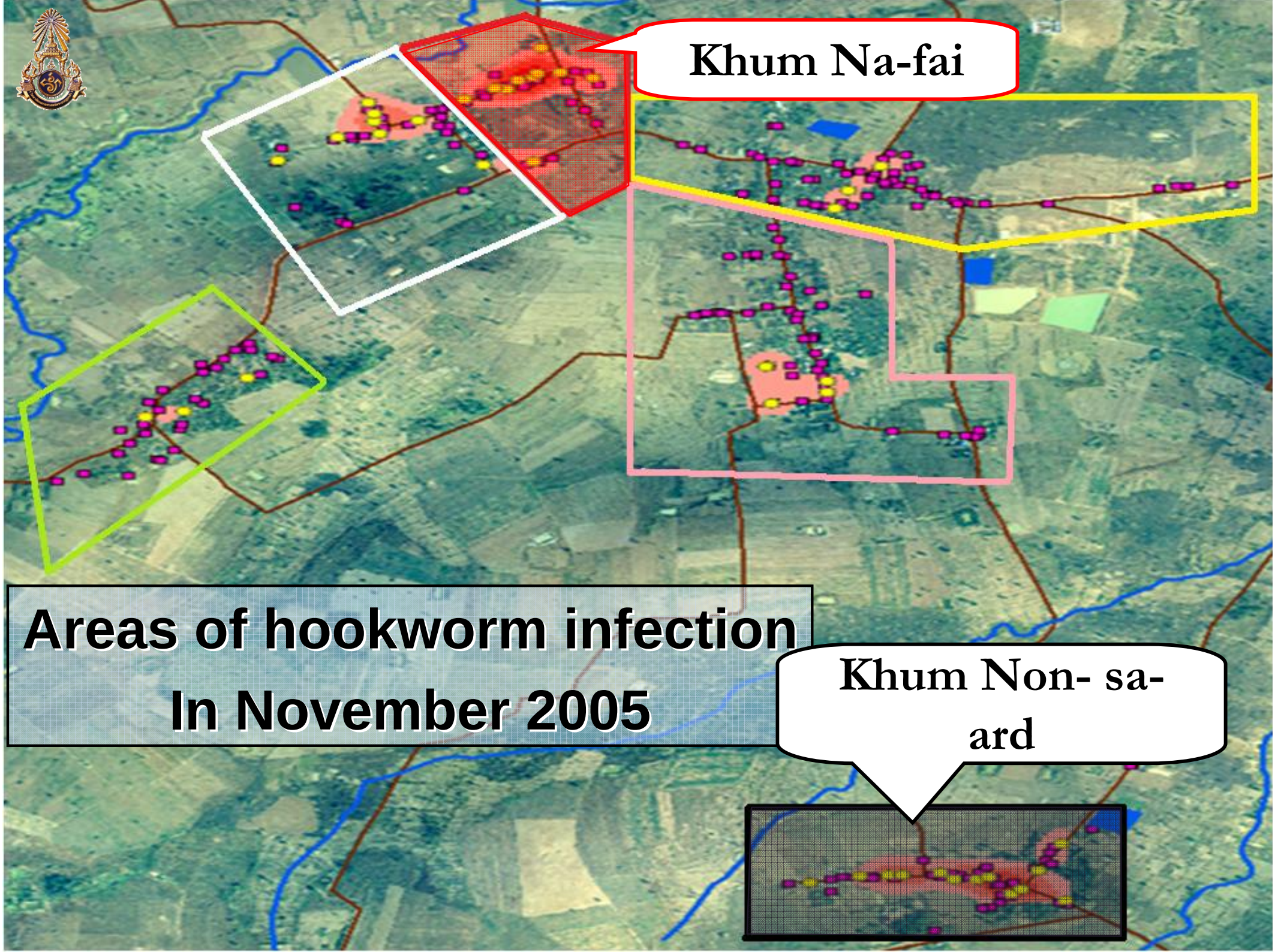
- Baseline survey, 515 hookworm negative subjects

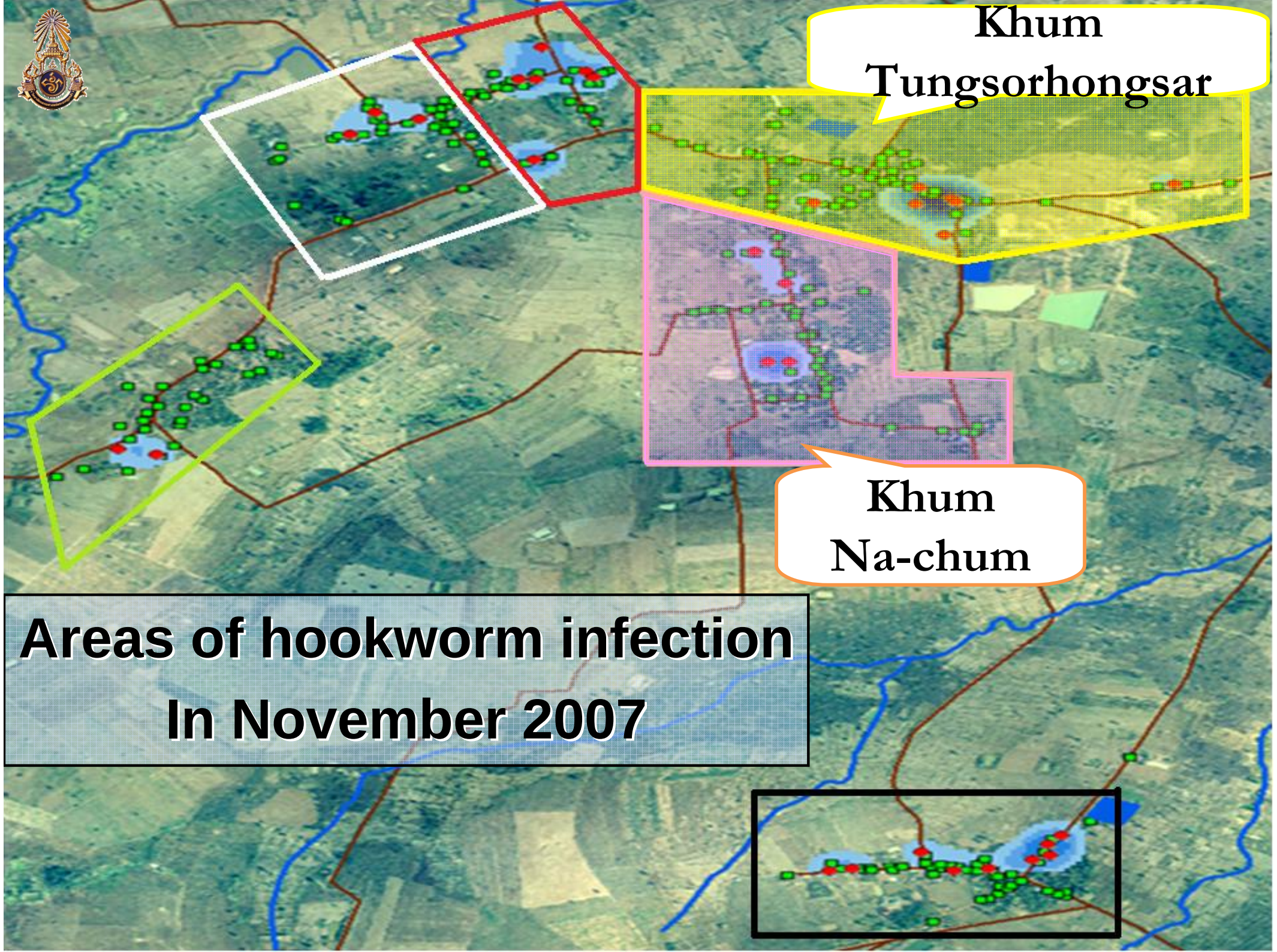
- 352 (68.34%) were enrolled into the follow-up study.
  - Migration caused the decrease in numbers of population.

Results

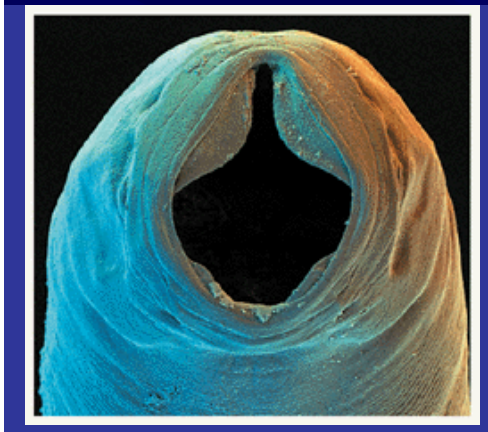
- 33 (9.37%) were positive for hookworm infection.
- The incidence rate of hookworm infection was 7.5/100 person-years.


Khum Na-udom
Khum Na-fai
Khum Tungsorhongsar
Khum Klongkham
Khum Na-chum
Khum Non- sa-ard

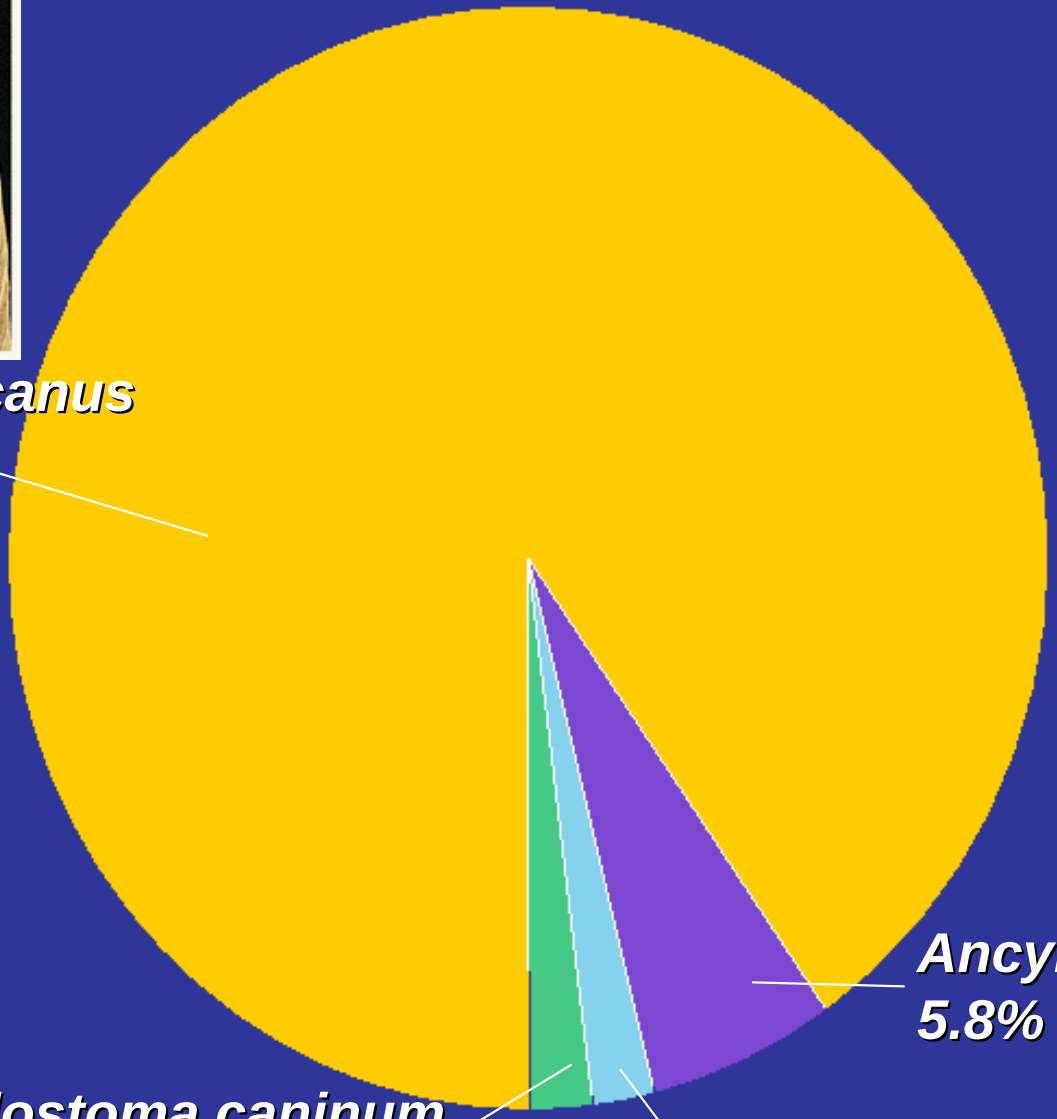

Khum Na-fai
Areas of hookworm infection
In November 2005
Khum Non- sa-
ard


Khum
Tungsorhongsar
Khum
Na-chum
Areas of hookworm infection
In November 2007

Species identification



Necator americanus
90.4%

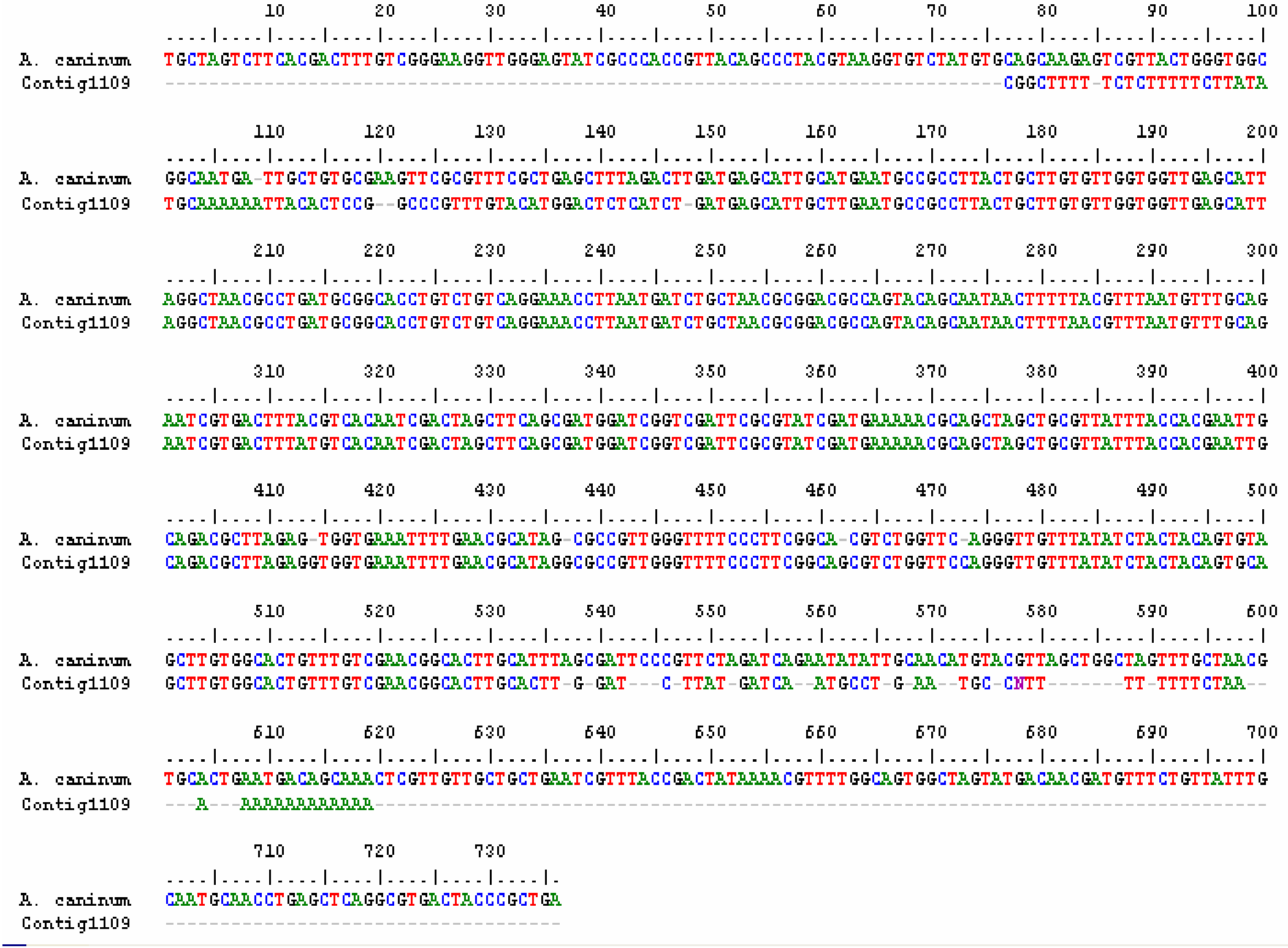


Ancylostoma ceylanicum
5.8%

Ancylostoma caninum
1.9%

Not determined 1.9%


A. ceylanicum
Contig620
Contig1176


10 20 30 40 50 60 70 80 90 100
A. carinum TGCTAGTCTTCACGACTTTGTCGGGAAGGTTGGGAGTATCGCCCACCGTTACAGCCCTACGTAAGGTGTCTATGTGCAGCAAGAGTCGTTACTGGGTGGC
Contig1109 CGGCTTTT-TCTCTTTTTCTTATA
110 120 130 140 150 160 170 180 190 200
A. carinum GGCAATGA-TTGCTGTGCGAAGTTCGCGTTTCGCTGAGCTTTAGACTTGATGAGCATTGCATGAATGCCGCCTTACTGCTTGTGTTGGTGGTTGAGCATT
Contig1109 TGCAAAAAATTACACTCCG--GCCCGTTTGTACATGGACTCTCATCT-GATGAGCATTGCTTGAATGCCGCCTTACTGCTTGTGTTGGTGGTTGAGCATT
210 220 230 240 250 260 270 280 290 300
A. carinum AGGCTAACGCCTGATGCGGCACCTGTCTGTCAGGAAACCTTAATGATCTGCTAACGCGGACGCCAGTACAGCAATAACTTTTTACGTTTAATGTTTGCAG
Contig1109 AGGCTAACGCCTGATGCGGCACCTGTCTGTCAGGAAACCTTAATGATCTGCTAACGCGGACGCCAGTACAGCAATAACTTTTAACGTTTAATGTTTGCAG
310 320 330 340 350 360 370 380 390 400
A. carinum AATCGTGACTTTACGTCACAATCGACTAGCTTCAGCGATGGATCGGTCGATTCGCGTATCGATGAAAAACGCAGCTAGCTGCGTTATTTACCACGAATTG
Contig1109 AATCGTGACTTTATGTCACAATCGACTAGCTTCAGCGATGGATCGGTCGATTCGCGTATCGATGAAAAACGCAGCTAGCTGCGTTATTTACCACGAATTG
410 420 430 440 450 460 470 480 490 500
A. carinum CAGACGCTTAGAG-TGGTGAAATTTTGAACGCATAG-CGCCGTTGGGTTTTCCCTTCGGCA-CGTCTGGTTC-AGGGTTGTTTATATCTACTACAGTGTA
Contig1109 CAGACGCTTAGAGGTGGTGAAATTTTGAACGCATAGGCGCCGTTGGGTTTTCCCTTCGGCAGCGTCTGGTTCCAGGGTTGTTTATATCTACTACAGTGCA
510 520 530 540 550 560 570 580 590 600
A. carinum GCTTGTGGCACTGTTTGTCGAACGGCACTTGCATTTAGCGATTCCCGTTCTAGATCAGAATATATTGCAACATGTACGTTAGCTGGCTAGTTTGCTAACG
Contig1109 GCTTGTGGCACTGTTTGTCGAACGGCACTTGCACTT-G-GAT---C-TTAT-GATCA--ATGCCT-G-AA--TGC-CNTT-------TT-TTTTCTAA--
610 620 630 640 650 660 670 680 690 700
A. carinum TGCACTGAAATGACAGCAAACTCGTTGTTGCTGCTGAATCGTTTACCGACTATAAAACGTTTTGGCAGTGGCTAGTATGACAACGATGTTTCTGTTATTTG
Contig1109 ---A---AAAAAAAAAAAAA----------
710 720 730
A. carinum CAATGCAACCTGAGCTCAGGCGTGACTACCCGCTGA
Contig1109 ----------

Univariate and multivariate analysis of the risk factors of hookworm infection

Factor	Subjects (Person-years)	No.of Hookworm Infection (Incidence rate)	Crude Incidence rate ratio (95% CI)	Adjusted IRR (95% CI)	P-value
Age				1.021	.113
				(.995-1.047)	
Wash hands before meal					
Yes	155	12(.077)	1.006	1.187	.754
No	260	20(.076)	(.448-2.161)	(.405-3.478)	
Wash hands after defecated					
Yes	315	22(.069)	.698	.639	.397
No	100	10(.10)	(.317-1.652)	(.227-1.799)	
Defecated in toilet					
Yes	351	26(.074)	.802	.656	.471
No	65	6(.092)	(.323-2.384)	(.209- 2.06)	
Barefoot					
Yes	296	28(.094)	2.837	11.686	.022*
No	120	4(.033)	(.992-11.134)	(1.431-95.378)	

Univariate and multivariate analysis of the risk factors of hookworm infection

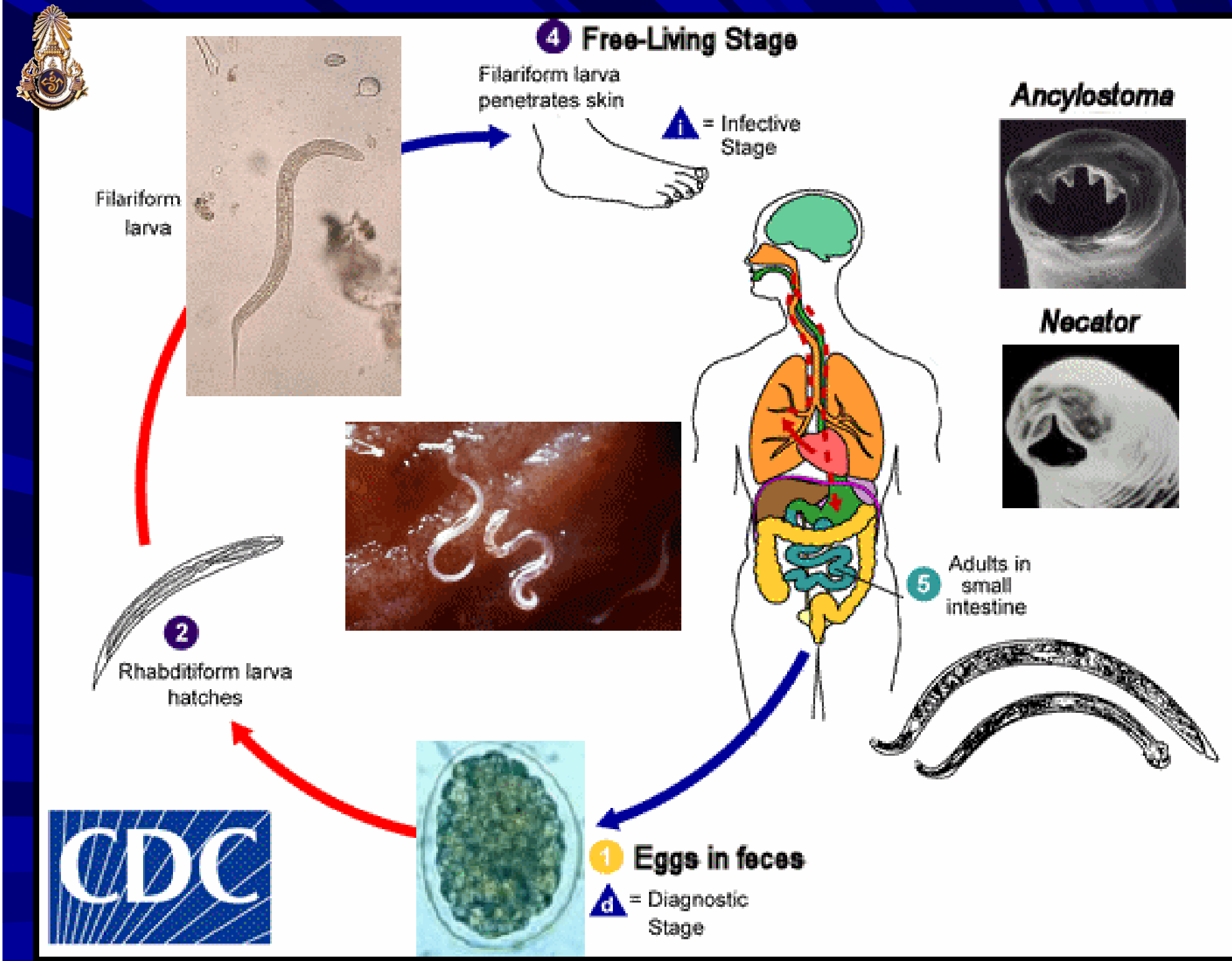
Factor	Subjects (Person-years)	No.of Hookworm Infection (Incidence rate)	Crude Incidence rate ratio (95% CI)	Adjusted IRR (95% CI)	P-value
Buffaloes					
Yes	15	6(.4)	6.539	10.103	.001*
No	376	23(.061)	(2.177-16.52)	(2.455-41.57)	
Duck					
Yes	14	1(0.071)	.961	2.58e-06	.989
No	377	28(.074)	(.02-5.817)	(0-.)	
Chicken					
Yes	315	26(.082)	2.091	2.433	.186
No	76	3(.039)	(.64-10.793)	(.65-9.105)	
Fish					
Yes	85	4(.047)	.576	.748	.619
No	306	25(.081)	(.145-1.668)	(.239-2.33)	

Univariate and multivariate analysis of the risk factors of hookworm infection

Factor		Subjects (Person-years)	No.of Hookworm Infection (Incidence rate)	Crude Incidence rate ratio (95% CI)	Adjusted IRR (95% CI)	P-value
Dog						
	Yes	285	22(.077)	1.168	2.155	.240
	No	106	7(.066)	(.482-3.239)	(.597-7.77)	
Cat						
	Yes	126	5(.039)	.438	.289	.101
	No	265	24(.090)	(.13-1.171)	(.065-1.273)	
Pig						
	Yes	45	3(.066)	.887	.551	.441
	No	346	26(.075))	(.171-2.894)	(.121-2.506)	
Cow						
	Yes	130	12(.092)	1.417	2.267	.077
	No	261	17(.065)	(.617-3.148)	(.14-5.623)	

Discussions

- The incidence of hookworm infection was high in this rural area of Thailand.
- Barefoot walking and having buffaloes raised by their houses increased risk of hookworm infection.


4 Free-Living Stage
Filariform larva penetrates skin
i = Infective Stage
Filariform larva
Ancylostoma
Necator
5 Adults in small intestine
2 Rhabditiform larva hatches
1 Eggs in feces
d = Diagnostic Stage
CDC

Discussions

Discussions

- From GIS, there were different levels of the intensity of hookworm infection in different areas.
- The areas showing high prevalence of hookworm infection were related with having buffaloes.

Conclusion & Suggestions

- Public health intervention should be addressed on the prevention of hookworm infection by ;
  - Sanitation
  - Changing habit of barefoot walking
  - Avoiding soil exposure in the areas with high rates of hookworm infection

Conclusion & Suggestions

- In addition , animal hookworms i.e. *A. ceylanicum* and *A. caninum* could be found in this population suggesting zoonotic infections.
- GIS showed different levels of the infection intensity which could guide the intervention program in each area.

Acknowledgement

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Collaborations

- Phramongkutklao College of Medicine
  - The fifth-year Medical cadets (29th)
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    - Vittaya Jiraanankul
    - Kuntaphol pengpan
    - Pipit Burasakan
    - Paiboon Kuhapensang
  - Department of Parasitology
    - Saovanee Leelayoova
    - Paanjit Taamasri
    - Mathirut Mungthin
    - Phunlerd Piyaraj
    - Rommanee Khositnithikul
    - Tawee Naaglor
  - Department of Military and Community Medicine
    - Ram Rangsin
    - Pote Aimpun
- School of Veterinary Science, the University of Queensland
  - Rebecca J Traub