

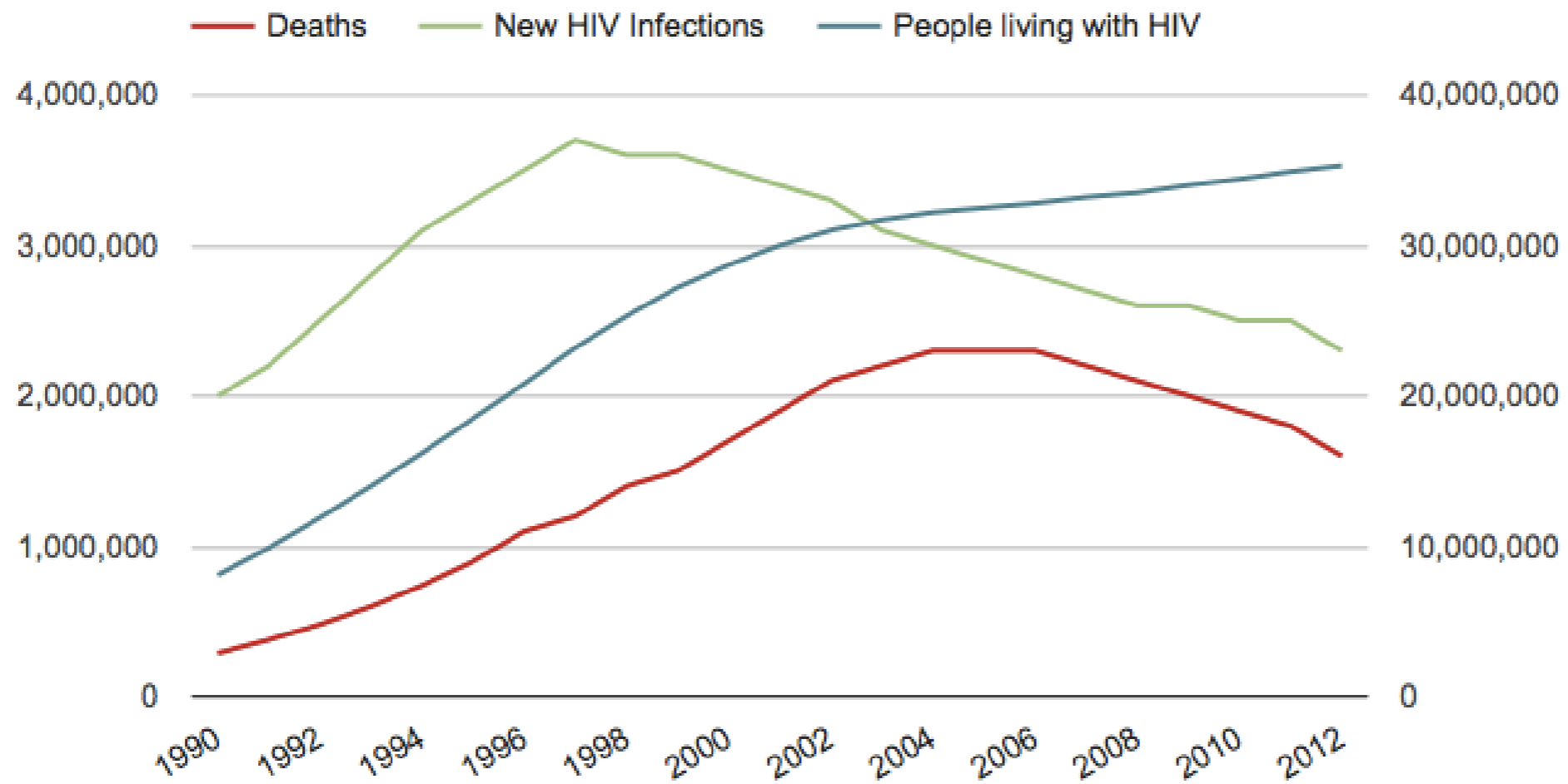
Molecular epidemiology of HIV-1 in central region of Nepal

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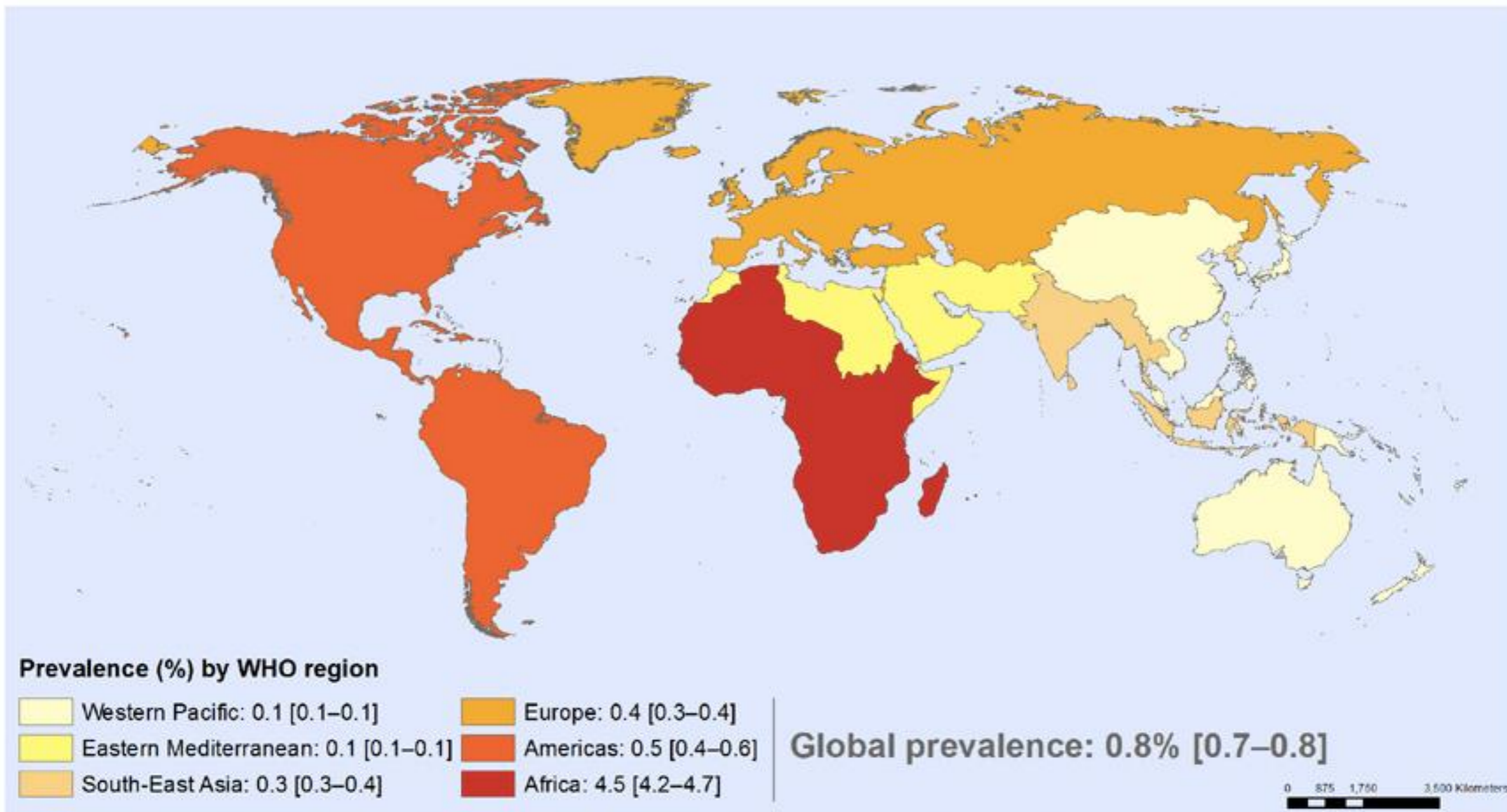
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Trend of global HIV Infection and Death 1990-2013



Adult HIV prevalence (15–49 years), 2013 By WHO region

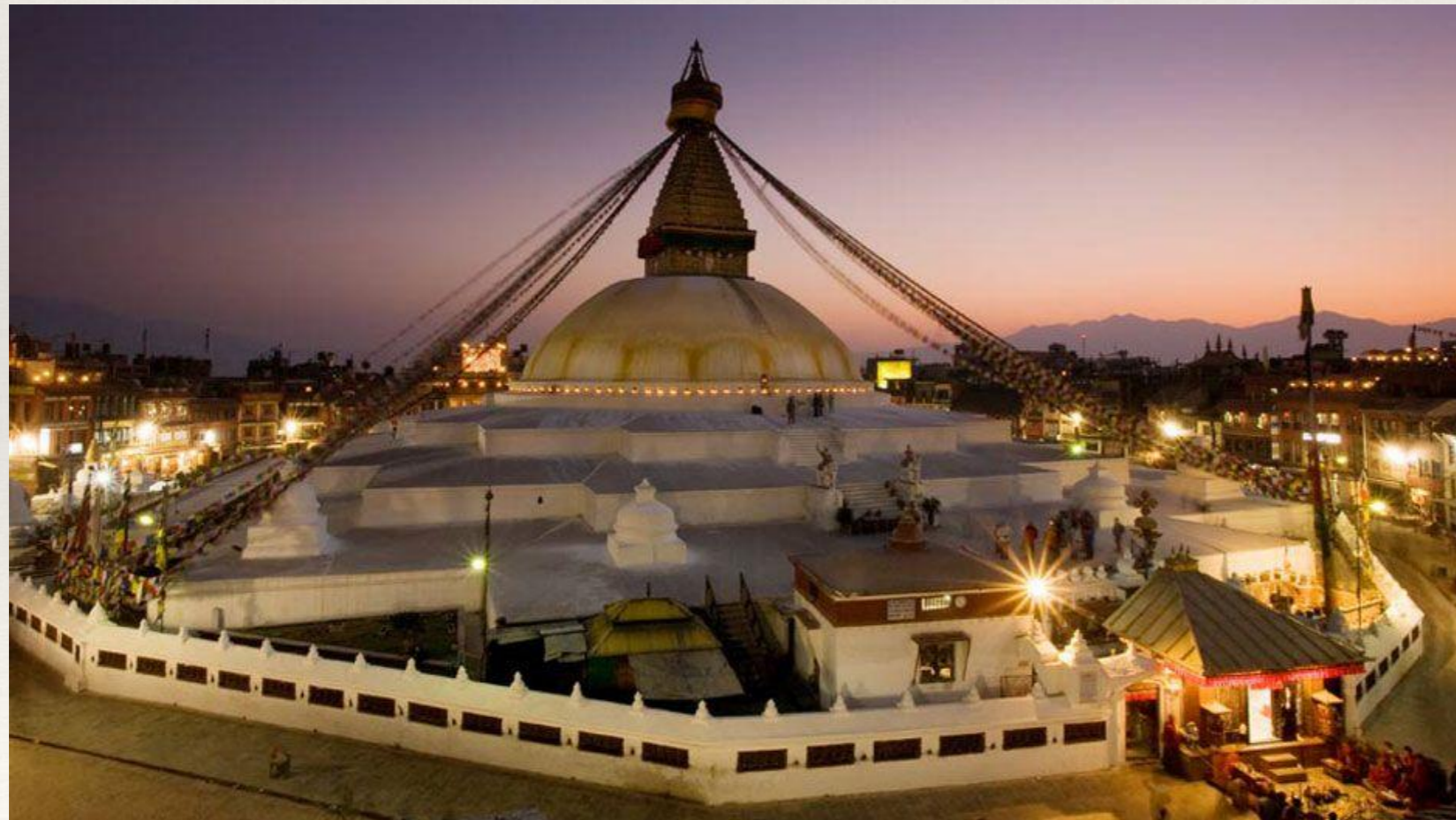
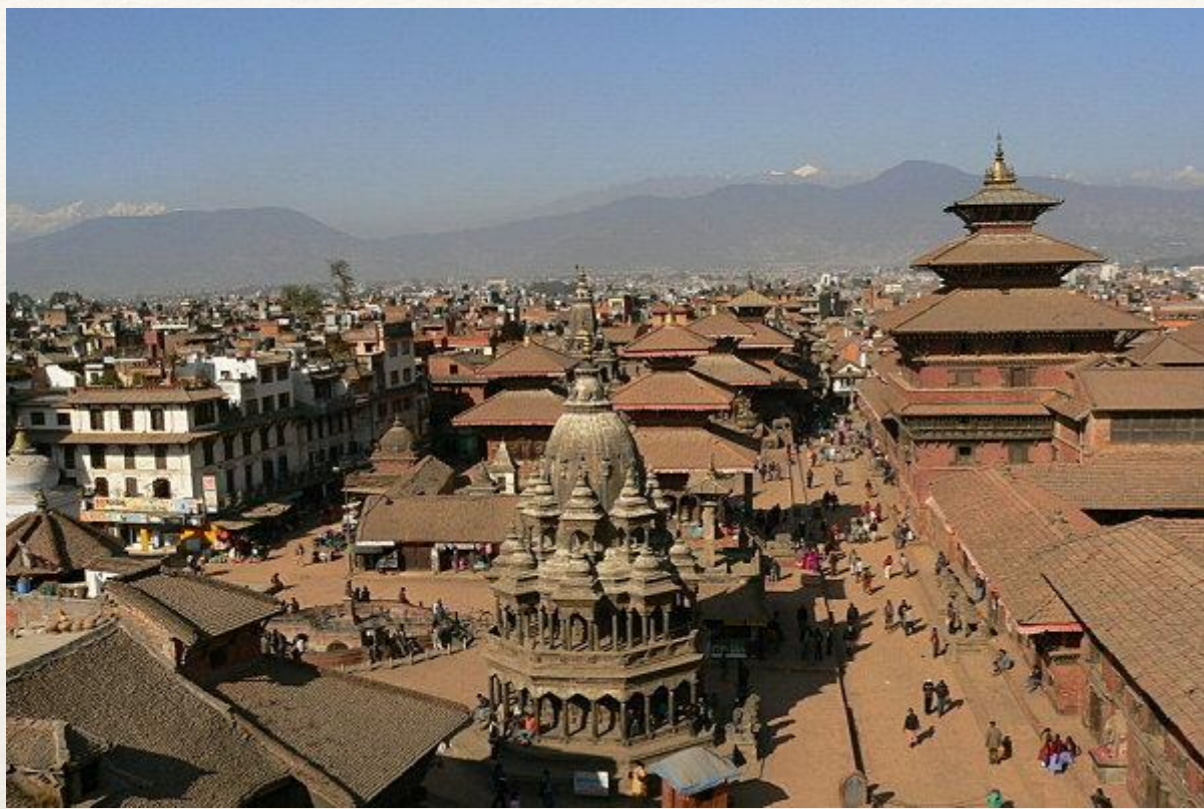


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Data Source: World Health Organization
Map Production: Health Statistics and Information Systems (HSI)
World Health Organization



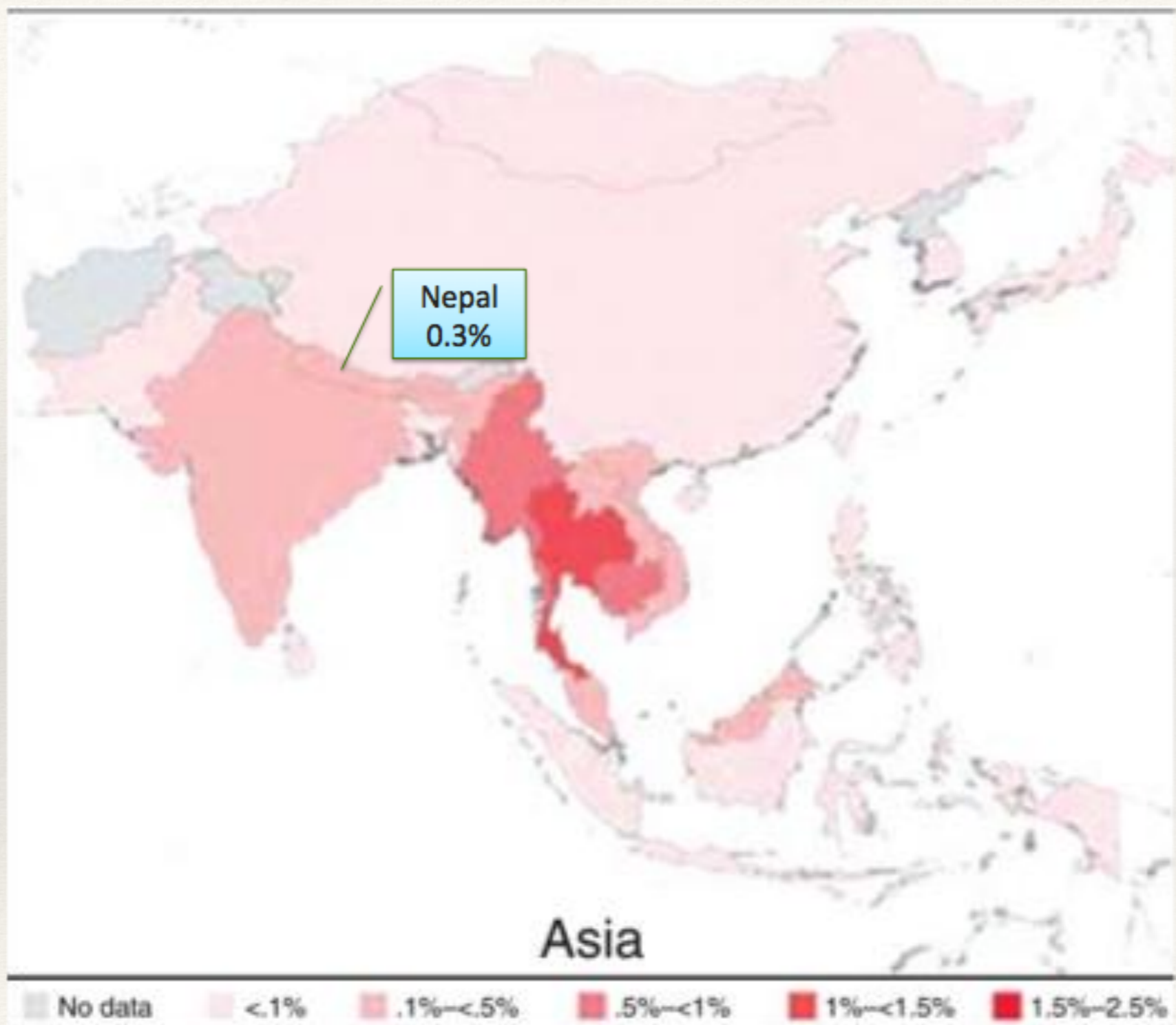
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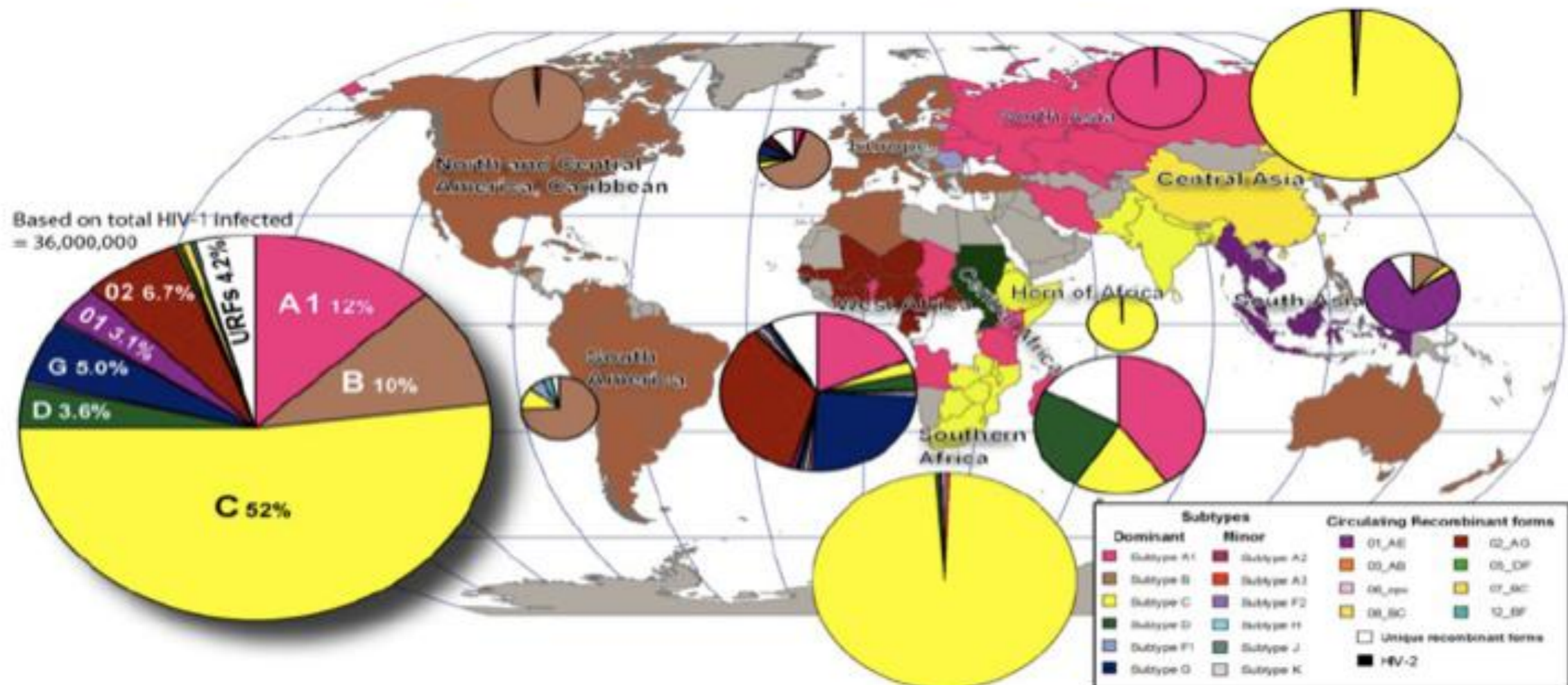


HIV Prevalence:

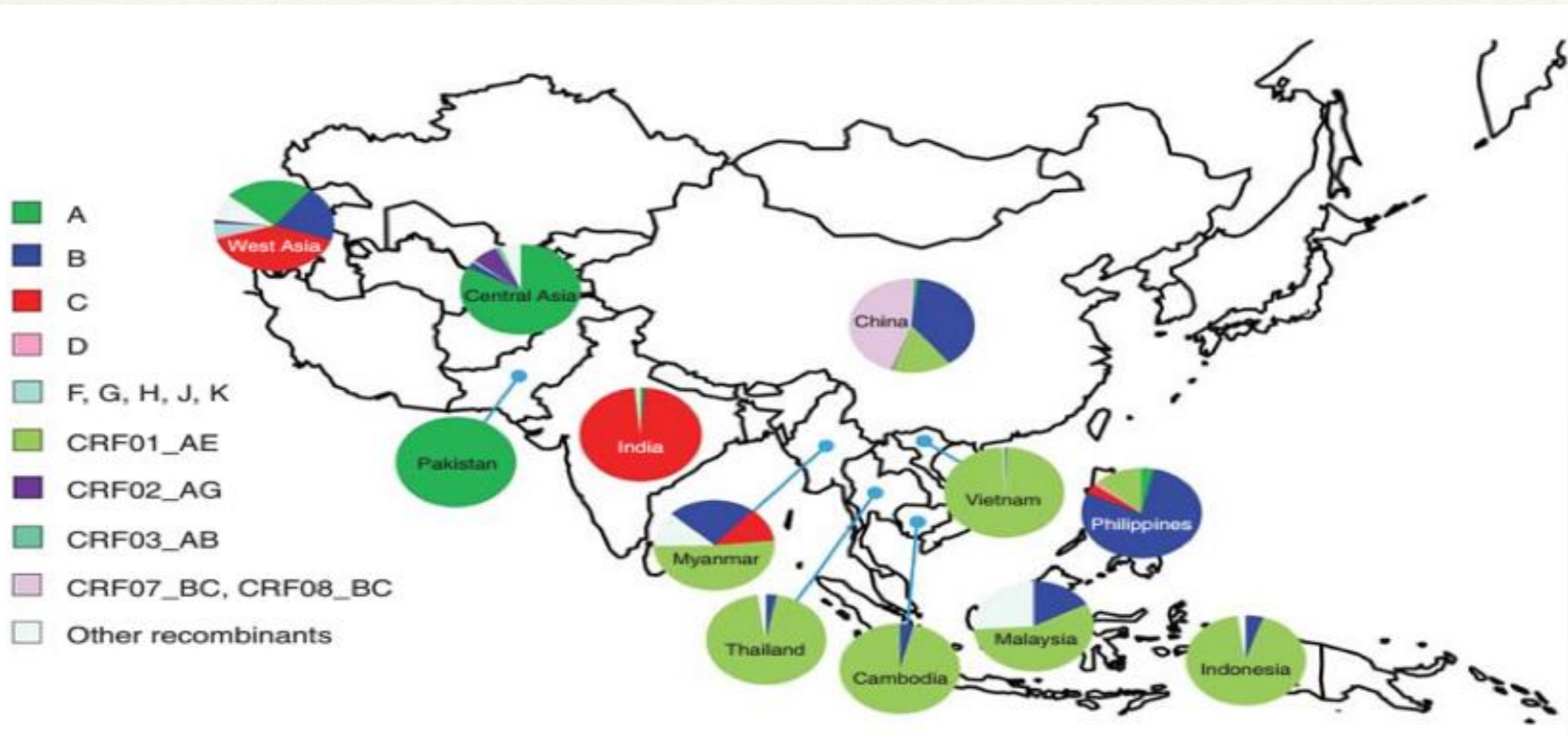


HIV-1 subtype and recombinant prevalence in the world

Subtype C is dominating the epidemic



Subtypes and CRFs distribution in Asia:



Epidemic snapshot of HIV in Nepal

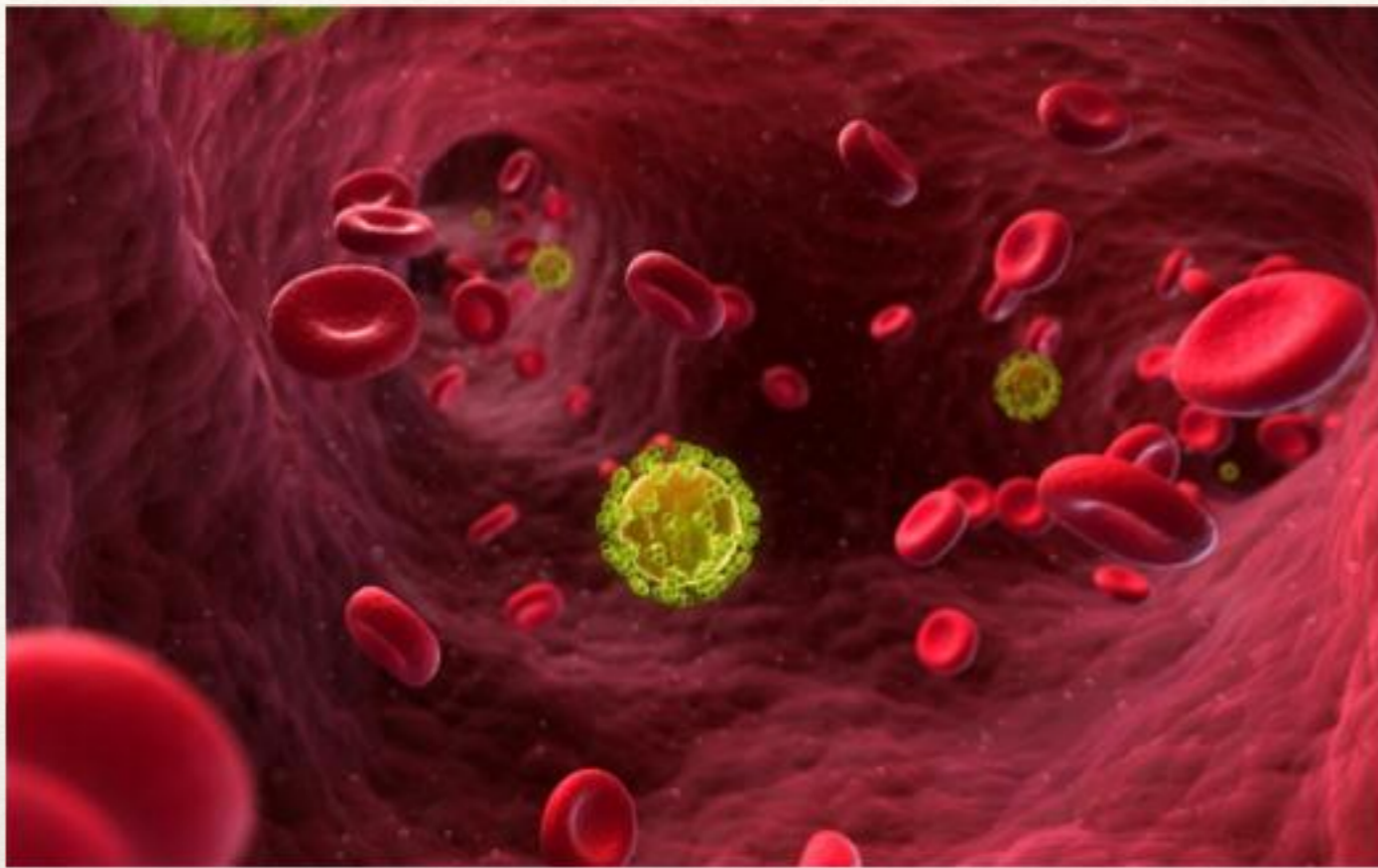


Adult prevalence rate 0.3%

Objectives:

1. To characterize the local subtype and CRF(s) distribution of HIV-1 in central region of Nepal

2. To study the phylogenetic relationship between circulating HIV-1 subtype in Nepal and in neighboring countries.



Material and Methods

Subjects

- Sample population
 - 10 samples (EDTA blood)
- Study area
 - Central** region of Nepal
- Sample collection duration
 - July – September** 2011



Clinical characteristics of selected sample

Sample I.D	Year of Infection	Location	Risk Group	Viral load	ART status
NP003	2007	Nawalparasi	HSR	3,685	NONE
NP004	2008	Chitwan	HSR	79,787	AZT,3TC,NVP
NP005	2008	Nawalparasi	HSR	5,942	NONE
NP007	2009	Chitwan	IDU	3,298	AZT,3TC,NVP
NP008	2011	parsa	MTCT	31,940	NONE
NP010	2001	Chitwan	HSR	10,413	AZT,3TC,NVP
NP014	2011	Chitwan	HSR	5,942	AZT,3TC,NVP
NP015	2011	Chitwan	HSR	63,485	NONE
NP016	2011	Chitwan	HSR	14,420	AZT,3TC,NVP
NP021	2011	Gorkha	HSR	4,599	AZT,3TC,EFV
NP023	2008	Chitwan	HSR	22,777	AZT,3TC,NVP
NP028	2011	Makwanpur	HSR	< 40	AZT,3TC,NVP
NP035	2011	Chitwan	MTCT	8,64,318	d4T,3TC,EFV
NP041	2010	Chitwan	IDU	4,599	AZT,3TC,NVP
NP046	2009	Chitwan	IDU	40,192	NONE
NP051	2010	Chitwan	HSR	159,488	AZT,3TC,NVP
NP054	2003	Chitwan	IDU	25,980	AZT,3TC,NVP
NP065	2010	Chitwan	MSM	2,176	NONE
NP068	2007	Gorkha	HSR	2,230	NONE
NP069	2011	Chitwan	MTCT	6,067	NONE
NP071	2009	Chitwan	IDU	51,218	AZT,3TC,NVP
NP074	2011	Nawalparasi	HSR	86,103	AZT,3TC,NVP
NP075	2009	Nawalparasi	HSR	7,314	NONE
NP076	2009	Nawalparasi	HSR	9,649	NONE
NP078	2011	Chitwan	MTCT	3,186	NONE

Clinical characteristics of selected sample

Sample I.D	Year of Infection	Location	Risk Group	Viral load	ART status
NP079	2011	Chitwan	HSR	9,649	NONE
NP080	2005	Nawalparasi	HSR	21,253	NONE
NP082	2011	Nawalparasi	HSR	32,235	NONE
NP084	2008	Chitwan	HSR	44,285	NONE
NP086	2011	Parsa	HSR	4,695	AZT,3TC,NVP
NP089	2001	Chitwan	HSR	60,480	NONE
NP091	2007	Chitwan	HSR	3,535	NONE
NP092	2006	Nawalparasi	MSM	21,265	AZT,3TC,NVP
NP093	2010	Chitwan	HSR	5,545	NONE
NP095	2011	Chitwan	HSR	<40	AZT,3TC,EFV
NP097	2009	Nawalparasi	MSM	1,819	NONE
NP100	2011	Nawalparasi	MTCT	9,625	NONE
NP101	2011	Chitwan	HSR	5,807	NONE
NP102	2010	Nawalparasi	HSR	31,794	AZT,3TC,NVP
NP103	2010	Chitwan	HSR	2,612	AZT,3TC,NVP
NP104	2010	Chitwan	HSR	159,488	NONE
NP107	2003	Chitwan	IDU	25,980	AZT,3TC,NVP
NP111	2010	Chitwan	HSR	2,176	NONE
NP113	2003	Gorkha	HSR	2,230	AZT,3TC,NVP
NP115	2011	Chitwan	MSM	6,067	NONE
NP119	2011	Chitwan	HSR	51,218	NONE
NP120	2008	Nawalparasi	HSR	86,103	AZT,3TC,NVP
NP122	2009	Nawalparasi	HSR	7,314	NONE
NP128	2009	Nawalparasi	HSR	9,649	NONE
NP131	2011	Chitwan	MTCT	3,186	NONE





Siriraj hospital in bangkok

Ethical issues

Institutional factors:

- Approved by Research ethics committee of Mahidol university
- Ethical clearance from NHRC(Nepal Health Research Council)
- Permission from Hospital establishment, of patient recruited

Sample population factor:

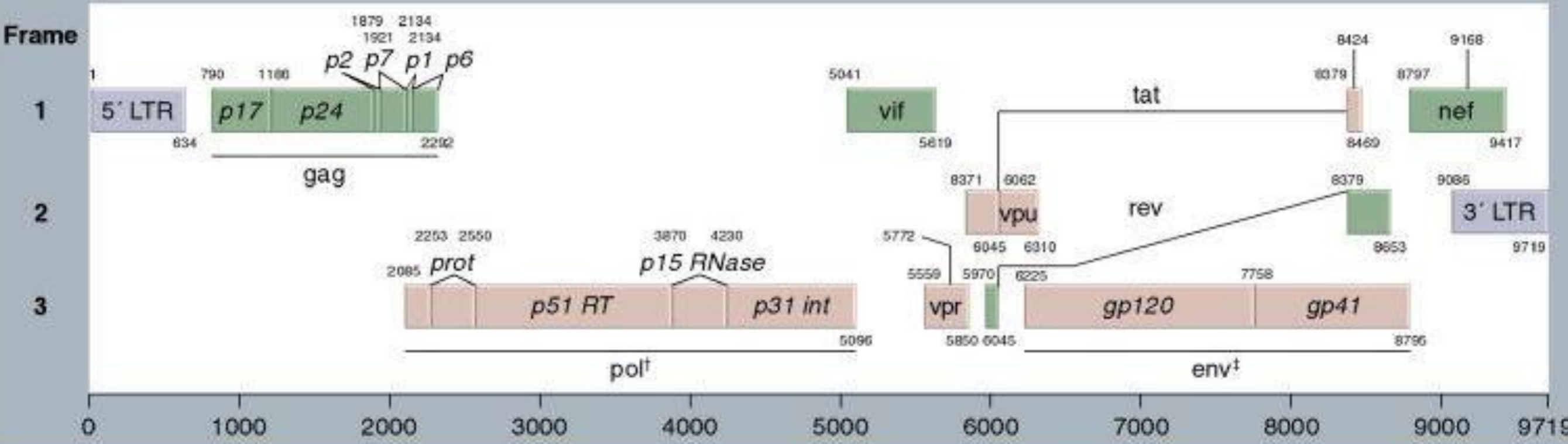
- Signed Informed consent taken from all participating patients before collecting demographic data and blood samples.
- Explanation of the study provided in local language

Baseline characteristics of 10 HIV-1 infected cases

Characteristics	Total patients	ART Naïve patients	ART experienced
	n	n	n
Number of patients	10	7	3
Male	6	4	2
Female	4	3	1
Transmission mode			
Heterosexual	5	4	1
Homosexual	1	1	0
MTCT	2	1	1
IDU	2	1	1

Viral load detection of all samples were done By m2000 Real Time HIV-1 automated system(Applied Bio system, CA, USA). After they were airlifted from Nepal.

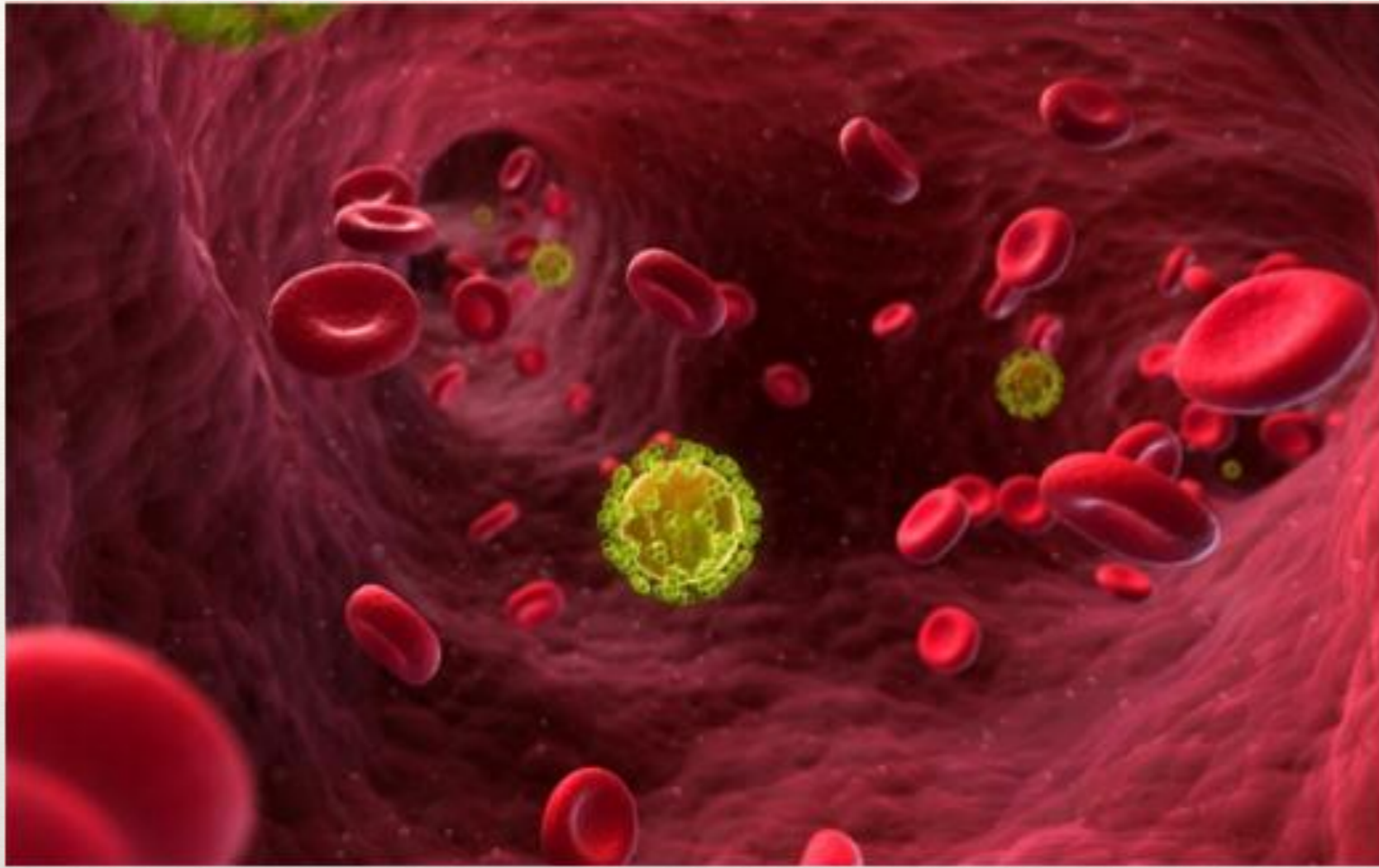
		min-max	median
		5-57	30
CD4 ⁺ T cells	365.5	8 - 1188	443
Viral load (log copies/ml)	3.3	1.60 – 6.00	3.61



Data Analysis

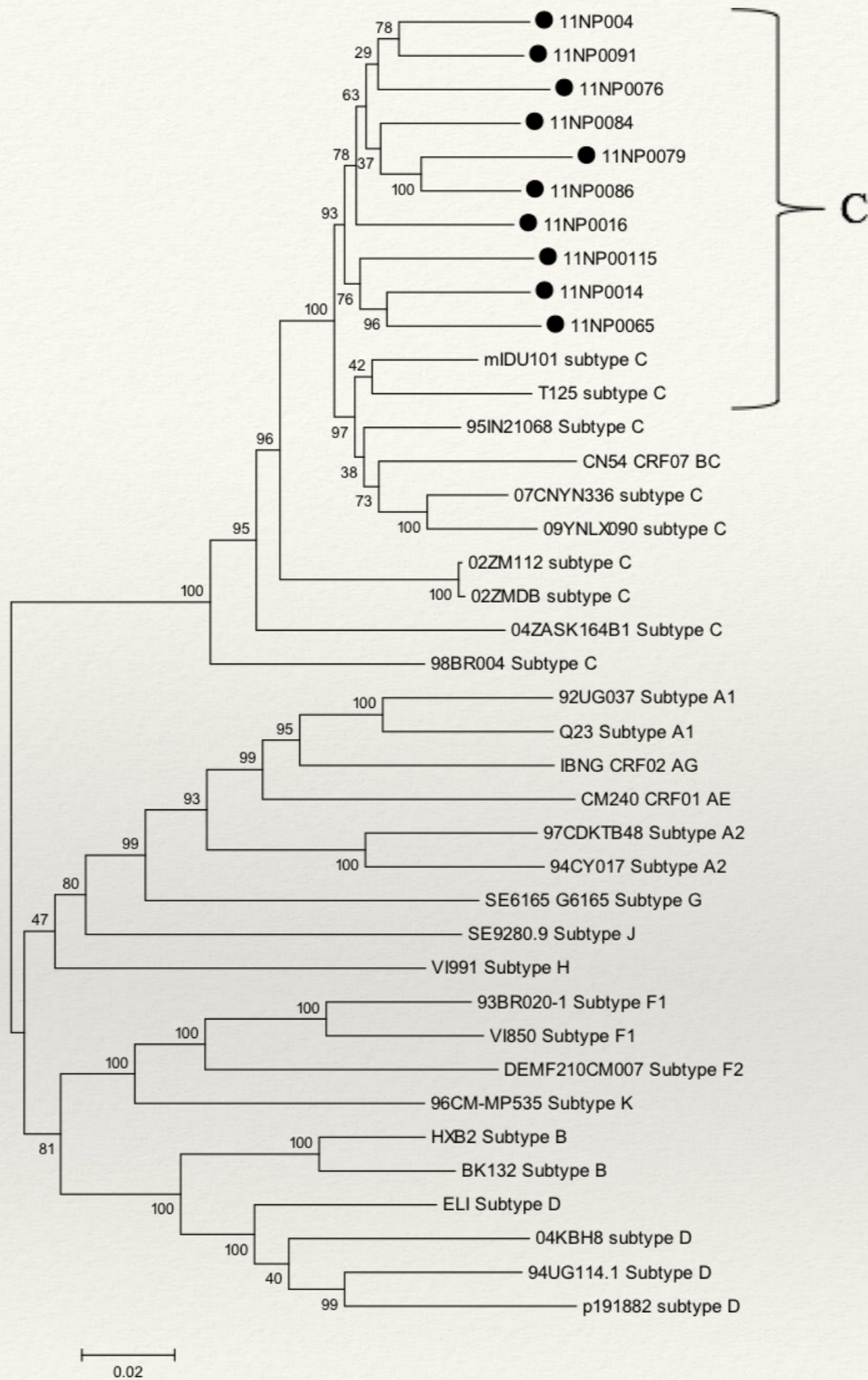
Nucleotide Sequences and Phylogenetic analysis

- **Ab1 file from Genetic Analyzer**
- ***env* and *gag-pol* : DNAbaser version 5.2.3 (HeracleBioSoft, Romania)**
- ***pol* : Viroseq software version 2.8 (Celera, USA)**
- **Multiple alignment : MEGA version 5.2 : 500 bootstraps replicate**
- **HIV-1 breakpoint of recombination : JpHMM soft ware**
- **Viral signature pattern analysis : VESPA software**
- **Phylogenetic tree: NJ method: MEGA5.2**



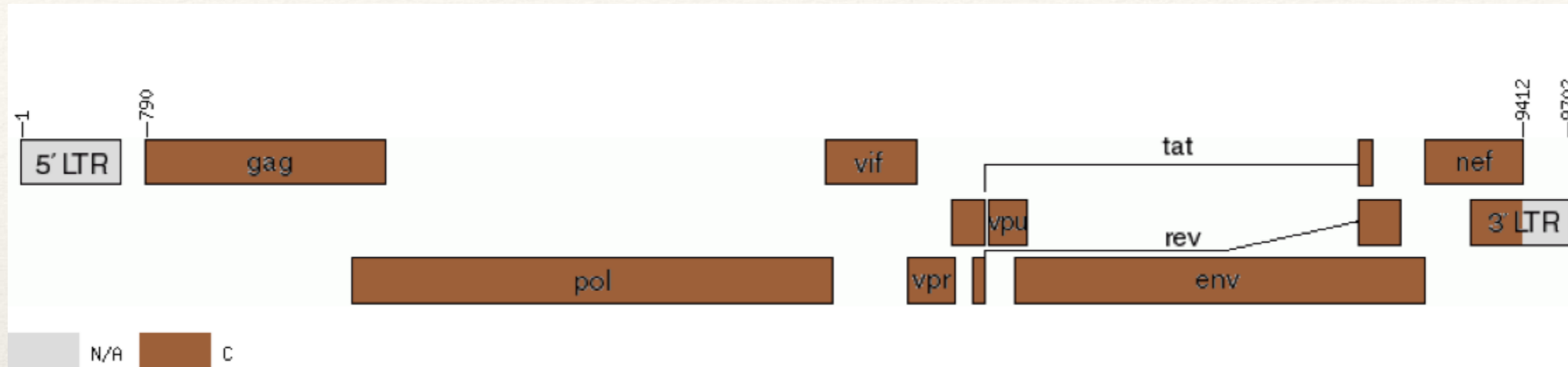
RESULTS





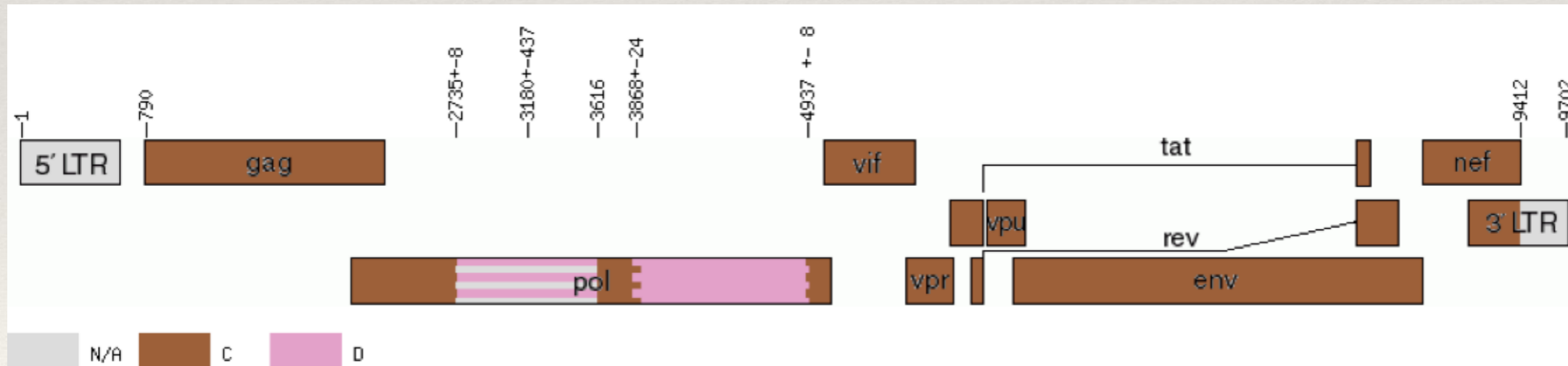
Phylogenetic analysis of 10 HIV-1 nearly full length nucleotide sequences

Subtype and recombination by jpHMM



9 samples :

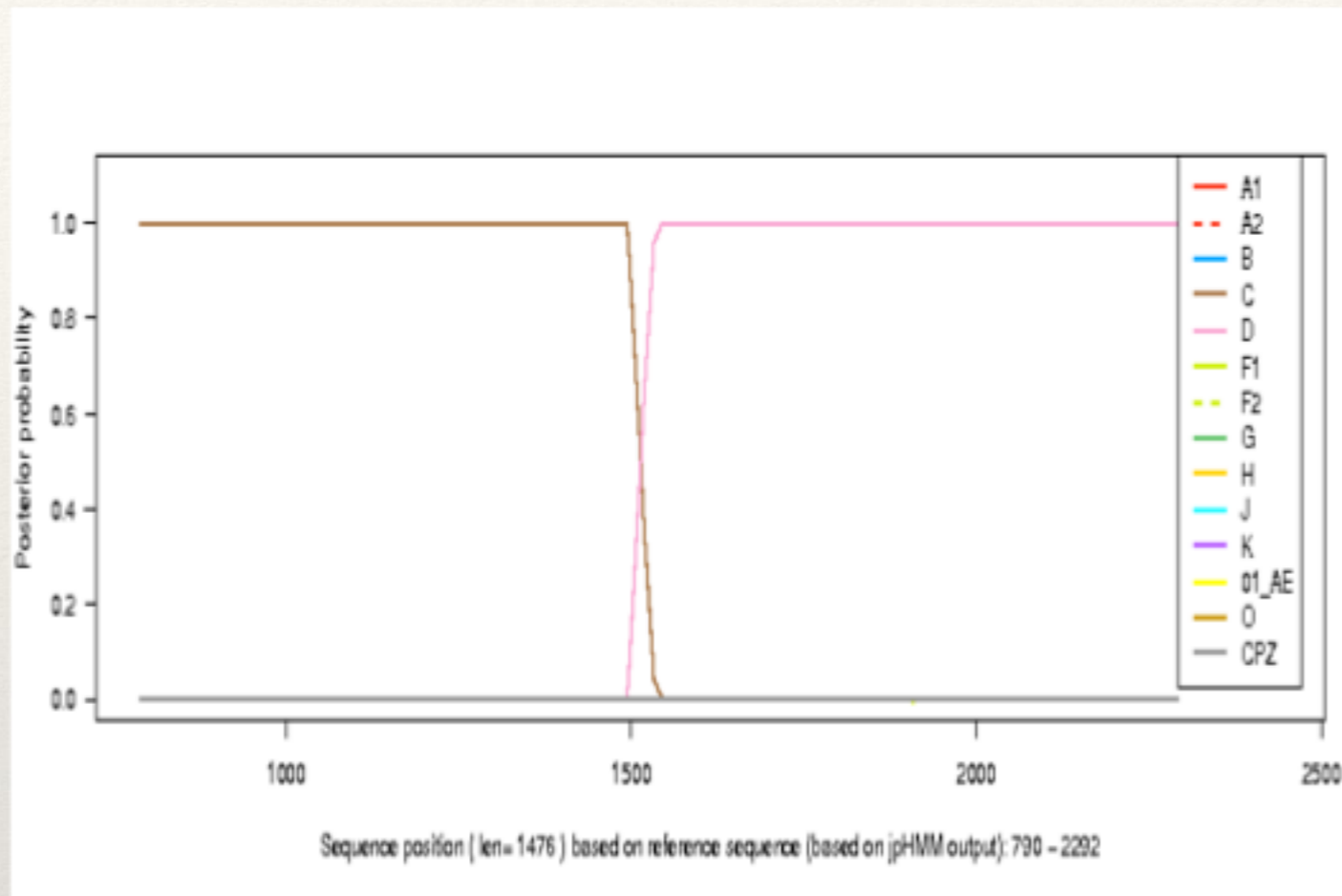
11NP004, 11NP014, 11NP016, 11NP065, 11NP076, 11NP084, 11NP086, 11NP091 and 11NP115



1 sample 11NP079

Fragment Start Position	Uncertainty Region Start - End	Breakpoint Interval Start - End	Fragment End Position	Fragment Subtype
Position in the original sequence [pred recombination] , [recombination incl UR and BPI] , [UR and BPI]				
1	-	-	789	N/A
790	2701 - 2716	-	2716	C
2717	2717 - 3590	-	3590	D
3591	3591 - 3597	3818 - 3865	3828	C
3829	-	-	4902	D
4903	-	-	9390	C
9391	-	-	9681	N/A
Position based on HXB2 numbering [pred recombination] [recombination incl UR and BPI] [UR and BPI]				

Posterior probability plot of Recombinant break points isolates



Quantity denotes probability base to subtypes in probabilistic recombinant model

intervals of expected breakpoint location.

NP079

790 - 2735	C
2736 - 3615	D
3868-4937	D
4937-9412	C

Mutation pattern of amino acids and drug resistance

Sample ID	HIV diagnosis year	Route of infection	Plasma HIV-1 RNA level (copies/ml)	HIV DRM			Drugs
				PI	NRTI	NNRTI	
11NP008	2011	MTCT	31,940	-	-	K103R	Efavirenz
11NP010	2010	HSR	10,413	-	-	V179D	Delavirdine
11NP068	2009	HSR	2,230	-	-	E138A	Etravirine
11NP071	2009	IDU	51,218	-	-	K103R	Efavirenz
11NP82	2010	HSR	32,325	A71V	-	-	Ritonavir
11NP103	2010	HSR	2,612	A71T	-	-	Indinavir
11NP104	2010	MTCT	159,297	-	-	V108I	Efavirenz
11NP122	2011	MSM	58,782	A71T	-	-	

Amino acid polymorphism in different subtypes

		↓	↓	↓		↓							↓		↓	↓	↓	↓				↓		↓	↓					
Position	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
B-cons	T	V	L	E	E	M	N	L	P	G	R	W	K	P	K	M	I	G	G	I	G	G	F	I	K	V	R	Q	Y	D
C-cons	V	S	.	.	.	K	R	E
NP008	D	I	D	Q	
NP015	N	D	H	

		↓	↓	↓	↓	↓					↓	↓	↓	↓		↓	↓	↓				↓												
Position	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99					
B-cons	A	I	G	T	V	L	V	G	P	T	P	V	N	I	I	G	R	N	L	L	T	Q	I	G	C	T	L	N	F					
C-cons	M					
NP008	I					
NP015	I					

Subtype C NP015 one major mutation at position 82 one minor at 60

CRF-08BC NP008 three minor mutation at position 36,60 and 77

Discussions

- HIV-1 infected patients from Nepal revealed the distinct cluster between HSR, IDU, MSM and MTCT transmission group.
- Nepalese sequences clustered very tightly indicating possible “founder effect”, i.e. infection had originated from single ancestral virus, possibly brought in either by commercial sex worker or migrating labor from India
- The identification of these subtypes and CFRs in the central region of Nepal is important in epidemiological tracking as well as surveillance program planning.

Conclusion

- ❑ This study is first look into
 - HIV-1 near full length **subtypes** circulating in Nepal
 - Recombination**
- ❑ Critical factor monitoring HIV **pandemics**,
vaccine design and HIV **treatment** outcome
- ❑ Country wide **Molecular Surveillance** needed

Acknowledgements

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- Dept. of Microbiology, Faculty of Medicine, Siriraj Hospital.
- Participating patients
- Staff of Bharatpur Hospital , Nepal

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THANK YOU !
For Your Attention



