



Phylogenetic analysis of Human Bocavirus isolates in Iran

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April 12, 2010

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Vienna–Austria



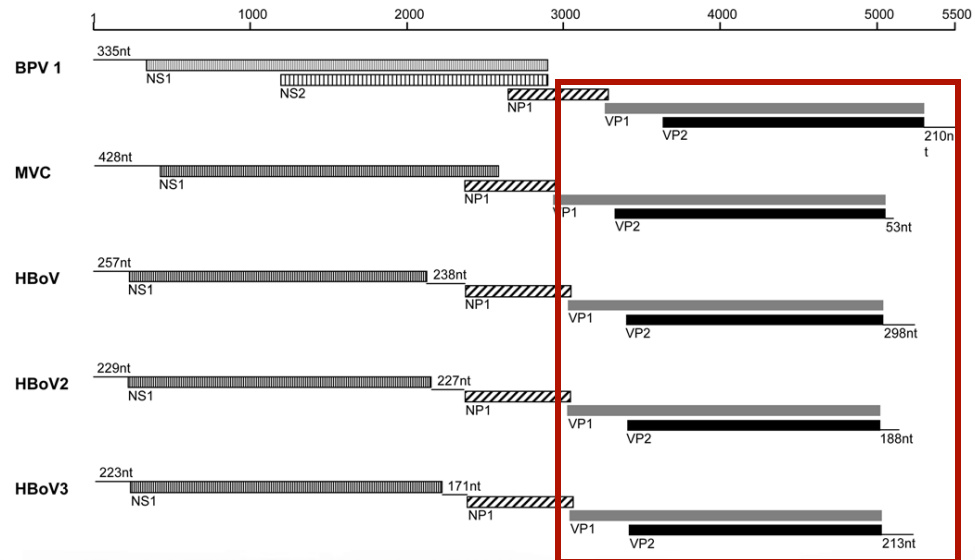
Human Bocavirus (hBoV)

- Acute respiratory infections and viral gastroenteritis are major causes of morbidity and mortality in children worldwide.
- In 2005, hBoV was discovered through a ‘molecular virus screening’ procedure.
- hBoV classified in
 - *Parvoviridae* family, *Parvovirinae* subfamily and Bocavirus genus.



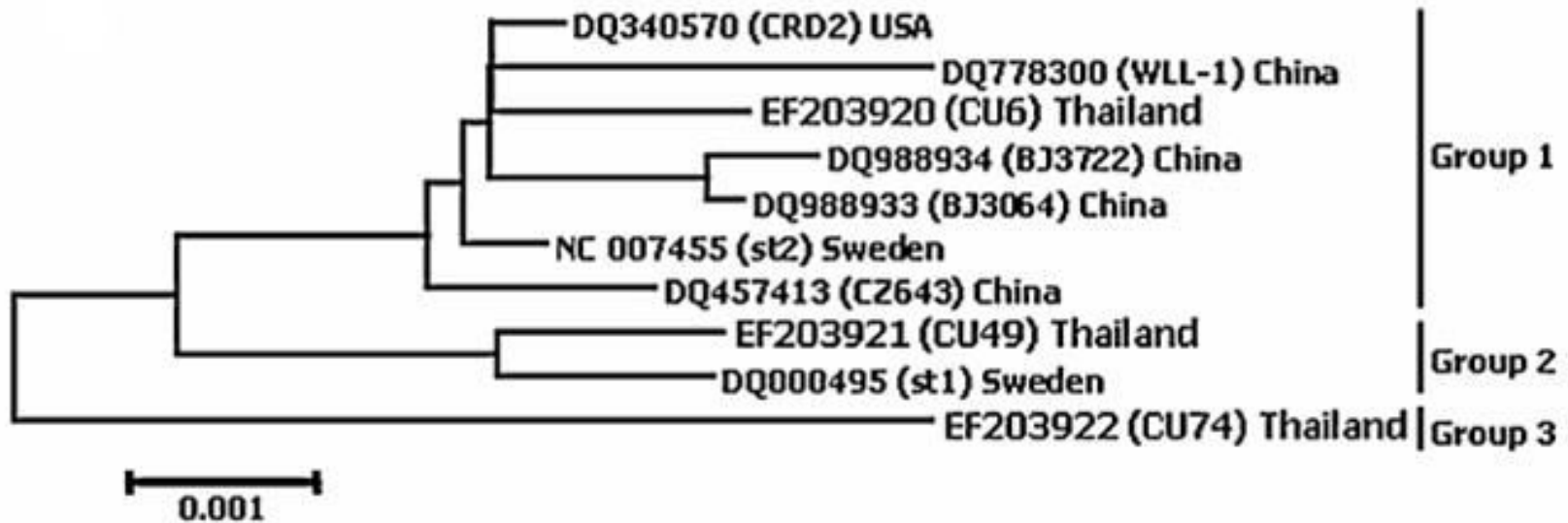
hBoV

- HBoV
 - circulating worldwide.
 - associated with both upper and lower respiratory tract infections and acute gastroenteritis
 - predominantly in infants and children.
- Genome of HBoV is conserved in all gene
 - a very infrequent nucleotide polymorphism restricted mainly to VP1 and VP2 genes.



PLoS Pathog 5(4): e1000391.
doi:10.1371/journal.ppat.1000391

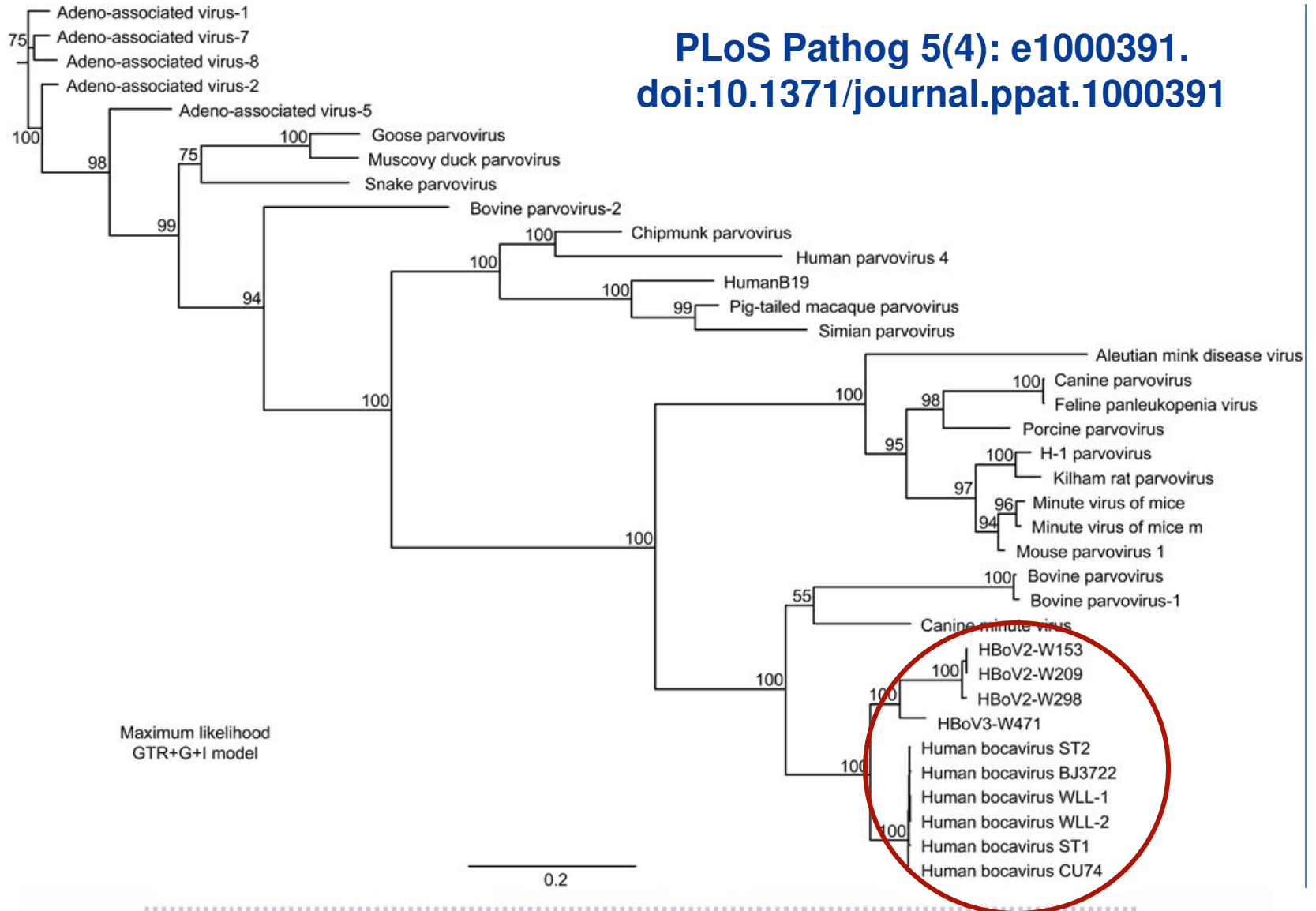
T. Chieochansin et al. / Virus Research 129 (2007) 54–57



Phylogenetic analyses of complete coding sequence of HBoV



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hBoV in Iran

- 1st report of hBoV in Iran published by Naghipour and coworkers
 - 8% frequency in Iranian children ≤ 5 y with acute respiratory infections in a northern city of Iran (RASHT) during the autumn and winter of 2003–2004 (5 months)
- The aim of the present study was to determine the prevalence of hBoV and to analyse hBoV–1 phylogeny in Iranian children suffering from acute respiratory tract illnesses and acute gastroenteritis in NRITLD.



Materials and methods

Clinical samples

- collected from children aged ≤ 17 y
 - admitted to the Masih Daneshvari Hospital, NRITLD, Tehran.
- 180 individuals
 - 133 nasopharyngeal swabs and nasal washings
 - 50 exacerbated asthma cases and 83 cases with respiratory tract illnesses
 - collected between October 2007 and September 2008
 - 47 stool samples from individuals with acute gastroenteritis
 - collected between February 2006 and October 2008.



Materials and methods

Screening and Sequencing analysis

- Screening
 - a nested PCR in the NS-1 coding region.
- Phylogenetic analysis
 - Sequencing of an 863-bp fragment in the VP1 /VP2 gene junction.
 - Sequence edited by BioEdit software program version 7.0.5.2.
 - molecular evolutionary analyses conducted by MEGA version 3.1.
 - neighbour-joining method/Tamura-Nei



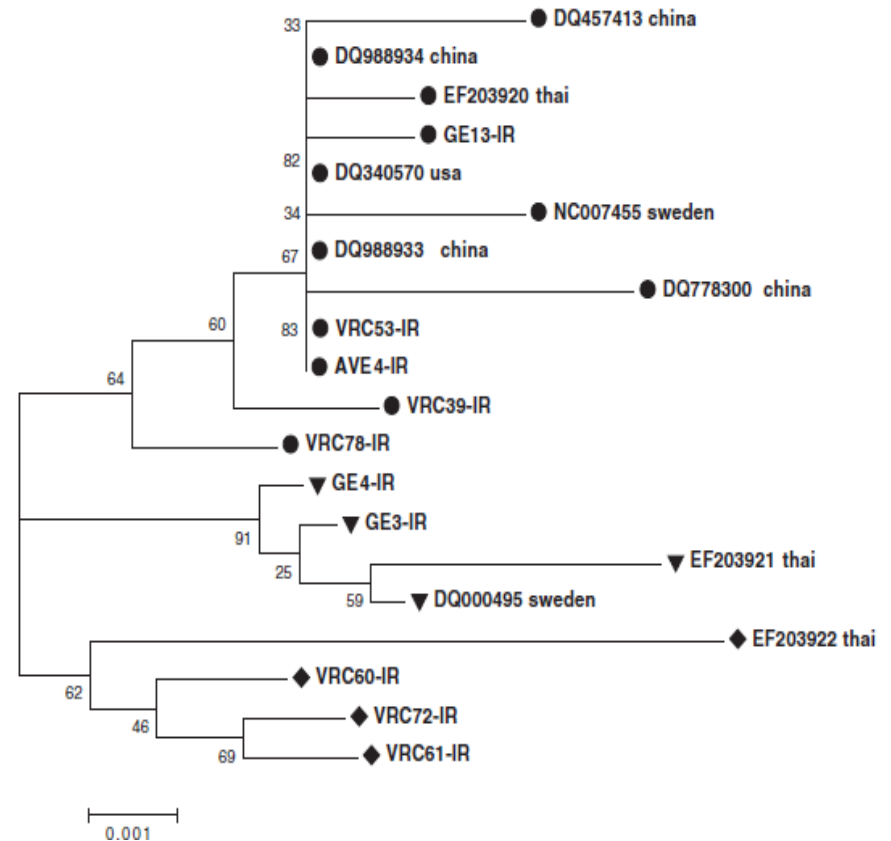
Results

Parameter	Exacerbated asthma (<i>n</i> = 50)	Respiratory illness (<i>n</i> = 83)	Gastroenteritis (<i>n</i> = 47)
Age (months), mean ± SD	66.74 ± 37.11	60.55 ± 41.72	30.26 ± 28.61
Gender, <i>n</i> (%)			
Female	14 (28)	25 (30.1)	19 (40.4)
Male	36 (72)	58 (69.9)	28 (59.6)
Season, <i>n</i> (%)			
Spring	5 (10)	12 (14.5)	15 (31.9)
Summer	6 (12)	3 (3.6)	6 (12.8)
Autumn	24 (48)	47 (56.6)	15 (31.9)
Winter	15 (30)	21 (25.3)	11 (23.4)
hBoV, <i>n</i> (%)			
Positive	<u>3 (6)</u>	<u>6 (7.2)</u>	<u>6 (12.8)</u>
Negative	47 (94)	77 (92.8)	41 (87.2)

Variable	Exacerbated asthma		Respiratory illness		Gastroenteritis	
	hBoV-positive (%)	No. of subjects	hBoV-positive (%)	No. of subjects	hBoV-positive (%)	No. of subjects
Age (y)						
≤1	0	3	0	6	4 (25)	16
2-3	3 (25)	12	4 (12.9)	31	2 (10)	20
4-6	0	13	1 (4.8)	21	0	5
≥7	0	22	1 (4)	25	0	6
<i>p</i> -Value	<u>0.031</u>		0.853		0.443	
Gender						
Female	0	14	1 (4)	25	3 (15.8)	19
Male	3 (8.3)	36	5 (8.6)	58	3 (10.7)	28
<i>p</i> -Value	0.364		0.411		0.465	
Season						
Spring	0	5	0	12	1 (6.7)	14
Summer	0	6	0	3	1 (16.7)	5
Autumn	2 (8.3)	24	6 (12.8)	47	0	15
Winter	1 (6.7)	15	0	21	4 (36.7)	7
<i>p</i> -Value	1.000		0.244		<u>0.024</u>	

Phylogenetic Tree of hBoV isolates

- Phylogenetic analysis of the VP1 /VP2 gene junction sequences in hBoV isolates.
- The tree was constructed by the neighbour-joining method using MEGA 3.1.
- The sequences of genetic groups 1, 2 and 3 are marked with black circles, triangles and diamonds, respectively.
- accession number of isolates deposited in GenBank database:
 - GQ891081-GQ891088, GQ906591 and GQ906592.



High nucleotide identity (99.18–100%) between all hBoV sequences

```
[ 1] #AVE4-IR
[ 2] #VRC53-IR
[ 3] #VRC39-IR
[ 4] #VRC60-IR
[ 5] #VRC72-IR
[ 6] #VRC78-IR
[ 7] #VRC61-IR
[ 8] #GE13-IR
[ 9] #GE3-IR
[10] #GE4-IR
```

	1	2	3	4	5	6	7	8	9	10
[1]										
[2]	<u>0.0000</u>									
[3]	0.0023	0.0023								
[4]	0.0058	0.0058	0.0058							
[5]	0.0070	0.0070	0.0070	0.0035						
[6]	0.0035	0.0035	0.0035	0.0047	0.0058					
[7]	0.0070	0.0070	0.0070	0.0035	0.0023	0.0058				
[8]	0.0012	0.0012	0.0035	0.0070	0.0082	0.0047	0.0082			
[9]	0.0058	0.0058	0.0082	0.0070	0.0082	0.0070	0.0082	0.0070		
[10]	0.0058	0.0058	<u>0.0082</u>	0.0070	0.0058	0.0070	0.0058	0.0070	0.0023	



99.138–100% amino acid identity between all hBoV sequences

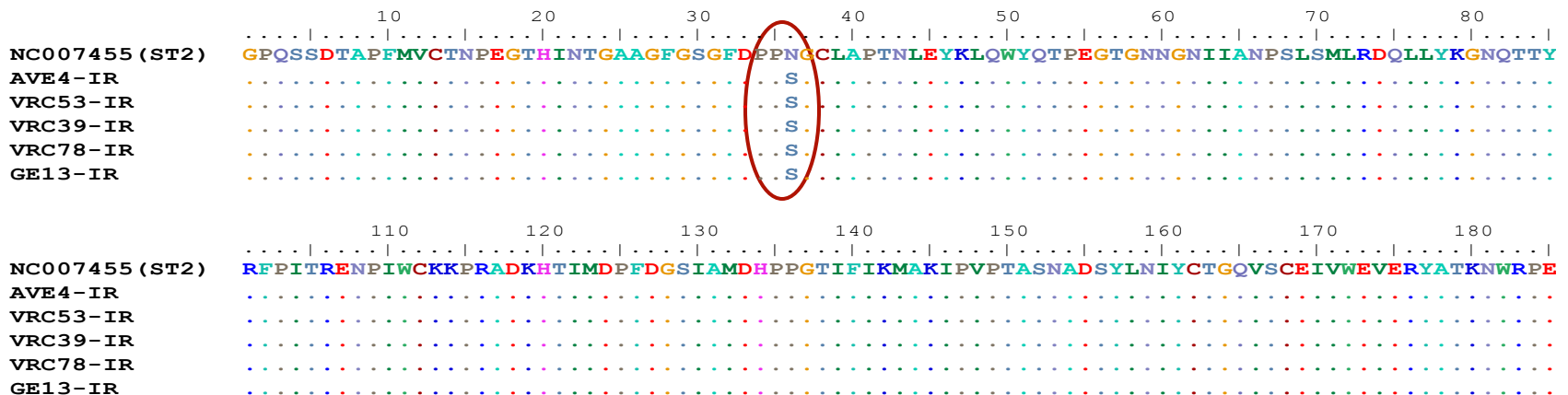
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[ 1] #AVE4-IR
[ 2] #VRC53-IR
[ 3] #VRC39-IR
[ 4] #VRC60-IR
[ 5] #VRC72-IR
[ 6] #VRC78-IR
[ 7] #VRC61-IR
[ 8] #GE13-IR
[ 9] #GE3-IR
[10] #GE4-IR
```

	1	2	3	4	5	6	7	8	9	10]
[1]										
[2]	<u>0.00000</u>									
[3]	<u>0.00000</u>	0.00000								
[4]	0.00430	0.00430	0.00430							
[5]	0.00000	0.00000	0.00000	0.00430						
[6]	0.00000	0.00000	0.00000	0.00430	0.00000					
[7]	0.00000	0.00000	0.00000	0.00430	0.00000	0.00000				
[8]	0.00000	0.00000	0.00000	0.00430	0.00000	0.00000	0.00000			
[9]	0.00430	0.00430	0.00430	0.00862	0.00430	0.00430	0.00430	0.00430		
[10]	0.00430	0.00430	0.00430	<u>0.00862</u>	0.00430	0.00430	0.00430	0.00430	0.00430	0.00000



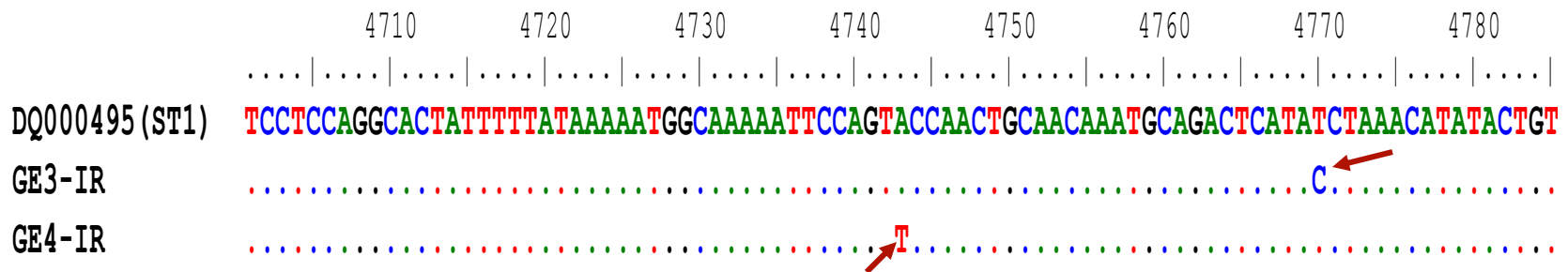
Genogroup 1

- nucleotide sequence identity of 99.42–99.77% with the reference isolate ST2
 - Seven nucleotide positions variable
 - All transitions
- Amino acid sequence identity of 99.57% with the reference isolate ST2
 - Only N36S amino acid change in all isolates



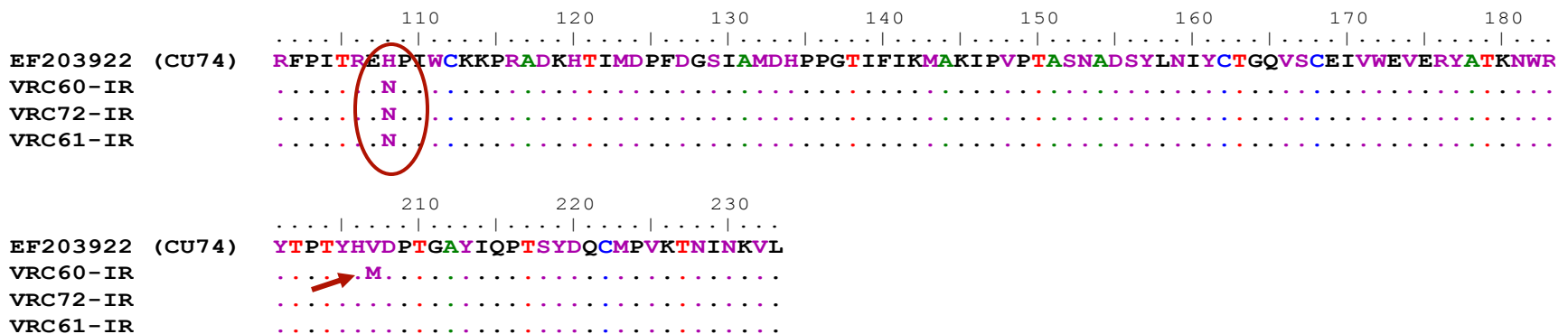
Genogroup 2

- 99.88% sequence identity between Iranian isolates and the reference isolate ST1
 - Two nucleotide positions variable
 - 1 transition and 1 transversion
- no amino acid change



Genogroup 3

- 98.95–99.15% nucleotide sequence identity with isolate CU74 (thai isolate).
 - Ten nucleotide positions variable (8 transitions and 2 transversions)
- Amino acid sequence identity of 99.138 – 99.57 % with the reference isolate CU74.
 - H108N change was detected in all studied isolates
 - V207M amino acid change only in VRC60-IR isolate.



Prevalence of hBoV– age group

- hBoV was detected in a range of 6% to 12.8% in the different clinical presentations.

Reasonable to include older children into prospective surveillance studies

- hBoV mostly affected children ≤ 24 months but older children may also be affected
 - In this study, hBoV statistically related to age group 2–3 y in exacerbated asthma cases
 - In other clinical presentation, most hBoV detected in children under 3 y old.
 - (15%) positive specimens in the study of Chung et al. obtained from children of 36 months of age.



Prevalence of HBoV– seasonality

- no obvious regular seasonal occurrence of hBoV has been reported
 - peak “respiratory season” varies from year to year
- In our study hBoV statistically related to cold season only in gastroenteritis cases
 - most hBoV detected in cold season.



Conclusion

- Existence of 3 different genetic groups among the hBoV isolates, which co-circulate in the Iranian population
 - highly conserved
 - no specific geographical variation



Thank you for your
patience 😊



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