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Emergence of a New Strain of Type O Foot-and-Mouth Disease Virus: Its Phylogenetic and Evolutionary Relationship with the PanAsia Pandemic Strain

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Abstract. In India, Foot-and-mouth disease virus (FMDV) serotype O has been associated with more than 75% of the outbreaks. Previous studies with this serotype have indicated that the viruses circulating in India belong to a single genotype. Recent (February 2001) FMD epidemics in Europe have focussed global attention on the source of the virus and have been traced to a strain, PanAsia (serotype O), which is present in India since 1990. In this study, to further characterize the isolates belonging to the PanAsian strain, we sequenced the complete VP1-encoding (1D) gene for 71 FMDV serotype O isolates from India recovered from the field outbreaks during the last 4 decades (1962–2001). All the isolates in the tree were distributed in to three major branches (designated as A, B and C); the branch C is further divided into four groups (I–IV), of which the group IV belongs to the PanAsia strain. Furthermore, we show that the PanAsia strain has been circulating endemically since 1982 (not 1990 as reported earlier) and has been the most dominant outbreak strain in the recent years and distributed at least in 17 states of the country. During the year 2001, another new group (group III) of virus with genetic divergence of 5.4–11.1% at nucleotide level from the PanAsia strain is found to co-circulate endemically, and is slowly replacing it. At amino acid level this strain differed from PanAsia strain at five amino acid positions in the VP1. Although these strains are divergent at nucleotide level, they maintained a good antigenic relationship with one of the vaccine strains (IND R2/75) widely used in the country. Given the ability of the PanAsia virus to persist, spread and to outcompete other strains, the present trend could be of serious concern as the newly emerging virus is replacing it. If this is true, then there is another equally divergent strain as PanAsia that may pose a serious threat to the global dairy and meat industries.

Key words: FMDV, PanAsia, serotype O, 1D gene

Introduction

Foot-and-mouth disease (FMD) causes an acute infection in cattle, pigs, buffaloes, sheep and goats leading to high economic losses to the countries harbouring it due to restriction of trade on animal products. The virus exists as seven distinct serological types (O, A, C, Asia 1, SAT 1, SAT 2 and SAT 3) and multiple subtypes [1]. Globally, type O is the most prevalent type compared to other serotypes. Within this serotype viruses belonging to distinct genetic lineages (genotypes) with more than 15% nucleotide divergence at the VP1 structural protein encoding (1D) gene specific to each geographical region exist [2,3]. These have been termed topotypes (geographically distinct evolutionary lineages) [2].

FMDV, like other RNA viruses, exhibits high mutation rate due to lack of proofreading mechanism. This leads to generation and amplification of related but non-identical mutant population termed virus

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quasispecies [4,5] which may manifest different competitive abilities, allowing a certain sequence to predominate in a given viral population [6,7]. So generated variant virus with favourable mutations can gain upper hand during infection and evade host's immune defense resulting in severe disease outbreaks [8,9]. This characteristic of the virus has been utilized in number of epidemiological studies [10–24] and to individually characterize and track the movement of FMDV strains across the international border [25]. More recently, by sequence analysis, a strain belonging to type O has been traced to originate from India which moved both eastward and westward, and by the beginning of 2001 it has caused serious outbreaks in Europe [25]. This pandemic strain has been named as 'PanAsia strain' [26] belonging to Middle East–South Asia topotype [2].

In India, FMD is endemic with 75% of the reported outbreaks are due to type O and PanAsia virus has been responsible for most of these outbreaks in the last five years (Anon, 1997–2001) [27]. By our own observation and from the report [26], it is now known that the pandemic strain has not only out-competed all other strains but also persisted for years. This paper describes the emergence of a new strain, which is now slowly overtaking the PanAsia strain in India. To this end we have conducted the retrospective study of the entire 1D gene of 71 FMDV type O isolates recovered from 12 states of India between 1962 and 2001 with special emphasis to PanAsia strain.

Materials and Methods

Virus

This laboratory maintains a National repository of FMDV, from which we have selected a subset of isolates belonging to different states, outbreaks and a period of 4 decades. The isolates used in this study are listed in Table 1. The isolates were passaged 3–5 times in Baby hamster kidney (BHK-21) monolayer culture.

Table 1. FMDV serotype O isolates used in the study

Isolate No.	Year of Isolation	Animal	Place of Isolation
IND 1/62*	1962	—	Moradabad, Uttar Pradesh
IND R2/75*	1975	Cattle	Tamilnadu
IND 53/79*	1977	—	Tamilnadu
IND 136/87	1987	Cattle	Guwahati, Assam
IND 4/88	1988	Cattle	Bareilly, Uttar Pradesh
IND 8/89	1989	Cattle	Bangalore, Karnataka
IND 6/90	1990	Cattle	Jallandhar, Punjab
IND 79/90	1988	Cattle	Jind, Haryana
IND 100/90	1989	Pig	Jallandhar, Punjab
IND 231/88	1982	Cattle	Kheda, Gujarat
IND 232/90	1990	Cattle	Cuttack, Orissa
IND 234/90	1990	Cattle	Cuttack, Orissa
IND 235/90	1990	Cattle	Cuttack, Orissa
IND 7/94	1993	Cattle	Bangalore, Karnataka
IND 27/95	1994	Cattle	Salem, Tamilnadu
IND 52/95	1995	Cattle	Bhiwani, Haryana
IND 50/96	1990	Cattle	Thane, Maharashtra
IND 162/97	1997	Cattle	Jalgaon, Maharashtra
IND 279/97	1997	Cattle	Nagaon, Assam
IND 289/97	1997	Cattle	Howrah, West Bengal
IND 313/97	1997	Cattle	Hisar, Haryana
IND 489/97*$_{(TNN24/84)}$	1984	—	Tamilnadu
IND 31/98	1997	Cattle	Jalpaiguri, West Bengal
IND 146/98	1998	Pig	Tumkur, Karnataka
IND 149/98	1998	Cattle	Ropar, Punjab
IND 413/98	1998	Cattle	Doddaballapura, Karnataka
IND 429/98	1998	Cattle	Anantpur, Andhra Pradesh

Table 1. Continued

Isolate No.	Year of Isolation	Animal	Place of Isolation
IND 141/99	1999	Cattle	Thirunelveli, Tamilnadu
IND 143/99	1999	Buffalo	Agra, Uttar pradesh
IND 209/99	1998	Cattle	Ahmednagar, Maharashtra
IND 246/99	1999	Cattle	Gujarat
IND 5/00	1999	Cattle	Kurnool, Andhra Pradesh
IND 30/00	1999	Sheep	Sirsa, Haryana
IND 34/00	2000	Cattle	Hisar, Haryana
IND 36/00	2000	Cattle	Sirsa, Haryana
IND 43/00	1999	Cattle	Chitradurga, Karnataka
IND 45/00	1999	Cattle	Doddaballapura, Karnataka
IND 74/00	2000	Cattle	Hassan, Karnataka
IND 75/00	2000	Cattle	Arakalgudu, Karnataka
IND 77/00	2000	Cattle	Arakalgudu, Karnataka
IND 90/00	2000	Cattle	Chitradurga, Karnataka
IND 91/00	2000	Cattle	Chitradurga, Karnataka
IND 111/00	1999	Cattle	Howrah, West Bengal
IND 112/00	2000	Cattle	Nadia, West Bengal
IND 151/00	2000	Cattle	Midnapore, West Bengal
IND 156/00	2000	Cattle	Nadia, West Bengal
IND 157/00	2000	Cattle	24 Parganas, West Bengal
IND 398/00	2000	—	Surendra Nagar, Gujarat
IND 402/00	2000	—	Jam Nagar, Gujarat
IND 40/01	2000	Cattle	Midnapore, West Bengal
IND 58/01	2001	Cattle	Mahadevpur, Karnataka
IND 64/01	2001	Cattle	Muzaffarnagar, Uttar pradesh
IND 78/01	2001	Cattle	Hoshiarpur, Punjab
IND 79/01	2001	Cattle	Hoshiarpur, Punjab
IND 83/01	2001	Cattle	Mandya, Karnataka
IND 86/01	2001	Cattle	Kolar, Karnataka
IND 93/01	2001	Cattle	Kolar, Karnataka
IND 96/01	2001	Buffalo	Ludhiana, Punjab
IND 97/01	2001	Cattle	Ambala, Haryana
IND 102/01	2001	Buffalo	Hatharas, Uttar Pradesh
IND 106/01	2001	Cattle	Hisar, Haryana
IND 111/01	2001	Cattle	Gurgaon, Haryana
IND 112/01	2001	Buffalo	Gurgaon, Haryana
IND 115/01	2001	Cattle	Kuruksetra, Haryana
IND 116/01	2001	Cattle	Narnaul, Haryana
IND 118/01	2001	Buffalo	Jaipur, Rajasthan
IND 119/01	2001	Cattle	Karnal, Haryana
IND 125/01	2001	Cattle	Faridabad, Haryana
IND 136/01	2001	Cattle	Baruch, Gujarat
IND 151/01	2001	Cattle	Kutch, Gujarat
IND 155/01	2001	Cattle	Baruch, Gujarat

*Vaccine strain, number in parenthesis is the accession number of Indian Immunologicals Ltd, Hyderabad.
—, not available.

RNA Extraction, RT, PCR

The infected cell culture supernatant was used for the extraction of viral RNA using Total RNA isolation kit (Qiagen). The cDNA was synthesized using negative-sense primer NK61 [28] and MMLV reverse transcriptase (Promega) as per the recommendation of the supplier. For amplification of the 1D gene, we used primers NK61/ARS4 [28] and the Hotstar Taq polymerase (Qiagen). The PCR conditions were: 1 cycle at 95°C for 15 min; 35 cycles of denaturation at 94°C for 30 s, primer annnealing at 55°C for 30 s and primer extension at 72°C for 1.5 min followed by final extension step of 10 min at 72°C.

Sequencing and Analysis

The PCR products were purified with the QIAquick gel extraction kit (Qiagen) and directly sequenced using Cy5-labelled primer NK72 [28] and the *fmol* DNA cycle sequencing system (Promega). The following thermal conditions were used: 1 cycle at 95°C for 2 min, 40 cycles each at 95°C for 30 s, 55°C for 30 s and 72°C for 1.5 min. The reaction was terminated by the addition of 4 µl of stop solution (Amersham Pharmacia Biotech). The reactions were resolved on ALF express II automatic DNA sequencer (Amersham Pharmacia Biotech). The sequences (nucleotide and amino acid) were aligned using the CLUSTAL W algorithm [29], available in the OMIGA 2.0 (Oxford Molecular Ltd., UK). Phylogenetic analyses were performed using the PHYLIP 3.5 c package [30]. The distance matrices were generated using the Kimura 2-parameter method present in NEIGHBOR program. The tree was plotted using the program Tree view v 1.5 [31]. The tree was statistically evaluated using 1000 bootstrap samples. The number of synonymous substitutions per synonymous site (K_S) was calculated with DnaSP [32] using the method of Nei and Gojobori [33].

Results and Discussion

Previous studies [19,22,34] on serotype O viruses from India showed the prevalence of a single genotype with multiple genetic groups. The same studies also showed that the strains from Europe are genetically different from the Indian isolates, and belong to a different genotype. Further, we have also noted the dominance of one of the genetic groups [22], which is now termed as PanAsia strain [26]. To characterize these strains further, we have sequenced and analysed complete 1D gene of FMDV type O isolates as old from 1962 to 2001. In the dendrogram, generated by UPGMA method, all the isolates were clustered in a single genotype with less than 11% nucleotide divergence among themselves (data not shown). To provide additional insight into the relatedness of the type O virus, a phylogenetic tree using the neighbour-joining algorithm was generated (Fig. 1). In the tree, the isolates could be distributed in three major branches (designated as A, B and C). The four vaccine strains (IND 1/62, IND R2/75, IND 53/79 and IND 489/97) were clustered separately (branches A and B) with few related isolates and were away from the branch C in the tree. The branch C is further subdivided in to 4 groups (I–IV) representing isolates from 1982 to 2001. In the previous phylogenetic analysis of FMDV type O [26], the Indian isolates (IND 6/90, IND 138/90, IND 17/96, IND 304/98 and IND 143/99) have been shown to cluster with PanAsia group. The PanAsia virus has been isolated in recent outbreaks from large geographical areas including Bhutan, Nepal, Republic of China, Korea, Japan, Middle East, Russia, South Africa and Europe [25]. In the present study, two isolates (IND 6/90 and IND 143/99) from the above five along with 28 more isolates from India, were clustered in this group. The PanAsia virus has been reported to be present in northern India since 1990 [26]. However, from the retrospective analysis of our isolates we now show its presence to as early as 1982. The oldest strain (IND 231/88) belonging to this group was isolated in a 1982 sample from the western state of Gujarat, which was submitted to this laboratory during 1988. However, its presence could not be confirmed in the following four years, as the laboratory lacked isolates collected between 1982 and 1986. Examination of limited number of isolates of 1987–1989 did not show its presence. Subsequently, after a gap of 8 years, in 1990, isolates belonging to this group were recovered from the northern India (Punjab, Haryana, Uttar Pradesh) western India (Maharashtra), eastern India (Orissa) and central India (Madhya Pradesh). However, none of the isolates sequenced of this period from southern India belonged to this group. In southern India, PanAsia virus was first isolated from the state of Kerala in 1994 indicating its entry to this region much later. Although, PanAsia virus was present in India since 1982, its predominance in field outbreaks was noticed only in 1996 and from then onwards it was isolated more frequently covering at least 17 states by the year 2000 [27].

Nucleotide substitution rates provide an important information for studying the DNA sequence evolution [35]. In order to see how the PanAsia virus evolved compared to others, the rate of evolution was calculated by linear regression analysis on the genetic distances of the isolates from the oldest isolate, and is plotted against the time of isolation (Fig. 2). Two separate calculations were made; (i) for the synonymous substitutions and, (ii) for the total substitutions (Kimura 2-prameter) among the pandemic strains in

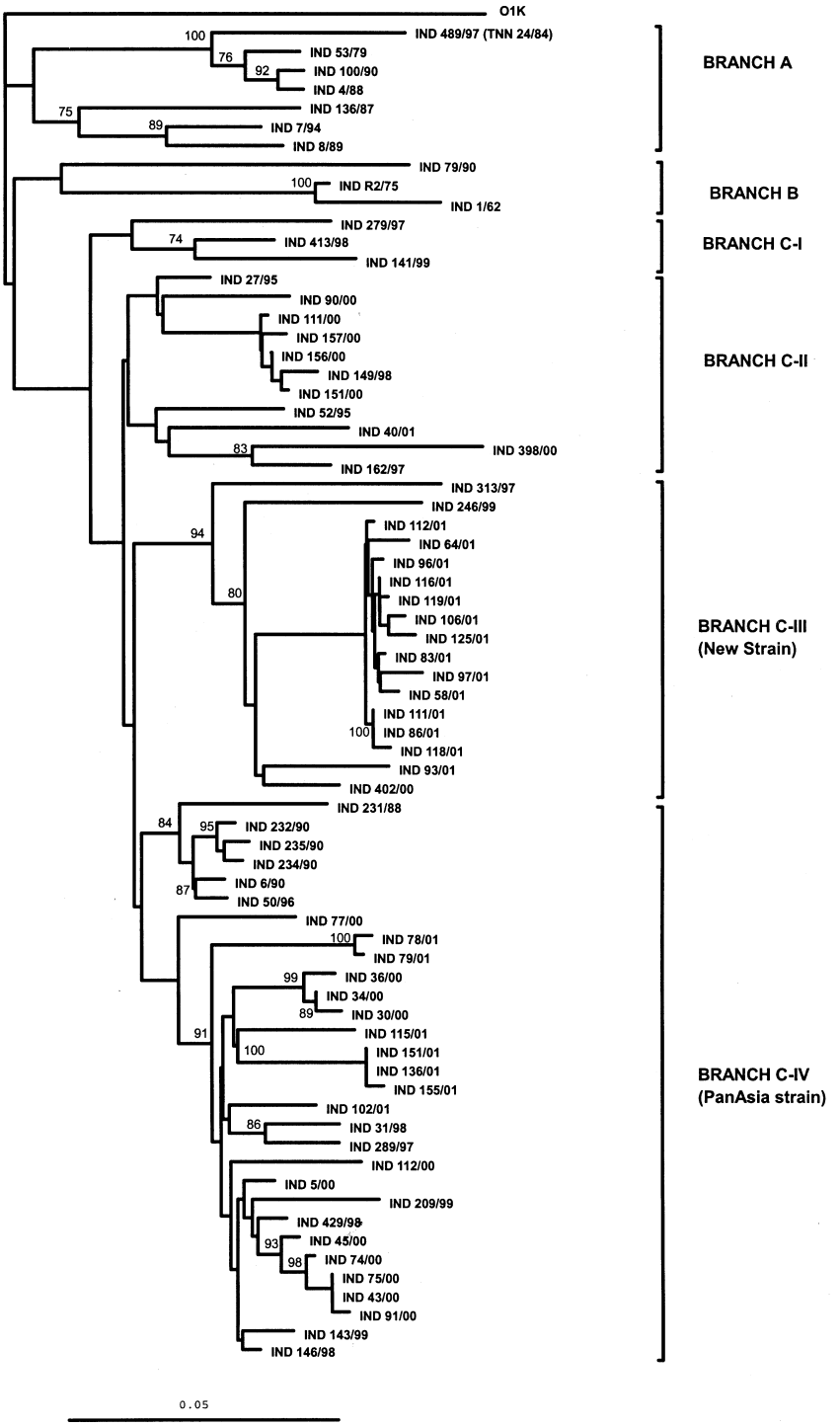

Fig. 1. Phylogenetic tree showing the relationships among the 71 FMDV type O isolates. Numbers near the node indicates bootstrap values (values >70% are given out of 1000 replicates). The scale bar represents the nucleotide changes. The branch length of the outgroup (O1K, GenBank accession number X00871) has been reduced by 50% to save the space.

the PanAsia group, and the remaining isolates in the tree (Fig. 2). The synonymous and the total substitution rates of the type O pandemic strain circulating in India was estimated to be 1.2×10^{-2} ($P < 0.05$) and 2.8×10^{-3} ($P < 0.05$) substitutions/nt/year, respectively. However, in all the remaining type O viruses, the synonymous rate (1.1×10^{-2} substitutions/nt/year) and the total substitution rate (2.0×10^{-3} substitutions/nt/year) was similar to that of the pandemic strain. The results obtained in this study are in conformity with that obtained for serotype C (1.4×10^{-3}) [13] and persistent cattle ($0.9–7.4 \times 10^{-2}$) [36].

Interestingly, in the year 2001, a new strain (group III), which is 5.4–11.1% divergent from the PanAsia strain (group IV) was found to cause FMD outbreaks. Out of the 32 isolates from the seven states sequenced, 23 belonged to this new group and are 0–8.2% divergent among themselves. Initial studies indicated that it was responsible for most of the recent outbreaks in five states viz., Haryana, Rajasthan, Uttar Pradesh, Punjab and Karnataka and to a limited extent in Gujarat and West Bengal. This is important considering the fact that in all the above-mentioned states, PanAsia strain caused most of the outbreaks in the past few years [27]. In the early part of the year 2001, the northern states of Punjab and Haryana experienced severe FMD outbreaks involving large number of animals. Although both the strains were involved, it was the new strain that was mostly responsible suggesting endemic cocirculation of both the strains. Endemic cocirculation of viruses belonging to different genetic groups has been reported earlier [22]. In order to trace the earliest outbreak due to this strain, a dendrogram was constructed using the partial VP1 sequences (165 nt at 3′end) generated earlier [19,22] by UPGMA method (Fig. 3). To our surprise, we could not find involvement of the new strain in outbreaks earlier to 2000 and again it was

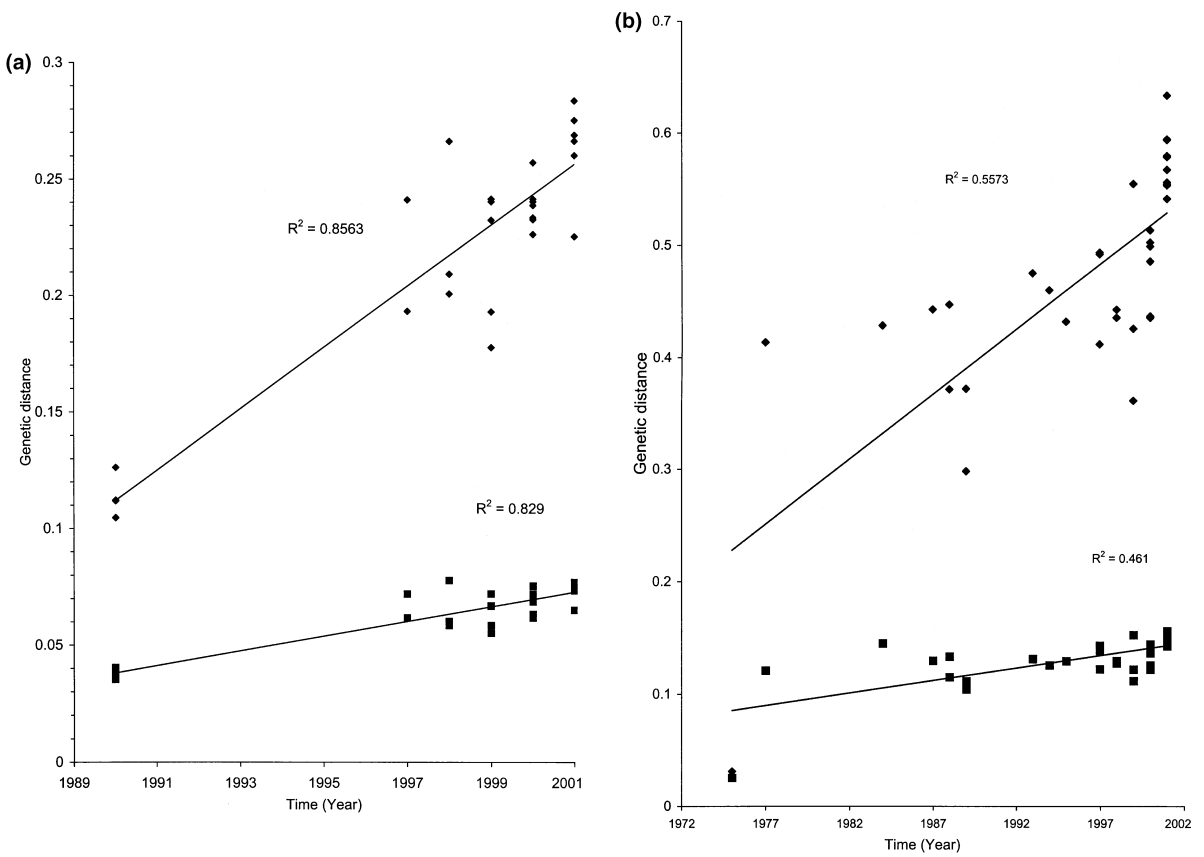

Fig. 2. Regression analyses of rate of evolution of the 1D gene sequence of type O FMDV (a) Pandemic strain and (b) overall field isolates. (◆) Synonymous substitution as per Nei and Gojobori [33]; (■) total substitutions calculated as per Kimura 2-parameter available in the Phylip package [30].

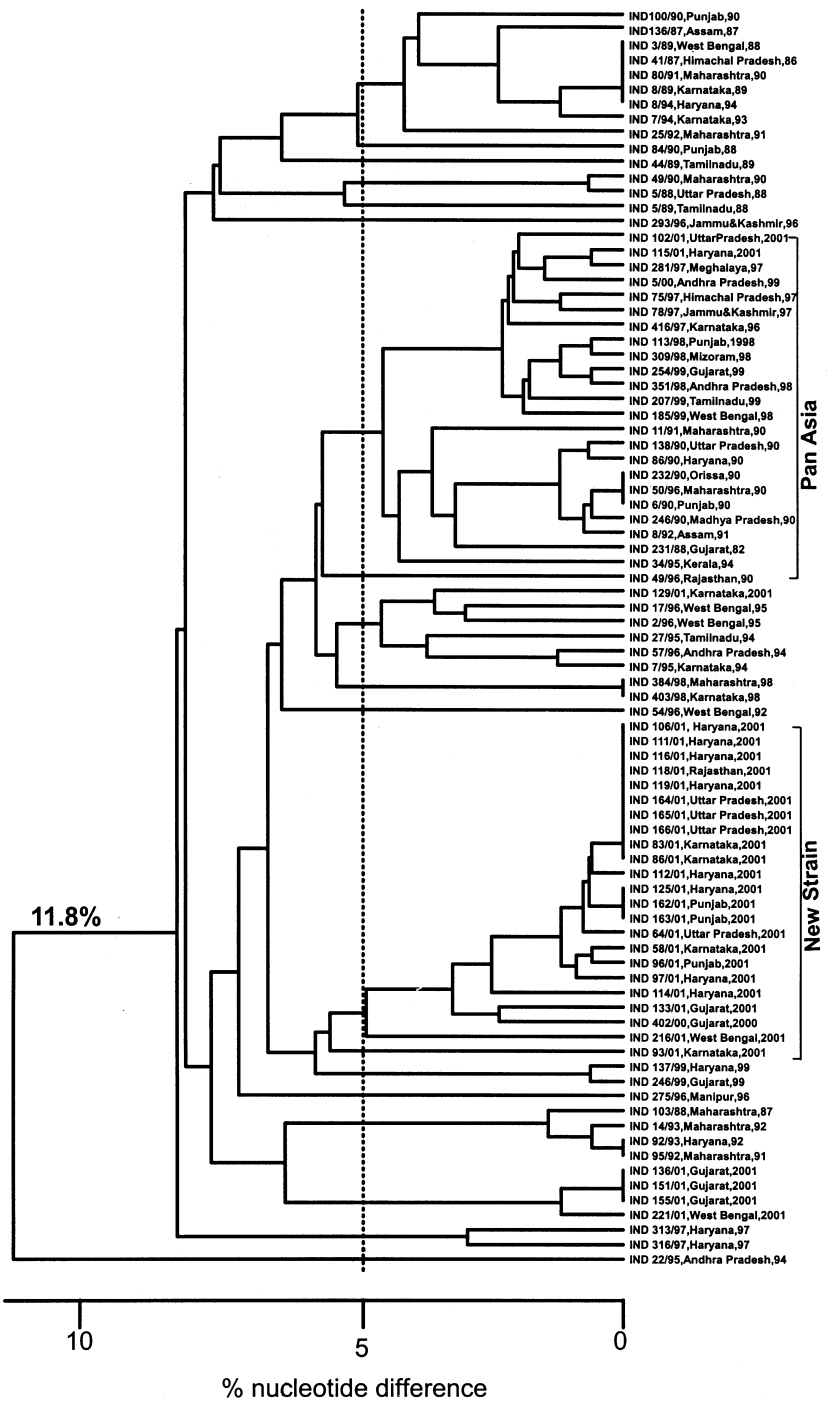

Fig. 3. UPGMA tree showing the genetic relationships among 85 FMDV type O isolates based on partial 1D gene sequence (165 nt from positions 475–639). State and the year of isolation are given after the isolate number.

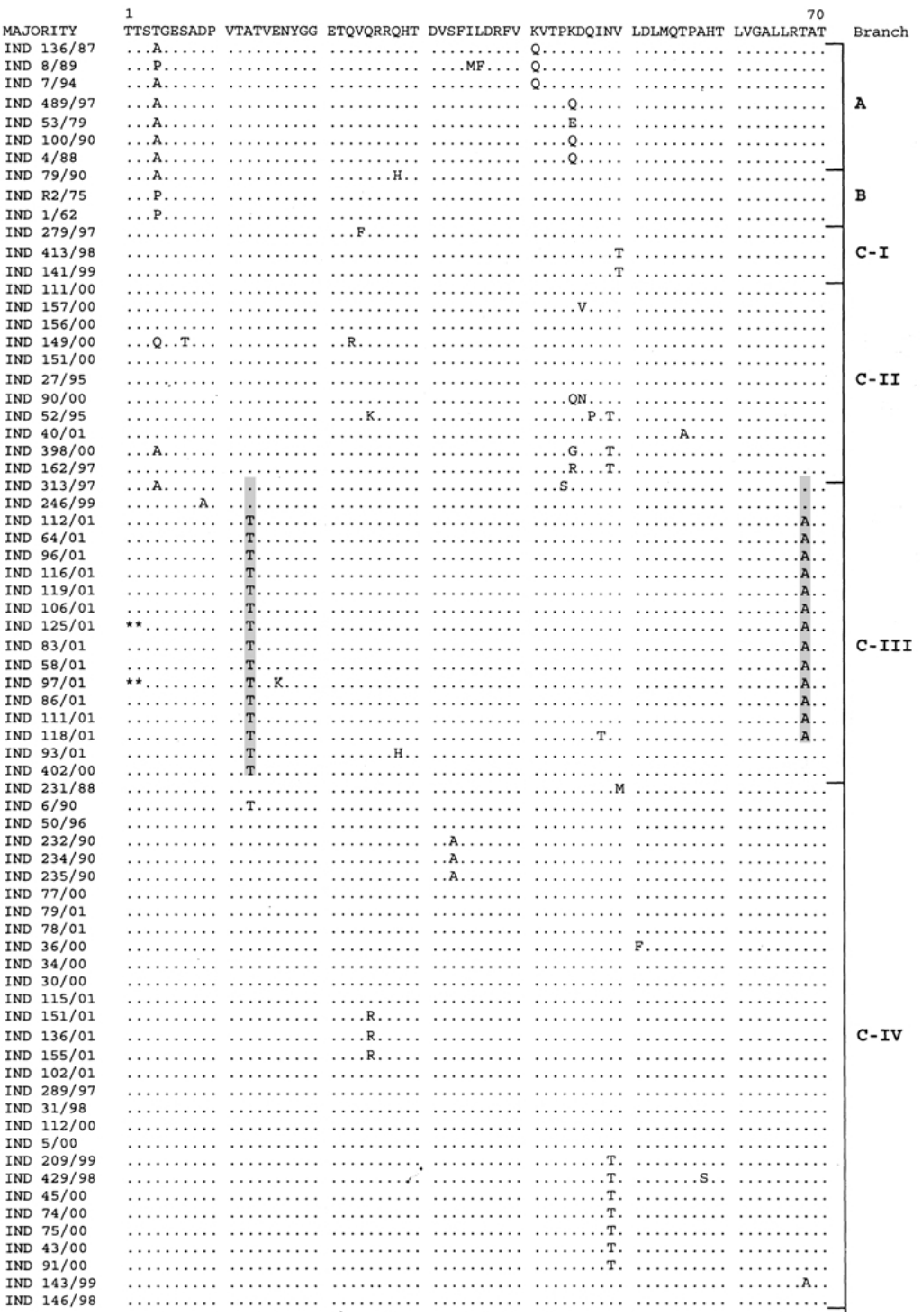

Fig. 4. Deduced amino acid sequence of type O FMDV VP1 protein. Dot (·) indicates sequence identity with the majority, Asterisk (*) represent region not sequenced. Characteristic amino acid substitutions specific to the new strain (group III of Fig. 1) are shaded. Branch/group designations (as in Fig. 1) of the isolates are shown to the right.

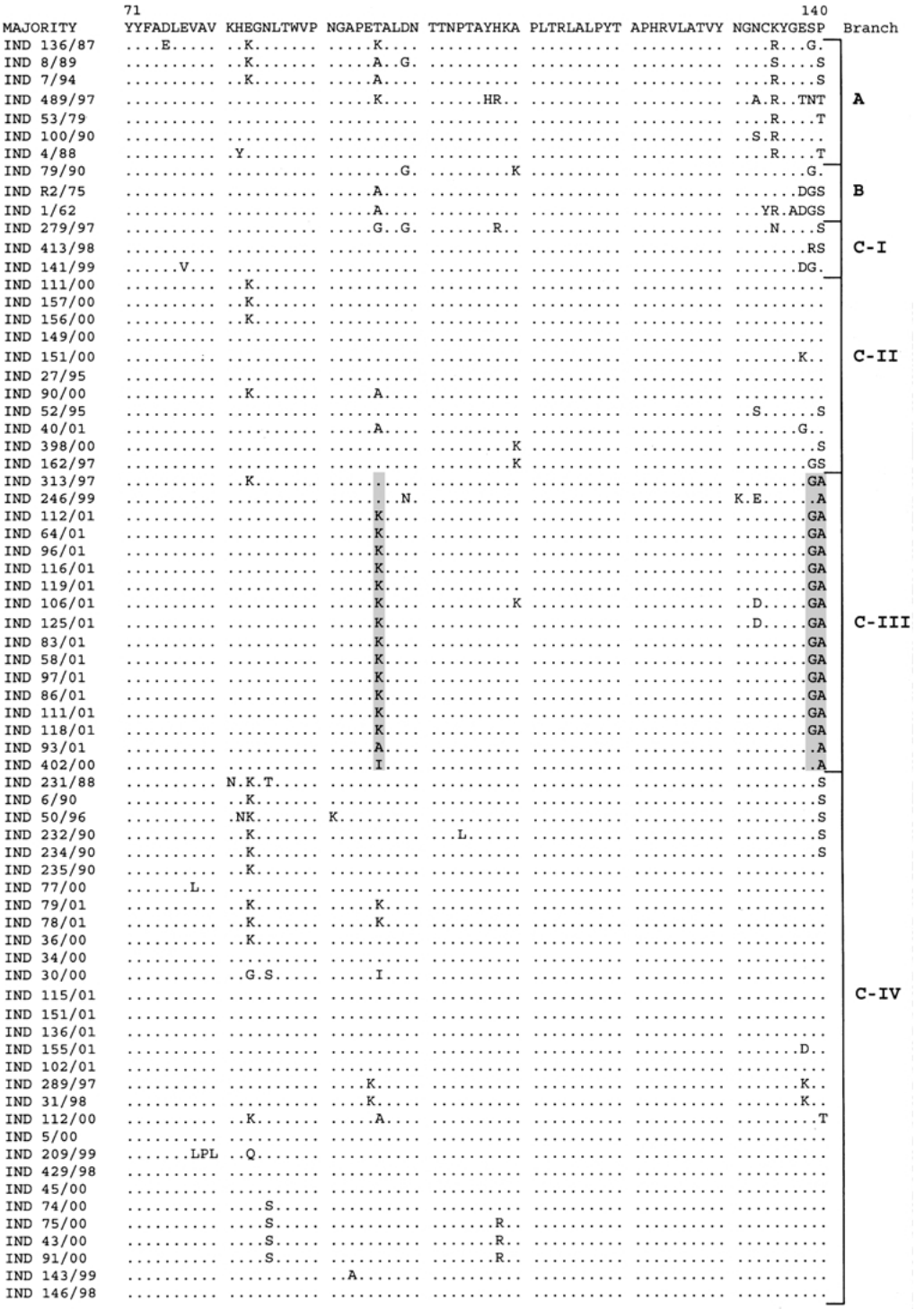

Fig. 4. Continued.

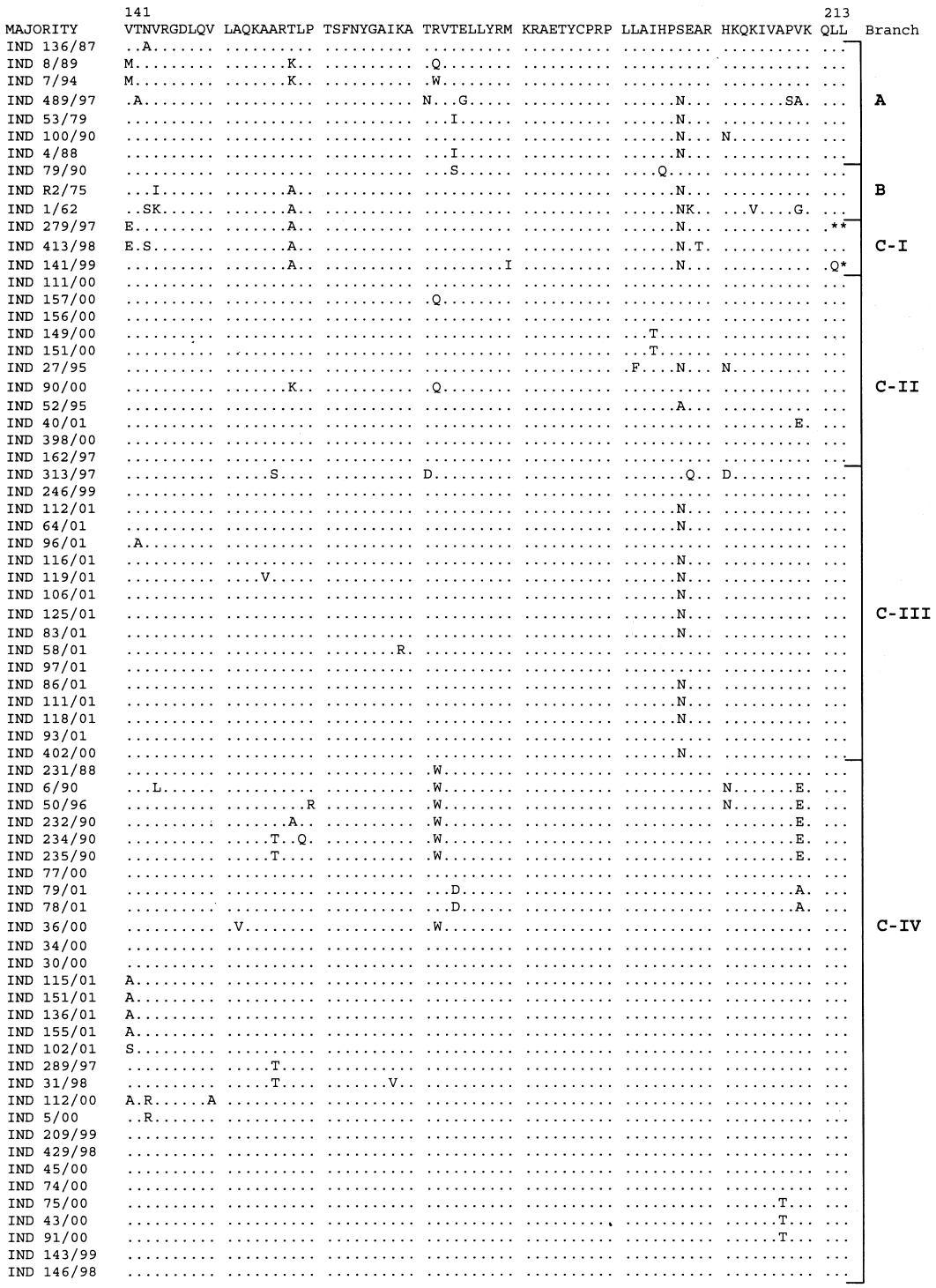

Fig. 4. Continued.

(IND 402/00) first isolated from Gujarat in the later part of that year. However, an isolate each from Haryana (IND 313/97) and Gujarat (IND 246/99) has shared an amino acid (A) at position 140 with the new strain while retaining other characteristics of the PanAsia strain. Hence, they may be regarded as intermediate isolates during the transition from PanAsia (group IV) to new group (group III) as they are phylogenetically related to the latter. Thus it is plausible that the PanAsia strain has given rise to the selection of new genetic variant. These results have to be evaluated in the context that the country has a vast population of FMD susceptible animals and outbreaks occur through out the year. As it often happens in the endemic region subclinical infection, low level of herd immunity and quasispecies nature of the virus might have played a role in emergence of the new strain.

To see how the new genetic variant differed from its ancestor (PanAsia strain), we have compared the amino acid sequences (Fig. 4). The isolates of the new strain have differed from their predecessor at 5 amino acid positions. In the VP1 protein, the residue A 12 is conserved (except IND 6/90 of PanAsia group) in all the remaining three groups (I, II and IV) of branch C as well as in branches A and B. It is replaced by T (barring the intermediate isolates IND 313/97 and IND 246/99) in the new strain. Similarly, at VP1 68, group III isolates have A whereas, all other groups (barring IND 143/99) and branches have T. Identical situation could be found at amino acid position 140 where group III isolates have A and rest of the groups have T/S/P. This Alanine replacement could be of recent origin, as none of the older isolates possessed it. From the amino acid comparisons across the groups, 5 different amino acids (T, K, A, G and I) are evident at position 96. Most of the isolates (13/17) of group III have K and most members (26/30) of group IV have T. At position 139, four different amino acids (S, N, R and G) are evident across the groups. Most (14/17) of the group III isolates have G and all the isolates (except IND 112/00) of PanAsia group have Serine.

The amino acid positions 43, 44, 45, 144, 148, 149, 154, 198 and 208 of the VP1 in type O have been implicated as critical in the formation of neutralizable antigenic sites [37–42]. None of the amino acid substitutions (at positions 12, 68, 96, 139 and 140) noticed in the new strain involved critical positions, nevertheless, two of them (at positions 139, 140) occurred at immunodominant (VP1 130–160) region. It is known that positive selection occurs at this region [43] and this is supported by the fact that, amino acid modification at VP1 139 is related to acquisition of serum neutralization resistance [44]. Apart from above two positions, VP1 68 and 96 have been shown to be the part of positions identified as under positive selection by using maximum likelihood model [45], although they are not at a significant level.

In India, two type O strains (IND R2/75 and IND 489/97) are presently used for manufacturing of FMD vaccine. To see how the widely used vaccine strain, IND R2/75 fared in the changing scenario, one-way antigenic relationship ('*r*' values) was measured in an indirect sandwich ELISA [46]. The results (not shown) indicated that most of the field isolates maintained close antigenic relationship ('*r*' 0.4–1.0) with it [27].

In conclusion, pandemic PanAsia strain has existed in India since 1982 and has been responsible for majority of the disease outbreaks in the recent years and very recently has given rise to the selection of a new genetic variant. Positive selection exerted on quasispecies population could lead to selection of variants endowed with selective advantage either for causing more severe clinical disease or increased ability to transmit between the susceptible species [45]. Given the ability of the PanAsia virus to persist, spread and outcompete other strains our results show that it is being over taken slowly and surely by a new strain. The newly emerging strain may pose a serious threat to the global dairy and meat industries in this changing scenario of international trade.

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References

1. Pereira H.G., Dev Biol Stand *35*, 167–174, 1977.
2. Samuel A.R. and Knowles N.J., J Gen Virol *82*, 609–621, 2001.
3. Sangare O., Bastos A.D.S., Marquardt O., Venter E.H., Vosloo W., and Thomson G.R., Virus Genes *22*, 345–351, 2001.

4. Domingo E., Martinez-Salas E., Sobrino F., de la Torre J.C., Portela A., Ortin J., Lopez-Galindez C., Perez-Brena P., Villnuera N., Najera R., Vande Pol S., Steinhauer D., De Polo N., and Holland J.J., Gene *40*, 1–8, 1985.
5. Holland J.J., Spindler K., Horodyski F., Grabau E., Nichol S., and Vandepol S., Science *215*, 1577–1585, 1982.
6. Gonzalez M.J., Saiz J.C., Laor O., and Moore D.M., J Virol *65*, 3949–3953, 1991.
7. Martinez M.A., Carillo C., Gonzalez-Candelas F., Moya A., Domingo E., and Sobrino F., J Virol *65*, 3954–3957, 1991.
8. Mateu M.G., Rocha E., Vicente O., Vayreda F., Navalpotro C., Andreu D., Pedroso E., Giralt E., Emjuanes L., and Domingo E., Virus Res *8*, 261–274, 1987.
9. Martinez M.A., Carillo C., Plana J., Mascarella R., Bergada J., Palma E.L., Domingo E., and Sobrino F., Gene *62*, 75–84, 1988.
10. Beck E. and Strohmaier K., J Virol *61*, 1621–1629, 1987.
11. Knowles N.J., Marquardt O., and Samuel A.R., Rpt Sess Res Gp Tech Comm Eur Comm Control of FMD, Prague, Czechoslovakia (FAO), Rome, pp. 149–154, 1988.
12. Carillo C., Dopazo J., Moya A., Gonzalez M., Martinez M.A., Saiz J.C., and Sobrino F., Virus Res *15*, 45–56, 1990.
13. Martinez M.A., Dopazo J., Hernandez J., Mateu M.J., Sobrino F., Domingo E., and Knowles N.J., J Virol *66*, 3557–3565, 1992.
14. Vosloo W., Knowles N.J., and Thomson G.R., Epidemiol Infect *109*, 547–558, 1992.
15. Saiz J., Sobrino F., and Dopazo J., J Gen Virol *74*, 2281–2285, 1993.
16. Ansell D.M., Samuel A.R., Carpenter W.C., and Knowles N.J., Epidemiol Infect *112*, 213–224, 1994.
17. Stram Y., Chai D., Fawzy H., Molad T., Meiri N., Van-Ham M., El-Kilani S., Fahamy F., Moussa A., and Yadin H., Arch Virol *140*, 1791–1797, 1995.
18. Samuel A.R., Knowles N.J., Kitching R.P., and Hafez S.M., Epidemiol Infect *119*, 381–389, 1997.
19. Pattnaik B., Venkataramanan R., Tosh C., Sanyal A., Hemadri D., Samuel A.R., Knowles N.J., and Kitching R.P., Virus Res *55*, 115–127, 1998.
20. Samuel A.R., Knowles N.J., and Mackay D.K.J., Epidemiol Infect *122*, 529–538, 1999.
21. Marquardt O., Rahman M.M., and Freiberg B., Arch Virol *145*, 149–157, 2000.
22. Hemadri D., Tosh C., Venkataramanan R., Sanyal A., Samuel A.R., Knowles N.J., and Kitching R.P., Epidemiol Infect *125*, 729–736, 2000.
23. Gurumurthy CB, Sanyal A, Venkataramanan R, Tosh C, George M, and Hemadri D, Arch Virol, *147*, 85–102, 2002.
24. Nayak B., Pattnaik B., Tosh C., Sanyal A., Hemadri D. Patil S., and Venkataramanan R., Acta Virol *45*, 13–21, 2001.
25. Knowles N.J., Samuel A.R., Davies P.R., Kitching R.P., and Donaldson A.I., Vet Rec *148*, 258–259, 2001.
26. Knowles N.J., Samuel A.R., Davies P.R., Kitching R.P., Venkataramanan R., Kanno T., Scherbakov A.V., Drygin V.V., Zhao Q.-Z., and Xie Q.-G., Rpt Sess Res Gp Tech Comm Eur Comm Control of FMD, Borovets, Bulgaria (FAO), Rome. pp. 20–31, 2000.
27. Anon., Annual reports of the Project directorate on FMD Mukteswar, Nainital, Uttaranchal, 1997–2001.
28. Knowles N.J. and Samuel A.R., Rpt Sess Res Gp Tech Comm Eur Comm Control of FMD, Vienna, Austria, (FAO), Rome. pp. 45–53, 1995.
29. Thompson J.D., Higgins D.G., and Gibson T.J., Nucleic Acids Res *22*, 4673–4680, 1994.
30. Felsenstein J., PHYLIP Version 3.5c. Department of Genetics, University of Washington Seattle, 1993.
31. Page R.D.M., Comp Appl Biosci *12*, 357–358, 1996.
32. Rozas J. and Rozas R., Bioinformatics *15*, 174–175, 1999.
33. Nei M. and Gojobori T., Mol Biol Evol *3*, 418–426, 1986.
34. Muthuchelvan D, Venkataramanan R, Hemadri D, Sanyal A, and Tosh C, Acta Virol, *45*, 159–167, 2001.
35. Kimura M., 1983 Cambridge University Press Cambridge, United Kingdom.
36. Gebauer F., de La Torre J.C., Gomes I., Mateu M.G., Barahona H., Tiraboschi B., Bergmann I., Auge de Mello P., and Domingo E., J Virol *62*, 2041–2049, 1988.
37. Xie Q.C., McCahon D., Crowther J.R., Belsham G.J., and McCullough K.C., J Gen Virol *68*, 1637–1647, 1987.
38. McCahon D., Crowther J.R., Belsham G.J., Kitson J.D.A., Duchesne M., Have P., Meloen R.H., Morgan D.O., and De Simone F., J Gen Virol *70*, 639–645, 1989.
39. Kitson J.D.A., McCahon D., and Belsham G.J., Virology *179*, 26–34, 1990.
40. Crowther J.R., Farias S., Carpenter W.C., and Samuel A.R., J Gen Virol *74*, 1547–1553, 1993.
41. Barnett P.V., Samuel A.R., Pullen L., Ansell D., Butcher R.N., and Parkhouse R.M.E., J Gen Virol *79*, 1687–1697, 1998.
42. Aktas S. and Samuel A.R., Rev sci tech Off int Epiz *19*, 744–753, 2000.
43. Martin M.J., Nunez J.I., Sobrino F., and Dopazo J., J Virol Meth *74*, 215–221, 1998.
44. Rojas E.R., Carillo E., Schiappacassi M., and Campos R., J Virol *66*, 3368–3372, 1992.
45. Haydon D.T., Bastos A.D., Knowles N.J., and Samuel A.R., Genetics *157*, 7–15, 2001.
46. Ouldridge E.J., Barnett P.V., Hingley P., Head M., and Rweyemamu M.M., Curr Top Vet Med Anim Sci *22*, 152–160, 1982.