

Contact Information

Oliver Laeyendecker

LIR, NIAID, NIH

Mail address:

Johns Hopkins University

720 Rutland Avenue Ross 1155

Baltimore, MD 21205

email: olaeyen@mail.jhmi.edu

Phone: 410-502-3268

Fax: 410-614-9775

761-W

Abstract

Background: A new epidemic of HIV-1 among injection drug users (IDU) in Guangxi Province, China was first noted in 1996. Two routes of the epidemic converge in Guangxi as documented by two circulating recombinant viruses (CRF) CRF08_BC from Yunnan and CRF01_AE from Vietnam. Previous studies on limited numbers have suggested striking homogeneity of viral sequences. In this study, samples from 106 HIV+ IDUs from two cities in geographically separated locations were assessed.

Methods: Heroin users were enrolled from two cities in Guangxi (Binyang, N= 329 and Pingxiang, N= 286), 80% of whom used intravenous drugs. Blood samples collected between 9/00 and 10/00 from HIV-1 positive IDUs (Binyang, N= 62 and Pingxiang, N= 44) were tested for HIV-1 RNA levels by Roche Amplicor v1.5. 24/106 subjects had seroconverted within the last nine months (Binyang N=16 and Pingxiang N=8). Of the 99 subjects with quantifiable RNA levels, 92 were further analyzed by extracting the equivalent of 6 μ l of sera and amplifying *pol* gene (nt 1831-3025 of HXB2) and *env* gene (nt 6883 -7535 of HXB2) by RT-PCR. For internal control 25% of the samples were re-amplified, sequenced and analyzed in a blinded fashion. Phylogenetic relationships were inferred using PHYLIP and inter subject variation was quantified using Hamming distances.

Results: The incidence rates in these cities were 9.5% and 5.3% per year for Binyang and Pingxiang for the year 2000 respectively. 99/106 subjects had quantifiable HIV-1 RNA levels with a median value of 2.4×10^4 copies/ml. There were no significant differences in median HIV RNA level city or subtype. 72/92 samples were *pol* RT-PCR positive in a concentration dependant manner with 57%, 83%, 100% and 100% of samples amplified with HIV-RNA titers of $>10^3$, 10^4 , 10^5 and 10^6 copies/ml, respectively. 75/92 samples were *pol* RT-PCR positive in a concentration dependant manner with 68%, 87%, 88% and 100% of samples amplified with HIV-RNA titers of $>10^3$, 10^4 , 10^5 and 10^6 copies/ml, respectively. 31/34 samples from Pingxiang were CRF01_AE with the remaining three being CRF08_BC. 48/50 samples from Binyang were CRF08_BC with the remaining two being a distinct new AE variant. There were no differences in viral load by genotype. Median inter-subject variation for the two CRF forms was *pol* 1.8%, *env* 6.3% for CRF01_AE and *pol* 1.0 %, *env* 3.1% for CRF08_BC infected subjects.

Conclusions: The HIV-1 epidemic in Guangxi is dominated by distinct CRF01_AE coming through Vietnam and CRF08_BC coming through Yunnan. CRF08_BC is newly introduced into Pingxiang. The lower diversity of CRF08_BC and the greater incidence of this strain may indicate a virus better at adapted parental transition.

Background

- HIV outbreak in Guangxi in 1996-1997
- Two subtypes converge in Guangxi, CRF_01 A/E from Vietnam and CRF_08 B/C from Yunnan province in China.
- Current inter-subject variation is extremely low comparable to other IDU based epidemics¹
- Previous studies have surveyed limited numbers of subjects (<14 per city)²⁻⁴

References

- 1) Yu, AIDS & Hum. Ret. 2002;18:167
- 2) Yu, JAIDS 1999 vol 22 p180
- 3) Piyasirisilp, JV 2000 vol 74 p11286
- 4) Yu, AIDS 2001 vol 15 p523

Location of Current Investigation

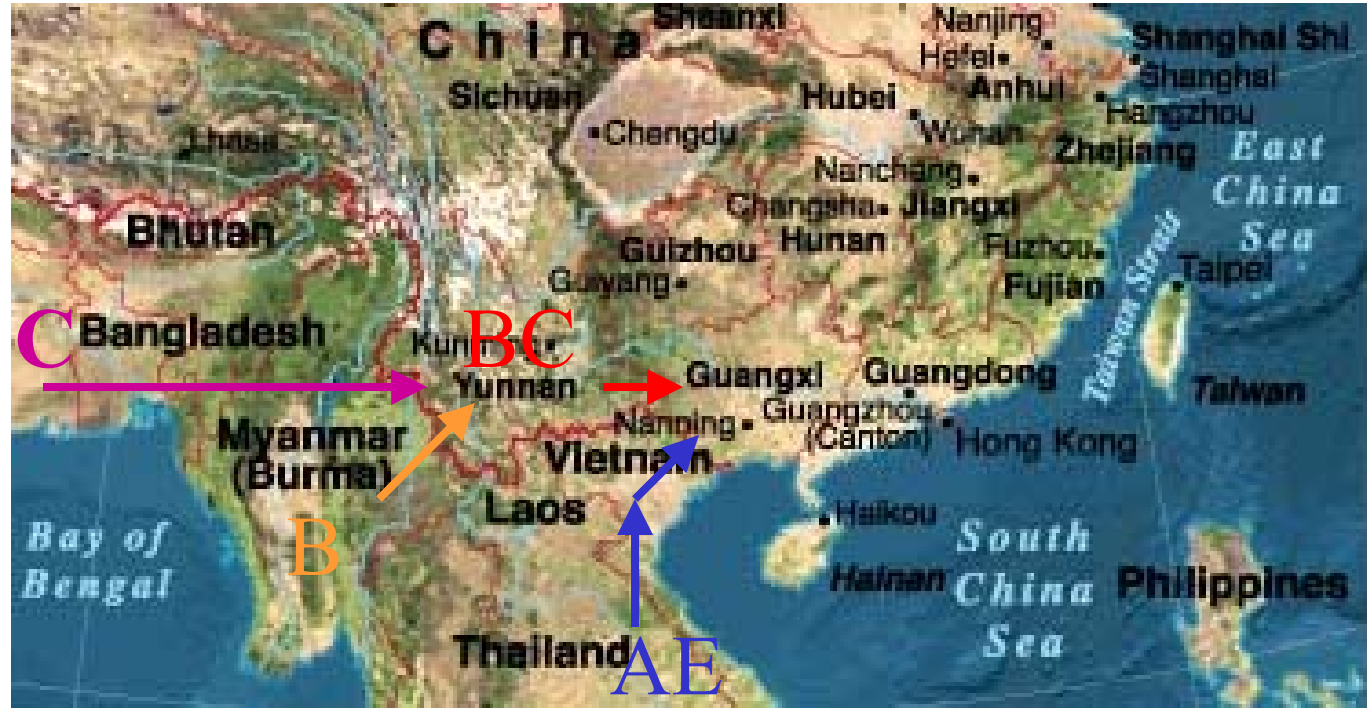


Progression of HIV Subtypes into Guangxi

Subtype **C**
from India¹

Subtype **B**
from Myanmar²

BC recombinant
formed in Yunnan³



AE recombinant from
Thailand through Vietnam⁴

References

- 1) Lole, JV 1999;73:152
- 2) Motomura, AIDS & Hum. Ret 2000;16:1831
- 3) Piyasirisilp, JV 2000;74:11286
- 4) Caumont, AIDS & Hum. Ret 2001;17:1285

Previous sequence data

References

- 1) Yu, JAIDS 1999 vol 22 p180
- 2) Piyasirisilp, JV 2000 vol 74 p11286
- 3) Yu, AIDS 2001 vol 15 p523

Baise¹
1996-1998
96-98CNGX6-9
N = 9, All BC

Baise²
Full length²
1997
97CNGX-6F }
97CNGX-7F }
97CNGX-9F }
Defined CRF_08BC

Pingxiang¹
1996 - 1998
96-98CNGX1-5
N = 14, all AE

Pingxiang²
full length
1997
97CNGX-2F
Both CRF_01AE



Tiayang³
1999
99NN001-5
N = 4, All BC

Tiandong³
1998
99NN001-5
N = 8, All BC

Binyang³
2000
99NN001-5
N = 3, All BC

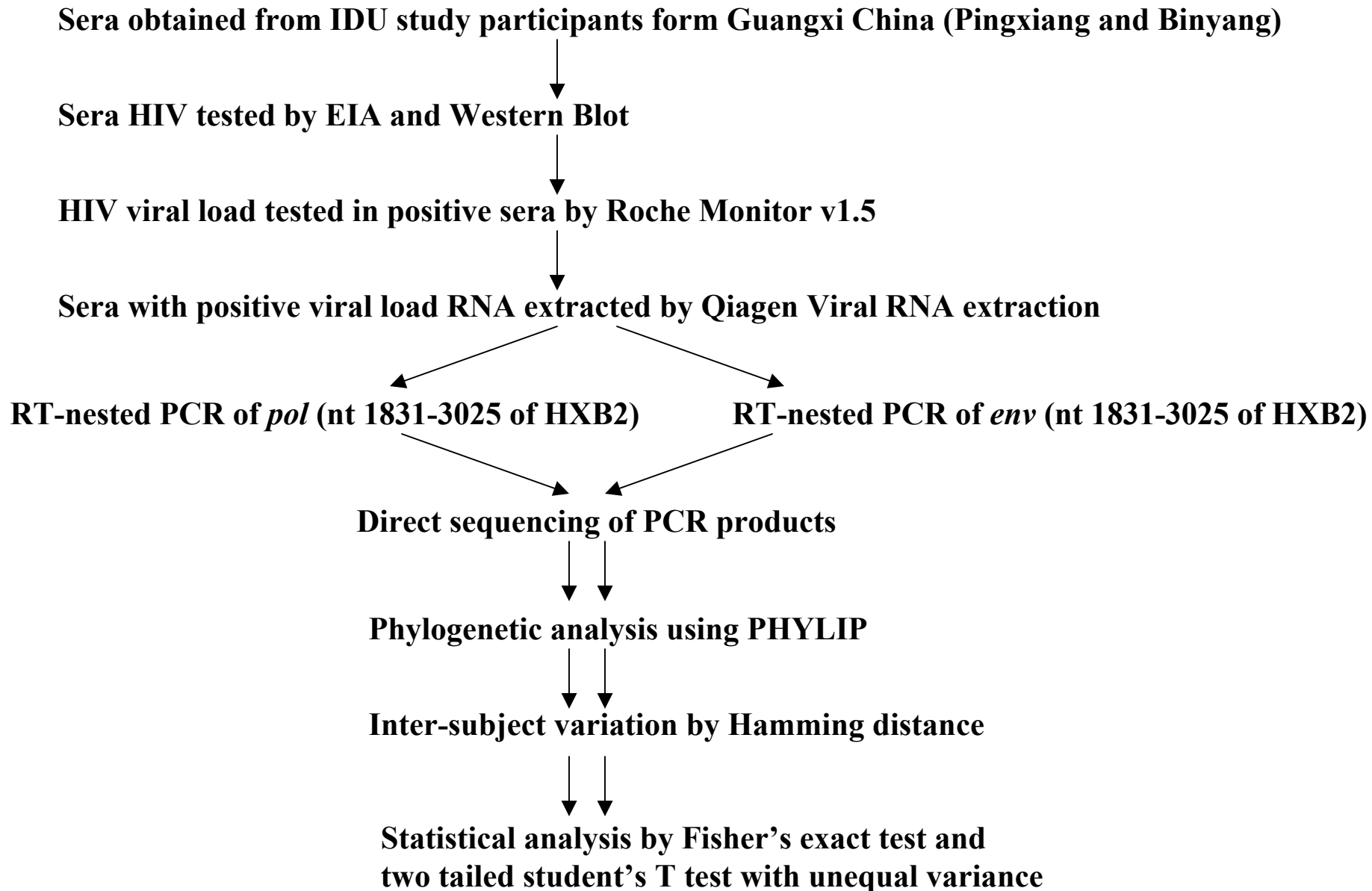
Nanning³
1999
99NN001-5
N = 3, All BC

Nanning² full length
1997, 97CNGX-11F
CRF_01AE

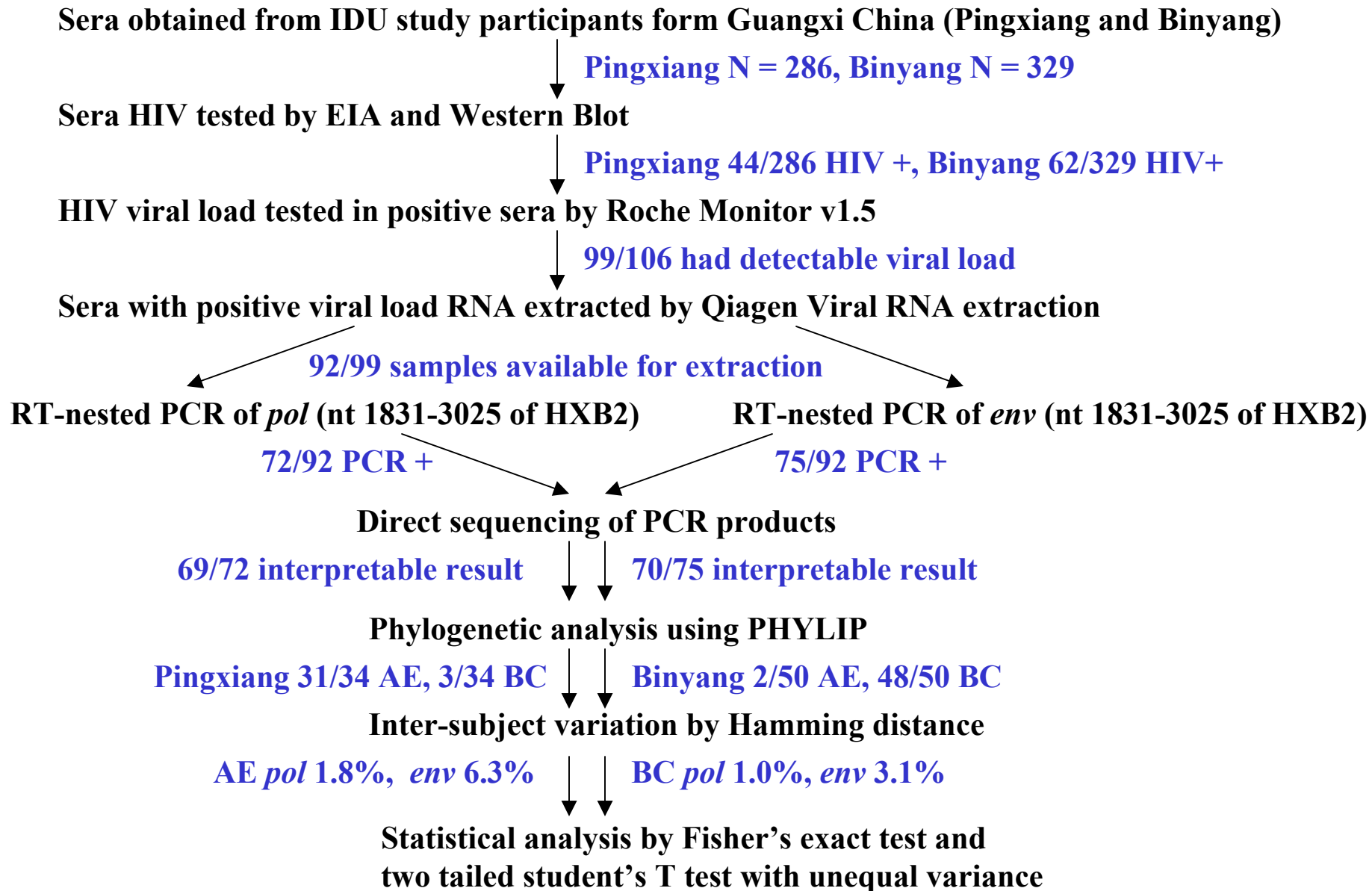
Objective

- Monitor the HIV epidemic in Guangxi China
 - Frequency of viral subtypes in Binyang and Pingxiang
- Determine differences in HIV subtype
 - Viral load set point
 - Inter-subject diversity of *env* and *pol*
- Develop a RT-PCR Protocol to obtain sequence data from limited volumes of sera and diverse HIV-1 subtypes

Procedure – Experimental Scheme



Experimental Scheme - Results



Results - viral load distribution by city

<u>City</u>	<u>N</u>	<u>minimum</u>	<u>Quartile</u>			<u>maximum</u>
			<u>1st</u>	<u>2nd</u>	<u>3rd</u>	
Pingxiang	40*	1.00 x10 ³	7.36x10 ³	3.24x10 ⁴	8.64x10 ⁴	4.31x10 ⁵
Binyang	60**	1.00 x10 ³	1.13x10 ⁴	2.40x10 ⁴	7.37x10 ⁴	1.83x10 ⁶

No difference $P > 0.05$

* Four subjects with viral loads <400 copies/ml

** Two subjects with viral loads <400 copies/ml

Results - viral load distribution by subtype

<u>Subtype</u>	<u>N</u>	<u>minimum</u>	<u>Quartile</u>			<u>maximum</u>
			<u>1st</u>	<u>2nd</u>	<u>3rd</u>	
AE	33	1.00 x10 ³	1.14x10 ⁴	3.65x10 ⁴	7.51x10 ⁴	1.83x10 ⁶
BC	53	1.00 x10 ³	9.37x10 ³	2.06x10 ⁴	8.64x10 ⁴	4.31x10 ⁵

No difference $P > 0.05$

Procedure – RNA extraction and RT-Nested PCR

30 μl of sera + 110 μl PBS extracted with QiaAMP viral RNA extraction kit



50 μl of RNA eluted, 10 μl used for RT-PCR (equivalent of 6 μl of sera/reaction)* in a 50 μl reaction



10 μl of the 1st round RT-PCR used as target for 2nd round 100 μl PCR



PCR product extracted with Qiaquick PCR purification kit, eluted with 30 μl

* A sample of 1×10^4 copies/ml would provide 60 targets

RT-PCR Results of *pol* and *env*

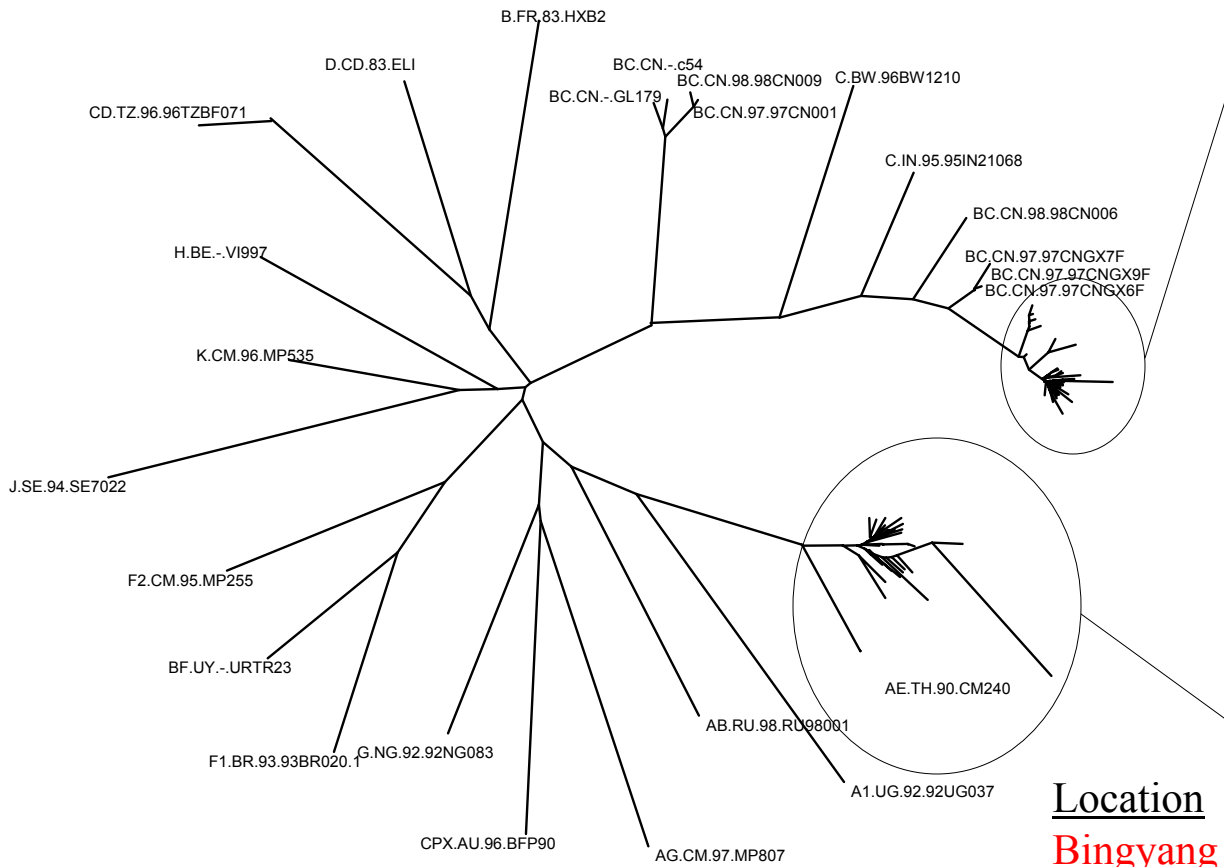
<i>pol</i> PCR results						
viral load	N	% positive	+	-	NA	ND
und	7	0.00	0	3		4
10 ³	29	0.57	16	12	1	0
10 ⁴	49	0.83	38	8	3	0
10 ⁵	19	1.00	16	0	3	0
10 ⁶	2	1.00	2	0	0	0
total	106		72	23	7	4

		<i>pol</i> PCR	
		+	-
<i>env</i> PCR	+	60	15
	-	12	5

<i>env</i> PCR results						
viral load	N	% positive	+	-	NA	ND
und	7	0.00	0	1		6
10 ³	29	0.68	19	9	1	0
10 ⁴	49	0.87	40	6	3	0
10 ⁵	19	0.88	14	2	3	0
10 ⁶	2	1.00	2	0	0	0
total	106		75	18	7	6

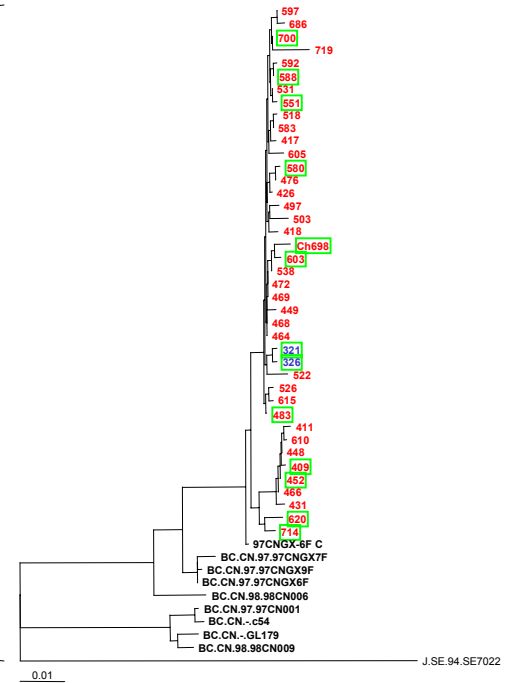
PCR <i>pol</i> / <i>env</i>	viral load			
	10 ³	10 ⁴	10 ⁵	10 ⁶
+/+	10	34	10	2
+/-	6	4	2	
-/+	9	6		
-/-	5			

Phylogenetic Analysis of HIV *pol* from Quangxi Province October 2000



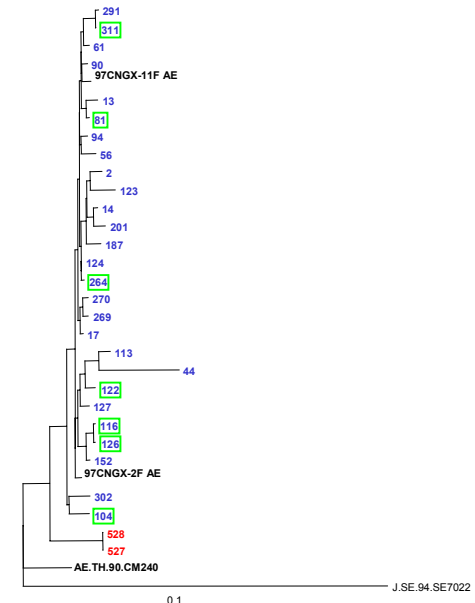
0.1

Recent seroconverters



0.01

J.SE.94.SE7022

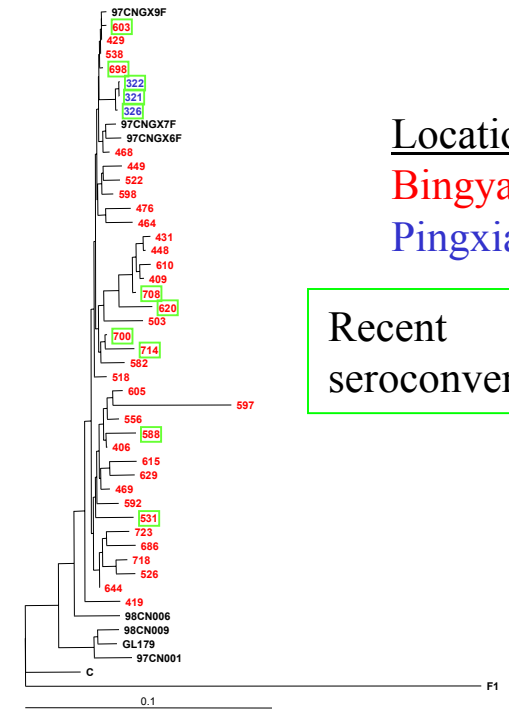
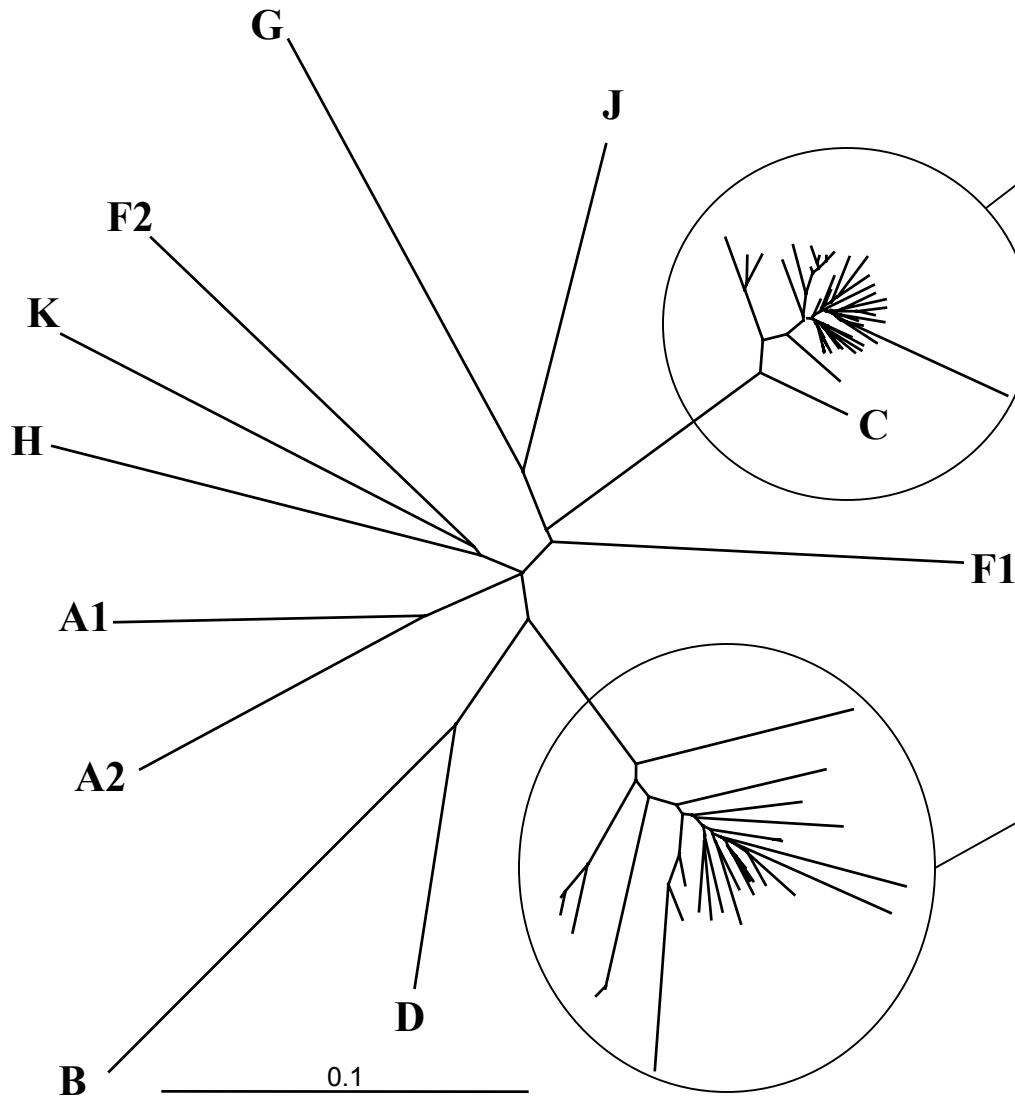


0.1

J.SE.94.SE7022

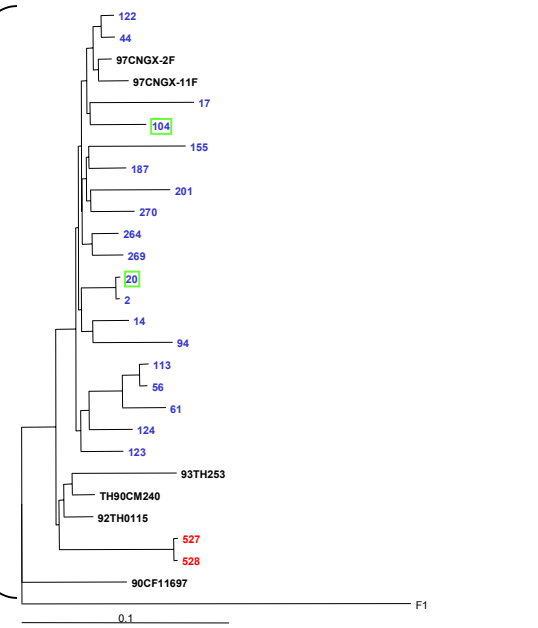
Location
Bingyang
Pingxiang

Phylogenetic Analysis of HIV *env* from Quangxi Province October 2000



Location
 Bingyang
 Pingxiang

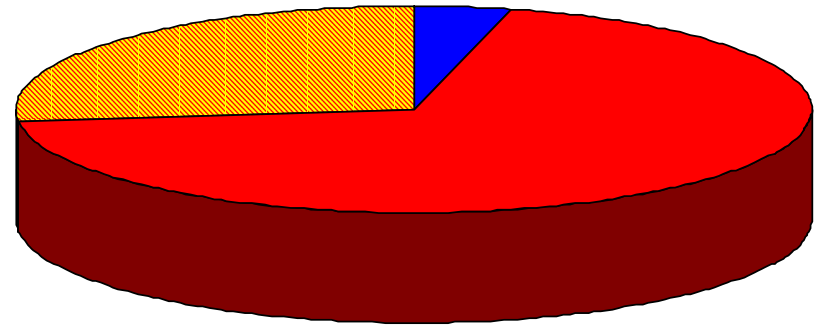
Recent seroconverters



HIV Subtype by City

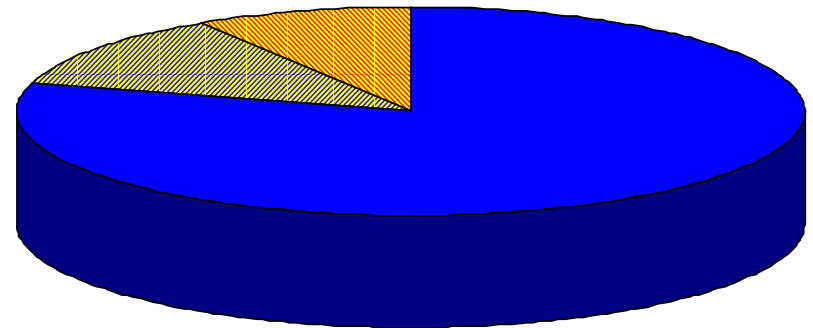
Binyang

14/14 recently infected BC
36/38 chronic BC
2/38 chronic AE



Pingxiang

4/7 recently infected AE
3/7 recently infected BC
27/27 chronic AE



Fisher's exact test shows a significant difference in Binyang vs. Pingxiang in recently infected subjects having a different subtype than in the prevalent population, $P = 0.026$

HIV Subtype

Chronic

● AE

● BC

Recently Infected

● AE

● BC

Results - *pol* Inter-Subject Hamming Distances

<u>Subtype</u>	<u>minimum</u>	Quartile				<u>SD</u>
		<u>1st</u>	<u>2nd</u>	<u>3rd</u>	<u>maximum</u>	
AE	0.00%	1.33%	1.84%	2.76%	6.46%	1.12%
BC	0.00%	0.75%	1.03%	1.50%	2.52%	1.03%

Significant difference $P \ll 0.001$

Results - *env* Inter-Subject Hamming Distances

<u>Subtype</u>	<u>minimum</u>	Quartile				<u>SD</u>
		<u>1st</u>	<u>2nd</u>	<u>3rd</u>	<u>maximum</u>	
AE	0.02%	4.68%	6.33%	8.44%	13.92%	2.51%
BC	0.04%	2.48%	3.11%	3.73%	7.90%	1.14%

Significant difference $P \ll 0.001$

Conclusions

- CRF08_BC is spreading more rapidly than CRF01_AE in this cohort of IDUs
- Introduction of CRF08_BC into Pingxiang
- Significant difference inter-subject diversity based on hamming between distances based CRF08_BC on CRF01_AE subtype
 - Less variation could indicate a stain better adapted to parental transmission
- No difference in viral load by subtype or city
- Effective PCR for generation of sequence data from small amount of sera