Genetic analysis of foot-and-mouth disease virus type O isolates responsible for field outbreaks in India between 1993 and 1999

D. HEMADRI1, C. TOSH1, R. VENKATARAMANAN1*, A. SANYAL1, A. R. SAMUEL2, N. J. KNOWLES2 AND R. P. KITCHING2

1 Central Laboratory, All India Coordinated Research Project on Foot-and-Mouth Disease, Indian Veterinary Research Institute, Mukteswar-Kumaon, Nainital 263 138, India
2 World Reference Laboratory for Foot-and-Mouth Disease, Institute for Animal Health, Pirbright Laboratory, Pirbright, Surrey, GU 24 ONF, UK

(Accepted 7 July 2000)

SUMMARY
Partial nucleotide sequence at the 3′ end of 1D (VP1-encoding) gene of 90 foot-and-mouth disease virus type O isolates recovered from field outbreaks in India between 1993–9 were determined. The sequences were compared with each other and reference viruses. The published sequences of 15 type O isolates recovered from different parts of Asia and one isolate (O1BFS) from Europe and one from Egypt (O1/Sharquia/Egypt/72) were also included in the analysis for comparison. On the basis of phylogenetic analysis the viruses could be grouped into four distinct genotypes (genotypes I–IV). All 90 isolates from India were genotype-I, as were the reference isolates from Bangladesh, China, Egypt, Iran, Saudi Arabia, Syria and Turkey. Genotype-I isolates were further subdivided into 16 sub-genotypes. The Indian isolates were found to be extremely heterogeneous in nature and clustered into 12 different genetic groups. In genotype-I, the nucleotide sequence difference seen between the isolates was 0–11.6%, while among the Indian isolates it is 0–8.8%. Viruses of similar genetic groups are circulating in India, Bangladesh and countries of the Middle East. Genotype-II and -III are represented by isolates from Lebanon (O1/South Lebanon) and Europe (O1-BFS), respectively. Genotype-IV is formed by isolates from China, Hong Kong and Taiwan. The present study reveals the occurrence of viruses belonging to multiple genetic groups over a short period of time and persistence of single genetic group in the same geographical area over several years. This is consistent with the endemic nature of the disease in the country.

INTRODUCTION
Foot-and-mouth disease causes serious economic losses to the livestock sector due to its effects on productivity. The causative agent, foot-and-mouth disease virus (FMDV), belongs to the genus aphthovirus of the family Picornaviridae, and exists as seven distinct serotypes (O, A, C, Asia1, SAT1, SAT2 and SAT3) and each serotype has, historically been divided into a number of subtypes [1]. In India the disease is endemic and outbreaks occur due to types O, Asia1, A and C. The majority (> 85%) of the outbreaks in the country are due to type O followed by types Asia1 and A; the last reported type C outbreak was during 1995 [2]. Though vaccination is an important step in the control of the disease, antigenic diversity of the virus in endemic areas where regular vaccination is followed reduces its effectiveness. Serological tests with virus neutralization (VN) and an enzyme-linked immuno-
Table 1. *Virus isolates used in the study*

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Isolates*</th>
<th>Date of isolation</th>
<th>Place of isolation (state)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INDR2/75†</td>
<td>00/00/75</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>2</td>
<td>IND53/79†</td>
<td>00/00/79</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>3</td>
<td>IND27/97</td>
<td>00/01/97</td>
<td>Uttar Pradesh</td>
<td>Bovine</td>
</tr>
<tr>
<td>4</td>
<td>IND28/97</td>
<td>00/01/97</td>
<td>Uttar Pradesh</td>
<td>Caprine</td>
</tr>
<tr>
<td>5</td>
<td>IND31/97</td>
<td>30/12/96</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>6</td>
<td>IND33/97</td>
<td>04/01/97</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>7</td>
<td>IND37/97</td>
<td>09/01/97</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>8</td>
<td>IND38/97</td>
<td>06/01/97</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>9</td>
<td>IND39/97</td>
<td>00/01/97</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>10</td>
<td>IND49/97</td>
<td>29/01/97</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>11</td>
<td>IND61/97</td>
<td>00/00/96</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>12</td>
<td>IND63/97</td>
<td>00/01/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>13</td>
<td>IND64/97</td>
<td>00/00/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>14</td>
<td>IND70/97</td>
<td>00/00/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>15</td>
<td>IND74/97</td>
<td>00/00/97</td>
<td>Haryana</td>
<td>Buffalo</td>
</tr>
<tr>
<td>16</td>
<td>IND75/97</td>
<td>28/11/97</td>
<td>Himachal Pradesh</td>
<td>Bovine</td>
</tr>
<tr>
<td>17</td>
<td>IND78/97</td>
<td>25/01/97</td>
<td>Jammu &amp; Kashmir</td>
<td>Bovine</td>
</tr>
<tr>
<td>18</td>
<td>IND79/97</td>
<td>17/02/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>19</td>
<td>IND82/97</td>
<td>10/02/97</td>
<td>Andhra Pradesh</td>
<td>Bovine</td>
</tr>
<tr>
<td>20</td>
<td>IND160/97</td>
<td>13/01/97</td>
<td>Maharashtra</td>
<td>Bovine</td>
</tr>
<tr>
<td>21</td>
<td>IND162/97</td>
<td>00/00/97</td>
<td>Maharashtra</td>
<td>Bovine</td>
</tr>
<tr>
<td>22</td>
<td>IND170/97</td>
<td>01/01/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>23</td>
<td>IND275/97</td>
<td>00/03/97</td>
<td>Haryana</td>
<td>Buffalo</td>
</tr>
<tr>
<td>24</td>
<td>IND278/97</td>
<td>11/02/97</td>
<td>Assam</td>
<td>Bovine</td>
</tr>
<tr>
<td>25</td>
<td>IND279/97</td>
<td>20/03/97</td>
<td>Assam</td>
<td>Bovine</td>
</tr>
<tr>
<td>26</td>
<td>IND281/97</td>
<td>21/03/97</td>
<td>Meghalaya</td>
<td>Bovine</td>
</tr>
<tr>
<td>27</td>
<td>IND289/97</td>
<td>19/04/97</td>
<td>West Bengal</td>
<td>Bovine</td>
</tr>
<tr>
<td>28</td>
<td>IND296/97</td>
<td>00/04/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>29</td>
<td>IND313/97</td>
<td>00/00/97</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>30</td>
<td>IND352/97</td>
<td>06/03/97</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>31</td>
<td>IND380/97</td>
<td>24/02/94</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>32</td>
<td>IND384/97</td>
<td>29/04/94</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>33</td>
<td>IND391/97</td>
<td>06/08/94</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>34</td>
<td>IND399/97</td>
<td>00/00/94</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>35</td>
<td>IND407/97</td>
<td>21/12/95</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>36</td>
<td>IND409/97</td>
<td>06/07/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>37</td>
<td>IND410/97</td>
<td>04/09/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>38</td>
<td>IND411/97</td>
<td>18/09/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>39</td>
<td>IND414/97</td>
<td>22/11/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>40</td>
<td>IND416/97</td>
<td>28/11/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>41</td>
<td>IND417/97</td>
<td>04/12/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>42</td>
<td>IND420/97</td>
<td>27/01/97</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>43</td>
<td>IND423/97</td>
<td>00/00/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>44</td>
<td>IND424/97</td>
<td>00/00/96</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>45</td>
<td>IND461/97</td>
<td>00/10/96</td>
<td>Haryana</td>
<td>Buffalo</td>
</tr>
<tr>
<td>46</td>
<td>IND463/97</td>
<td>00/11/96</td>
<td>Haryana</td>
<td>Buffalo</td>
</tr>
<tr>
<td>47</td>
<td>IND464/97</td>
<td>00/12/96</td>
<td>Haryana</td>
<td>Ovine</td>
</tr>
<tr>
<td>48</td>
<td>IND465/97</td>
<td>00/12/96</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>49</td>
<td>IND485/97</td>
<td>00/00/93</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>50</td>
<td>IND489/97</td>
<td>NA</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>51</td>
<td>IND47/98</td>
<td>31/12/97</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
</tbody>
</table>
sorbent assay (ELISA) using defined serum/monoclonal antibodies are useful in showing antigenic diversity, but they are unable to individually characterize strains and cannot be used to trace the origin and course of epizootics. Nucleotide sequence analysis has now replaced serology as the method of choice for FMDV strain identification and epidemiological investigation [3]. New molecular techniques such as reverse transcription-polymerase chain reaction and cycle sequencing has improved the speed and accuracy of these studies. The phylogenetic grouping correlates with geographical origin of isolates and provides a powerful tool for epidemiological surveillance [4]. Recently several authors [5–9] including us [10] have used the nucleotide sequence within the 1D gene to make phylogenetic grouping of FMDV isolates [11].

In the present communication we have determined and compared the nucleotide sequence at the 3’ end of the 1D (VP1-encoding) gene of 90 FMDV type O isolates recovered from different outbreaks in India during the last 7 years (1993–9) including two of the vaccine viruses (INDR2/75 and IND53/79) used in

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Isolates*</th>
<th>Date of isolation</th>
<th>Place of isolation (state)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>52</td>
<td>IND48/98</td>
<td>31/12/97</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
<tr>
<td>53</td>
<td>IND54/98</td>
<td>00/01/98</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>54</td>
<td>IND55/98</td>
<td>16/01/98</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
<tr>
<td>55</td>
<td>IND56/98</td>
<td>29/01/98</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
<tr>
<td>56</td>
<td>IND57/98</td>
<td>01/02/98</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
<tr>
<td>57</td>
<td>IND64/98</td>
<td>11/02/98</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
<tr>
<td>58</td>
<td>IND65/98</td>
<td>12/02/98</td>
<td>Punjab</td>
<td>Bovine</td>
</tr>
<tr>
<td>59</td>
<td>IND66/98</td>
<td>12/02/98</td>
<td>Punjab</td>
<td>Buffalo</td>
</tr>
<tr>
<td>60</td>
<td>IND81/98</td>
<td>25/03/98</td>
<td>Uttar Pradesh</td>
<td>Bovine</td>
</tr>
<tr>
<td>61</td>
<td>IND304/98</td>
<td>18/05/98</td>
<td>Meghalaya</td>
<td>Porcine</td>
</tr>
<tr>
<td>62</td>
<td>IND307/98</td>
<td>05/06/98</td>
<td>Assam</td>
<td>Bovine</td>
</tr>
<tr>
<td>63</td>
<td>IND309/98</td>
<td>03/07/98</td>
<td>Mizoram</td>
<td>Bovine</td>
</tr>
<tr>
<td>64</td>
<td>IND427/98</td>
<td>03/11/98</td>
<td>Andhra Pradesh</td>
<td>Bovine</td>
</tr>
<tr>
<td>65</td>
<td>IND469/98</td>
<td>00/12/98</td>
<td>Uttar Pradesh</td>
<td>Ovine</td>
</tr>
<tr>
<td>66</td>
<td>IND23/99</td>
<td>13/12/98</td>
<td>Kerala</td>
<td>Bovine</td>
</tr>
<tr>
<td>67</td>
<td>IND81/99</td>
<td>21/12/98</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>68</td>
<td>IND141/99</td>
<td>24/01/99</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>69</td>
<td>IND143/99</td>
<td>00/00/99</td>
<td>Uttar Pradesh</td>
<td>Buffalo</td>
</tr>
<tr>
<td>70</td>
<td>IND146/99</td>
<td>04/02/99</td>
<td>Andhra Pradesh</td>
<td>Buffalo</td>
</tr>
<tr>
<td>71</td>
<td>IND148/99</td>
<td>04/02/99</td>
<td>Andhra Pradesh</td>
<td>Buffalo</td>
</tr>
<tr>
<td>72</td>
<td>IND153/99</td>
<td>00/02/99</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>73</td>
<td>IND160/99</td>
<td>11/02/99</td>
<td>Karnataka</td>
<td>Bovine</td>
</tr>
<tr>
<td>74</td>
<td>IND164/99</td>
<td>29/12/98</td>
<td>Assam</td>
<td>Bovine</td>
</tr>
<tr>
<td>75</td>
<td>IND174/99</td>
<td>18/01/99</td>
<td>Mizoram</td>
<td>Bovine</td>
</tr>
<tr>
<td>76</td>
<td>IND175/99</td>
<td>18/01/99</td>
<td>Assam</td>
<td>Porcine</td>
</tr>
<tr>
<td>77</td>
<td>IND178/99</td>
<td>09/09/98</td>
<td>West Bengal</td>
<td>Bovine</td>
</tr>
<tr>
<td>78</td>
<td>IND185/99</td>
<td>21/12/98</td>
<td>West Bengal</td>
<td>Bovine</td>
</tr>
<tr>
<td>79</td>
<td>IND195/99</td>
<td>15/10/98</td>
<td>Orissa</td>
<td>Bovine</td>
</tr>
<tr>
<td>80</td>
<td>IND205/99</td>
<td>01/03/99</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>81</td>
<td>IND207/99</td>
<td>09/03/99</td>
<td>Tamilnadu</td>
<td>Bovine</td>
</tr>
<tr>
<td>82</td>
<td>IND208/99</td>
<td>00/09/98</td>
<td>Maharashtra</td>
<td>Bovine</td>
</tr>
<tr>
<td>83</td>
<td>IND209/99</td>
<td>00/09/98</td>
<td>Maharashtra</td>
<td>Bovine</td>
</tr>
<tr>
<td>84</td>
<td>IND210/99</td>
<td>00/11/98</td>
<td>Maharashtra</td>
<td>Bovine</td>
</tr>
<tr>
<td>85</td>
<td>IND246/99</td>
<td>00/00/99</td>
<td>Gujarat</td>
<td>Bovine</td>
</tr>
<tr>
<td>86</td>
<td>IND249/99</td>
<td>24/01/99</td>
<td>Gujarat</td>
<td>Bovine</td>
</tr>
<tr>
<td>87</td>
<td>IND256/99</td>
<td>07/02/99</td>
<td>Gujarat</td>
<td>Bovine</td>
</tr>
<tr>
<td>88</td>
<td>IND282/99</td>
<td>10/06/99</td>
<td>Kerala</td>
<td>Bovine</td>
</tr>
<tr>
<td>89</td>
<td>IND285/99</td>
<td>00/00/99</td>
<td>Haryana</td>
<td>Bovine</td>
</tr>
<tr>
<td>90</td>
<td>IND287/99</td>
<td>00/00/99</td>
<td>Haryana</td>
<td>Porcine</td>
</tr>
</tbody>
</table>

* Laboratory accession number; † Vaccine virus; NA, not available.
the country. The published sequences of 17 type O isolates from Asia and Europe were also included in the analysis.

METHODS

Viruses

The type O FMDV isolates studied are listed in Table 1. They were initially isolated on primary calf kidney cells and subsequently grown in BHK-21 cells. The infected cell culture supernatant was used for RNA extraction.

Nucleotide sequencing

Total RNA was extracted from infected cell culture supernatants using TriReagent (Sigma) according to the manufacturer’s protocol. Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously [12] using two oligonucleotide primers (NK61, Sense, 5’-GACATGTCCCTCTG-CATCTG and ARS4, anti-sense, 5’-ACCAACCT-CTTGATGTTGG) [13]. The PCR products were purified using the Wizard PCR Preps (Promega). Cycle sequencing of the PCR products was performed using Silver Sequencing kit (Promega) with an anti-sense primer (NK72, 5’-GAAGGGCCAGGGTTGATCT) [13]. The reaction was carried out on a thermocycler (Hybaid) using the following thermal conditions: 94 °C for 2 min, 1 cycle; 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, 60 cycles. The sequencing products were resolved in 6% polyacrylamide gel containing 7.5 M urea. The sequencing gel was stained with silver nitrate using silver staining kit (Promega).

Sequence analysis

Nucleotide sequences of two Bangladesh strains (BAN/3/96, BAN/5/96) [14] and one European strain (O1-BFS) [15] were used for comparisons. In addition to these strains, the following 14 sequences of type O strains from outside of India were obtained from the GenBank and included in the analysis (GenBank accession numbers are given in parenthesis): China/GD/86, China/A/58, HK/93 (AJ131468, AJ131469, AJ131470), Golan/Israel/81 (Z21860), O1/South Lebanon (S77354), SAU/1/88, SAU/100/94, SAU/2/95, SAU/5/95 (AJ004659, AJ004660, AJ004661, AJ004662), O1/Sharquia/72, Iran/3/87, Syria/1/87, O1/Manisa/Turkey/69 (AJ004655, AJ004656, AJ004657, AJ004658), and TL/Taiwan/97 (AF030259). The nucleotide sequences of individual isolates were analysed on a personal computer using the programme ‘Seqprog’ written by one of the authors (N.J.K). All pairwise comparisons were performed by giving each base substitution equal statistical weight (ambiguities were ignored). A phylogenetic tree was constructed using the UPGMA method as implemented in the computer programme Neighbor and dendrogram plotted using the programme DRAWGRAM both from the PHYLIP 3.5c phylogeny package [16]. The UPGMA method constructs a tree by successive (agglomerative) clustering using an average-linkage method of clustering. The scale below the tree was drawn from the nucleotide sequence distance matrix.

RESULTS

On the basis of nucleotide sequence analysis at the 3’ end of 1D (VP1-encoding) gene, we have classified the type O FMDV in to four distinct genotypes. A divergence of >15% in nucleotide sequence distinguishes genotypes [17] and strains which differ by <5% are considered to be closely related [8]

We determined the nucleotide sequence within the 1D region (165 nucleotides, positions 475–639) of 90 FMDV type O isolates including two vaccine strains (Table 1) recovered from different parts of India (Fig. 1a). In addition to these strains recovered from India, we have also included 17 more type O exotic sequences in our analysis. The result is shown in the form of a dendrogram (Fig. 1b). On the basis of nucleotide sequence divergence, the FMDV isolates included in the study were clustered into four different genotypes. All the Indian isolates along with isolates from Bangladesh, Egypt, Israel, Saudi Arabia, Syria, Turkey and one isolate (China/A/58) from China were grouped in genotype-I. The genotype-I was further subdivided into 16 different sub-genotypes/groups (groups 1–16, groups are numbered randomly). The criteria used for defining sub-genotypes/groups was 5% nucleotide sequence divergence. The Indian isolates were distributed in 12 different groups, reflecting the heterogeneous nature of the viruses causing the outbreaks in the field. Sequences of viruses isolated from different regions/states, on different occasions are identical. This situation was observed between IND48/98, IND56/98, IND57/98 and IND65/98 isolated from the state of Punjab.
Fig. 1. (a) Distribution within India of FMDV type O field isolates used in the study. (b) Dendogram showing genetic relationship between FMDV type O strains.
during late 1997 and early 1998 and IND81/98, isolated from Bhagpat (Uttar Pradesh) during March 1998. These isolates along with a Gujarat isolate (IND249/99) share 100% nucleotide similarity. A similar result was observed with IND61/97, isolated from Haryana during December 1996 and IND289/97, isolated from West Bengal during April 1997 which had a 100% nucleotide sequence identity. Yet another, similar observation was made with the isolates IND210/99, recovered from Maharashtra in November 1998 and IND153/99, recovered from Tamilnadu in February 1999.

Group 2 is the second largest genetic group formed by 9 (of 90) Indian isolates, along with 3 (SAU/100/94, SAU/2/95, SAU/5/95), 2 (BAN/3/96, BAN/5/96) and 1 (Golan/81) isolates from Saudi Arabia, Bangladesh and Israel, respectively. The remaining one isolate from Saudi Arabia (SAU/1/88) formed a distinct group (group 13), with approximately 7-5% (average) nucleotide sequence difference from the former three Saudi Arabian isolates. The Saudi Arabian isolates are closely related to an isolate from Karnataka (IND380/97) recovered during February 1994.

Group 3 is the third largest genetic group comprising field strains isolated between 1994–9. Members of this group are mainly confined to four southern states of Andhra Pradesh, Karnataka, Tamilnadu and Kerala. This genetic group is still responsible for FMD outbreaks in this region. In this group IND160/97 is the only isolate from Maharashtra state which neighbours the southern states of Andhra Pradesh and Karnataka. IND57/96 isolated during 1994 from the state of Andhra Pradesh is the earliest outbreak strain in this genetic group. The nucleotide sequence difference seen in this group varies from 0-6–4-5%.

Group 4 comprises seven isolates from the state of Karnataka (Fig. 1b), all isolated from bovines between 1994–6. Two isolates, IND410/97 and IND411/97, from the same district, but isolated within 15 days intervals, showed nucleotide divergence as high as 5-4% between them. Group 5 comprises of six isolates including one vaccine strain (INDR2/75). These isolates are related to each other by 0–5-2%. Group 6 consists of three isolates from Gujarat and Haryana, which share more than 99% sequence homology over the region sequenced. Group 7 consists of isolates IND27/97 and IND279/97 from Uttar Pradesh and Assam, respectively, and show approximately 95% nucleotide sequence homology. Groups 8, 9, 10, and 11 are represented by single isolates. Group 8 is formed by IND485/97, which was isolated from Tamilnadu in 1993. Isolates representing groups 9 (IND79/97) and 10 (IND313/97) are from the state of Haryana, with a nucleotide difference of > 8%.

Another vaccine virus IND53/79 used in the country forms group 11.

**DISCUSSION**

In India FMD is endemic and occurs throughout the year. Of the four serotypes O, Asia1, A and C prevalent in India, type O accounts for nearly 85–90% of the outbreaks, followed by Asia1 and A. Disease due to type C has not been recorded since 1995. The country has a very large number of susceptible animals (> 470 million) comprising cattle, buffaloes, sheep, goats and pigs besides a large number of wild ungulates. Very few animals (less than 2%) are regularly vaccinated, mostly on the larger farms. The disease situation is further complicated by the unrestricted movement of animals. FMD samples included in this study were collected over a large geographical area of the country (Fig. 1a) and provide a clearer picture of the epidemiology of FMD in India. There is no restriction on movement of animals across the state boundaries which may explain the lack of correlation between geographical origin and genetic groups. Group 1, which is the largest genetic group in the genotype-I, is formed by 45 (of 90) Indian isolates from 15 different states of the country. This group is formed by isolates recovered in the past 3 years (between 1996–9). A nucleotide difference of 4-95% was the highest observed among the isolates in this group. These results, which show the same strain in locations over 100 km apart, suggest the long distance movement of infected animals. A similar situation was observed in SAT-type FMDV in Kruger National Park and other regions of Southern Africa [7], where 100% homology was found between isolates collected from different areas; in this instance it was thought due to fragmentation of animal herds, after a culling programme. The isolate IND81/98, was recovered from Bhagpat in Uttar Pradesh and there was a history of animals being brought from Punjab in the midst of a FMD outbreak to villages near to Bhagpat.

The isolates IND47/98, IND48/98, IND55/98 to IND57/98, IND64/98 to IND66/98 were all recovered from different districts of Punjab during an FMD epidemic. It started in December 1997 and
Among the Indian isolates varied from 0 to 8–8% in nucleotide sequence from the vaccine strains, they showed close antigenic relationship in enzyme-linked immunosorbent assay (unpublished observation) with the vaccine strain INDR2/75.

The results of this study show that in India during the last 6-year period there were at least 12 genetic groups active. Group 1 seems to be the dominant genetic group as it is responsible for the majority of the outbreaks. But this genetic group did not remain confined to any particular region of the country and was widespread. This is not unexpected considering the fact that no restriction is imposed on animal movement at the state boundaries. Furthermore, this genetic group was not restricted to any particular species of animal, instead, it caused disease outbreaks in large ruminants, small ruminants and pigs. Members of this genetic group showed 92% sequence homology with the vaccine viruses used in the country.

The appearance of different genetic groups in the same state/region within a period of one year is evident in the state of Karnataka, during 1994 and 1996 when outbreaks were caused by viruses belonging to three different genetic groups and, in the state of Haryana, where outbreaks due to four different genetic groups (groups 1, 3, 9 and 10) were evident during 1997. In contrast to these observations, there are examples of outbreaks due to the same genetic groups over a long period of time. Group 3 isolates from the state of Karnataka and Andhra Pradesh, were prevalent for a period of 4 (1994–7) and 6 (1994–9) years respectively. The conservation of genetic groups for such a long period of time may be due to persistence of virus in the cattle [19].

All the Indian type O isolates are not related to the European type O virus (O1 BFS) as reported earlier by us [10]. The genetic relatedness of Indian field isolates with those from Nepal and Saudi Arabia are reported earlier [8, 10]. The results of the present study also revealed a similar situation. O1 Manisa which is widely used in Asia as a vaccine strain and originated in Turkey is related to the Indian isolates in genotype I by 88%. Although there is considerable variation of strains within India, they all fall in to one genotype (genotype I) and are serologically related to the vaccine strain. Occurrence of epidemiologically unconnected outbreaks over a short period is consistent with the endemic nature of the disease and uncontrolled animal movement. The present study shows a clear picture of the epidemiological situation of the disease due to type O, which accounts for more
than 85% of the outbreaks in India. Such continuous monitoring of the field strains help in selection of vaccine strains and evolving a proper control strategy for the disease.

ACKNOWLEDGEMENTS

We are thankful to the Director, Indian Veterinary Research Institute and Indian Council of Agricultural Research for providing necessary facilities to carry out this work.

REFERENCES