Table 1. Gamma ray intensity in counts minute g and the monazite content in fine fraction

Station no	Sample no	Number of counts	Percentage of monazite
1	F'	234 2	0 88
	F"	171.2	0 46
	B	219 2	0.40
	B"	153 2	0 27
2	F'	231.2	0.56
	F"	177.2	0 33
	B'	215.2	0.51
	B"	149.2	0.26
3	F'	221.2	0.54
	F"	186 2	0.50
	B'	157.2	0.38
	B"	143.2	0.30
4	F'	217.2	0.53
	F"	160.2	0 46
	B'	169 2	0.47
	B"	119.2	0.16
5	F"	201.2	0.39
	F"	157.2	0 42
	B'	160.2	0 44
	B"	117.2	0.19

F : Foreshore–surface; F": Foreshore–bottom; B': Backshore–surface; B": Backshore–bottom.

sediments of east coast was estimated to range from 0.5% to 3%. However, monazite concentration of the nearby beach[10] is found to contain 9.05% of ThO_2 and 0.264% of U_3O_8. The plot between percentage of monazite versus radioactive intensity (Figure 2) shows an increase in radioactive intensity with increase of monazite level, indicating a good correlation between them. It is therefore concluded that in the present study the radiation energy obtained by the gamma counter may be attributed to the natural radioactive mineral, monazite, which occurs along the coast, as other sources are not significant in the locality. The average value of radiation found to be 178 counts/min (Table 1) would have been much lower if considered for the bulk sediment samples instead of its heavy mineral fraction which constitutes only 10–11% of the bulk sediment. This is because the monazite concentration accounting for the radioactivity gets reduced in the bulk sediment samples. Thus it is inferred that the radioactivity of the main channel of coast of Chilka lake is much lower than the normal background radiation of a beach[2]. The level of radiation on this coast is appreciably small compared to the Kerala[1] coast where the level of radiation varies from 250–2000 counts/min.

Thus it can be concluded that the radiation of the main channel coast of Chilka lake is below the normal background radiation of a beach and is not health hazardous and safe for the inhabitants from the point of view of radioactive pollution. Further, it is inferred that occurrence of monazite may be the locus of radioactivity in the study region and the coast is not suitable for mining the natural radioactive elements like thorium, uranium, etc.

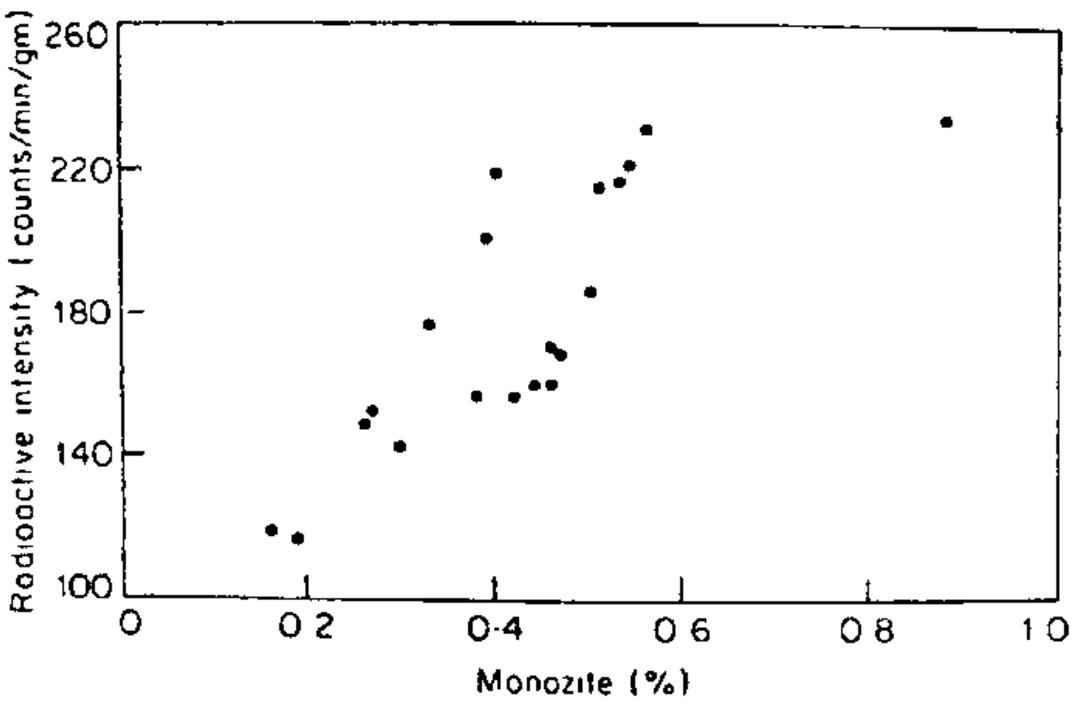

Figure 2. Plot of percentage of monazite vs radioactive intensity.

1. Mahadevan, C. and Poornachandra Rao, M., Symposium on Mineral Raw Materials and their By-products, 1953.
2. *Frontline*, April 14–27, 1990, pp. 114–123.
3. Mahadevan, C. and Aswathanarayana, U., *A. U. Mem.*, 1954, **1**, 36–52.
4. Sastry, A. V. R. and Mahadevan, C., *J. Sci. Ind. Res.*, 1957, **169**, 429–431.
5. Kshira Sagar, T. V. S. R and Rao, B. N., *Indian J. Mar. Sci.*, 1989, **18**, 8–10.
6. Aswathanarayana, U. and Mahadevan, C., Symposium on Mineral Raw Materials and their By-products, 1953, pp. 21–27.
7. Sahu, B. K., Pradhan, B. B. and Mishra, S. B., *Indian J. Mar. Sci.*, 1990, **19**, 226–227.
8. Carver, R. E., *Procedures in Sedimentary Petrology*, John Wiley and Sons, New York, 1971, pp. 69–589.
9. Rao, A. T. and Prakash Rao, S. Ch., *Indian J. Mar. Sci*, 1980, **9**, 214–216.
10. Subbarao, J., Unpublished Annual Report for the field season 1958–59, Eastern Circle, AMD, DAE, 1959

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Characterization of aphthovirus type Asia-1 isolates of Indian origin using monoclonal antibodies

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Two monoclonal antibodies (MAbs) directed against the major antigenic viral polypeptide VP1 of Asia-1 vaccine strain were used to characterize 17 Asia-1 isolates from different regions of India, by neutralization assays. Six of these field isolates were further characterized with polyclonal antibodies as well as MAbs by neutralization assays *in vitro* and passive mouse protection assay (*in*

***vivo*). The variations that had occurred in the major antigen of Asia-1 field isolates as detected by MAbs and their significance are discussed here. The occurrence of isolates with low, medium and high reactivity with MAbs and the isolates that resisted neutralization with MAbs; a possible mutation and also phenomenon of opsonization enhanced phagocytosis in the *in vivo* neutralization are also discussed.**

FOOT-and-mouth disease virus (FMDV), a member of aphthovirus genus in the Picornaviridae family, has an ss RNA genome of 8.5 kb. Seven serotypes and many subtypes complicate the antigenic differentiation. The hybridoma technique for production of monoclonal antibodies (MAbs)[1] and their use made a significant contribution in FMDV characterization. Type Asia-1 is of particular epidemiological importance and the second most prevalent type causing outbreaks in India[2]. The reported work on this type is scanty, and antigenic characterization of field isolates of Asia-1 is of great significance.

The first report[3] on development of MAbs against aphthovirus dates back to 1982. In the next six years different laboratories[4-13] in Europe and USA developed MAbs against FMDV type O, A and C. In India, Butchaiah and Rao[14] produced MAbs against type Asia-1 directed against VP1. Recently Reddy *et al.*[15] reported production of MAbs against subtype A22.

Aphthovirus surface is a mosaic of epitopes. The location of the epitopes and characterization FMDV type O and A based on antigenic sites recognized by MAbs were reported[12,13,16-18]. The mechanism of virus neutralization by MAbs was studied by McCullough *et al.*[19,20]. Thomas *et al.*[21] identified the epitopes of serotype A virus by RNA sequencing of neutralization escape mutants. Saiz *et al.*[22] identified neutralizing antigenic sites on VP1 and VP2 of type A FMDV defined by neutralization-resistant variants. A modest attempt at characterizing FMDV type Asia-1 isolates of Indian origin with neutralizing MAbs was made in this study.

Baby hamster kidney (BHK) clone-13 cell line was used for virus growth, titration and neutralization assays. Eagles's minimum essential medium (MEM) containing glutamine (2 mM) and sodium bicarbonate (5.5%) was used. The medium was supplemented with bovine serum or foetal calf serum for cell growth and neutralization assays respectively. The culture supernatants of hybridoma clones IIC/2 and IIC/5 directed against VP1 of FMDV vaccine strain India 63/72 generated by Butchaiah and Rao[14] were used. A batch of six healthy male guinea pigs was inoculated each with 30 μg of binaryethylenimine (BEI) inactivated 146 S particles of Asia-1 vaccine strain adjuvanted with Freunds complete adjuvant. Serum collected at 28 days post-inoculation, pooled and heat inactivated served as the polyclonal guinea pig immune serum.

Asia-1 strain India 63/72 used for vaccine production at this institute was used. All the Asia-1 field isolates were obtained from the central FMD typing laboratory, Mukteswar-Kumaon. These were adopted to grow in BHK21 clone-13 monolayer cell line by limited passage. The history of these aphthovirus Asia-1 isolates originally isolated from cattle is shown in Table 1.

The virus dilution, serum/MAb constant method of neutralization similar to that described by Forman[23] was used to assay the neutralizing activity of the antibodies against all the virus isolates. Briefly, $\log_{10}$ dilutions of virus were allowed to react with a constant dilution of MAb (1:4) and the virus neutralization was assayed in 96-well cell culture plate using BHK21 monolayer cells. The fifty per cent end points of virus in the presence and absence of MAbs were calculated according to the established procedures[24]. Some selected Asia-1 virus field isolates were subjected to virus constant and varying serum/MAb dilution method neutralization tests, wherein constant amount of virus (100 $TCID_{50}$) was allowed to react with serial two-fold dilutions of the MAbs in the medium. The mixtures were allowed to react for 1 h and BHK21 cells in growth medium were added and incubated at 37°C in 5% carbon dioxide tension for 48 h. The tissue culture infectivity neutralization end points were calculated as described earlier[24].

Suckling BALB/C mice (5 day old) were inoculated intra muscularly (i/m) 100 μl each with serial two-fold dilutions of culture supernatants of hybridomas (MAbs). After 24 h the mice were challenged i/m with 100 μl of 200 LD_{50} dose of the virus (mouse adopted virus). The highest dilution of MAbs that protected at 50% end point against the challenge virus was determined.

The infectivity titres of virus isolates neutralized by the MAb II/C2 are presented in Table 2. There was a

Table 1. History of aphthovirus Asia-1 strains isolated from cattle

Strain	History of vaccination	Field sources	Year
India 21/80	Yes	Hyderabad AP	1980
India 4/86	Yes	Bhutan	1986
India 10/86	Yes	Haryana	1986
India 57/86	No	Jammu and Kashmir	1986
India 58/86	No	Jammu and Kashmir	1986
India 59/86	No	Assam	1986
India 60/86	No	Assam	1986
India 61/86	No	Meghalaya	1986
India 66/86	No	Assam	1986
India 67/86	No	Assam	1989
India 68/86	No	Assam	1986
India 71/86	No	Assam	1986
India 75/86	No	Assam	1986
India 81/86	No	Manipur	1986
India 82/86	No	Manipur	1986
India MFB/86	Yes	Uttar Pradesh	1986
India GDG/87	No	Karnataka	1987

Table 2. Neutralization indices of virus strain against monoclonal antibody (II/C2)

Strain	Virus titre in absence of antibody ($log_{10}TCID_{50}$)	Virus titre in the presence of antibody (log_{10} $TCID_{50}$)	Virus titre neutralized ($log_{10}TCID_{50}$)
India 139 85	4.70	3.50	1.20
India 4 86	3.75	2.50	1.35
India 10 86	7.50	7.50	0.00
India 57 86	5.50	4.70	0.80
India 59 86	4 66	3.50	1.16
India 60 86	5.00	5.25	0.00
India 61 86	6.25	3.75	2.50
India 66 86	3.75	3.50	0.25
India 67 86	4.66	2.50	2.16
India 68 86	4.75	2.50	2.25
India 74 86	5.00	4.00	1.00
India 75 86	6.50	4.70	1.80
India 81 86	7.50	4.70	2.80
India 82 85	6.25	5.00	1.25
India MFB	3.75	3.75	0.00
India 21 80	5.00	1.50	3.50
India GDG	6.50	6.50	0.00
India 63,72 (reference strain)	4.75	2.50	2.25

Table 4. Neutralization of Asia-1 strains

Strain	Log_{10} antibody titre		
	II/C2	II/C5	Polyclonal serum
Reference strain	1.35	1.35	1.95
India 21/80	1.35	0.45	1 80
India MFB/86	0.00	0 00	1 65
India 4/86	0 45	0.00	1.65
India 10/86	0.00	0.45	1.50
India 66/86	0.30	0.00	1.65
India GDG/87	0.00	0.00	1.80

Table 5. Comparison of *in vitro* and *in vivo* neutralization activities of monoclonal antibody II/C2

Strain designation	Neutralization activity (log_{10})	Passive protection titre (log_{10})
Reference strain	1.35	1.95
India 21/80	1.35	1.35
India MFB/86	0.00	0.75
India 10/86	0.00	0.00
India 66/86	0 30	0.75
India GDG/87	0.00	0.00

high variability in the neutralizing ability of the MAbs towards different field strains ranging from zero for strains 10/86, 66/86, MFB and GDG to a value of 3.5 against strain 21/80. Accordingly the strains were grouped as those having low (<1.00), medium (1.00–2.00) and high (>2.00) reactivities with this MAb. Thomas *et al.*[21] followed a similar criterion and classified the strains as having low reactivity (0.5 to 1.5) and high reactivity (>1.5). Based on the observations, six field isolates were selected for further studies, the details of which are given in Table 3. The selected isolates were further characterized by *in vitro* virus neutralization studies against MAbs II/C2 and II/C5 and also guinea pig polyclonal serum. These results are presented in Table 4. The strains were also compared by their reactivity to MAb *in vitro* and also in *in vivo* studies (i.e. passive mouse protection tests) and the neutralization pattern *in vitro vis-a-vis in vivo* are shown in Table 5.

The variations in aphthovirus strains occur within the key amino-acid sequences of neutralizing epitope

Table 3. Particulars of selected Asia-1 strains

Designation	Source	Virus titre neutralization within MAb II/C2
Reference strains	IVRI, Bangalore	2.25
India 21/86	Hyderabad, AP	3.50
India 4,86	Bhutan	0.25
India 10/86	Hissar, Haryana	0.00
India MFB/86	Bareilly, Uttar Pradesh	0.00
India 66/86	Kamrup, Assam	0.00
India GDG/87	Dharwad, Karnataka	0 00

and hence the application of virus neutralization and passive protection tests using MAbs directed against these epitopes is the best method of virus characterization. In the virus neutralization tests with MAb II/C2, the neutralization activity ranged from 0 to 3.5. The virus isolates GDG, MFB and 10/86 were not neutralized at all, whereas the isolates 4/86 and 66/86 showed low neutralization values (1.25 and 0.25 respectively), isolate 21/80 had a value higher than that of vaccine strain against which the MAbs had been raised. This could probably be due to repetition of the key amino-acid sequence comprising the neutralizing epitope on VP1 of the virus, very similar to that described by McCullough *et al.*[18]. The epitope in the VP1 might have undergone a change in the virus isolates GDG, 10/86 and MFB and this could be the reason why they resisted neutralization.

In the cross-neutralization tests with MAb IIC/2 and IIC/5 and also with guinea pig immune serum, it could be observed that virus isolates MFB, 10/86 and GDG were not neutralized at all by MAbs IIC/2, whereas isolates 4/86 and 66/86 had low neutralization titres against this MAb. The obvious conclusion was that isolates 66/86 and 4/86 had probably undergone only minor alterations in this neutralizing epitope and therefore had a low degree of variation, whereas the isolates 10/86, GDG and MFB had significant variations from the vaccine strain. The neutralization pattern against the other MAb namely IIC/5 was similar for all the virus isolates except 10/86. The latter were the only isolates neutralized by the MAb among the variants such as GDG and MFB, suggesting that isolate 10/86 had variation in one epitope, whereas in MFB and GDG the variations involved both epitopes

recognized by these two MAbs. In contrast, polyclonal serum antibody neutralized all the isolates equally well although its titre was marginally less against 10/86 (Table 4). The findings were similar to those obtained earlier[12,19,21,22]. Majority of neutralizing MAbs were reportedly directed against VP1 though some may also react with other structural proteins[12,18,26]. The MAbs used in the present study were known to be directed against VP1 of Asia-1 vaccine strain therefore, variations observed by them were on VP1.

Regarding passive protection tests performed in suckling mice using MAb IIC/2, the degree of protection was similar to the *in vitro* neutralization. But in respect of virus isolates MFB, 4/86 and 66/86, the MAbs conferred passive protection at dilutions at which they were not neutralized *in vitro*. This might be due to the opsonization enhanced phagocytosis. The strains 10/86 and GDG which were not neutralized *in vivo* also, probably had a greater degree of variation than the isolates 4/86, MFB and 66/86. These findings were similar to those obtained by McCullough *et al.*[19,20]. Another interesting observation was the difficulty in adaptation and lesser pathogenicity of isolate GDG to baby mice. This isolate failed to produce characteristic symptoms and mortality in the first passage and the observation that the isolates had a lesser pathogenicity to BHK21 (Glasgow) monolayer cells in the first two passages is proof that it might have had undergone mutation. Dave and King[27] observed in a SAT-1 virus strain, altered mouse virulence and BHK21 cell pathogenicity due to mutation in VP1 which they regarded as mis-sense mutation. Further studies with a panel of MAbs directed against the major antigenic epitopes by cross neutralization assays and also the RNA sequencing of variants would help understand precisely the epitopes against which the MAbs were directed and also the strain variations in the hitherto less studied and more important apthovirus serotype Asia-1.

1. Kohler, G. and Milstein, C., *Nature*, 1975, **256**, 495–497.
2. Ramarao, D. and Rao, B. U., *Rev. Sci. Tech. Off. Int. Epiz.*, 1988, 7, 357–364.
3. McCullough, K. C. and Butcher, R. N., *Arch. Virol.*, 1982, **74**, 1–9.
4. Brocchi, E., DeSimone, F., Mellano, F. and Panina, G. F., *Atti. Soc. Ital. Sci. Vet.*, 1982, **36**, 576–578.
5. Brocchi, E. and DeSimone, E., 44th Meeting of TUMEA Italian Soc. Microbiol, Teramo, Italy, 3-5 December, 1982.
6. Yavin, I., Lalazar, A., Zeelon, A., Panina, G. F., Brocchi, E., DeSimone, F., Spier, R., Clarke, J., Gorecke, P. and Aviv, H., XVI Conf. OIE, Paris, 14–17 September, 1982.
7. McCullough, K. C., Butcher, R. N. and Parkinson, D., *J. Biol. Stand.*, 1983a, **11**, 171–181.
8. McCullough, K. C., Butcher, R. N. and Parkinson, D., *J. Biol. Stand.*, 1983b, **11**, 183–194.
9. McCullough, K. C., Crowther, R. and Butcher, R. N., Eur. Comm. Cont. F. M. D. Lelystad, The Netherlands. 7-16, FAO, Rome, 1983.
10. Duchesne, M., Cartwright, T., Crespo, A., Boucher, F. and Fellourd, A., *J. Gen. Virol.*, 1984, **65**, 1559–1566.
11. Ouldridge, E. J., Barnet, P., Parry, N. R., Syred, A., Head, M. and Rweyemamu, M. M., *J. Gen. Virol.*, 1984, **65**, 203–207.
12. Stave, J. W., Card, J. L. and Morgan, D. O., *J. Gen. Virol.*, 1987, **67**, 2083–2092.
13. Grubman, M. J. and Morgan, D. O., *Virus Res.*, 1987, **6**, 33–43.
14. Butchaiah, G. and Rao, B. U., *Acta Virol.*, 1989, **33**, 121–130.
15. Reddy, P. S., Sakkubai, P. R., Rao, B. U., Prabhudas, K. and Butchaiah, G., *Curr. Sci.*, 1992, **63**, 94–98.
16. Baxt, B., Morgan, D. O., Robertson, B. H. and Timpone, C. A., *J. Virol.*, 1989, **51**, 298–304.
17. Xie, Q. C., McCahon, D., Crowther, J. R., Belsham, C. J. and McCullough, K. C., *J. Gen. Virol.*, 1987, **68**, 1637–1647.
18. McCullough, K. C., Crowther, J. R., Carpenter, W. C., Broochi, E., Cappucci, L. and DeSimone, F., *Virology*, 1987, **157**, 516–522.
19. McCullough, K. C., Crowther, J. R., Butcher, R. N., Carpenter, W. C., Brocchi, E., Cappucci, L. and DeSimone, F., *Immunology*, 1986, **58**, 421–428.
20. McCullough, K. C., Parkinson, D. and Crowther, J. R., *Immunology*, 1988, **65**, 187–191.
21. Thomas, A. A. M., Woortmeijer, R. J., Pujik, W. and Barteling, S. J., *J. Virol.*, 1988, **62**, 2782–2789.
22. Saiz, J. C., Gonzales, J. J., Borca, M. V., Sobrino, F. and Moore, D. M., *J. Virol.*, 1991, **65**, 2518–2524.
23. Forman, A. I.,.*J. Hyg.*, 1975, **74**, 215–232.
24. Reed, L. J. and Muench, H. A., *Am. J. Hyg.*, 1938, **27**, 493–497.
25. Barteling, S. J., Boerke, J., Woortmeizer, R. and Thomas, A., O. I. E. 7th Conf., FMD commission, 1986, pp. 41–49.
26. Pfaff, E., Theil, H. J., Beck, E., Strohmeier, K. and Schaller, F., *J. Virol.*, 1988, **63**, 2033–2040.
27. Dave, P. S. and King, A. M. Q., *Arch. Virol.*, 1983, **76**, 117–126.

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Far-red light–ethylene interaction in seed germination of *Caesulia axillaris* Roxb.

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Absolute requirement of light for seed germination in *Caesulia axillaris* Roxb. manifested itself into a biphasic fluence-response. Ethylene, which was little effective in dark, interacted significantly with both red light (R) and far-red light (FR) to promote germination. Vegetation canopy, besides reducing photon flux in PAR, also lowered the R:FR ratio (ξ). Ethylene, a natural component of soil environment, appears to modify the photosensitivity of *Caesulia* seeds, thus promoting the germination of high P_{fr}/P_{tot} (φ) requiring seeds under an otherwise unfavourable light environment.

FAR-red light (FR) is known to promote seed germination in a few plant species[1–3]. Yet no published work is available on Indian species where the criteria set[4,5] and light source-filter combinations recommended[6,7] were employed to confirm FR induction of