



The Molecular Characterization of *Giardia duodenalis* infection in Thailand



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Giardia duodenalis

- ◆ ***Giardia intestinalis, Giardia lamblia***
- ◆ **A flagellated unicellular eukaryotic microorganism**
- ◆ **No mitochondria or peroxisomes**
- ◆ **Commonly causes diarrheal disease called “Giardiasis”**
- ◆ **Worldwide distribution**

Significance of Giardiasis

- ◆ ***Giardia duodenalis* has a global distribution causing an estimated 280 million cases per annum (Lane and Lloyd, 2002).**
- ◆ **In Asia, Africa and Latin America, and the developed countries, about 200 million people have symptomatic giardiasis (WHO, 1996).**

Morphological Features of *Giardia*

Trophozoite



Cyst



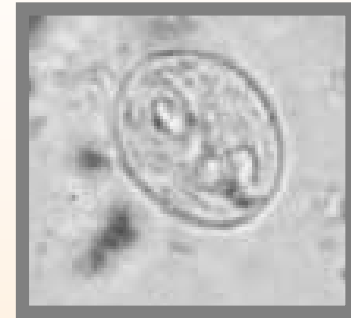
Clinical outcomes of *Giardia* infection

- **Asymptomatic carriers**
- **Symptomatic patients**
 - ◆ **Acute diarrhea**
 - ◆ **Chronic diarrhea**



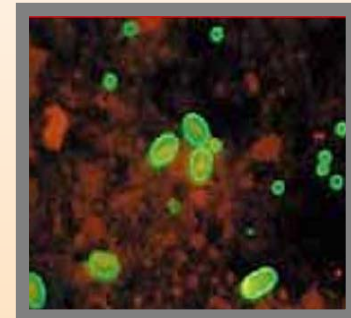
Diagnosis and Detection

- ◆ **Microscopic methods**



- ◆ **Immunodiagnosis**

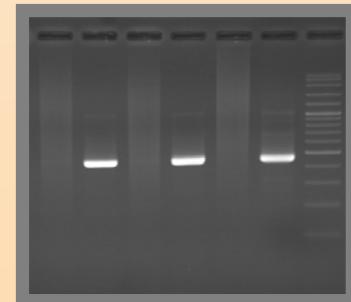
- ◆ **Direct immunofluorescence**



- ◆ **Enzyme immunoassays (EIAs)**

- ◆ **Molecular techniques**

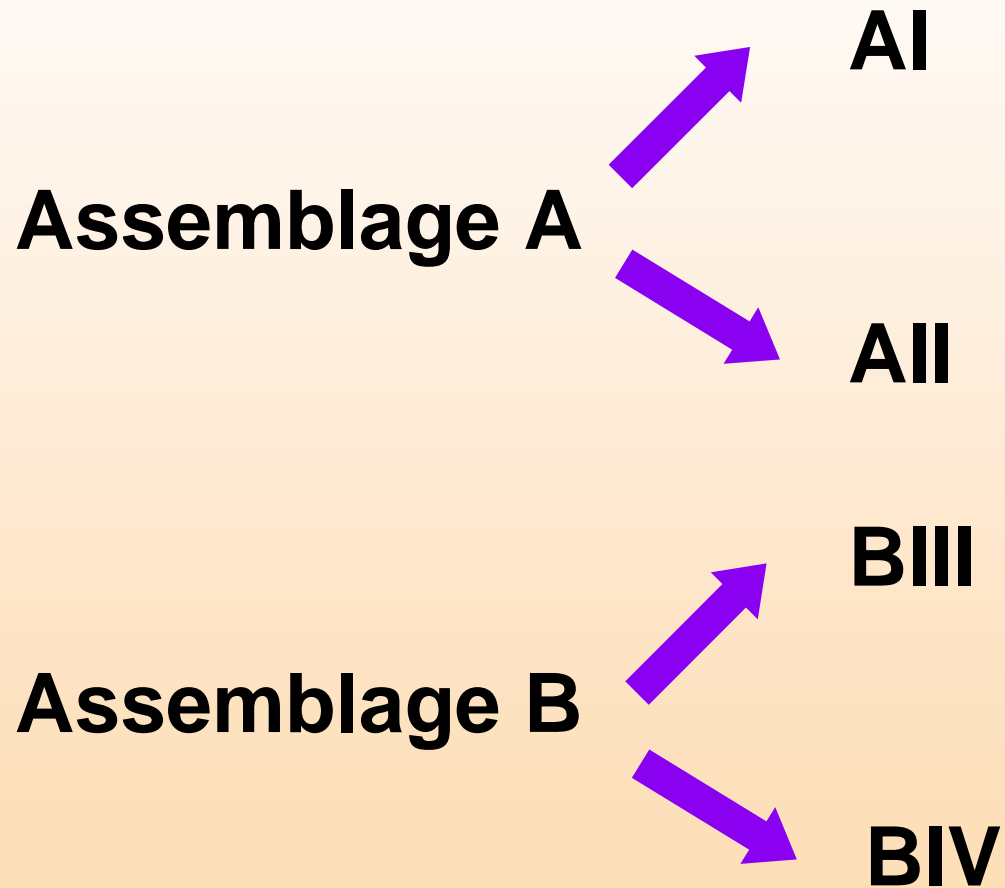
- ◆ **PCR-based procedures**



Genotype and host range of *Giardia duodenalis*

Assemblages	Host range
A	Humans, livestock, cats, dogs, beavers, guinea pig, slow loris
B	Humans, slow loris, chinchillas, dogs, beavers, rats, siamang gibbon
C,D	Dog
E	Cattle, sheep, pigs
F	Cat
G	Domestic Rats
Muskrats/Vole	Wild rodents

Subgenotypes within assemblage A and B



Giardiasis in Thailand

Summary of intestinal parasite studies in Thailand

Study	Province	Population		<i>G. lamblia</i> detected
Chavalittamrong et al., 1978 ¹⁵	Bangkok/urban	Children in low socioeconomics	Asymptomatic	18.18%
			Symptomatic	18.58%
Chavalittamrong and Jirapinyo, 1984 ¹⁹	Bangkok/urban	Children in low socioeconomics	Symptomatic	6.1%
Saksirisampant et al., 2003 ¹³	Pathum Thani/rural	Pre-school orphans	Asymptomatic	37.7%
Mungthin et al., 2001 ¹²	Bangkok/urban	Children/orphans Phayathai BH	Asymptomatic	None
		Childcare workers	Asymptomatic	None
Termmathurapoj et al., 2000 ¹¹	Bangkok/urban	Pre-school children/orphans Phayathai BH	Asymptomatic	None
		Childcare workers	Asymptomatic	None
Janoff et al. 1990 ¹⁰	Bangkok/urban	Pre-school children/orphans	Asymptomatic	18%
			Symptomatic	2%
Jongwitiwes et al., 1990 ¹⁸	Nonthaburi/urban	Children/orphans	Asymptomatic	6.4% overall
			Symptomatic	
Bunnag et al., 1982 ²¹	Phichit/rural	Village dwellers		7%
	Bangkok/urban	Slum dwellers		10%
Sornmani et al., 1973 ²²	Khon Kaen/rural	Village dwellers on periphery of a lake		7.2%
Waikagul et al., 2002 ²⁵	Nan/rural	School children		5.3%
Kasuya et al., 1989 ²⁴	Chiang Mai/rural	Primary school children		7.7%
Echeverria et al., 1989 ¹⁴	Bangkok/urban	Pre-school children	Asymptomatic	1.3%
			Symptomatic	2%
Boonchai et al., this study	Kanchanaburi/rural	Pre-school children	Asymptomatic	23.3%
			Symptomatic	13.6%

G. Giardia; E. Entamoeba; B. Blastocystis; BH Babies' Home; N/A not applicable.

Canine parasitic zoonoses in Bangkok temples.

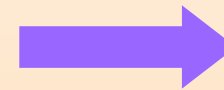
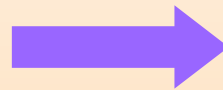
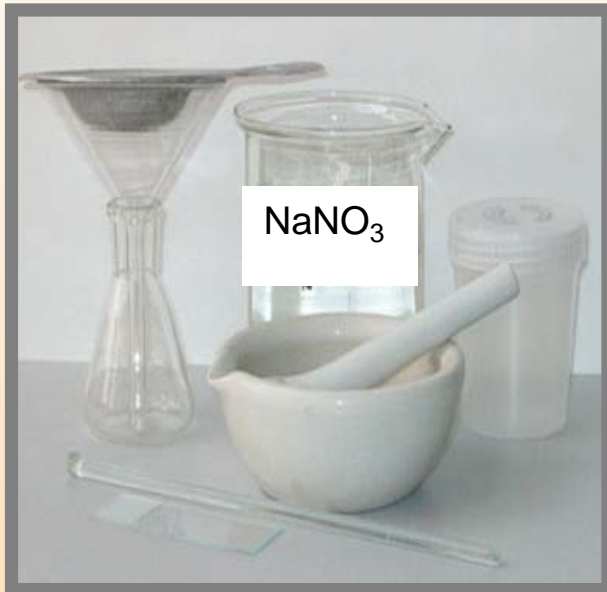
Inpankaew T, Traub R, Thompson RC, Sukthana Y

Southeast Asian J Trop Med Public Health 38 (2007).

Objectives

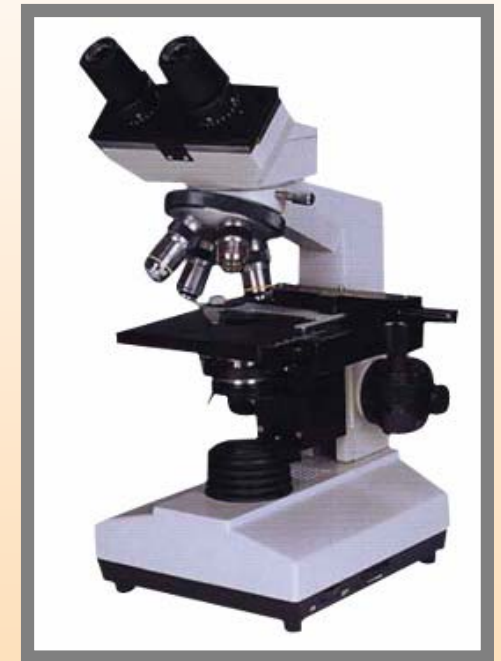
- ◆ To compare the sensitivities of two primer sets for glutamate dehydrogenase (*gdh*) gene amplification
- ◆ To investigate the distribution of subgenotypes in different groups of Thai population

Floatation Technique



PBS

Cysts



DNA Extraction Technique

QIAmp Stool Mini Kit

FTA filter paper



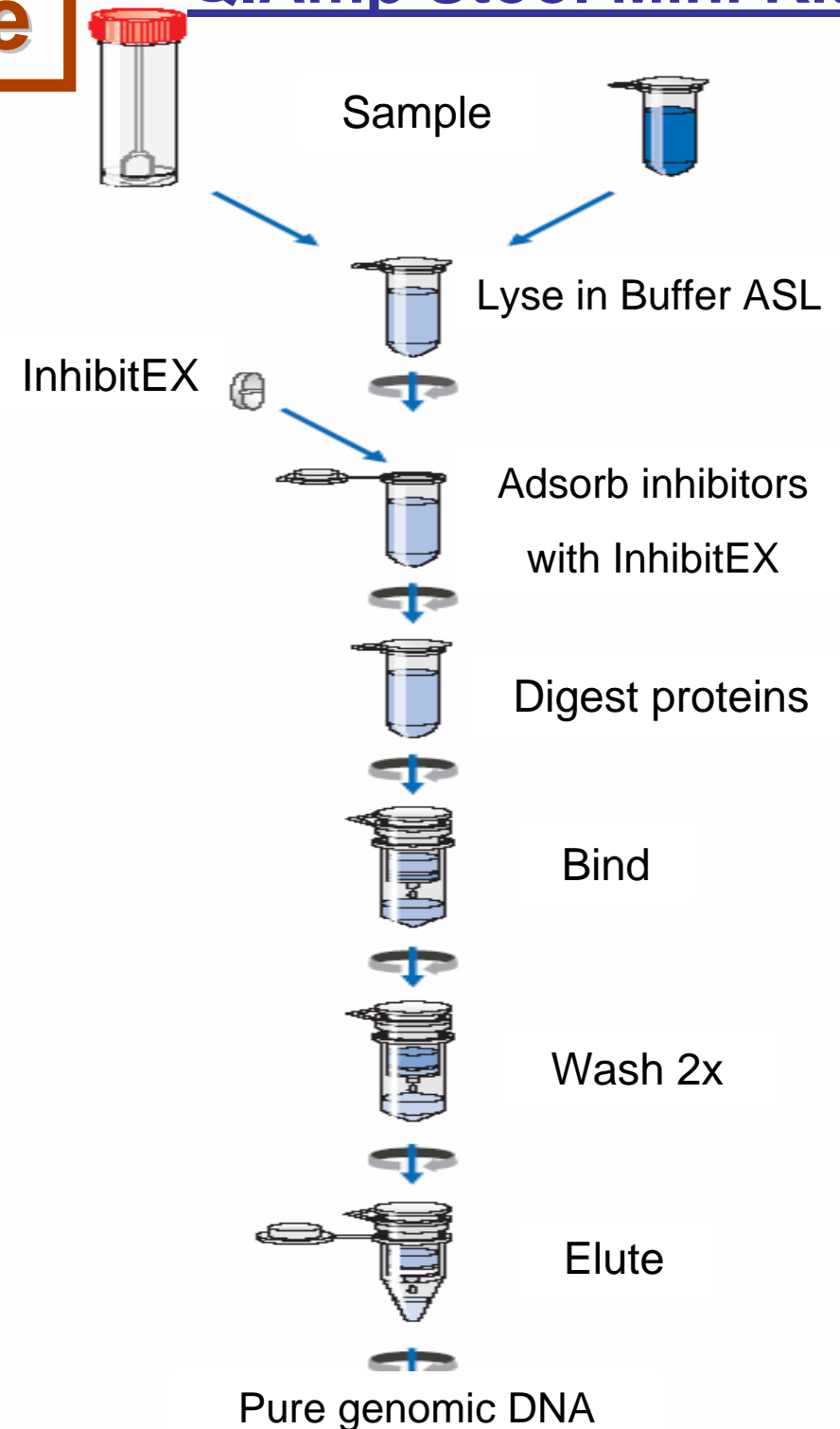
Remove disc

Place disc in microcentrifuge tube. Apply stool sample and allow to dry completely. Wash disc with FTA Purification Reagent.

Wash disc with TE⁻¹ Buffer

Air-dry the disc

Add PCR master mix directly to the disc and proceed to PCR



Specific genes for genotyping of *Giardia duodenalis*

Target gene loci	Sensitivity for cyst DNA	Subgroup genotyping
SSU rDNA	+++++	AI, AII
β -giardin	+	AI, AII
Glutamate dehydrogenase	+++	AI, AII, BIII, BIV
Triosphosphate isomerase	++	AI, AII, BII, BIV
Elongation factor 1-alfa	+	AI, AII

Modified PCR amplification

Name	Primers	Target Gene	References
GDH1 GDH4 GDHeF GDHiR	5'ATC TTC GAG AGG ATG CTT GAG3' 5'AGT ACG CGA CGC TGG GAT ACT3' 5'TAC ACG TYA AYC GYG GYT TCC GT3' 5'GTT RTC CTT GCA CAT CTC C3'	Glutamate dehydrogenase (GDH)	Homan <i>et al.</i> 1998 Read <i>et al.</i> 2004

Genotypic Characterization

RFLP analysis

Nla IV

5'...GGN[▼]NCC...3'
3'...CCN[▲]NGG...5'

Rsa I

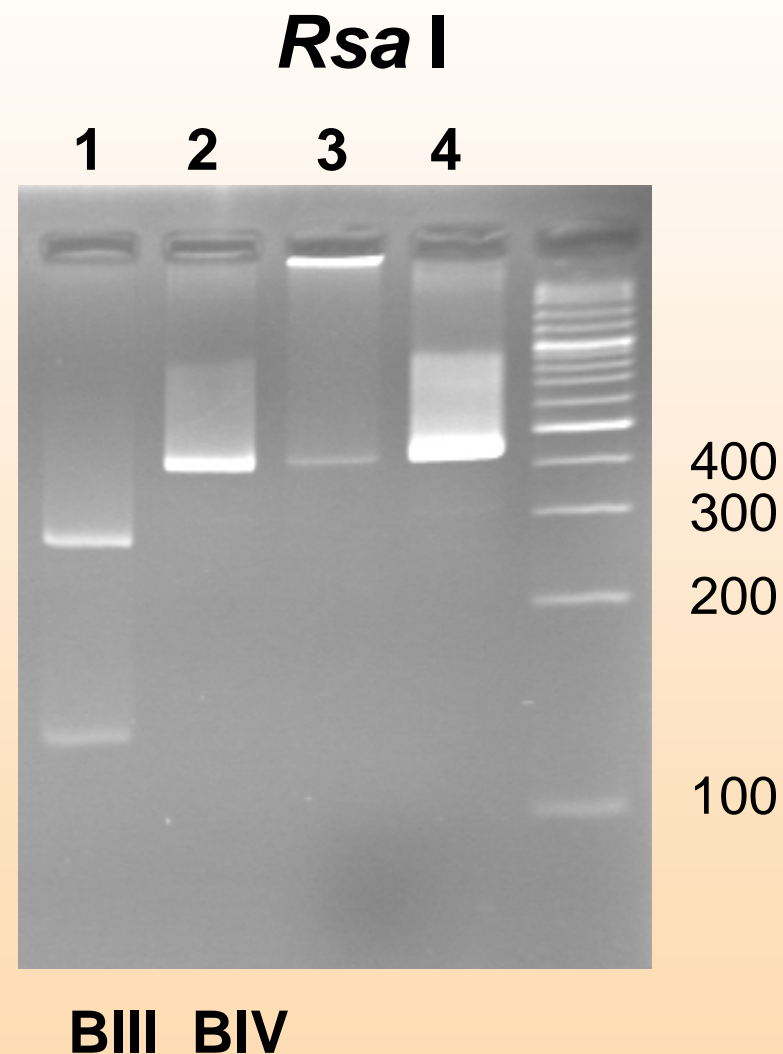
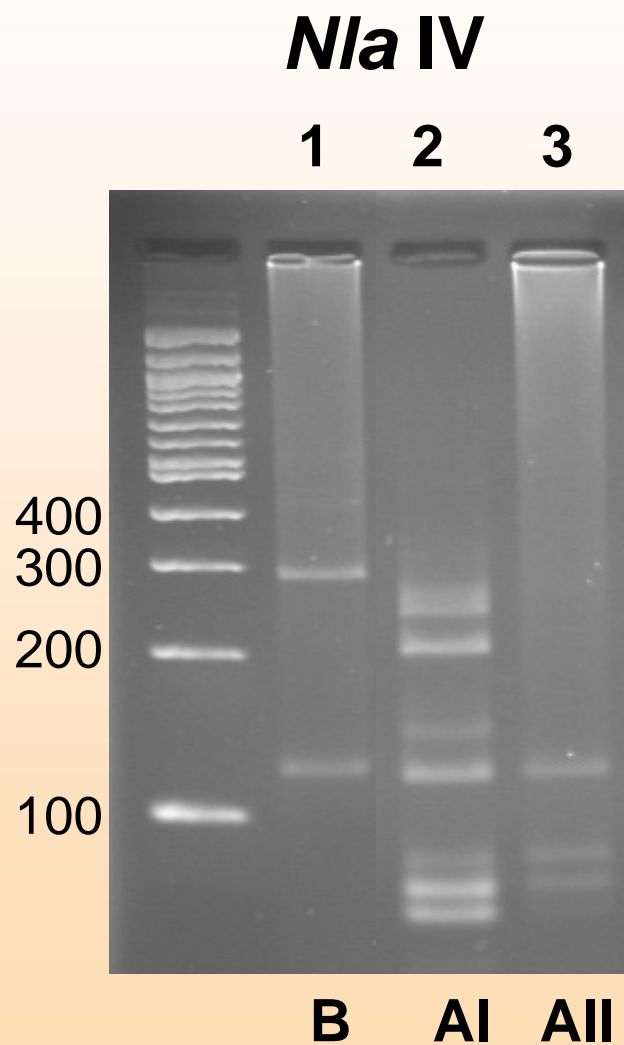
5'...GT[▼]AC...3'
3'...CA[▲]TG...5'

RFLP analysis

Predicted fragment sizes (bp) and diagnostic genotyping profile (bp) of *G. duodenalis* genetic assemblages when using primers GDHeF and GDHiR

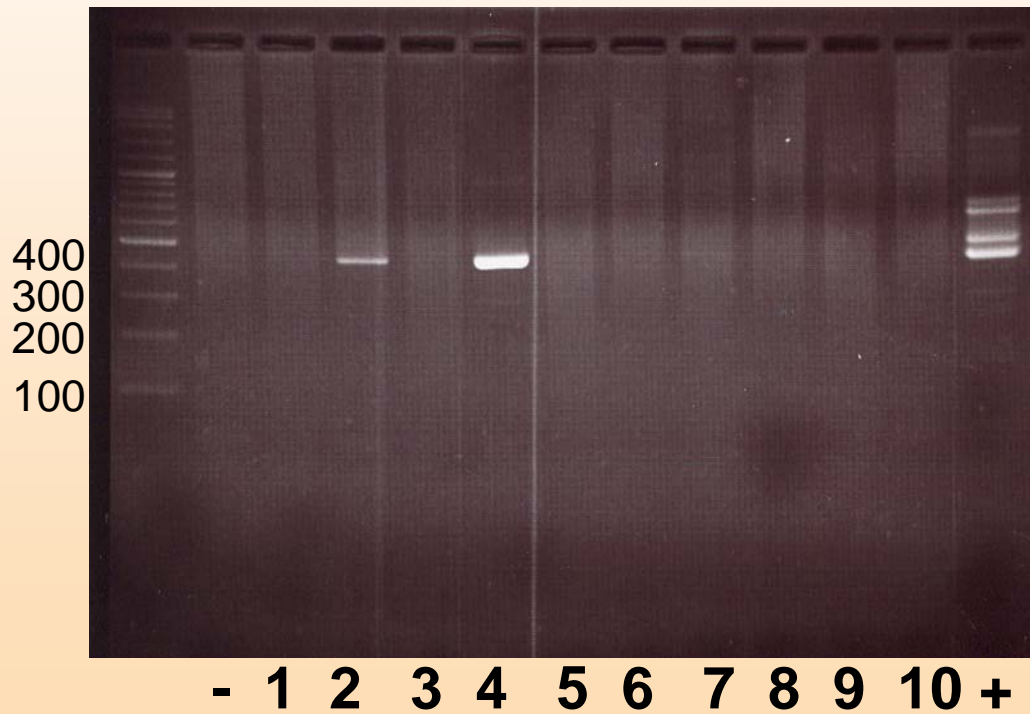
Assemblage	Enzyme	Predicted fragment sizes	Diagnostic genotyping profile
AI	<i>NlaIV</i>	149, 123, 87, 44, 39, 16	149, 123, 87
AII	<i>NlaIV</i>	123, 87, 77, 72, 44, 39, 16	123, 87
BIII	<i>NlaIV</i>	291, 123, 44	291, 123
	<i>RsaI</i>	297, 133, 28	297, 133
BIV	<i>NlaIV</i>	291, 123, 44	291, 123
	<i>RsaI</i>	430, 28	430

Genotypic Characterization

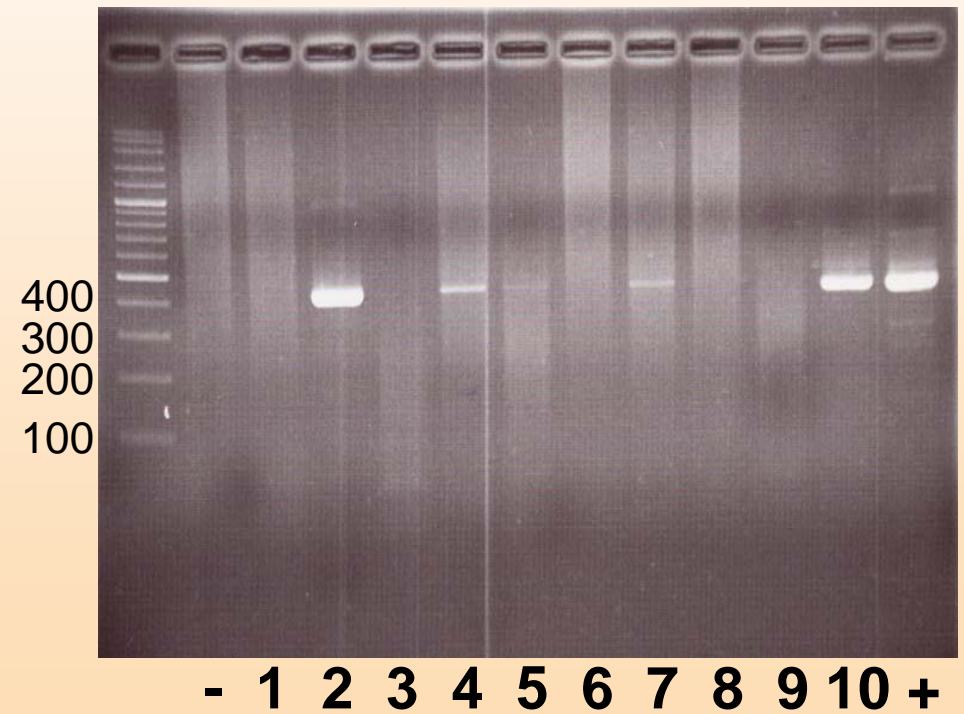


PCR amplification

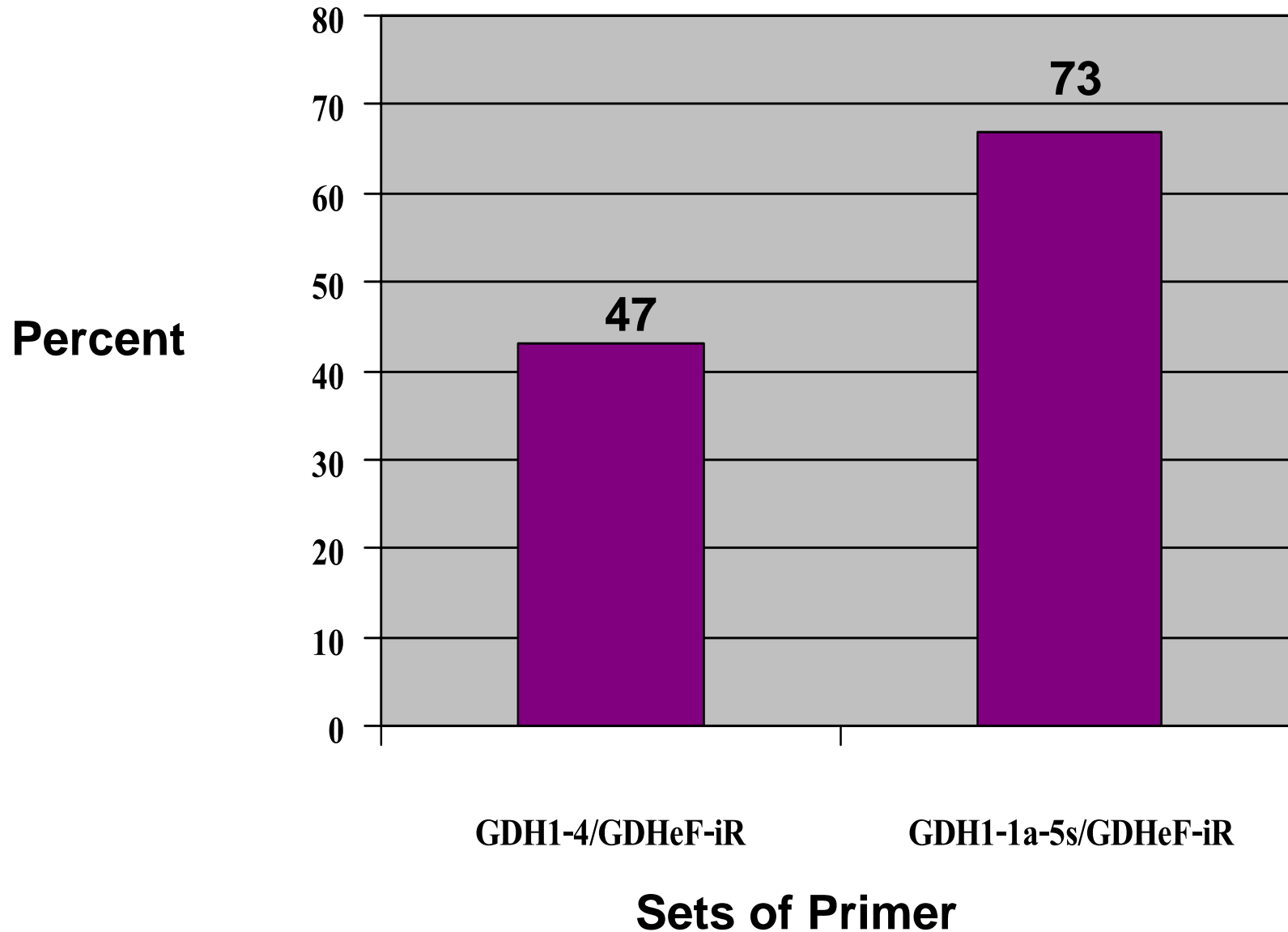
GDH1-4/GDHeF-iR
(Homan *et al.* 1998)



GDH1-1a-5s/GDHeF-iR
(This study)



Results and Discussion



Significant difference among two primer sets, $p < 0.050$

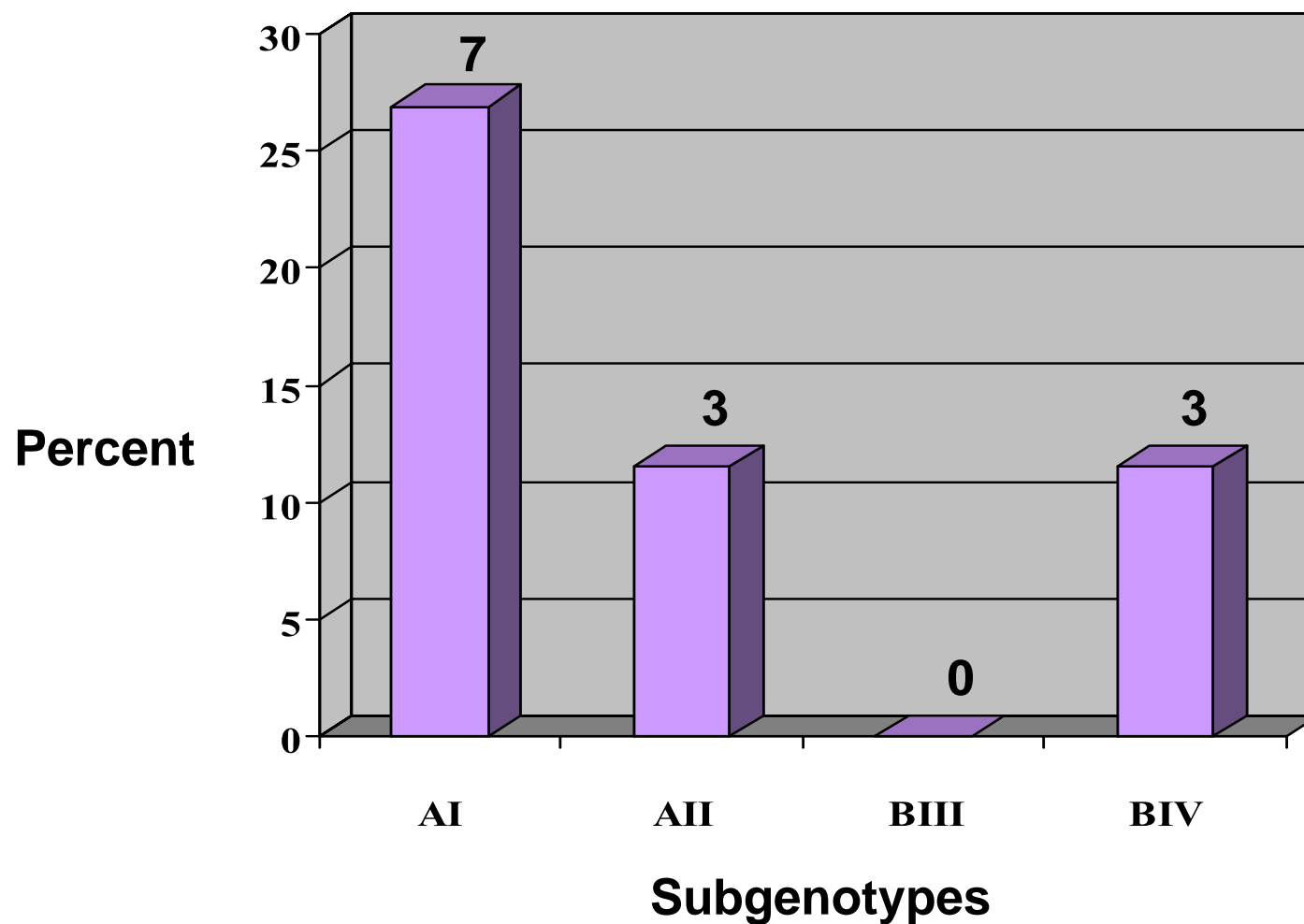
**Application of PCR-RFLP analysis
to determine the genotypic
characterization of *Giardia duodenalis*
in different groups of Thai population**

Studied population

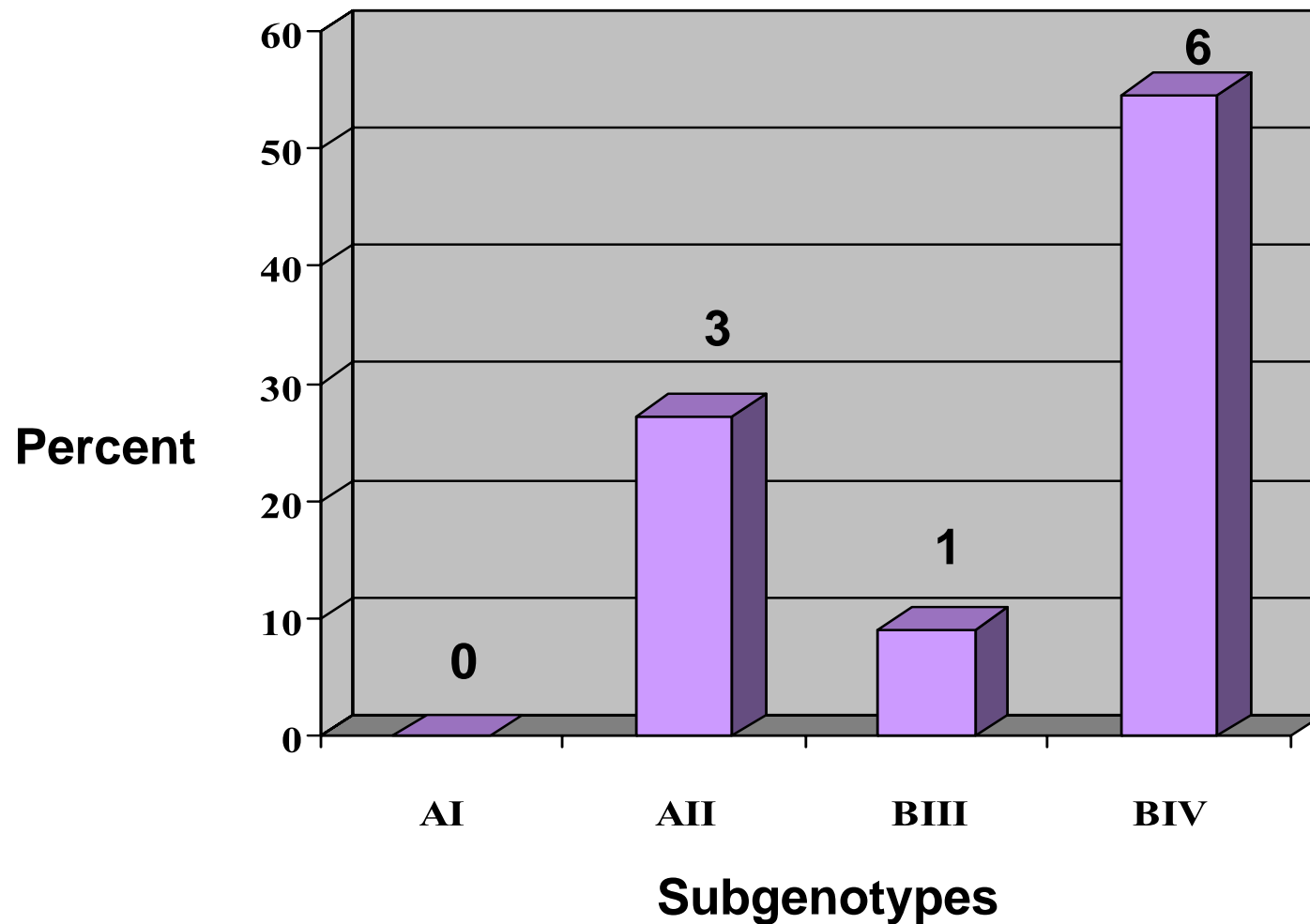
Total 109 samples

- **Orphans (26)**
- **Pre-school children (11)**
- **Primary-secondary school children (20)**
 - **School children, Chachoengsao Province (10)**
 - **Hill tribe children, Chiangmai Province (10)**
- **Adults over 20 years old (52)**
 - **Villagers, Chachoengsao Province**

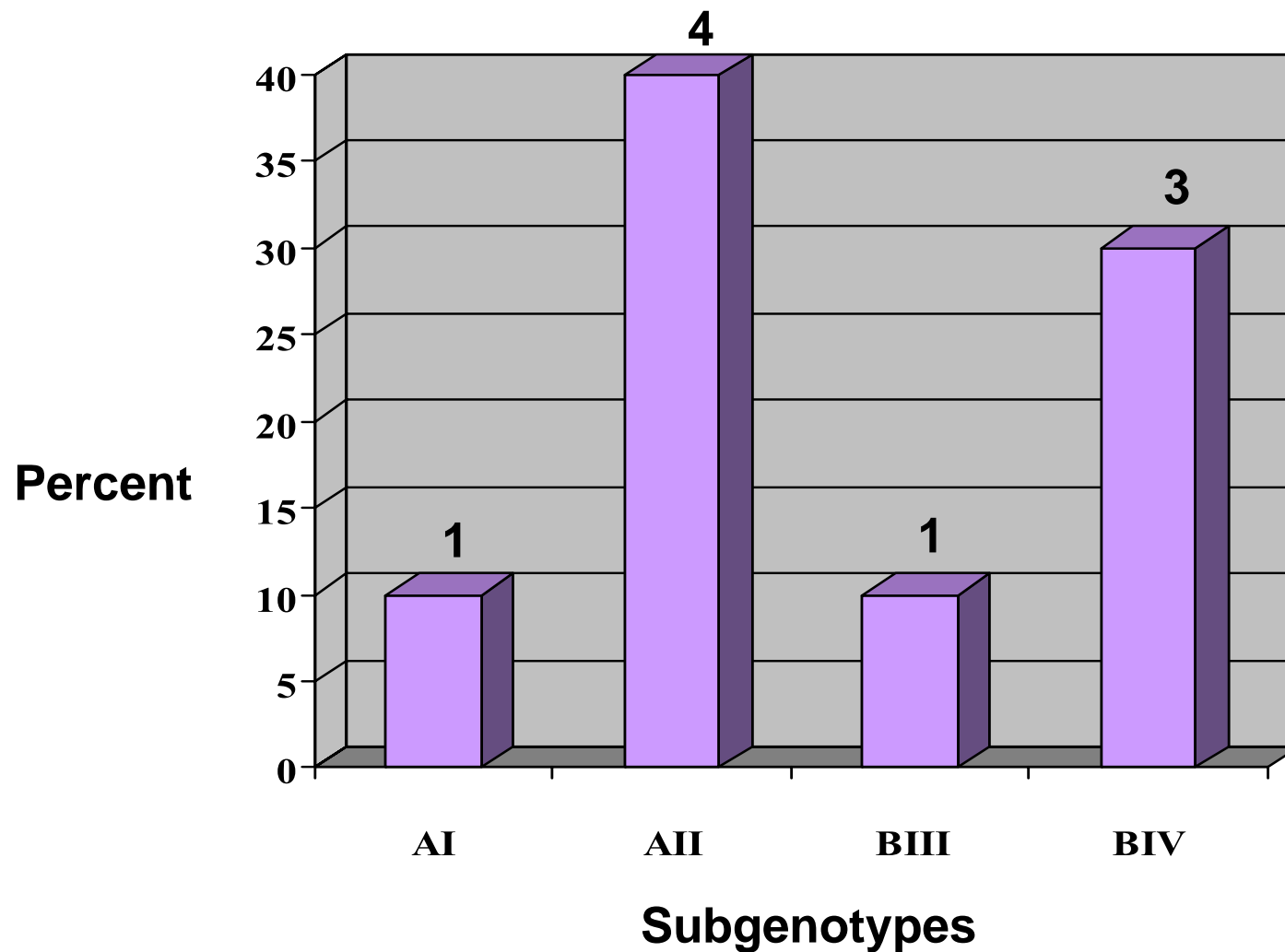
Subgenotype of *G. duodenalis* infected orphans



Subgenotype of *G. duodenalis* infected pre-school children

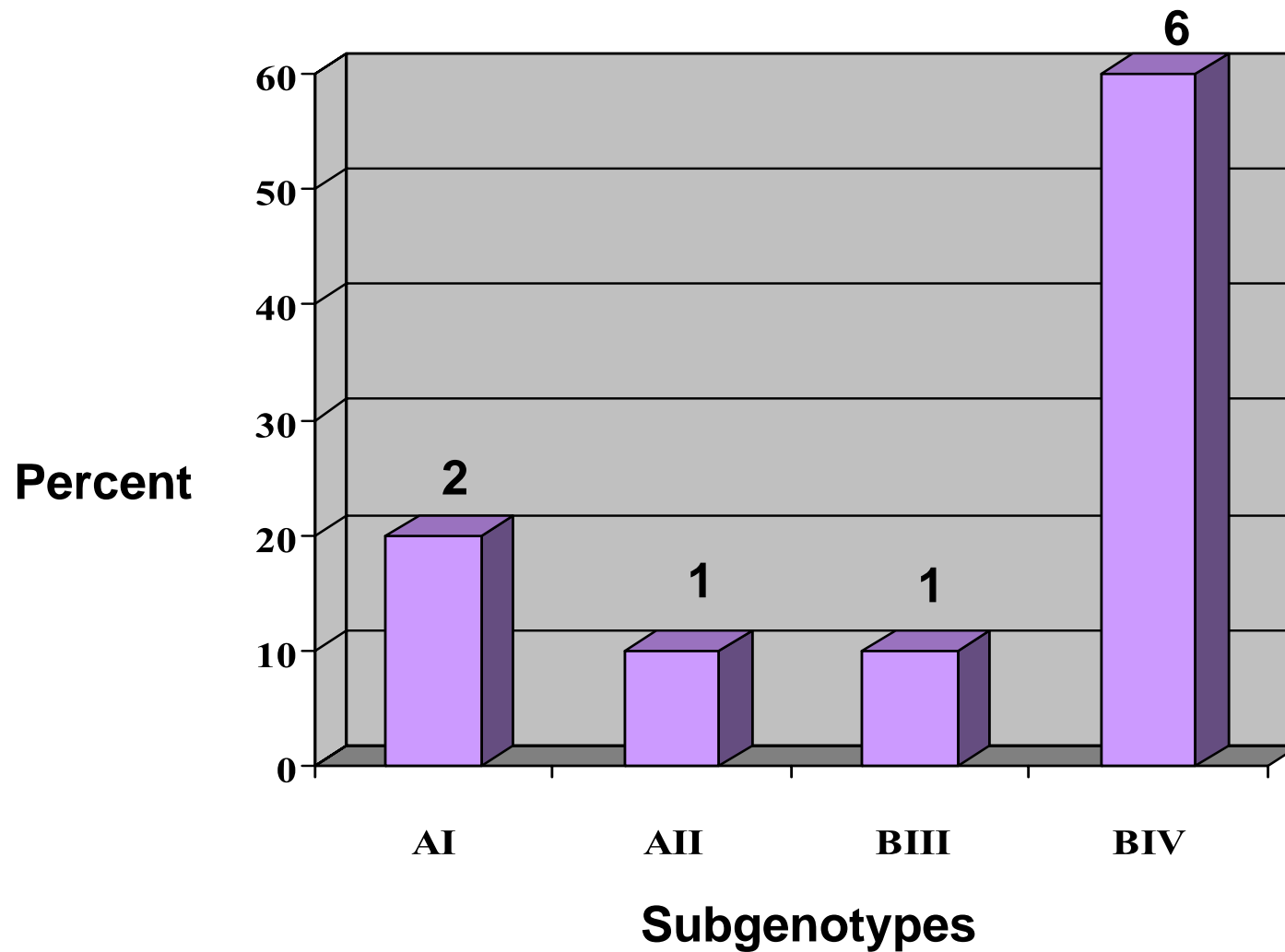


Subgenotype of *G. duodenalis* infected school children

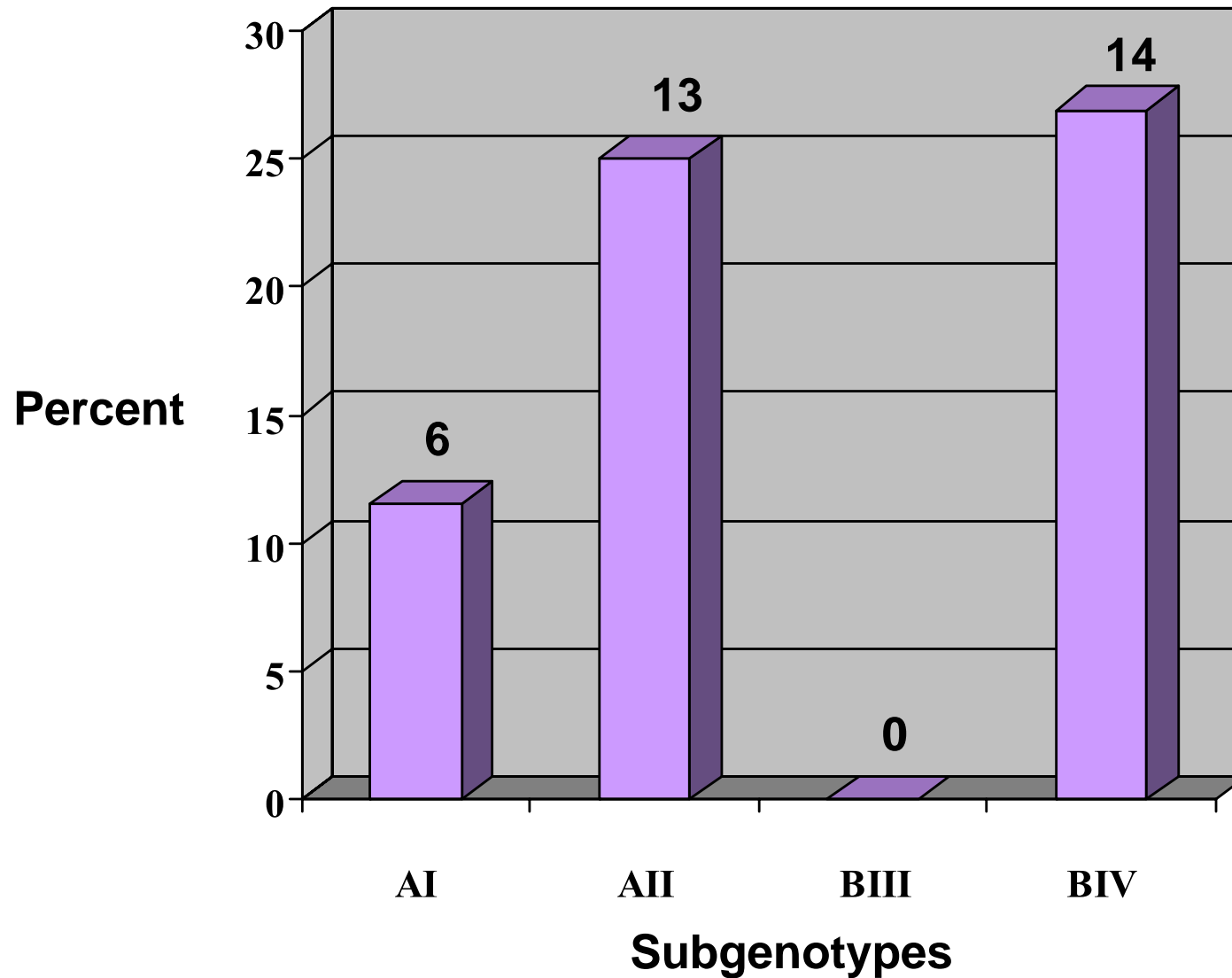


Subgenotype of *G. duodenalis* infected

Hill tribe children



Subgenotype of *G. duodenalis* infected adults



Discussions

- **The Molecular Characterization of *G. duodenalis* infection in Thailand using PCR-RFLP analysis revealed all of Assemblages AI, AII, BIII and BIV.**
- **The Most frequent Assemblage was BIV, followed by AII, AI and BIII, respectively.**
- **The Assemblage AI was the most frequent subgenotypes in orphans different from other groups of Thai population.**

Conclusions

- ◆ **The association between potential risk factors and *Giardia duodenalis* infection in each group of population has to be assessed statistically.**
- ◆ **The results from this study can be the baseline data to determine the transmission dynamic of this infection.**
- ◆ **Then the control strategies can be established.**

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