Studies on Comparative Efficacy of Different Techniques for the Detection of Foot and Mouth Disease Virus

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE)

PUSA (SAMASTIPUR)

By

Pramod Kumar

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(MICROBIOLOGY)

POST GRADUATE DEPARTMENT OF VETERINARY MICROBIOLOGY

BIHAR VETERINARY COLLEGE

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DEDICATED TO MY PARENTS

Department of Veterinary Microbiology
Bihar Veterinary College, Patna
RAJENDRA AGRICULTURAL UNIVERSITY, BIHAR

CERTIFICATE - I

This is to certify that the thesis entitled " Studies on comparative efficacy of different techniques for the detection of 'foot and mouth disease' virus " submitted in partial fulfilment of the requirement for the Degree of Master of Veterinary Science (Microbiology) of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar is the record of bonafide research carried out by Dr. Pramod Kumar under my supervision and guidence. No part of the thesis has been submitted for any other Degree and Diploma.

It is further certified that such help or informations received during the course of this investigation and preparation of the thesis have been duly acknowledged.

29.5.84

(C.B.Prasad)
Associate Professor & Head,
Department of Veterinary
Microbiology.

CERTIFICATE-II

We, the undersigned, member of the Advisory Committee of Dr. Pramod Kumar a candidate for the Degree of Master of Veterinary Science with major in Microbiology have gone through the manuscript of the thesis and agree that the thesis entitled " Studies on comparative efficacy of different techniques for the detection of 'foot and mouth disease' virus " may be submitted by Dr. Pramod Kumar in partial fulfilment of the requirements for the Degree.

29.5.84

(C.B.Prasad)
Chairman, Advisory Committee

Members of the Advisory Committee

1 K. C. P. Singh

2 Md. Murtuza

CERTIFICATE-III

This is to certify that the thesis entitled " Studies on comparative efficacy of different techniques for the detection of 'foot and mouth disease' virus " submitted in partial fulfilment of the requirements for the Degree of Master of Veterinary Science (Microbiology) of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on 15.6.1984.

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15.6.84
(C.B.Prasad)
Chairman, Advisory Committee

(S.Prasad) 15/6/84
External Examiner

Members of Advisory Committee

1 K.C.P. Singh -

2 Md. Murtuza -

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The last few decades have been an extremely exciting period in the realm of foot and mouth disease research , particularly to its applied side. Admittedly, bold new ideas bearing on fundamental as well as applied aspects of foot and mouth disease have intered into a new horizon. Much of our thinking of past had to undergo critical reappraisal and pass through application of new concept in terms of more accurate and absorbing analysis of epidemiological factors which influences the malady! In presenting an interpretive account of " comparative efficacy of different serological test for the detection of foot and mouth disease virus " which by no means was easy task for the present scholar to complete without help of many people with whom the author came in contact in course of samples collection, ecquiring and investigation In fact, several persons drawn from various strata of the society, Govt. officers and huge band of employees working in numberous establishment not to speak of scores of individuals both known and unknown but surely the illiterate virtually mute but resourceful peasant of the locality, came with their helping hands to assist me to complete field work, on the spot observation, collection of clinical samples and epidemiological data. I very gratefully acknowledge their gracious outlook and endeavour in lending facheal information which immensely helped me to build up initial edifice of the present investigation.

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I fail to find any suitable word to express my thanks to Mr. Raju Kumar, Typist, Department of Microbiology, Bihar Veterinary College, Patna for his day and night untiring labour without which it would be impossible to complete the thesis in time.

I wish to record my deep appreciation for my parents for their constant encouragement, sacrifice and devotion without which it would not have been possible to carry out the present piece of work and also I can not forget the help rendered to me by my brothers and sister.

P.K.

C O N T E N T S

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I N T R O D U C T I O N

Foot and Mouth Disease (FMD), a viral disease of cloven footed animals, is endemic, widespread and has been occurring over past centuries in India. This disease though not highly fatal, invariably leaves the survivors in extremely debilitated condition usually neither good for production nor for work. This malady has been recognised as a serious health hazard for the livestock development programme. Losses due to this terrible affliction, which stiffles milk production, lames drought animals, causes sterility and sparks of abortion, are more than Rs. 400 crores annually (Adlacka & Sharma, 1982).

There are altogether seven serotypes of FMD virus with plurality of subtypes of which four types 'O', 'A', 'C' and 'Asia-1' are prevalent in this country. A prerequisite to any viable control programme for FMD would be timely recognition of prevalent serotypes and subtypes of FMD virus associated with the outbreak in question. A variety of serological tests are currently in vogue for routine FMD virus typing; but a simple,reliable and reproducible among them has to be selected which can furnish the quickest and correct information about the serotype of the virus involved in the outbreak.

The Complement Fixation Test (CFT) is the most widely employed technique in typing and subtyping of

FMD virus and offers the advantage of simplicity, versatility and rapidity of results and can be used to type inactivated as well as infectious virus preparations (Rweyemamu et al.,1978). The test,however fails to give definite typing results on number of occasions on account of antigen preparations being either anticomplementary or not containing sufficient quantity of antigen. Disquieting aspect of FMD typing works with CFT has been attributed to occasional failure of unweaned mice to yeild FMD virus or irregular mortality pattern in suckling mice (Tewari et al., 1980).

In recent past tissue culture system has been adopted as an alternative to suckling mice for the recovery of FMD virus (Patty et al.,1965; Tewari et al.,1980) but it requires repeated passage before it is adapted. Other serological tests less widely used for FMD virus typing include immunodiffusion (Bodon, 1955; Brown & Crick,1958; Sharma & Dutta,1969), neutralization test (Forman, 1975; Rweyemamu, Booth and Pay,1977), plaque reduction (Capstick Sellers and Stewart, 1959), fluorescent antibody technique (Sugimura & Eissner, 1976; Rai, 1980) and enzyme linked immunosorbent assay (Crowther et al., 1976; Rai, 1980). Each of these tests has got one drawback

or another and hence workers on FMD research are still in search of a serological test which may prove to be foolproof, less time consuming and effective under field conditions.

Studies on epidemiological factors associated with the occurrence of the disease is equally important for evolving an effective control measure. Identification of epidemiological factors responsible for the spread and dessimination of FMD virus in the population has attracted the attention of animal health custodians and efforts are being made in this direction in a coordinated manner all over the country. So far, some of the epidemiological factors directly associated with the spread of disease have been pinpointed which include cattle fair, movement of animals as well as humen beings, introduction of new animals, congregation and intermixing of animals at a common place as seen during the flood and famine for grazing, transportation of animals from long distance, sex, crossbred and exotic animals and carrier status. However, sufficient epidemiological data are yet to be generated to contain this malady and to fill up the gap in our knowledge and understanding of such factors.

Keeping in view the above facts, the

present study has been undertaken with the following objectives:

1. To study the comparative efficacy of different serological tests for the detection of FMD virus types.
2. To study the suitability of tissue culture over suckling mice for the recovery of FMD virus from field samples.
3. To study the effects of different epidemiological factors like topography, season, age, sex and species on the incidence of FMD in the state of Bihar.

It is sincerely hoped that the data generated during the present investigation would result in pinpointing the most efficient serological test for detecting the serotypes of FMD involved in an outbreak in the least possible time under field condition, and in detecting still unidentified epidemiological factors so as to control this dreadful disease more effectively.

REVIEW OF LITERATURE

The literature on serological tests for the detection of FMD virus, propagation of clinical samples for recovery of FMD virus and effect of different epidemiological factors on the spread of disease have been reviewed in some details on the following pages under three sub-headings.

I. SEROLOGICAL TESTS

Serological tests which are currently used for the typing of FMD virus are micro-CFT, agar gel diffusion test, serum neutralization test, conglutinin adsorption test, radioimmuno assay, radial immunohaemolysis, fluorescent antibody technique and enzyme linked immunosorbent assay.

Traub and Mohlmann (1943) applied the complement fixation test for typing of foot and mouth disease virus. A good result was obtained by the use of an antigen prepared from primary tongue lesions in animals experimentally inoculated with the virus strain to be tested and a serum from guineapig. The result of CFT was compared to that of cross immunity test.

Aramburu (1947) conducted the preliminary titration of complement in presence of antigen, a 1:10 suspension of bovine epithelium. A minimum dose

(1.5) of complement was used in the test. The test might be carried out in an incubator or in waterbath at 37°C. The sensitized cell suspension, containing the unusually small amount of MHD in each dose was added after 15-20 minutes incubation and final reading was made 30 minutes later.

Palacios and Rodriguez (1949) developed the technique to remove anticomplementary effect by treating the antigen with ether overnight at room temperature. Alternative treatment mentioned was the addition of 10% superol to the antigen and its removal by centrifugation. Fixation was carried out at room temperature for one hour followed by keeping overnight at -5 to -10°C. Test was then thawed at room temperature and haemolytic system was added after two hours. Two units of complement were used on the basis of 100% haemolysis.

Brooksby (1952) described in detail the technique of complement fixation test applied to the study of foot and mouth disease. The method advocated was based on an abbreviated complement fraction referred to the standard of 50% haemolysis. With suitable adjustment of number of different doses of complement used, the accuracy varied to meet the needs of routine typing test. The preparation of

antisera for use in complement fixation was in guinea pig since bovine serum was found to be generally of low titre. The fixation of complement with the FMD virus and its antiserum increased with prolonged fixation at 18-24 hours at 4°C or at 37°C in 2-4 hours. The test was specific as it differentiated the FMD virus from vesicular stomatitis. Overlapping fixation among types was distinct from fixation by a mixed antigen with sera of more than one type. The test was considered to be reliable for the differentiation of virus types in routine practice.

Bodon (1955) demonstrated the type specific precipitation reaction of FMD virus and differentiated 'O', 'A' and 'C' types from each other on the basis of agar gel diffusion test.

Kornyei (1955) introduced the technique of CFT, using a 9 mm plexi glass plate containing a series of holes of 0.15 ml capacity. The components were added by means of metal tip tubes delivering 0.025 ml. Serial loops. The test was claimed to be very accurate, easily read and was useful in typing unknown antigen which were available in small quantity.

Marucci (1957) developed a new technique

to provide optimum condition of complement activity by adding traces of magnesium and calcium to the buffer solution, pH 7.4. Gelatin (0.5%) was added to reduce non specific loss of reacting components,. Ambocepter and complement were standardized spectrophotometerically. The test was carried out in the cold and the fixation period was 18 to 20 hours at 2 to 5 ^{o}C.

Korn (1957) reported the isolation of FMD virus from 7 of 41 samples from oral mucus membrane (including palate) and 24 of 51 samples from other parts (nose, muzzle and trachea).

Brown and Crick (1958) affirmed that two precipitation lines were produced with homotypic immune serum in agar gel diffusion.

Hobohm et al. (1960) introduced an immuno chromatographical technique for typing FMD virus.

Hobohm and Riverson (1960) demonstrated the precipitation between FMD virus and specific immune sera by paper chromatography.

Graves (1960) described a procedure for quantitative determination of small antigenic differences among three subtype samples of type 'O' FMD virus.

Capstick et al. (1960) suggested that neutralization should be carried out for 90 minutes at 37°C, 4 hours at 26°C or 24 hours at 4°C. Comparison of the plaque method and inoculation of unweaned mice showed that the titre of mouse inoculation was higher.

Martin and Chapman (1961) described a tissue culture colour test, using pig kidney cells for titration of FMD virus and its antibody.

Cowan and Graves (1966) reported that FMD virus gave two precipitation bands with agar gel diffusion technique. One band was attributed to 23 μ infectious particles and the other 7 μ protein subunits.

Sabko et al. (1967) introduced fluorescent antibody technique for the detection and typing of FMD virus . Antigen was in the form of infected cultures of kidney cells from cattle and rabbits. Antigen was first detected six hours after infection of a culture and it was present in the greatest amount after 12-15 hours of infection. However, the test did not prove suitable to type the virus.

Darbyshire et al. (1972) compared the macro technique in tube and micro technique in plastic plate for CFT using strains of three subtypes of 'Asia-1' FMD virus. The results was found to be

comparable and the micro technique was considered to be economic with reagent and capable of similar accuracy.

Prasad and Singh (1972) employed the conglutinin complement adsorbent test for FMD virus typing.

Forman (1974) established that the two systems, tube and microplate CFT, were comparable, although greater reproducibility was obtained with tube test. He observed that microplate test was satisfactory for differentiation of strains and tube test provided a more precise method for the identification of small antigenic differences.

Chemyaev and Sobko (1974) applied accelerated complement fixation reaction for FMD virus typing from field samples using a spectrophotometer to evaluate the results. The tubes were incubated at 38°C until cloudiness appeared in the first two or three tubes, after addition of haemolytic system. Haemolysis was then interrupted by centrifugation at 800-1000 g for 7-10 minutes and the supernatent fluid was examined in the spectrophotometer for haemoglobin content. The result was obtained within 45 minutes and the technique was found much more

sensitive than the ordinary CFT.

Sutmoller and Cowan (1974) compared three immunoperoxidase techniques (direct, indirect and peroxidase antiperoxidase) for potentiating FMD research. Each technique was shown to offer a simple and efficient means for detecting FMD virus types and virus infection antigen in infected cells.

Smertin and Sviridov (1975) proposed a modification of the neutralization test. The main feature was an increase in the volume of reactants to 5 ml, comprising 2.5 ml diluted bovine serum and 2.5 ml of viral suspension. There was no need to add maintenance medium to the monolayer cell culture. The test was as accurate as the standard method, was simpler and reduced the rate of technical error.

Forman (1975) compared some of the immunological methods, the guinea pig protection test and kinetic neutralization test. The author found these tests to be capable of differentiating FMD virus strains.

Crowther (1976) studied the application of radio immuno assay (RIA) in FMD virus research and indicated its usefulness in the examination of a large number of field strains of the virus.

Ferreira (1976) described a modification of micro neutralization test for FMD virus typing. The work was comparable with tube neutralization test. Principal advantage of this modification was saving of reagents.

Sugimura and Eissner (1976) employed fluorescent antibody technique for typing of FMD virus. The globulin fraction of sera was conjugated with fluorescein-isothiocynate and used in direct fluorescent antibody technique.

Rweyemamu et al. (1977) reported that rate of neutralization in most cases was not rectilinear. Treatment of virus antibody mixture with antispecies globulin enhanced the rate of neutralization of homologous and heterologous reactions without altering the reaction between the two.

Sarma et al. (1977) studied some of the cross reacting isolates of FMD virus obtained from field outbreaks. The cross reacting behaviour of these isolates was detected by CFT as well as by serum neutralization test. The possible causes of cross reactions of the isolates were studied primarily with CFT, serum neutralization test and plaque assay in calf kidney cell culture. On the basis of experimental

findings, a simple mixture of two or more types of the virus was suggested to be the possible cause of cross reactions in these isolates.

Abu Elzein & Crowther (1977) introduced the enzyme labelled immunosorbent assay (ELISA) technique in FMD virus research. The indirect ELISA technique was successfully applied to measure antibodies against FMD virus in cattle serum. The test showed that reproducible results could be obtained.

Rai and Rao (1977) employed BHK 21 cell culture and micro complement fixation test for the isolation and typing of FMD virus.

Rweyemamu et al. (1978) applied micro neutralization test for serological typing and subtyping of FMD virus strains. The test was found to be more sensitive and specific than CFT. About 90% of the samples was found to be positive by micro neutralization test as compared to only 50% found positive by CFT. The test was also susceptible to minimal quantities of heterotypic viral contamination.

Sobko et al. (1978) used concentrated antigen for radial immunodiffusion test for identifying FMD virus antigens and antibodies. They confirmed the sensitivity and specificity of the test in comparison with the CFT.

Arbelaez et al. (1979) standardised the micro neutralization technique for the detection of FMD antibody using BHK-13 cell line with buffered MEM F-11 medium. Serum samples were run in 6 replicates against 50 medium infective doses (ID_{50}) of the FMD virus. No significant difference in replicability were detected in the range of 10-180 ID_{50}.

Crowther and Abu Elzeine (1979a) developed enzyme labelled immunosorbent assay (ELISA) to detect and quantify FMD virus using flexible plastic microtitre plates. The method was successful for the specific detection of FMD virus and proved 50-100 times more sensitive than CFT. The authors (1979b) again reported the detection and identification of FMD virus types employing indirect ELISA. The higher sensitivity of ELISA test was evident from the fact that all the 19 samples in this study were found positive whereas only 8 samples could be typed by CFT, that, too, could be possible after further tissue culture passage before CFT typing was obtained. The ELISA test remained unaffected by anticomplementary factors as was seen for 5 samples using the CFT before passage. Cross reactions with the samples were encountered in CFT which probably reflected the

cross reactivity of the guinea pig antisera containing antibody to 12 S and VIA antigen with these components in crude infected tissue culture fluids. The cross reaction was found to be greatly reduced in ELISA test as this test detected whole particle (140 S) antigen in the presence of tissue culture and 12 S materials.

Rai et al. (1980) employed the fluorescent antibody technique using guinea pig globulin against FMD virus type 'O' as well as indirect FAT using conjugated rabbit antiguinea pig globulin. The FMD virus antigen was demonstrated in the smears of infected tissue of cattle tongue epithelium, heart muscle, rumen mucosa, abomasal mucosa and pancrease as well as infected BHK-21 cell culture monolayer. This method could be valuable in identifying specimen of FMD from which either virus could not be recovered or isolate could not be typed by conventional CFT due to low titre of antigen.

Rai and Prasad (1980) applied indirect immunoperoxidase technique (IPT) to detect FMD virus antigen in infected BHK-21 cell culture, oesophage-pharyngeal fluid (OP) and smears of tongue epithelium. The possibility of its use in screening large number of animals for their carrier status was suggested.

Rai (1980 a) observed that the optimum amount of 2.5 units of complement in the micro CFT was capable of causing 50% haemolysis. The author (1980 b) again made a comparative study of the MCFT and micro neutralization test for subtyping of FMD virus. He found serum neutralization test to be more sensitive than the CFT.

Shazhko et al. (1981) made a comparative study on neutralization and radial immunodiffusion tests for FMD virus typing; the former was found to be positive in 100% of infected pigs and the latter in 95.6% of the cases. The procomplementary activity of pig serum was eliminated by heat treatment at 56°C for 40 minutes or by formaldehyde.

Rai et al. (1981) standardised indirect technique of a micro enzyme labelled immunosorbent assay and found it efficient in detecting FMD virus antigen in cell culture fluids, mouse carcass and cattle tongue epithelium as well as in determining serum antibody. The authors (1981 b) further employed indirect micro ELISA for subtyping of FMD virus strains recovered from field outbreaks.

Abu Elzein and Crowther (1981) described a simple solid phase ELISA for the detection of antibod-

classes showing activity against FMD virus in bovine serum.

Ahuza et al. (1982) made an attempt to detect FMD virus through direct immunoperoxidase technique.

Lombard & Petermann (1982) employed an enzyme linked immunosorbent assay, using paper discs to hold the antigen. He found the test suitable to detect antibodies to apthovirus in the serum of vaccinated cattle. The test was simple, fast and reliable. The SNT and ELISA gave similar results.

Rweyemamu & Ouldrize (1982) used two dimentional micro neutralization and indirect ELISA tests to examine serological variations among the type 'O' FMD virus.

Perrin et al. (1982) applied ELISA test to 15 serum samples from cattle positive to neutralization test and 19 negative serum samples. Purified apthovirus antigen gave better results in ELISA than crude antigen.

Elzein and Crowther (1982) compared the apthovirus isolates by a solid phase competition and indirect micro ELISA tests. Similar relationship

between the isolates were obtained with indirect ELISA and CFT. The competition assay was found to be more discriminatory and results did not always correlate with the other two assays.

Rao et al. (1983) standardized a modified MCFT for typing of FMD virus in which 25μ each of antiserum, antigen complement and haemolytic system were used making a total of 0.1 ml of the reagents. The complement and haemolysin were titrated together in a checker board system in the microplate. Using of $2HD_{50}$ units of complement and 4 MHD/ml of haemolytic system, the test was found to be highly sensitive and convenient to handle large number of field samples.

Suryanaryana (1983) employed the sandwitch technique of ELISA for the rapid typing of FMD virus in field condition. The plates were charged with rabbit virus specific antibodies as catching antibodies. These plates were carried to the field for FMD virus typing.

Dumanova et al. (1983) applied the ELISA technique to apthovirus vaccine strains 'O' 'W','A5F' and Si and found this technique to be 50 times more sensitive than the passive haemagglutination and 100 times more sensitive than CFT.

II. MOUSE PASSAGE AND TISSUE CULTURE STUDIES

Failure of isolation and detection of FMD virus by the serological tests on account of low titre of virus in antigen facilitated the necessity of propagation through some living system for the recovery of virus. Various living system as cattle, guinea pigs, rabbits, mice, rats and tissue culture were employed for the purpose.

Skinner (1951) introduced a technique for the successful propagation of six strains of FMD virus in unweaned 7-10 day old mice. Paralysis of muscles of hindquarter and respiratory distress were produced. On post-mortem examination, degeneration of muscle fibres was observed in the skeletal and heart muscle. A comparison was made with other species of animals in which heart lesion occurred in FMD and with other virus such as coxsackie. The application of these findings to virus titration and serum neutralization were discussed.

Giroud & Ciaccio (1952) found young mice to be suitable for passage of FMD virus. Intracerebral route was effective even for type 'C' previously passaged cutaneously in guinea pig. The entire body of the young mice was used for preparation of vaccine.

Ciaccio et al. (1954) passaged a guinea pig strain of type 'C' virus 35 times in unweaned mice aged 20-30 days. Susceptibility diminished with age. Cortisone treatment did not increase the number of passages in adult mice. Virus could be isolated from two cortisone treated mice which remained apparently healthy.

Sellers (1955) investigated the application of calf and pig kidney monolayer tissue cultures to FMD virus using two strains, M 11 and C 997. Both strains behaved similarly on calf kidney monolayers producing small cluster of necrotic tissue surrounded by healthy cells. Destruction was not sufficient for plaque production. In pig kidney cultures, both strains destroyed all epithelial cells but not fibroblasts.

Kotsche (1955) employed the mice of gradually increasing age. He found that FMD virus type 'O' was adapted in adult mice after intraperitoneal serial passage of the virus. The musculature of the pelvic girdle was the most suitable for the recovery of virus.

Petermann et al. (1956) reported that the tissue culture of calf kidney cells were suitable for the large scale cultivation of FMD virus.

Schneider (1956) passaged FMD virus type O_2 and 'C' through intraperitoneal route in unweaned mice. The author observed that the virulence increased for mice and diminished for cattle.

Mackowiak & Lang (1958) observed a parallel result when the virus was grown on trypsinized porcine kidney and titrated simultaneously on young mice and on tissue culture. There was a slight difference in values of succesive titration of the same virus on tissue culture. Titration in tissue culture was found to be more sensitive, quicker, constant and accurate than mice.

Heartley et al. (1960) found mice to be more susceptible to FMD virus through I/M route than I/P route.

Graves and Poppensick (1960) reported that 5 to 14 day old mice were equally susceptible to FMD virus. Resistance to the lethal effect of virus increased rapidly after sixteenth day .

Capstick et al. (1962) reported the adaptation of a cloned strain of hamster kidney cells (BHK-21, clone 13) to grow in suspension culture and the susceptibility to the FMD virus. These cells have since been grown in deep suspension culture on an

since been grown in deep suspension culture on an industrial scale and are being used for virus production in the preparation of inactivated FMD virus vaccine.

Mowat and Chapman (1962) reported that BHK-21 was capable of supporting FMD virus growth. Cells were grown as monolayer culture in 4-OZ prescription bottles, Roux bottles and petridishes. Following infection with FMD virus, a cytopathic effect was observed similar to that seen with infected primary cultures of pig kidney cells. The cells become rounded and granular and detached from glass surface. Infection with cattle passaged virus produced a well marked cytopathic effect in 24 hours, but destruction of cell sheet was not complete in some experiments until approximately 72 hours after infection.

Khera & Dhillon (1962) described that epithelial cells and fibroblasts were susceptible to the degenerative effects of FMD virus. They retracted, became irregularly spherical and stained intensely with acid dyes. The nuclei of infected cells became ecentric and there was margination of chromatin. They were contorted or became rectangular dentate, crecentric or half moon shaped . There was pronounced pyknosis, karyorrhexis and karyolysis.

Khera and Dhillon (1963) infected goat kidney single cell culture with FMD virus which manifested a cytopathic effect on primary and all subsequent passages. They also carried out neutralization test with standard antisera and found three of the four strains to be related to type 'O' and the fourth was 'Asia-1'.

De Castro (1964) affirmed the susceptibility of the IB-RS-2 pig kidney cell line and of clones derived from this cell line to FMD virus.

Patty et al. (1965) studied the comparative assay of FMD virus in cattle, mice and primary cultures of bovine kidney cells . Superiority of bovine kidney cell culture over mice was reported. Lowest value of virus infectivity were obtained in mice while in cattle it was 4 times more than in mice. Highest values were obtained in cell cultures where infectivity was about 2.5 times to those in cattle and about 11 times more than in mice.

Snowdon (1966) described the preparation and infection with FMD virus of calf thyroid (CT) monolayer cultures resulting in cytopathic changes (CPE) and its comparison with other systems. First CPE changes were observed in single cells or group

of cells, which contracted, became irregular in shape but remained attached to neighbouring cells by long thin processes. As incubation proceeded the cells became rounded and detached from the glass. Higher titre was obtained in CT cultures than pig or baby hamster kidney cultures, unweaned mice and cattle tongue epithelium. The infected tissue culture fluid was a satisfactory antigen for CFT.

Singh et al. (1968) recorded the cytopathic changes in buffalo embryo monolayer cell cultures after 16 hours of FMD virus adsorption. Both fibroblast as well as epithelial cells were affected. In most cases the cytopathic changes were characterized by hydropic degeneration and cytoplasmic vacuolization. Few cells also showed nuclear degenerative changes.

Sehgal (1969) reported that the inoculated mice died after 72 hours without exhibiting any macroscopic lesion on post mortem examination, whereas control mice remained normal. The mice antigen was tested for infectivity in guinea pigs which developed both primary and secondry lesions. Serial passages were then conducted in baby mice which on subsequent passages generally died in 24 to 30 hours exhibiting muscular paralysis of hind parts and respiratory distress prior to death.

Singh et al. (1969) adapted the FMD type 'O' virus to lamb kidney monolayer cell culture after five serial passages. A fairly constant cytopathic effect was produced, begining with cell agglutination at about 4 hours, half of the cells were lysed by 9 hours and nuclei of other cells showed pyknosis, karyorrhexis and karyolysis. The whole monolayers were found to be disintegerated after 18-24 hours of inoculation. The use of monolayers in identifying FMD virus was suggested.

Chapman and Ramshaw (1971) described the adaptation of pig kidney cell line IB-RS-2, clone 60 to grow in suspension culture. Replicate virus titrations in monolayers prepared from suspension adapted cells, IB-RS-2 monolayer cells, BHK monolayer cells and in suckling mice showed that the suspension cells had retained sensitivity to FMD virus. The sensitivity of the IB-RS-2 suspension cells to the FMD virus strains used was slightly higher than that observed in BHK cells.

Patty (1971) enumerated environmental factors which affect the susceptibility of porcine kidney cells and described methods for increasing the susceptibility of PK cells to infection with

seven types of FMD virus not fully adapted to cell culture.

Patty (1972) observed in increase in susceptibility of primary cultures of bovine kidney (BK) cells and of porcine kidney (PK) cells to infection with FMD virus by addition of Eagel's none essential amino acids to the growth medium of the cells.

Sen et al. (1972) studied strains of FMD virus in the cells obtained from bovine kidney, bovine testicle, lamb kidney, baby hamsters kidney (primary), goat testicle, goat pancreas, goat thyroid, goat heart and monkey kidney cell lines. The result indicated the possibility of using the heart cells along with goat kidney cells in the production of virus for vaccine production.

Singh et al. (1973) successfully propagated the 'O' strains of FMD virus in lamb kidney cell culture and lamb testicle cell culture and found the former to be better than the latter in respect of growth and cytopathogenicity.

Syusyukevi'a et al. (1974) noted that adaptation of virus to tissue culture, new born rabbits or new born mice was accompanied by an increase in plaque size.

Serodzher (1975) found a line of sheep kidney cells in its 149th -200th passage to be suitable for preparing monolayer cultures for the propagation of FMD virus. The best nutrient medium was 0.5% bovine serum.

Tewari et al. (1980) observed superiority of bovine kidney cell culture (BKCC) over unweaned mice for isolating FMD virus. BKCC yielded FMD virus in 20% of 100 samples from cattle, buffaloes, sheep, goats and yalks which were negative or produced irregular mortality in mice. Further, he suggested that at least non viable or erratic samples from unweaned mice should be processed in BKCC to improve the rate of virus recovery in laboratories dealing with FMD virus research.

Clarke & Spier (1980) described variations in the susceptibility of BHK population and cloned cell lines to three strains of FMD virus.

House et al. (1982) studied the ability of apthovirus serotypes 'A', 'O' and 'C' isolated from bovine tongue to replicate in seven different types of cell cultures. Primary and secondry calf thyroid cells were equivalent in susceptibility to bovine kidney cell culture passaged upto five times.

Calf thyroid cells lost their susceptibility after two passages. Susceptibility to FMD virus seroypes 'C' was most variable among the cells tested. Lamb testicle and porcine kidney were susceptible to apthovirus while goat and calf testicle and calf lung cells were resistant.

III. EPIDEMIOLOGICAL STUDIES

A number of epidemiological factors are involved in the origin, development and spread of FMD outbreaks, of which some have already been pinpointed while others still remain unidentified. Control of FMD is most intriguing and complex and will continue to remain so unless these factors are identified at the earliest. Several workers on FMD research have dedicated themselves from the very begining which have been reviewed on the following pages.

De Mello (1946) recorded foot and mouth disease in Portgal on the Spanish frontier in 1944 which persisted till 1946. The disease was of mild nature, spreading more rapidly in summer than in winter and affected cattle, sheep, pigs and goats. Control was affected by segregation and vigorous measures of disinfection.

Datta (1951) carried out the epidemiological studies of FMD in India under a research scheme started at Mukteshwar. He reported the susceptibility of cattle, buffaloes and wild mammals. Natural outbreaks among Indian sheep and goats were not frequent but they usually remained unnoticed due to the mild course of the disease. Even when infected artificially by inoculation on the tongue, no generalization of disease was observed. Experimentally goats appeared to be more susceptible than sheep. The possibility of transmission of disease from sheep and goat to cattle and vice versa was investigated.

Seetharaman & Datta (1951) made an analysis of the various types of FMD virus encountered in India. Apart from the standard strains 'O', 'A' and 'C' and their variants, one atypical strain was observed. Type 'O' was found to be preponderant in India.

Gupta et al. (1962) reported that in 1960, despite stringent precautions, FMD due to 'O' type virus spread from cattle to 229 imported white pigs. Lesions in feet developed earlier than in mouth and youngs were more affected than older. Mortality due to primary infection was 7% and for secondry infection 14%.

Huq and Khan (1963) reported the prevalence of FMD in Pakistan between the month of December to April.

Gajapati and D'souza (1968) made an attempt to analyse epidemiological data on rainfall, atmospheric temperature and incidence of haemorrhagic septicemia (HS), Black quarter (BQ) and FMD. Thr result indicated a significant influence of climatic factor in the incidence of HS and FMD. In Madras the FMD was more prevalent in the month of November to February.

Burrows (1968) have described that sheep infected with FMD strains of different origin developed a carrier state which persisted in the majority of animals for 1-5 months. The site of virus persistence and multiplication in the convalescent animals were identified by titration of suspension of mucosa and epithelia taken during post mortem. Subclinical symptoms of disease is also discussed.

Smith and Hug -Jones (1969) examined the initial stages of FMD epidemics in Hampshire (1967), Northumberland (1966), OS werty (1961) and Chestir (1952) in relation to the prevailing weather conditions. It was readily app arent that wind direction and the amount and duration of rainfall, particularly at

night, very largely determined the severity of epidemic. The distance between sources and secondry outbreaks was under 20 km in 91% of the cases. It appeared that airborne transmission of virus was crucial during epidemics of this disease and was largely controlled by climatic condition.

Sellors et al. (1971) described that FMD virus can persist for a maximum of 48 hours in the nasal chambers of personnel handling infected animals. An exposure to natural aerosol of infected personnel for 2½ minutes per animal was sufficient to dessiminate the disease to susceptible animals.

Plotnikov et al. (1972) made an attempt to correlate the incidence of FMD with the month of its occurrence using statistics collected in Uzbekistan, Kazakhstan, Western Siberia and neighbouring areas during the year 1947-1958 and 1962-1968. They concluded that there were two major peaks of disease incidence, one in autumn-winter and other in summer.

Uppal et al. (1972) reported an outbreak of FMD at Haryana Agricultural University Farm. Cattle, buffaloes, sheep and goats were affected while swine remained unaffected. 20 out of 37 goats examined showed vesicles on the tongue, gum, hard palate and

feet. Among 100 sheep only 8 showed mouth lesions and non had feet lesions. The virus was identified as type 'O'. Sheep and goats with only slight lesions could spread the disease without their infection being noticed.

Singh & Murty (1972) described in detail an epidemic of FMD in the piggery at Aligarh farm due to type 'O' virus. The epidemic started due to introduction of some newly purchased pigs which were already infected with this virus. 1000 out of a stock of 1456 animals were affected with an overall morbidity rate of 93.37% while the mortality rate was only 10.4%.

Falconer (1972) described the epidemiology of FMD in Botswana with reference to both domestic stock and free game population. The possible prepetuation of disease through the interrelationship of domestic stock and wild life was suggested. The clinical symptoms in small stock were less marked than in cattle. Excessive salivation was not apparent, the tongue lesions healed more quickly and lameness was not so marked.

Hug -Jones (1972) reported an epidemic of FMD in England and Wales during 1967-68. The disease behaved differently in different parts of the country.

The herd attack rate increased with number of dairy cows and heifers per 100 acres of crops and grass. The attack rate increased with density of population of livestock.

Khukhorov et al. (1973) analysed the epidemiological, clinical and serological data collected from 3956 dairy cattle in 6 herd in rural areas in U.S.S.R. with endemic of FMD due to serotype 'A-22'. The rate was higher among heifers (40%) and calves, 4-12 month old (23%) and lowest among cows (5.6%) and among calves under 4 months of age. The limited occurrence of FMD in calves upto 4 months of age was attributed to colostral antibody. On the other hand higher serum antibody level were required to limit the carrier state.

Sellers & Forman (1973) postulated that virus was present seven days before first FMD outbreak in Hampshire in January and February, 1967. It was suggested that the disease occurred initially in pigs fed on infected meat and that the virus was subsequently disseminated to four farms by movement of animals, slaughter waste, people or vehicle. Airborne spread was correlated with wind direction.

Sosov & Taranova (1973) described that

relative humidity was probably a more important climatic factor than air, temperature and rainfall but quantity and virulence of virus excreated seemed to be more important in transmission than any of the climatic factors investigated.

Boyakhchyan et al. (1975) demonstrated that chronic gastroenteritis with wasting among lambs was caused by FMD virus. Two isolated type 'O' strain of FMD virus differed from typical strain in virulence.

Gibbs et al. (1975) have described FMD in British deer and transmission of virus to cattle, sheep and deer. Clinical disease was typical and severe in roe and muntjac deer with some animals dying, less severe in Sika deer and usually subclinical in the fallow and red deer.

Pyakural et al. (1976) reported an outbreak of FMD in Indian elephants, the disease affected over half a group of 30 elephants. The source of infection was probably affected water buffaloes.

Ahuja and Rai (1977) reported the occurrence of FMD in an organised cattle and pig farms in Assam affecting some of the vaccinated animals. The disease spread from animals maintained by nearby owners

through attendents. Isolation of type 'C' and 'O' FMD virus indicated the possibility that the field strains responsible for outbreak was different from strains incorporated in the vaccine.

Sharma and Asthana (1978) studied an epidemic of FMD at pig farm, Aligarh. Mortality was higher in unweaned piglets (22%) than in pigs aged 2-6 months (16.8%).

Pustiglione et al. (1978) isolated FMD virus from pigs affected with swine fever and pneumonia while there was no history of FMD.

Hajela and Sharma (1973) reported the presence of haemorrhagic gastroenteritis in the FMD affected buffalo calves. Type 'A' FMD virus was isolated from 41% cattle and 61% buffaloes in mixed herd. The clinical course of disease was more severe in buffaloes. Typical oral and feet lesions appeared in the adult cattle and buffaloes but all the 18 affected buffalo calves showed severe diarrhoea and small vesicles in mouth.

Chakrabarty et al. (1979) described the seasonal prevalence of FMD in Assam. The disease was more prevalent in the winter (December-February) and

during the monsoon (June-September). There was no apparent seasonal influence on the occurrence of any particular type of FMD virus. Winter epidemic were attributed to weather condition favouring survival, and monsoon epidemics to movement of animals.

Singh and Sharma (1980) reported a severe outbreak of FMD at the Central Sheep and Wool Research Station in Doon valley. Mortality was 1.16% .Morbidity was highest in animals above 1 year of age (57.2%) as compared with 20% in animals between 6-12 months of age and 32.65% in sheep less than 6 month old. The morbidity rate was higher in females (58.7%) than male (14.7%). When breeds were compared, morbidity was found to be 77.9% in Nali, 47.6% in R ambuillet and 46.3% in cross breed.

Sellers & Gloster (1980) recorded involvement of movement of animals, people and vehicles in the spread of FMD. Besides, air borne spread was in general between 1 to 8 kms and on two occassions as much as 20 kms.

Singh et al. (1981) reported lesions in eye among the exotic and cross breed cattle which formed a focus for further infection to other susceptible animals. Vaccination of animals with

killed polyvalent vaccine in the vicinity probably precipitated the disease in the vaccinated cattle. Females, milking cows and cow with recent parturition were found to be more susceptible than males and dry cows. Presence of lesions in teats and udders among recently affected crossbred and exotic cows was found to be a characteristic feature.

Rauf et al.(1981) made a typographical study of FMD virus in Pakistan. Type 'A' and 'O' virus was recovered of which type 'O' caused more severe lesions in cows than buffaloes. Maximum outbreak were recorded during the month of June and January over the period of two years.

Sellers et al. (1981) studied the spread of foot and mouth disease during the epizootic in Malta in 1975. The effect of control measures, vaccination and meteriological conditions were studied. The outbreaks were attributed to contact of pigs with swill, offal garbage, people and contiguity to an infected farm. A combination of control measures, vaccination and adverse meteriological conditions contributed to decline of the epizootic.

Prasad et al. (1981) studied the FMD in

North-West region of India. August to December were the months during which the higher number of outbreaks of FMD occurred in the years in which flood, movement of animals and humen beings were larger during flood.

Ahuja et al. (1981) recorded 1268 outbreaks of FMD between year 1972-78. Two third of the outbreaks occurred between 1976 and 1978 when flood caused widespread movement of livestock. They confirmed the enhancement of spread of the disease by cattle fairs, common grazing, seasonal migration and movement of technical personnel working with diseased animals.

Glosler et al (1981) described that under field condition inhalation is the more likely route of infection in cattle, there is little deposition of the virus to the ground within the first 10 km from the source.

Mishra and Ghel (1983) reported a severe outbreak of FMD in a flock of 97 goats. The morbidity rate was 82.47% while the mortality rate was 8.24%. Apthovirus 'A-22' was involved in the outbreak.

Dutta et al. (1983) reported 615 outbreaks of FMD in North East India during the period of 1973-81 involving buffaloes in 70 outbreaks. The morbidity rate was 14%. FMD virus type 'Asia-1', 'O' and 'C' were found to be responsible for the outbreak.

MATERIAL AND METHODS

Part-I

Serological Studies

I. SAMPLES

A total of 131 samples were collected from FMD affected animals kept under village condition as well as farm condition. Studies on farm animals during present investigation were confined to livestock farm, R.A.C.,Kanke, Ranchi and Government Cattle Farm, Gauriakarma in Hazaribagh district. The samples included oral epithelium (Tongue, gum, palate lesions), feet epithelium as well as vesicular fluid. In few instances affected eye tissues were also collected. These samples represented the different geographical regions of Bihar State. Table-1 depicts the details of samples collected during the present study.

Methods of Collection

Clinical materials for FMD virus isolation and typing from suspected cases were collected at the height of reactions and comprised affected oral epithelium or feet epithelium but preferably the first. The affected tissues, specially the feet epithelium were washed with soap and water. The following methods were followed for collection of different tissues.

(1) Oral lesions

The epithelial covering of the

Table-1

Source and nature of samples collected for typing of FMD virus

Sl. No.	Source of sample	Total	Nature of sample				
			Feet epith	Tongue epith	Gum lesion	Pallate	Eye lesion
1.	From FMD affected animals under village condition						
	(a) North Bihar	52	20	21	5	4	2
	(b) South Bihar Plain	53	21	26	4	2	-
	(c) Chotanagpur Plateue	17	8	7	2	-	-
2.	Under Farm condition						
	(a) R.A.C.Livestock Farm, Ranchi.	3	2	1	-	-	-
	(b) Govt. Cattle Farm Gauriakarma, Hazaribagh	6	4	2	-	-	-
	Total :-	131	55	57	11	6	2

vesicles from tongue, gums, palate and of oral cases were collected by scrapping with the help of sterile scalpel. In the case of ruptured vesicles, the epithelial covering was collected by cutting the tissue after holding with the sterile forceps. About 2-3 gms of the tissues were collected from each animal and preserved in a vial containing 30 ml of 50% glycerine phosphate buffer. Materials collected from one or more animals of the same species at one place comprised one sample, while materials from the other species constituted second sample. While collecting samples, particular of animals viz., species, age, sex and breed were noted.

(2) Feet epithelium

The epithelial covering of the recently developed vesicles between the cleft of hoof were collected with the help of sterile forceps and scissors and preserved in a vials containing 50% glycerine phosphate buffer (vide infra). About 2-3 gm of materials were collected from each animals.

(3) Eye lesions

A few animals showed eye lesions associated with the FMD. The keratinised tissues on the eye lids were collected with the help of forceps and preserved in 50% glycerine phosphate buffer.

50% Glycerine Phosphate Buffer

Solution A

Disodium hydrogen phosphate (Na_2HPO_4)	21.390 g
Distilled water	3000 ml

Solution B

Dihydrogen potassium phosphate (KH_2PO_4)	2.725 g
Distilled water	500 ml
pH	7.4

Equal parts of phosphate buffer was mixed with neutral glycerine and then autoclaved at 15 lbs pressure for 30 mimutes. The pH was checked and solution was distributed in small vials which was used to store at $4^{\circ}C$ till used.

II. TYPING OF ORIGINAL MATERIALS BY MCFT

The samples collected from FMD affected animals for FMD virus typing were stored at $4^{\circ}C$ untill further used. Each clinical samples were divided in three parts; the first part was subjected directly to typing by MCFT, the second unmanipulated part was used for mouse passage while the third part was used for ELISA test. Portion of the sample specially collected from vaccinated animals was used to be sent to Calcutta/

I.V.R.I., Mukteshwar and Banglore for confirmation.

First part of the each sample was subjected to the micro complement fixation test (MCFT) as per the method followed by Rai (1980). The procedures of the micro complement fixation test were as noted below:

(A) Pre-test procedures

(1) Preparation of Antigen

FMD virus antigen were prepared from the field samples collected from FMD affected animals as per the method of Rai (1980).

(i) 1 g of the collected epithelium was washed thrice in phosphate buffer saline (PBS), pH 7.2 in order to remove the glycerine buffer.

(ii) The epithelium was then triturated with pestle and mortar in PBS sufficient to prepare approximately a 10% suspension.

(iii) The above suspension was centrifuged at 3000 rpm for 30 minutes in a referigerated centrifuge.

(iv) The supernatent was separated with the help of pipettes.

(v) Equal amount of chloroform was added and then centrifuged again at 3000 rpm for 30 minutes.

(vi) There were three layers, chloroform layer at the bottom, virus containing aqueous layer at the top and a middle slimy thick layer consisting of debris and lipid. The solution at the top was separated with a sterile pipette and used as antigen.

Phosphate buffer saline (PBS)

PBS solution A

Sodium chloride	64 g
Potassium chloride	1.6 g
Disodium hydrogen phosphate ($Na_2 HPO_4$)	17.36 g
Potassium dihydrogen phosphate (KH_2PO_4)	1.6 g
Distilled water*	6400 ml

PBS Solution B

Calcium chloride ($Cacl_2\ 2H_2O$)	1.06 g
Distilled water	800 ml

PBS Solution C

Magnesium chloride ($Mgcl_2\ 6H_2O$)	1.70 gm
Distilled water	800 ml

*:- The double glass distilled water was used every where mentioned as distilled water.

All the three solutions were autoclaved separately at 15 lbs pressure for 30 minutes.

Final preparation

After cooling, solution B was added to A and then solution C was mixed with B-A: 1,00,000 I.U. of crystaline penicillin (1,00,000 I.U.) and streptomycin (100 mg) per liter of PBS were added with sterile precautions. The solution was stored at 4°C till used.

(2) Guinea pig anti-FMD virus hyperimmune serum

The hyperimmune serum was supplied by the Central Laboratory of All India Coordinated Research Project for Epidemiological Studies on Foot and Mouth Disease, Indian Veterinary Research Institute, Hebbal, Banglore (India).

(3) Dilution and inactivation of hyperimmune sera

The hyperimmune sera against the virus types 'O', 'A', 'Asia-1' and subtype 'A-22' were diluted as per the direction on the vials. The diluent used was veronal buffer . Then all the diluted sera were inactivated at 56°C in water bath for 30 minutes before use.

Veronal Buffer (Brooksby,1952)

The stock solution was prepared as follows:

Sodium chloride	85.00 g
5.5 diethyl barbituric acid	5.75 g
Sodium 5.5 diethyl barbiturate	3.75 g
Magnesium chloride ($Mgcl_2 6H_2O$)	1.68 g
Calcium chloride ($Cacl_2\ 2H_2O$)	0.37 g

The barbituric acid was first dissolved in 500 ml of hot glass distilled water. Then the solution was made to 2000 ml by adding glass distilled water after disolving the other ingredients. It was distributed in 200 ml amounts and autoclaved at 15 lbs pressure for 20 minutes. The solution was stored at $4^{\circ}C$. In order to make it working solution, it was diluted in 1:5 in sterile distilled water.

(4) The complement

The fresh guinea pig serum was used as the source of complement in complement fixation test.

Collection of sera

Male guinea pig in good health weighing about 500-700 gm were allowed to fast overnight. Next morning the guinea pigs were bled by cardiac puncture with the help of sterile 20 gauge needle and syringe. The blood was collected in clean sterile tubes and

left at room temperature for one hour for clotting. The clot was broken and allowed to stand at $4^{o}C$ for several hours. The sera was separated by centrifugation at $4^{o}C$ and stored in amber coloured ampoules.

Preservation

Richardson (1941) method was used to preserve the complement using Richardson's solution which was prepared as follows:

Richardson solution

Two stock solutions were prepared

Solution A

Boric acid ($Na_3 BO_3$)	0.93 g
Borax ($Na_2 B_4 H_7 10H_2O$)	2.29 g
Sorbitol	11.74 g

The above ingredients were dissolved in saturated sodium chloride solution.

Solution B

Borax	0.57 g
Sodium Azide* ($Na N_3$)	0.81 g

The above ingredients were dissolved and the volume was made upto 100 ml with saturated sodium chloride solution.

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*:- Toxicity comparable to KcN.

In order to preserve, the complement was mixed with Richardson's solution as follows:

Richardson's solution A	1 part
Richardson's solution B	1 part
Serum complement	8 part

Titration of complement

i. One ml Richardson preserved complement was added to 7 ml distilled water in order to prepare 1:10 dilution. From 1:10 stock further dilutions 1:20, 1:25, 1:30, 1:40, 1:45 and 1:60 were prepared using veronal buffer as diluent.

ii. Complement in a volume of 25 ml of each dilution were added in the microtitre plates keeping three wells for each dilution.

iii. Veronal buffer (50 ml) was added in each of the above wells containing complement dilutions. In three separate wells of microtitre plates without complement, 75 ml of veronal buffer was added to act as RBC controls.

iv. The plates were sealed with the adhesive tape and incubated in the waterbath at 37°C for 30 mimutes.

v. Then 50 ml of sensitized haemolytic system was added in each of the wells and incubated at 37°C for 30 minutes. The plates were then kept in the refrigerator at 4°C overnight in order to allow the RBC to settle completely.

Observation

The degree of haemolysis was ascertained by the degree of settling of unlysed RBC in the bottom of wells and scored in the scale of 0 to 4 as given below:

0 = No RBC settled (100% haemolysis)
1 = 25% RBC settled(75% haemolysis)
2 = 50% RBC settled(50% haemolysis)
3 = 75% RBC settled(25% haemolysis)
4 = 100% RBC settled(No haemolysis)

The diameter of the button formed by settling of RBC control (without complement) was given a score of 4. With this control, other wells were compared and given appropriate scores. The highest dilution giving a score of 2 was considered to contain 1 haemolytic dose 50 (HD_{50}) of complement or one unit.

In the test proper three units of complement was used.

(5) Haemolytic system

(i) Sheep erythrocyte suspension

The blood from the jugular vein of a sheep in good health was collected with the help of bleeding canula in equal volume of Alserver's solution. It was mixed thoroughly and stored at $4^{\circ}C$.

For preparing erythrocyte suspension, the Alsever's preserved sheep blood was centrifuged at 2000 rpm for about 10 minutes, and the supernatent was removed. Normal saline solution (0.85%) was added, mixed with pipette and again centrifuged at 2000 rpm for 10 minutes. The washing of RBC with normal saline solution was repeated once more and finally centrifuged at 3000 rpm for 15 minutes to pack the cells in bottom.

Alerver's solution (Newman, 1967)

Dextrose	20.50 g
Sodium citrate	8.00 g
Sodium chloride	4.20 g
Citric acid	0.55 g
Distilled water	1000 ml
pH	6.1

It was sterilized by autoclaving at 10 lb pressure for 15 minutes.

(ii) Haemolysin

(a) Preparation of Haemolysin

The standard procedure for preparation of haemolysin has been described by Komer et al. (1952) and Kebat & Mayer (1961). During the present investigation, the method followed at the Central Laboratory of All India Coordinated Research Project for Epidemiological Studies on Foot and Mouth Disease, Banglore was followed for preparation of haemolysin.

Freshly collected sheep erythrocytes were washed in normal saline solution and then diluted as to make 20% suspension in normal saline. Then a group of 4 healthy rabbits weighing about 600-800 g each with prominent ear vein were inoculated by I/V route as per the schedule given in Table - 2.

On the 9th day of the last inoculation, test bleeding by cardiac puncture was done and the sera were inactivated at 56°C for 30 minutes in water bath. The sera were screened for the presence of haemolytic activity by CFT utilising Brooksby technique (1952).

If the titre was found to be satisfactory, all the rabbits were exanguinated and sera collected and pooled.

Table-2

Inoculation schedule of rabbits for the preparation of haemolysin

Sl. No.	Days	Inoculum size	Route	Remarks
1.	0	1.0 ml	I V	20% of sheep erythrocytes
2.	5	i) 0.5 ml	S C	
		ii) 1.5 ml	I V	After 15 minutes of (i)
3.	10	i) 0.5 ml	S C	
		ii) 2.0 ml	I V	After 15 minutes of (i)
4.	15	i) 0.5 ml	S C	
		ii) 2.5 ml	I V	After 15 minutes of (i)
5.	20	i) 0.5 ml	S C	
		ii) 3.0 ml	I V	After 15 minutes of (i)

I V : Intra venous

S C : Sub-cutaneous

(b) Preservation

The pooled haemolytic sera was inactivated at 56°C for 30 minutes in order to inactivate the complement present in the sera and then mixed with equal volume of analytical grade of neutral glycerine and stored at 4°C till used.

(c) Haemolytic system preparation

The haemolytic system contained haemolysin and sheep erythrocytes in veronal buffer as follows:

Haemolysin	4 MHD/ml
Sheep erythrocytes	1%
Diluent	Veronal buffer

The haemolytic system thus prepared was incubated at 37°C for 30 minutes in water bath for sensitization. The system was kept at 4°C for 5 days without further sensitization.

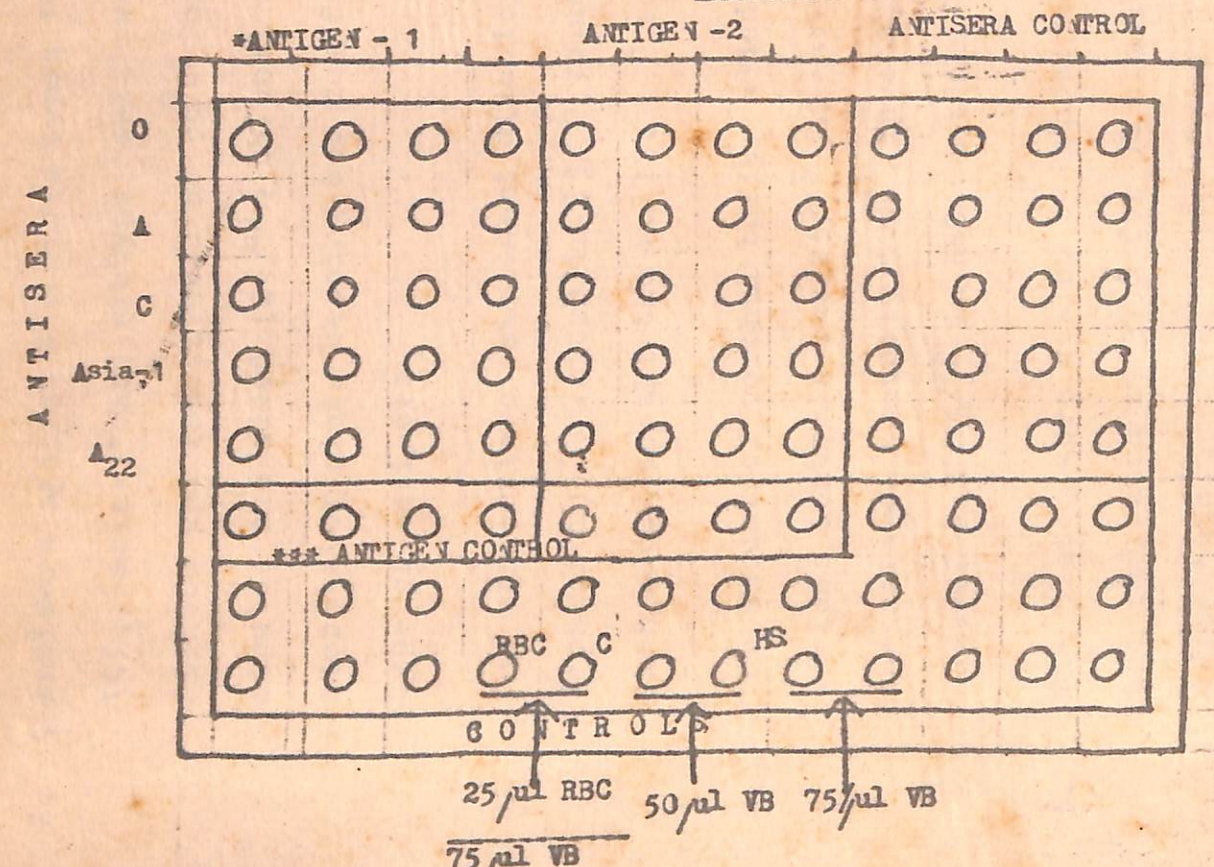
(B) Test Procedure

The microcomplement fixation test was conducted as per the method of Rai (1980) using microtiter plates.

1. The 25 µl antigen was added to all the wells keeping 4 wells for each serum as shown in Figure-1.

SCHEMATIC REPRESENTATION OF MICRO-COMPLIMENT FIXATION

TEST.



PHASE I : Ag Ab C'
25µl + 25µl + 25µl.

INCUBATION AT 37°C FOR 30 MTS.

PHASE II: HS (50µl) INCUBATION AT 37°C FOR 30 MTS.

REFERENCES

Ag = ANTIGEN.

Ab = ANTISERA.

C' = COMPLEMENT.

HS = HAEMOLYTIC SYSTEM.

VB = VERONAL BUFFER.

*(a) Ag - 25µl.
Ab - 25µl.
C' - 25µl.
(b) HS - 50µl.

** ANTISERA CONTROL.
(a) Ab - 25µl.
VB - 25µl.
C' - 25µl.
(b) HS - 50µl.

***ANTIGEN CONTROL.
(a)
Ag - 25µl
VB - 25µl.
C' - 25µl.
(b) HS - 50µl.

FIGURE - 1

2. Antisera against FMD virus types 'O', 'A', 'C', 'Asia-1' and subtype 'A-22' were diluted and inactivated at 56°C for 30 minutes and added (25/µl) to well of microtitter plate keeping one row for each type of antisera.

3. The appropriate complement dilution (containing 3 units) in 25/µl volume was then added to all the wells.

4. The amount of veronal buffer added to various controls was 25/µl in antisera and antigen control, 50/µl in complement control and 75/µl in RBC control.

5. The plates were sealed by adhesive tapes and incubated at 37°C for 30 mimutes in water bath.

6. 50 ml of haemolytic system was added to each well after thorough mixing and plates were incubated at 37°C for 30 mimutes after sealing.

7. The plates were left at 4°C overnight to allow settling of RBC.

Interpretation of MCFT

The diameter of button of erythrocytes settled in the RBC control wells was scored as 4 i.e. 100% RBC settled and no haemolysis. The other wells were compared with the RBC control. The row of wells giving no haemolysis and settled RBC indicated the presence of that type of virus against which the antisera was used in that well. If the button of RBC was observed in the well of either antigen or antisera, that antigen or antisera was declared as anticomplementary and the test was repeated with best possible effort to eliminate the effect.

III. PASSAGE OF SAMPLES IN MICE

The samples failing to yield foot and mouth disease virus by direct MCFT was passaged through the unweaned suckling mice for recovery of the virus. A total of 78 samples were passaged through the mice and then subjected to MCFT to ascertain the FMD virus types.

The virus inoculum for mouse passage was prepared as per the method mentioned above under preparation of antigen except that treatment with the chloroform was ommitted. Penicilline 1000 units/ml, streptomycin 1 µg/ml and mycostatin 250 units/ml was added to the antigen preparation and allowed to remain

at room temperature for one hour. For inoculation of suspension for FMD virus isolation, unweaned suckling mice (Albino) 5-7 day-old were used as host.

Inoculation

One litter was used for each of the samples for virus passage of which one mouse was kept uninoculated as control whose tip of the tail was cut for immediate identification. The mice were inoculated with 0.1 ml of the virus suspension intramuscularly in thigh region. The mice were observed daily upto 7 days for the development of paralysis of hind quarters. Usually by 3rd day, mice died showing paralytic symptoms followed by death. Any death occurring within 24 hours post inoculation was taken as non specific. The mice carcass was cleaned of the appendages, viscera and skin. Only skeletal portion was collected and used as antigen after processing as per the method mentioned above under preparation of antigen.

MCFT of the Mouse Passage Antigen

The mouse carcass antigen was then subjected to MCFT to ascertain the FMD virus type from the samples which could not give positive result in direct MCFT. The samples, still failing to yield FMD virus types were passaged further for 2nd or 3rd

time. A total of 45 and 36 samples, which failed to yield virus types were allowed to 2nd and 3rd passage respectively. The samples yielding low fixation during the MCFT were used to be confirmed by the tube CFT as per the method of Brooksby (1952) .

IV. PASSAGE OF SAMPLES IN CELL CULTURE

The samples failing to yield FMD virus types even after three passages through the unweaned mice, were passed through the cell culture for the recovery of the virus. A total of 34 field materials were processed for cell culture passage.

Cell line used

1. BHK-21 C1-13
2. IB-RS-2

Media

BHK media was used for both the cell lines.

Composition

Sodium chloride	128 g
Potassium chloride	8.0 g
Calcium chloride ($Cacl_2$ $2H_2O$)	5.28 g
Magnesium sulphate ($MgSO_4$ $7H_2O$)	4.00 g
Sodium dihydrogen phosphate	2.80 g
Dextrose	90.00 g

Ferrous sulphate	0.002 g
L-glutamin	5.84 g
Penicillin	20 lakhs
Streptomycin	2 g
Phenol red	30 ml
Distilled water	10 litres
Sodium bicarbonate	55.00 g
Amino acids	1000 ml
Vitamins	80 ml
Distilled water	8890 ml

Flow of CO_2 sterilized by filteration.

Phenol red solution

Phenol red powder	1 g
N/10 sodium hydroxide	30 ml
Distilled water	70 ml

Autoclaved at 15 lb pressure for 15 minutes.

Amino acid stock solution

Arginine	8.4 g
Cystine	4.8 g (dissolved in 50 ml NaoH)
Histidine	3.84 g
Isoleucine	10.48 g
Leucine	10.48 g

Lysine	14.62 g
Phenyl alanine	6.60 g
Threonine	9.52 g
Tyrosine (N/1 NaoH 70 ml)	9.52 g
Valine	9.36 g
Methionine	3.00 g
Inositol	0. 70 g
Phenol red 1%	0.4 ml
Distilled water	10,000 ml

It was used to store frozen at -20°C.

Stock solution of vitamins

Choline chloride	0.50 g
Folic acid (in N/1 NaoH)	0.50 g
Nicotinamide	0.50 g
D-Pantothenic acid	0.50 g
Pyredoxal Hcl	0.50 g
Thiamine Hcl	0.50 g
Riboflavin	0.50 g

All the above vitamins were dissolved in 100 ml double distilled water and stored frozen till used.

Tryptose phosphate broth

Tryptose phosphate broth	59 g
Distilled water 2	2 litres

Autoclaved at 15 lb pressure for 15 minutes.

Trypsine-Versene-Glucose solution (TVG)

i. Trypsin 2%

Trypsin	2 g
Distilled water	100 ml

Sterilization by filtration.

ii. Versene 0.2%

Versene	0.2 g
Distilled water	100 ml

Autoclaved at 15 lb pressure for 15 minutes.

iii. Glucose 10% solution

Glucose	10 g
Distilled water	100 ml

Autoclaved at 15 lb for 15 minutes.

Final preparation of TVG

PBS (Calsium & Magnesium free)	840 ml
2% Trypsine solution	50 ml
0.2% Versene solution	100 ml
10% Glucose solution	5 ml
1 Phenol red solution	1 ml

Phosphate Buffer Saline (Calcium & Magnesium free

Sodium chloride	80 g
Potassium chloride	2 g
Potassium dihydrogen phosphate	2 g
Disodium hydrogen phosphate	11.5 g
Distilled water	1000 ml

A drop of chloroform was added and stored at $4^{\circ}C$.

Final preparation of growth medium

BHK media	80 ml
TPB	10 ml
Calf serum	10 ml
	100 ml

Maintenance media

BHK media	95 ml
TPB	5 ml
	100 ml

Procedure for sub-culture

The bottles with confluent growth of monolayers were selected, the medium were discarded and then cell sheets were washed with Trypsin-

Versene-Glucose (TVG) solution. Fresh TVG solution was added and incubated at room temperature for about one minute with contimuous shaking. The TVG solution was discarded and the bottles were kept at room temperature till the cells were released from the surface. The cells were mixed thoroughly in 10 ml of growth medium. The desired split of cells were inoculated in to fresh sterile bottles and tubes with growth medium, sealed tightly and incubated at 37°C. The bottles and tubes were examined after 24 hours upto 48 hours. The virus was inoculated when the monolayer was complete.

Inoculation of monolayers

The field samples which proved negative after mice passage were triturated with mortar and pestle, suspended in 3 ml of PBS and then treated with streptomycin 100/µg per ml and penicillin 100 units per ml. The antigen was centrifuged and then supernatent was collected. Three tubes of monolayers were used for inoculation of each samples and three tubes were kept as uninoculated control with the maintenance medium. The tubes were inoculated with virus inoculum with a volume of 0.2 ml per tube and then left for adsorption for one hour. After adsorption , two ml of the maintenance medium was added to each tube. The

tubes were observed for the cytopathic effect (CPE) for as long as the control tubes did not show signs of digeneration. This period was about 24 hours to 48 hours with BHK-21 cells and upto 72 hours with IB-RS-2 1 cell lines.

When the CPE appeared or the control cells showed degenerative changes, then the inoculated tubes were harvested. The harvested materials were frozen and thawed twice before inoculation for second passage into another set of monolayers. The morbidity pattern of CPE was recorded. The cell culture adopted antigens were then subjected to different tests to ascertain the FMD virus types.

V. SERUM NEUTRALIZATION TEST (SNT)

The serum neutralization test of the cell culture adapted viruses from field samples was performed with BHK-21 monolayer cultures grown in tubes.

(1) Preparation of Virus dilution

The diluent(4.5 ml) was distributed in tubes marked from 10^{-1} to 10^{-7}. Then 0.5 ml of stock virus suspension was added to the Ist tube and mixed well. A 0.5 ml volume of the Ist tube was transferred to the second and so on. Thus the ten fold dilutions of antigen were prepared. (Table-3)

Table-3

Showing the Ten fold dilutions of cell culture antigen

Virus	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Diluent* (in ml)	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Antigen (in ml)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

*: Diluent : Maintenance media for BHK-21 cells

(2) Preparation of dilutions of serum and its inactivation

The hyperimmune serum raised in guinea pig was used in a constant dilution (1:5). The sera were inactivated at 56°C for 30 minutes in water bath before use.

(3) Preparation of serum virus mixture

The cell culture passaged antigen was mixed with equal amount of antisera against FMD virus types 'O', 'A', 'C', 'Asia-1' and subtype 'A-22' separately and incubated at 37°C for 30 minutes. The serum virus mixture was prepared as presented in Table-4.

Test procedure

The protocol for conducting serum neutralization test has been depicted in Table-5.

Three tubes of BHK-21 monolayer cultures were inoculated with serum virus mixture (.2 ml) of each dilution. After 30 minutes of adsorption, 2 ml of maintenance medium was added to each of the tubes. These tubes were incubated at 37°C and examined at different intervals for evidence of CPE. Three tubes of BHK-21 monolayer cultures were kept as uninfected control with 2 ml of maintenance medium only. Besides, antisera controls against each of the FMD virus types were also maintained.

Table-4

Procedure depicting preparation of Serum Virus Mixture

Sl. No.	Virus types / Antisera	Virus dilutions in ml						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
1.	TCV * antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Type 'O' serum	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2.	Antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Type 'A' serum	0.5	0.5	0.5	0.5	0.5	0.5	0.5
3.	Antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Type 'C' sera	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4.	Antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Type 'Asia-1' sera	0.5	0.5	0.5	0.5	0.5	0.5	0.5
5.	Antigen	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Sub-type 'A-22' sera	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* : Tissue culture adopted virus dilution (unknown type)

Table-5

Protocol for conducting Serum Neutralization Test

Sl. No.	Log 10 of dilutions	Tube number	Materials to be inoculated				
			V + S 'O'	V + S 'A'	V + S 'C'	V + S 'Asia-1'	V + S 'A-22'
1.	1	1	0.2	0.2	0.2	0.2	0.2
		2	0.2	0.2	0.2	0.2	0.2
		3	0.2	0.2	0.2	0.2	0.2
2.	2	1	0.2	0.2	0.2	0.2	0.2
		2	0.2	0.2	0.2	0.2	0.2
		3	0.2	0.2	0.2	0.2	0.2
3.	3	1	0.2	0.2	0.2	0.2	0.2
		2	0.2	0.2	0.2	0.2	0.2
			0.2	0.2	0.2	0.2	0.2
4.	4	1	0.2	0.2	0.2	0.2	0.2
		2	0.2	0.2	0.2	0.2	0.2
		3	0.2	0.2	0.2	0.2	0.2
5.	5	1	0.2	0.2	0.2	0.2	0.2
		2	0.2	0.2	0.2	0.2	0.2
		3	0.2	0.2	0.2	0.2	0.2

i. 30 minutes adsorption time was allowed
ii. Whole set incubated at 37°C
iii. Three tubes left uninoculated (controls) were examined for CPE at different intervals
iv. Serum as well as medium controls were also kept.

VI. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

The micro ELISA was applied to the tissue culture passaged samples as well as original materials to ascertain the FMD virus types. In order to ensure the best condition for attachment of test antigen, a double sandwitch test was conducted as per the method of Crowther & Elzein (1979).

(1) Preparation of anti-guinea pig globulin in rabbit

(i) Separation of globulin from guinea pig serum

The healthy guinea pig serum was collected and precipitated with equal volume of saturated ammonium sulphate. This was centrifuged at 3000 rpm for 30 minutes in order to sediment the precipitate. The above procedure was repeated 3 times and then was dialysed against the PBS (pH 7.2). The globulin concentration was adjusted to 10 mg/ml and emulsified with equal volume of Freundt's complete adjuvant.

(ii) Inoculation schedule of rabbits

For preparing the anti-guinea pig globulin in rabbits each of four rabbits was immunized by inoculating guinea pig globulin with Freundt's complete adjuvant as per the schedule presented in Table-6.

Table-6

Inoculation schedule in rabbits

Sl. No.	Days	Route of inoculation	Dose of inoculation	Remarks
1.	0	I. M.	0.4 ml	0.1 ml in each of the legs
2.	7	I. M.	0.4 ml	- do -
3.	14	I. M.	0.4 ml	- do -
4.	21	I. M.	0.4 ml	- do -
5.	28	I. M.	0.4 ml	- do -
6.	35	I. M.	0.4 ml	- do -
7.	56	S. C.	0.4 ml	- do -

I. M. : Intra mascular with Freund's complete adjuvant

S. C. : Sub-cutaneous with Freund's complete adjuvant

The serum was collected after 10 days of the last injection. This serum was again precipitated with ammonium sulphate and dialysed. The serum was separated and tested for anti-guinea pig globulin by gel diffusion method.

(2) Peparation of conjugate (Horseradish peroxidase labelled anti-guinea pig immunoglobulin

Conjugation of enzyme with anti-guinea pig immunoglobulin raised in rabbit was done as per the method given below:

i. Horseradish peroxidase, 4 mg was dissolved in 0.1 ml of double glass distilled water.

ii. Sodium meta-periodate (NaI_4) in volume of .1 ml (0.1 M) was added and stirred for 20 minutes.

iii. It was dialysed against acetate buffer, pH 4.2 (0.001 M) overnight at $4^{o}C$.

iv. Immunoglobulin (8 mg) was dissolved in 0.1 ml of 0.01 M carbonate buffer pH 9.5. It was immediately added to dialysed activated enzyme after adding 20 ml of 0.2 M carbonate buffer, pH 9.5 and stirred for 2 hours at room temperature.

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*:- Horseradish peroxidase (type VI) Activity-250-300 units/mg Sigma Chemical Co., St. Louis,MO U.S.A.

v. The 0.1 ml sodium borohydrate (4 mg/ml water) was added and kept in refrigerator at 4°C till it was passed through columns.

vi. This conjugate was then passed through sephadex G-200 equilibrated with PBS (pH 7.4) and the fractions were collected.

vii. Absolute readings of all fractions were taken by spectrophotometer at 280 nm and 403 nm. The fraction covering two peaks were collected, pooled and dialysed against PBS. Bovine serum albumin was added to make it 1%. Then it was stored in ampoules.

(3) Dilution of conjugate

Conjugate was diluted to 1:2000 (as determined by checkerboard titration) in PBS, pH 7.2 containing 1% BSA and 0.5 per cent tween - 20.

(4) Virus type specific rabbit antisera

The virus type specific antisera against FMD virus types 'O', 'A', 'C' and 'Asia-1' and subtype 'A-22' were received from Central

Laboratory, Foot and Mouth Disease, Indian Veterinary Research Institute, Banglore. IgG fractions of antisera were prepared as described by Abu Elzein and Crowther (1978) and concentration was calculated after measuring the OD value at 278 nm. Samples were stored at -20°C. These antibodies were used as catching antibodies.

Dilution

The virus specific antibodies were diluted in carbonate buffer, pH 9.6 (0.01 M) to the final concentration of 10 µg per ml.

(5) Antigen dilution

The tongue or feet epithelium from field samples were triturated and diluted in PBS buffer, centrifuged and the supernatent was used as antigen. The stock 10% virus suspension in PBS was diluted to 1:10 in PBS tween containing 1% bovine serum albumin (BSA) and was used in the test proper.

(6) Dilution of guinea pig hyperimmune serum

The hyperimmune serum types 'O', 'A', 'C', 'Asia-1' and subtype 'A-22' were diluted to 1:300 in PBS (pH 7.2) containing 1 per cent BSA and 0.05% tween -20.

(7) Preparation of substrate

O-Dianisidine dihydrochloride was used as substrate. Acetate buffer (0.2 M), pH 5.0 containing 720 mg EDTA/L and 1% triton-x-100 was prepared and kept at $4^{\circ}C$ as stock solution. At the time of use the buffer was diluted 1:4 in distilled water, 5 mg of O-Dianisidine hydrochloride was added to 50 ml of above diluted buffer, 25 ml of H_2O_2 was added to the above and then used as substrate.

(8) Test proper

This test was performed as per the method of Crowther and Elzein (1979) as well as Rai et al (1980) for the identification of FMD virus.

Polysterin micro ELISA plates were used and coated in the following way:

i. Each test plate in a single row received each virus type specific rabbit IgG in a concentration of 10/μg per ml at the rate of 0.2 ml per well. The plate was incubated at room temperature overnight.

ii. Each well of the plate was washed three times with PBS tween (0.05%). For this purpose wells were filled with buffer

and allowed for 3 minutes and then discarded. This was repeated thrice.

iii. The wells were air dried.

iv. Each well was saturated with 1% BSA and incubated at 37°C for one hour and then discarded. This charged plate was kept at 4°C till used.

v. A volume of 0.2 ml of optimally diluted (1:10) antigen in PBS tween was added to each row of the plate. It was allowed to react for one hour at 37°C.

vi. The plate was washed thrice with PBS tween.

vii. Each well received 0.2 ml of guinea pig typing serum, optimally diluted (1:300), of the same serotype as the rabbit IgG originally attached to the row. Plates were incubated for 1 hour at 37°C.

viii. Plates were washed thrice with PBS tween and air dried.

ix. Enzyme labelled antiguinea pig rabbit globulin was then added (0.2 ml per well) at a suitable pretitrated dilution

(1:300) in PBS tween containing 1% BSA. The plates were incubated at 37°C for one hour.

x. Step (ii) and (iii) were repeated to remove excess of enzyme.

xi. Finally, the amount of enzyme to the wells were determined by adding 0.2 ml of substrate to each well and incubated at room temperature for 30 minutes. Then the reaction was stopped with 10/$^{\mu l}$ of 5 N Hcl.

Interpretations

The positive reaction was indicated by the development of different shades of yellow colour depending upon the titre of the antigen. The reaction was evaluated visually.

Control reaction for ELISA

In all the test positive, controls were run simultaneously in the same plate.

Buffer

1. Carbonate buffer (pH 9.6)

Sodium carbonate 1.59 g

Sodium bicarbonate	2.93 %
Sodium azide ($Na N_3$)	0.2 g
Distilled water	1000 ml

2. PBS Tween buffer (pH 7.4)

Sodium chloride	8.0 g
Potassium dihydrozen phosphate	0.2 g
Disodium hydrogen phosphate	2.9 g
Potassium chloride	0.2 g
Tween-20	0.5 ml
$Na N_3$	0.2 g
Distilled water qs ad.	1000 ml

3. Acetate buffer

Solution A acetic acid 0.2 M (11.55 ml glacial acidic acid/litre)

Solution B sodium acetate -0.2 M(Sodium acetate, $3H_2O$ - 27.28/L).

pH 5.00

148 ml of solution A was added to 352 ml of solution B.

Part -II

Epidemiological Studies

The epidemiological data of FMD outbreaks during the year 1981-83 in Bihar were taken for the present study. The data were consolidated and analysed statistically to see the influence of various epidemiological factors on the incidence of FMD.

(1) Effect of Topography

The Bihar state was divided into three conventional geographical regions-North Bihar, South Bihar Plains, and Chotanagpur Plateau. The data on the geographical regions were analysed statistically to see the influence of topography. The district wise distribution of the incidence of FMD was also studied.

(2) Effect of Species

The data based on species wise incidence of FMD among cattle, buffaloes, sheep, goats and pigs were analysed statistically.

(3) Seasonal Influence

The seasons were designated on the basis of meteorological data as Summer (March-May), South West Monsoon (June-September), Post Monsoon (October-November) and Winter (December-February). The month-wise distribution of FMD data were consolidated and analysed statistically to study the influence of seasons on the incidence of FMD.

(4) Influence of Age

To study the influence of age on the incidence of FMD, data were consolidated for three age groups as 0-6 months, 6 to 12 months and animals above one year of age.

(5) Influence of Sex

The data on sex-wise incidence of FMD were consolidated and analysed to see the effect of sex on the incidence of FMD.

For statistical analysis, X^2-test of significance were employed; the formulas used was:

$$X^2 = \sum \frac{(O-E)^2}{E}$$ whereas, E = Expected value

O = Observed value

Degree of freedom was (r-1) (c-1).

The value of X^2 calculated by the above formula was compared with the tabulated value at P 0.05 and P 0.01 for the required degree of freedom.

(6) Typographical study

The epidemiological data were also consolidated to study the district wise, sex wise, age wise, species wise distribution of different types of FMD virus.

R E S U L T S

Part-I

Serological Studies

A total of 131 samples from FMD affected animals were collected from 45 outbreaks during the years 1981 to 1983. It would be seen from Table-7 that out of 131 samples, which were subjected to different serological tests for typing, 111 (84.73%) were found to be positive for FMD virus. Amongst the collected materials from the FMD affected animals under field conditions, 37 out of 49 feet epithelium and 51 out of 54 tongue epithelium revealed the presence of FMD virus. All the 11 gum tissues and 2 samples from eye lesions were found to be positive for FMD virus. Amongst the 6 samples collected from the palate lesions, only one yielded the presence of FMD virus. It was interesting to note that all the tongue and feet samples collected from FMD affected animals under farm condition were found to be positive for FMD virus. In all 20 samples out of 131 failed to yeild the presence of FMD virus by all serological tests employed in this work.

The results of different serological tests of isolation of FMD virus through mouse and tissue culture passage and result of statistical analysis of epidemiological factors like influence of topography, season, species, sex and age of the animals on the incidence of FMD outbreaks and typographical studies are being presented in details on the following pages.

Table-7

Showing result of typings of foot and mouth disease materials collected from various sources

Source of samples	Nature of samples	Total number of samples tested	Number of samples samples found positive	Percent of positive samples
FMD affected animals under village condition	Feet epithelium	49	37	75.51
	Tongue epithelium	54	51	94.44
	Gum tissue	11	11	10.00
	Pallate tissue	6	1	16.66
	Eye lesion	2	2	100.00
FMD affected animals under Farm condition				
Ranchi Livestock Farm, Ranchi	Feet epithelium	2	2	100.00
	Tongue epithelium	1	1	100.00
Govt. Cattle Farm, Gauriakarma, Hazaribagh	Feet epithelium	4	4	100.00
	Tongue epithelium	2	2	100.00
Total :-		131	111	84.73

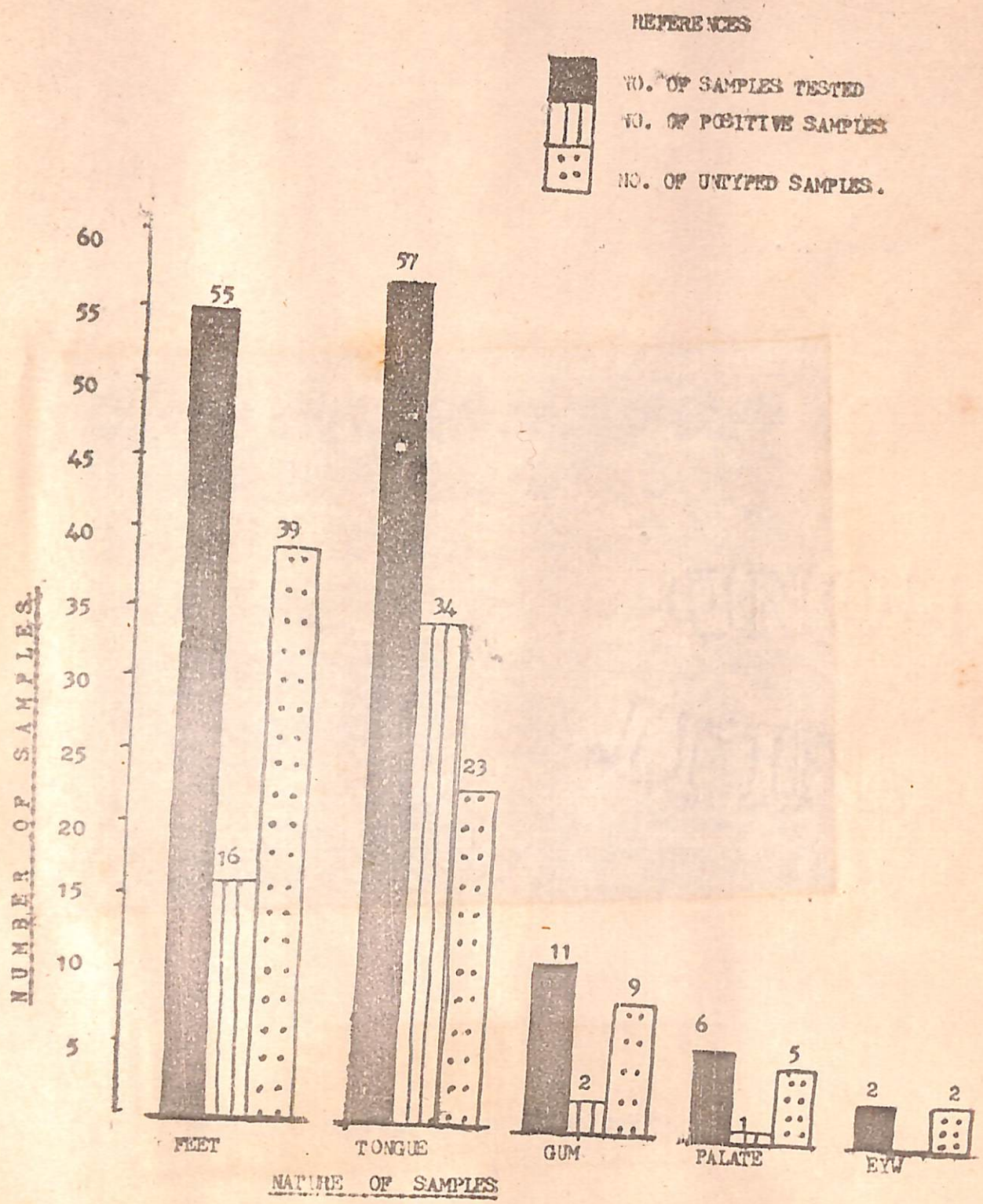
I. TYPING OF ORIGINAL SAMPLES BY MICRO COMPLEMENT FIXATION TEST

Altogether 131 samples, which included feet epithelium (55), tongue epithelium (57), gum lesion (11), palate lesion (6) and eye lesions (2) were subjected to micro complement fixation test (MCFT). Out of 131 samples, 53 (40.46%) revealed the presence of FMD virus whereas 78 (59.54%) samples were found to be negative. Among the untyped samples, 41 (52.56%) showed the anticomplementary effect. Graph-1 shows the details of the samples collected and its typing results.

The recovery of FMD virus from different tissues were 59.65% from tongue materials, 29.09% from feet epithelium, 18.18% from gum materials and 16.16% from the samples of palate lesions. Besides, both the samples collected from eye lesions of the affected cattle failed to yield the presence of FMD virus. Of the 53 samples found positive by MCFT, 29 (54.72%) type 'O', 4 (7.55%) type 'A', 2(3.77%) type 'C', 8(15.09%) type 'Asia-1', 5(9.43%) subtype 'A-22' and the remaining 5 (9.43%) were found to be cross reacting ('Asia-1'/'O') type (Table-8).

II. MOUSE PASSAGE

The duplicate field samples found negative



RESULT OF FMD VIRUS TYPING BY MCFT OF ORIGIVAL MATERIALS

Graph - 1

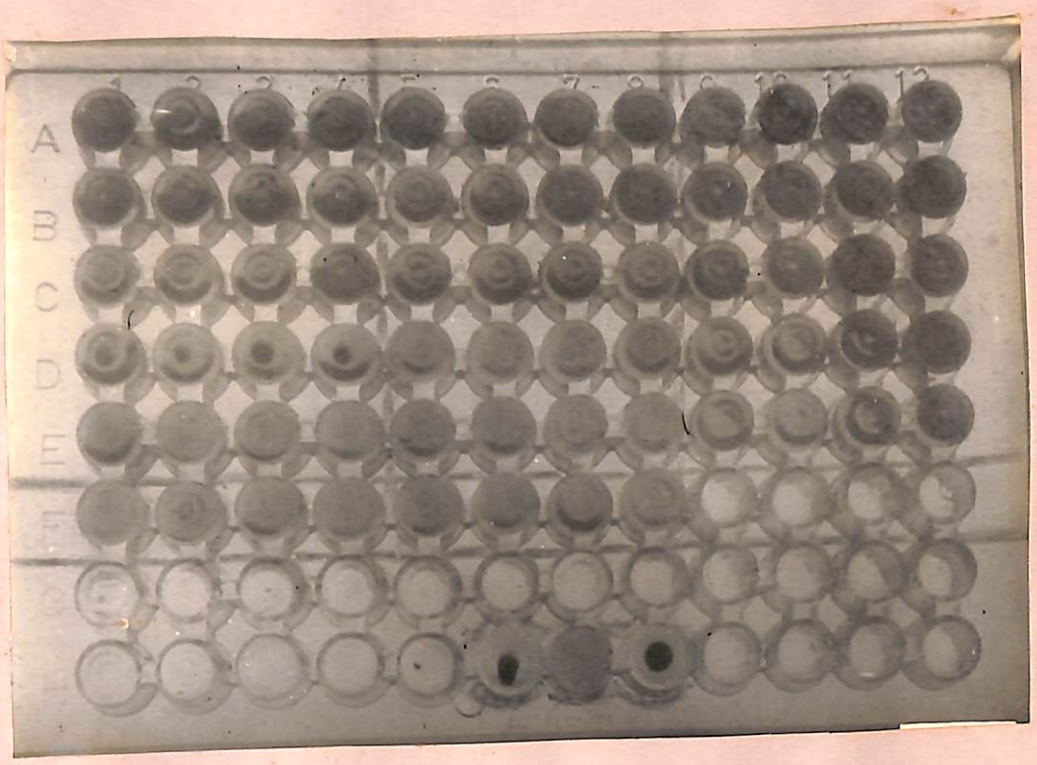


FIG: 2: Showing the result of MCFT

Table-8

Showing the details of result of virus typing of original samples by Micro Complement Fixation Test

Sl. No.	Nature of samples	Total No. of samples tested	Number of positive samples yielding serotypes							No.of untyped samples		
			'O'	'A'	'C'	Asia-1	A_{22}	Cross reacting.	Total	Anticomplementary.	Negative typing	Total
1.	Affected feet epith.	55	9	1	-	1	3	2*	16 (29.09)	23 (58.97)	16 (41.03)	39 (70.91)
2.	Affected tongue epith.	57	19	3	2	5	2	3*	34 (59.64)	14	9	23 (40.36)
3.	Affected gum lesions	11	-	-	-	2	-	-	2 (18.18)	2 (22.22)	7 (77.78)	9 (81.81)
4.	Affected pallate lesions	6	1	-	-	-	-	-	1 (16.66)	1 (20)	4 (80)	5 (83.34)
5.	Affected eye lesions	2	-	-	-	-	-	-	-	1 (50)	1 (50)	2 (100)
	Total :-	131	29	4	2	8	5	5	53 (40.47)	41 (31.29)	37 (28.24)	78 (59.53)

*F M D virus type Asia-1 cross reacting with serotype 'O'

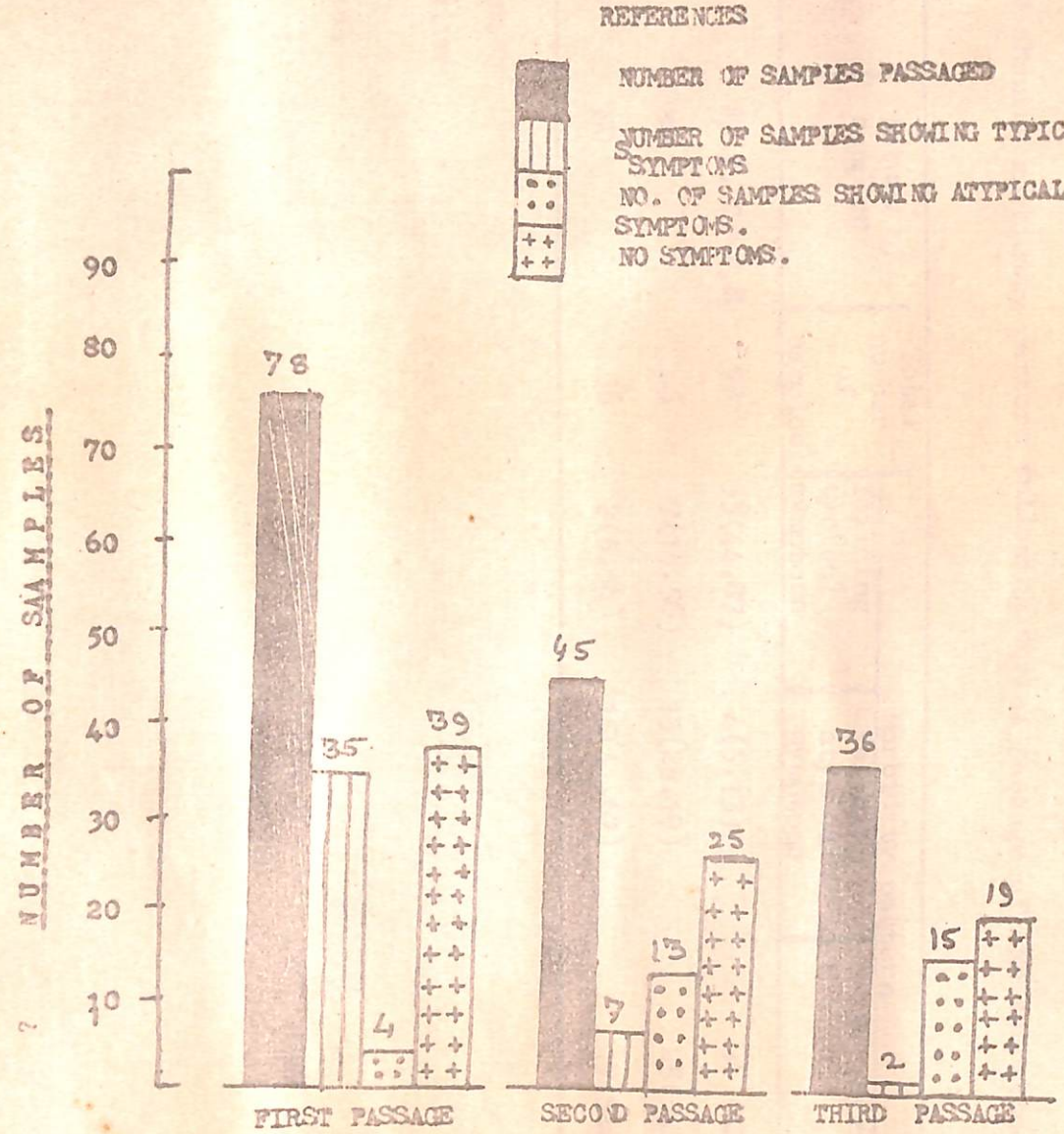
Figures in paren thesis represents the percentage of data.

by MCFT were passed through unweaned mice for the recovery of virus. Graph-2 depicts the morbidity pattern yeilding typical or atypical symptoms in mice. It would be seen from Table-9 that a total of 78 MCFT-negative samples were passaged through unweaned mice, out of which 35 samples exhibited the typical symptoms of paralysis of hind parts followed by death within 48 to 78 hours. Only 4 samples out of 78 behaved irratically by showing late development of paralysis which persisted long before death occurred. The remaining 39 samples failed to exhibit any symptom in mice.

The 45 samples which behaved either irratically or yeilded no symptom in mice were passaged for the second time. Out of 45 samples, 7 exhibited typical symptoms, 13 atypical symptoms and 25 failed to yeild any symptoms in mice. Again 36 samples showing atypical symptoms or no symptom in the second passage were processed for the third passage of which only two exhibited typical symptoms of FMD virus in mice, 15 samples gave atypical symptoms and 19 samples exhibited no symptom in mice, (Graph-2). It was noted that the number of samples yeilding irregular symptoms was less in the first passage level and maximum in third passage level. The samples which yeilded atypical

MORBIDITY PATTERN IN MICE FOLLOWING INOCULATION WITH

MCFT NEGATIVE SAMPLES



Graph - 2

Table-9

Mice showing morbidity pattern following serial passage of FMD suspected samples

Sl. No.	Passage	Nature of samples	Total number of samples	Morbidity pattern		
				Yeilding typical symptoms	Yeilding atypical symptoms	No symptoms
1.	Ist passage	F, T, G, P,E	78	35(44.87)	4(5.13)	39 (50)
2.	IInd passage	MC	45	7(15.56)	13(28.19)	25(55.56)
3.	IIIrd passage	MC	36	2(5.56)	15(41.67)	19(52.78)

F : Feet epithelium

T : Tongue epithelium

G : Gum material

P : Pallate material

E : Eye lesions

MC: Mouse carcass

symptoms in the first passage, continued to yeild the same type of atypical symptoms in second and third consecutive passages.

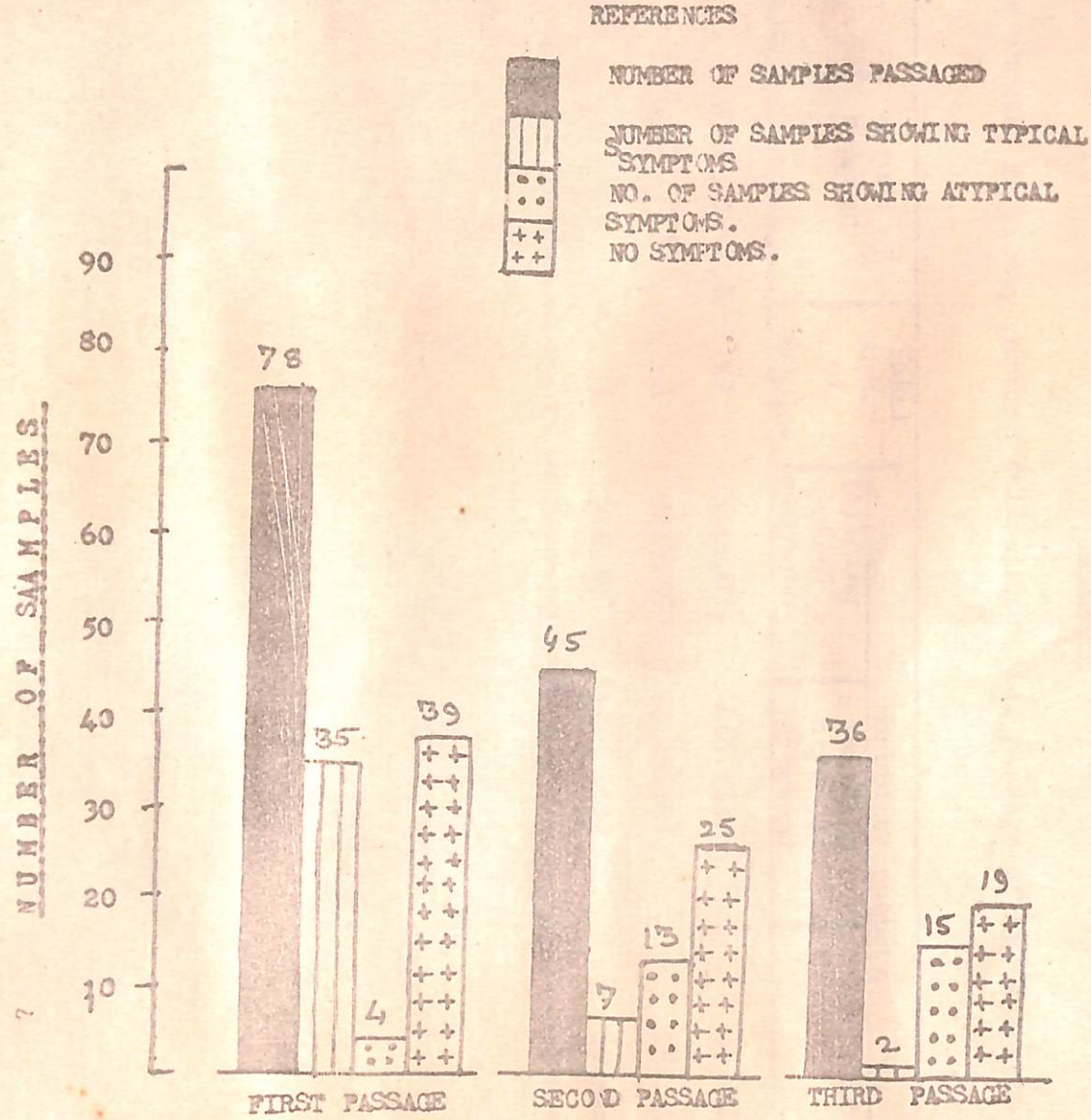
III. VIRUS TYPING RESULT OF MOUSE PASSAGED SAMPLES BY MCFT.

All the 78 samples passaged in mice were subjected to MCFT, of which 33 yeilded the presence of FMD virus after the first passage. A total of 45 samples were tested by MCFT after second passage, of which 9 samples were found to be positive for FMD virus. Out of 36 samples passaged for the third time, only 2 samples yeilded the presence of virus. After three passages of 78 samples in suckling mice the samples were tested by MCFT of which only 44 (56.41%) revealed the presence of FMD virus and 34 samples were declared as negative. The details of serotyping are summarised in Table-10.

It is evident from the Table-10 that virus could be recovered from 22 samples out of 39 feet epithelium and 11 out of 23 tongue epithelial samples. All the samples from eye and gum lesions were found to be positive for the presence of FMD virus whereas none of the samples from palate lesions yielded virus.

It would also be seen from the Table-10 that of the 44 positive samples revealing the presence of

MORBIDITY PATTERN IN MICE FOLLOWING INOCULATION WITH MCFT NEGATIVE SAMPLES



Graph - 2

Table-9

Mice showing morbidity pattern following serial passage of FMD suspected samples

Sl. No.	Passage	Nature of samples	Total number of samples	Morbidity pattern		
				Yeilding typical symptoms	Yeilding atypical symptoms	No symptoms
1.	Ist passage	F, T, G, P,E	78	35(44.87)	4(5.13)	39 (50)
2.	IInd passage	MC	45	7(15.56)	13(28.19)	25(55.56)
3.	IIIrd passage	MC	36	2(5.56)	15(41.67)	19(52.78)

F : Feet epithelium

T : Tongue epithelium

G : Gum material

P : Pallate material

E : Eye lesions

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It would also be seen from the Table-10 that of the 44 positive samples revealing the presence of

Table-10

Showing the details of result of FMD virus typing of samples after mouse passage

Sl. No.	Passage No.	Nature of samples	Total number tested	FMD virus type recovered							
				'O'	'A'	'C'	'Asia-1'	'A-22'	Cross reacting	Total	Number of negative samples
1.	Ist passage	F	39	10	1	2	2	2	1*	18	21
		T	23	5	-	-	3	1	-	9	14
		G	9	2	-	-	2	-	-	4	5
		P	5	-	-	-	-	-	-	-	5
		E	2	-	-	-	2	-	-	2	-
2.	IInd passage	F	21	3	-	-	-	-	-	3	18
		T	14	1	-	-	-	-	-	1	13
		G	5	2	1	1	1	-	-	5	-
		P	5	-	-	-	-	-	-	-	5
3.	IIIrd passage	F	18	1	-	-	-	-	-	1	17
		T	13	1	-	-	-	-	-	1	12
		P	5	-	-	-	-	-	-	-	5
Total :-			78**	25	2	3	10	3	1	44	34+

*:- FMD virus type 'O' cross reacting with 'A' **:- Total no. of samples tested.+:-Total no.of -ve sample

F :- Feet epithelium, T:- Tongue epithelium, G:- Gum samples, P:- Pallate samples, E:-Samples from eye lesion

FMD virus the maximum (25) belonged to type 'O' followed by type 'Asia-1' (10), type 'C' (3), subtype 'A-22' (3), type 'A' (2) and one sample was found to be cross reacting type ('O'/'A').

IV. CELL CULTURE PASSAGE

These 78 samples (original) which failed to yeild the presence of virus by direct MCFT was processed for passage in BHK-21 and IBRS-2 cell lines.

Passage in BHK-21 cells

The samples consisted of tissues from affected lesions numbering 39 feet epithelium, 23 tongue epithelium, 9 samples from gum epithelium, 5 palate epithelium and 2 eye lesions. The tongue epithelium samples proved worthy for the recovery of FMD virus in BHK-21 cell culture as it produced cytopathic effect (CPE) within 24 hours of inoculation. The CPE was characterised by almost complete destruction of cell sheet and roundening of the remaining cells. In the methanol fixed and eosine haematoxiylene stained coverslip preparations of virus infected BHK-21 cells granulation and areas of degeneration were seen in some of the cells after 18 hours of inoculation in the third passage (Figure 3-5). In 18-24 hours old virus infected culture preparations, roundening of cells

with deeply stained cytoplasm, clumping and vaccuolation of cells were observed (Figure 6-8). The CPE was compared with the uninfected BHK-21 cell monolayer culture (Graph-9).

Out of 78 samples, 54 (69.23%) samples were able to produce CPE in the cell culture. Table-11 depicts the morbidity pattern in the BHK-21 cell line. Twenty out of 23 tongue epithelium samples and 23 out of 39 feet epithelium revealed the presence of virus by producing the typical CPE. All the samples from the palate lesions failed to produce CPE in this cell line.

Passage in IBRS-2 cell line

Figure-10 shows the uninfected IBRS-2 cell monolayer cultures. The CPE in infected IBRS-2 cell line could be seen in the figure-11-12 . Further study with this cell line could not be carried out as the BHK-21 cell culture revealed more smooth recovery of FMD virus from the original samples.

V. SERUM NEUTRALIZATION TEST

All 54 samples which produced CPE in BHK-21 cell culture were subjected to SNT in BHK-21 cell line. The typing results are presented in Table-12. It is evident from the data presented in the table that all the 54 tissue culture passaged virus preparations could

FIG: 3: Showing CPE in BHK-21 cell culture after 18 hours of infection with FMD virus.

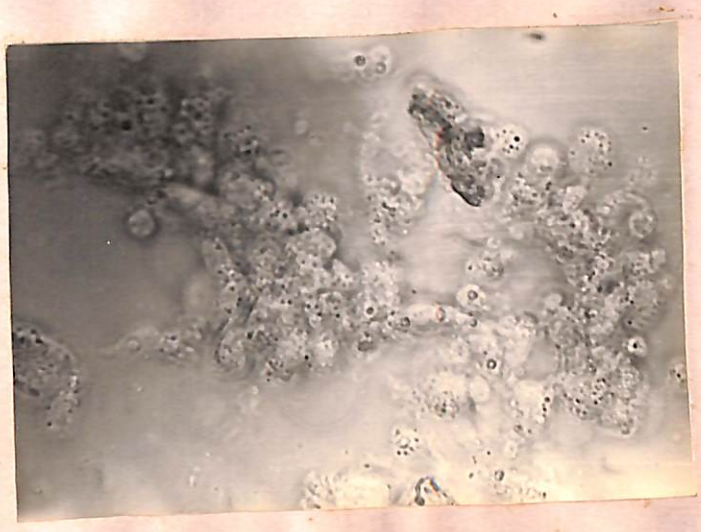


FIG: 4: Unstained BHK-21 cells showing CPE after 18 hours of infection with FMD virus.

FIG: 5: Showing degeneration of BHK-21 cells after 18 hours of infection with FMD virus.

FIG: 6: Showing granulation of cells after 16 hour of infection with FMD virus.

FIG: 7: Showing vaccuolation of BHK-21 cells after 18 hours of infection with FMD virus.

FIG: 8: Showing cell clumping and deeply stained cytoplasm of cells after 18 hours of infection with FMD virus.

FIG: 9: Uninfected BHK-21 cell monolayer culture (control).

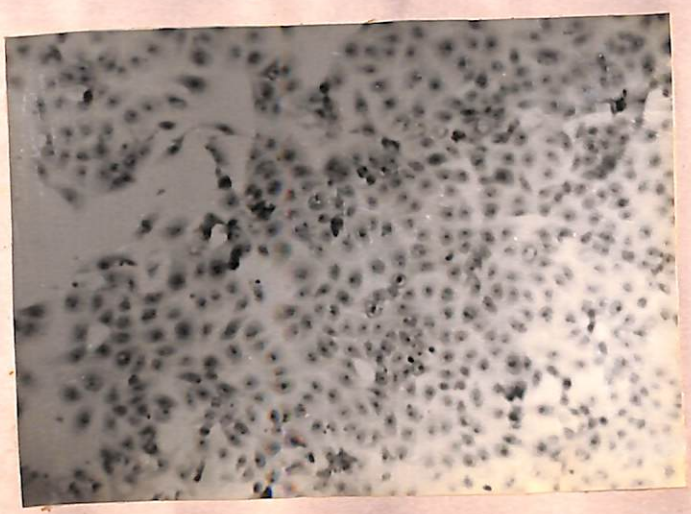


FIG:10: Uninfected IBRS-2 cell monolayer culture (control).

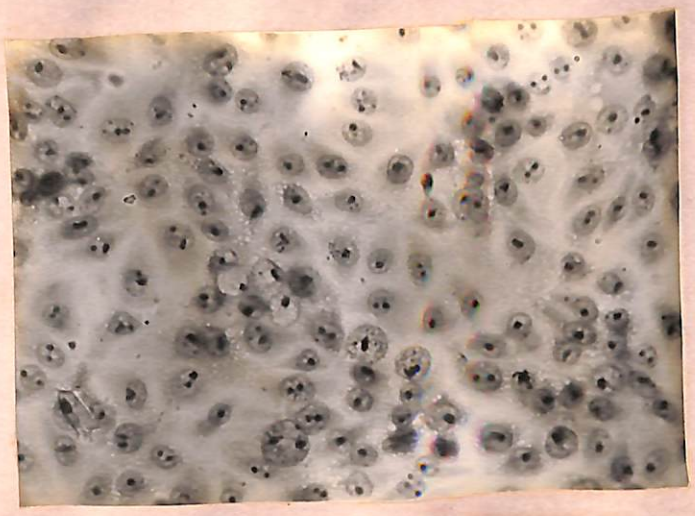


FIG:11: CPE in IBRS-2 cell after 40 hours of infection with FMD virus.

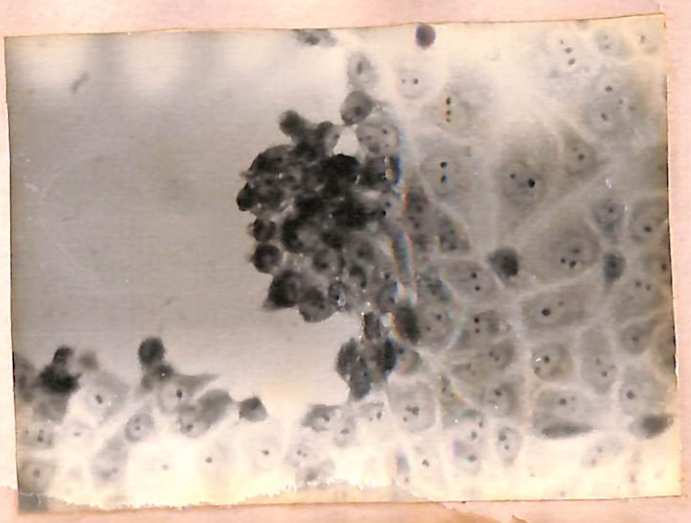


FIG:12: Cell clumping in IBRS-2 cell after 40 hours of infection with FMD virus.

Table-11

Showing pattern or cytopathic effect in BHK-21 cell culture following passage of CFT negative original samples.

Sl. No.	Sample No.	Nature of sample	BHK-21 passage and pattern of cytopathic effect																
			Ist passage						IInd passage						IIIrd passage				
			20 - 24 Hrs.		40 - 48 Hrs				20 - 24 Hrs			40-48 Hrs.			20-24 Hrs.			40-48 Hrs.	
1	2	3	4		5				6			7			8			9	
1.	BVC - PAT 1	F	+	+	+	+	+	+	+	+	+	++	++	++	++	+	++	+++	+++	+++
2.	SPR - CHM 1	F	±	±	±	+	+	+	+	±	+	+	+	++	++	+	++	+++	++	++
3.	SDC - PAT 3	T	+	+	+	++	++	++	++	++	++	+++	++	++	+++	+++	+++		-	
4.	MAN - MON 3	F	±	+	±	+	+	C	+	+	+	++	+	++	+	++	++	+++	+++	+++
5.	AD - BEG 3	F	+	+	±	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++
6.	AD - BEG 4	F	+	+	+	+	+	+	"			"			"			"		
7.	AD - BEG 5	G	±	±	±	+	+	+	+	+	+	++	+	++	++	++	++	+++	+++	+++
8.	KP - SIT 1	F	+	+	+	++	+	++	+	+	++	++	++	+++	++	++	++	+++	+++	+++
9.	KP - SIT 2	F		"			"			"			"			"			"	
10.	KP - SIT 5	G	±	±	±	±	+	±	+	+	+	+	++	+	++	++	++	++	+++	++
11.	KP - SIT 7	E	±	±	±	±	±	±	+	+	+	+	++	++	++	++	++	+++	++	++
12.	BH - SIT 1	T	+	+	+	++	+	+	+	++	++	++	++	+++	+++	+++	+++		-	
13.	BH - SIT 3	T		"			"			"			"			"			"	
14.	BH - SIT 6	G	±	±	±	±	C	±	+	±	+	++	+	++	++	++	++	+++	+++	+++
15.	BH - SIT 9	E	±	±	±	C	+	+	+	+	+	+	++	++	++	++	+	+++	++	++
16.	SAR - MUZ 2	T	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++		-	

1	2	3	4	5	6	7	8	9
17.	MD - MUZ 2	T	+ + +	++ ++ ++	++ ++ ++	+++ +++ +++	+++ +++ +++	-
18.	PHL - PAT 2	F	+ + +	+ + ++	+ + +	++ ++ ++	++ ++ ++	+++ +++ +++
19.	HAR - SAMT 3	G	+ + +	+ + ++	++ + +	+++ ++ ++	+++ +++ +++	-
20.	HAS - NAL 1	F	± ± +	+ + +	+ + +	++ ++ ++	++ ++ ++	+++ +++ ++
21.	" 2	F	"	"	"	"	"	"
22.	" 6	G	"	"	"	"	"	"
23.	BHA - NAL 1	T	+ + +	++ ++ ++	++ ++ ++	+++ +++ +++	+++ +++ +++	-
24.	" 3	G	"	"	"	"	"	
25.	BHD - PAT 1	F	± ± ±	+ + C	+ + +	++ ++ ++	++ ++ ++	+++ ++ ++
26.	" 2	T	+ + +	++ + +	++ ++ ++	+++ +++ +++	+++ +++ +++	-
27.	" 3	T	+ + +	++ + +	++ ++ ++	+++ +++ +++	+++ +++ +++	-
28.	KHR - PAT 1	T	"	"	"	"	"	-
29.	BR - AOR 1	F	- ± -	± + ±	+ + +	++ + ++	++ ++ ++	+++ ++ C
30.	BV - PAT 3	T	± + ±	+ + +	+ + +	++ ++ ++	+++ +++ +++	-
31.	" 4	T	"	"	"	"	"	-
32.	BV - PAT 6	G	- - ±	± ± C	+ + +	+ ++ +	++ ++ ++	+++ +++ +++
33.	RAC - RAN 1	T	+ + +	++ + +	++ ++ ++	+++ +++ ++	+++ +++ +++	-
34.	PAK - NAW 1	F	"	"	"	"	"	-
35.	PAK - NAW 2	T	+ + +	++ + ++	++ ++ ++	+++ ++ ++	++ +++	-

1	2	3	4			5			6			7			8			9		
36.	PAK - NAW 3	F	-	±	±	±	+	+	+	+	+	++	+	+	++	++	++	+++	++	+++
37.	MHL - NAW 1	T	+	+	+	++	+	+	++	++	++	+++	++	++	+++	+++	+++		-	
38.	MHL - NAW 2	F	±	+	+	+	+	+	+	++	++	++	+	+	++	++	++	+++	+++	+++
39.	GAUR - HAZ 4	F		"			"			"			"			"			"	
40.	" 5	T	+	+	+	++	+	+	++	++	++	+++	+++	+++	+++	+++	+++		-	
41.	" 6	F	±	+	±	+	+	C	+	+	+	++	++	+	++	++	++	+++	+++	+++
42.	DEO - HAZ 3	G	+	+	+	+	+	++	+	++	++	++	++	+++	+++	+++	+++		-	
43.	" 4	F	±	±	±	+	+	+	+	+	+	++	+	+	++	++	++	+++	+++	+++
44.	RAM - HAZ 1	T	+	+	+	++	+	++	++	+	++	+++	++	+++	+++	+++	+++		-	
45.	PTR - RAN 3	G		"			"			"			"			"			-	
46.	RAG - SIG 1	F	-	-	±	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++
47.	RAG - SIG 2	F	±	±	-	+	+	C	+	+	+	+	++	+	++	++	++	+++	+++	+++
48.	DEO - DEO 1	T	+	+	+	+	++	++	++	++	+	++	++	++	+++	+++	+++		-	
49.	DEO - DEO 2	T		"			"			"			"			"			-	
50.	RIG - SIT 3	F	-	-	±	±	+	+	+	+	+	++	+	++	++	++	++	+++	+++	+++
51.	BRG - MDB 1	F	±	-	+	C	+	+	+	+	+	+	++	++	++	++	++	+++	++	++
52.	BNR - MUZ 2	F	+	+	+	+	+	+	++	+	+	++	+	+	+++	++	+	+++	+++	+++
53.	DK - MUZ 1	T	+	+	+	++	+	++	++	++	++	+++	+++	+++	+++	+++	+++		-	
54.	DK - MUZ 2	T		"			"			"			"			"			-	
55.	*(24 sample)	FTP		-			-			-			-			-			-	

SAS-ROH 1 & 2, RAJ-MUZ 1 & 2, DHR-MUZ 1 & 2, MUS-PAT 1 & 2, TP-PAT 1 & 2, KNP-MUN-1, MGL-PAL 1, RAC-RAN 1 & 2, JUG-SIG 1 & 2, RAM-SIT 1 & 2, BHG-1, AD-BEG 6, KP-SIT 6, BH-SIT, 7 & 8, BV-PAT 7. - = Negative ; ± doutful for CPE; + : 25 CPE ; ++ = 50% CPE ; +++ Complete CPE F = Feet epith.; T = Tongue epith. ; P: Palate lesion ; G = Gum tissue ; C = Contaminated.

Table-12

Showing the details of Serum Neutralization Test

Sl. No.	Sample No.	Nature of sample	Log 10 of highest dilution of virus exhibiting CPE after 20 hrs	Log 10 of highest dilutions of serum-virus mixture exhibiting CPE after 20 hours.					Virus type determined
				O	A	C	Asia-1	A_{22}	
1	2	3	4	5	6	7	8	9	10
1.	BVC - PAT 1	F	6	3	6	6	6	6	O
2.	SPR - CHM 1	F	6	2	6	6	6	6	O
3.	SDC - PAT 3	T	5	2	5	5	5	5	O
4.	MAN - MON 3	F	6	2	6	6	6	6	O
5.	AP - BEG 3	F	6	6	6	3	6	6	C
6.	AP - BEG 4	F	6	6	6	2	6	6	C
7.	AP - BEG 5	G	5	5	5	2	5	5	C
8.	KP - SIT 1	F	6	6	6	6	3	6	Asia-1
9.	KP - SIT 2	F	7	7	7	7	3	7	Asia-1
10.	KP - SIT 5	G	6	6	6	6	3	6	Asia-1
11.	KP - SIT 7	E	6	6	6	6	3	6	Asia-1
12.	BH - SIT 1	T	6	6	6	6	2	6	Asia-1
13.	BH - SIT 3	T	6	6	6	6	3	6	Asia-1
14.	BH - SIT 6	G	5	5	5	5	2	5	Asia-1
15.	BH - SIT 9	E	5	5	5	5	2	5	Asia-1
16.	SAR - MUZ 2	T	6	6	6	6	6	2	A_{22}
17.	MD - MUZ 2	T	6	6	3	6	6	6	A
18.	PHL - PAT 2	F	6	6	3	6	6	5	A
19.	HAR - SAMT 3	G	5	5	2	5	5	5	A
20.	HAS - NAL 1	F	6	2	6	6	6	6	O
21.	HAS - NAL 2	F	6	3	6	6	6	6	O
22.	HAS - NAL 6	G	5	2	6	6	6	6	O
23.	BHA - NAL 1	T	6	3	6	6	6	6	O
24.	BHA - NAL 3	G	5	2	6	6	6	6	O
25.	BHD - PAT 1	F	6	3	6	6	6	6	O

1	2	3	4	5	6	7	8	9	10
26.	BHD - PAT 2	T	6	3	6	6	6	6	0
27.	BHD - PAT 3	T	6	3	6	6	6	6	0
28.	KHR - PAT 1	T	6	6	3	6	6	6	A
29.	BR - AUR 1	F	6	6	3	6	6	6	A
30.	BV - PAT 3	T	6	6	6	6	3	6	Asia-1
31.	BV - PAT 4	T	7	6	6	6	3	6	Asia-1
32.	BV - PAT 6	G	5	5	5	5	2	5	Asia-1
33.	RAC - RAN 1	T	6	2	3	6	6	6	O/A
34.	PAK - NAW 1	F	6	2	6	6	6	6	0
35.	PAK - NAW 2	T	6	2	6	6	6	6	0
36.	PAK - NAW 3	F	6	2	6	6	6	6	0
37.	MHL - NAW 1	T	6	3	6	6	6	6	0
38.	MHL - NAW 2	F	5	2	6	6	6	6	0
39.	GAUR - HAZ 4	F	5	2	5	5	5	5	0
40.	GAUR - HAZ 5	T	6	2	6	6	6	6	0
41.	GAUR - HAZ 6	F	4	2	4	4	4	4	0
42.	DEO - HAZ 3	G	6	3	6	6	6	6	0
43.	DEO - HAZ 4	F	6	3	6	6	6	6	0
44.	RAM - HAZ 1	T	7	3	7	6	6	6	0
45.	PTR - RAN 3	G	5	2	5	5	5	5	0
46.	RAG - SIG 1	F	6	3	6	6	6	6	0
47.	RAG - SIG 2	F	6	3	6	6	6	6	0
48.	DEO - DEO 1	T	6	3	6	6	6	6	0
49.	DEO - DEO 2	T	6	3	6	6	6	6	0
50.	RIG - SIT 3	F	5	5	5	5	5	2	A_{22}
51.	BRG - MDB 1	F	5	2	5	5	5	5	0
52.	BNR - MUZ 2	F	5	5	5	5	5	2	A_{22}
53.	DK - MUZ 1	T	6	3	6	6	6	6	0
54.	DK - MUZ 2	T	6	3	6	6	6	6	0

F = Feet epith. , T = Tongue epith.

G = Gum lesion , P = Palate .

be typed by SNT. Amongst the typed samples,31 (57.41%) were of FMD virus type 'O', 11 (20.37%) type 'Asia-1' 5(9.26%) type 'A', 3(5.56%) type 'C', 3 (5.56%) subtype 'A-22' and 1(1.85%) cross reacting FMD virus type 'O' with 'A'.

VI. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

All the 78 original samples, found negative by direct CFT, were subjected to ELISA test for serotyping. Out of 78 sample, 58 (74.36%) could be typed by this test. The results of virus serotyping by ELISA test are summarised in Table-13. It is evident from the result that ELISA test proved suitable to type FMD virus from all types of clinical materials like feet, tongue, gum and eye except palate epithelium. Among the original samples, the maximum percentage (100) were obtained from gum and eye epithelium in which all the 9 and 2 samples respectively were positive followed by tongue epithelium 20(86.96%) and feet epithelium 27 (69.23%). However, none of the affected palate epithelium proved positive for FMD virus. Out of 58 samples typed by ELISA, 33 proved to be of FMD virus type 'O' while 11 were typed as 'Asia-1', 5 type 'A', 3 type 'C', 3 subtype 'A-22' and 3 cross reacting type.

Table-13

FMD virus typing result of original samples by Enzyme-linked Immunosorbent Assay

Sl. No.	Nature of samples	No. of samples tested	No. of samples positive	Percentage of positive samples	Virus types recovered					
					O	A	C	Asia-1	A_{22}	Cross reacting
1.	Feet epith.	39	27	69.23	17	2	2	2	2	2
2.	Tongue epith.	23	20	86.96	12	2	-	4	1	1
3.	Gum epith.	9	9	100	4	1	1	3	-	-
4.	Palate	5	-	-	-	-	-	-	-	-
5.	Eye tissue	2	2	100	-	-	-	2	-	-
	Total :-	78	58	74.36	33	5	3	11	3	3

VII. COMPARATIVE EFFICACY OF CFT, SNT & ELISA TEST

Comparative typing results of the 78 CFT negative sample by MCFT after mouse passage, by SNT after tissue culture passage and by ELISA of original samples are presented in Table-14. It would be seen that out of 78 samples, 44 (56.41%) could be typed by CFT after mouse passage, 54 (69.23%) by SNT after tissue culture passage and 58 (74.36%) by ELISA test. Serotyping result of these three tests are also presented in the same Table. The FMD virus serotype 'O' identified by CFT, SNT and ELISA were 25%, 31% and 33% respectively, 'Asia-1' identified were 10, 11 and 11 respectively, type 'C' 3 in each, subtype 'A-22' 3 in each, and cross reacting FMD virus type 'O' with 'A' 1 in CFT and 2 in ELISA respectively.

VIII. FINAL FMD VIRUS TYPING RESULTS

Altogether 131 samples were studied for serotyping of FMD virus by three serological tests. Table-15 depicts the final typing results of the FMD virus by different tests. A maximum of 64 samples (48.85%) revealed the presence of FMD virus serotype 'O' followed by 19(14.50%) type 'Asia-1', 8 (6.11%) type 'A-22' , 7 (5.34%) type 'A', 5(3.82%) type 'C' and 8 were of cross reacting type ('O'/A & 'O'/'Asia-1). A total of 20(15.27%) samples were found to be negative by all the serological test employed in the present work.

Table-14

Comparative efficacy of CFT, SNT and ELISA tests for the detection of FMD virus in clinical materials

Sl. No.	No. of Tests	F	T	G	P	E	Total	Virus type recovered					
								O	A	C	Asia-1	A_{22}	Cross reacting
1.	Total no. of samples	39	23	9	5	2 (100)	78						
2.	No. of positive samples by CFT	22 (56.41)	11 (47.83)	9 (100)	-	2 (100)	44 (56.41)	25	2	3	10	3	1
3.	No. of positive samples by SNT	23 (58.97)	20 (86.96)	9 (100)	-	2 (100)	54 (69.23)	31	5	3	11	3	1
4.	No. of positive samples by ELISA	27 (69.23)	20 (86.96)	9 (100)	-	2 (100)	58 (74.36)	33	5	3	11	3	3

Figures in parenthesis represents percentage value.

Table-15

Showing final FMD virus typing result obtained by combination of all the 3 serological tests

Source of sample	Nature of sample	Total No. of samples tested	FMD virus types recovered						No. of negative sample
			O	A	C	Asia-1	A_{22}	Cross reacting	
FMD affected animals under village condition	Feet epith.	49	22	2	2	3	5	2	13
	Tongue epith.	54	31	4	2	9	3	3	2
	Gum lesion	11	4	1	1	5	-	-	-
	Palate tissue	6	1	-	-	-	-	-	5
	Eye lesion	2	-	-	-	2	-	-	-
FMD affected animals under Farm condition									
a) RAC livestock Farm, Kanke, Ranchi.	Feet epith.	2	-	-	-	-	-	2	-
	Tongue epith.	1	-	-	-	-	-	1	-
b) Govt. Cattle Farm Gauriak-arma, Hazaribagh.	Feet epith.	4	4	-	-	-	-	-	-
	Tongue epith.	2	2	-	-	-	-	-	-
Total :-		131	64 (48.85)	7 (5.34)	5 (3.82)	19 (14.50)	8 (6.11)	8 (6.11)	20 (15.27)

Part -II

Epidemiological Studies

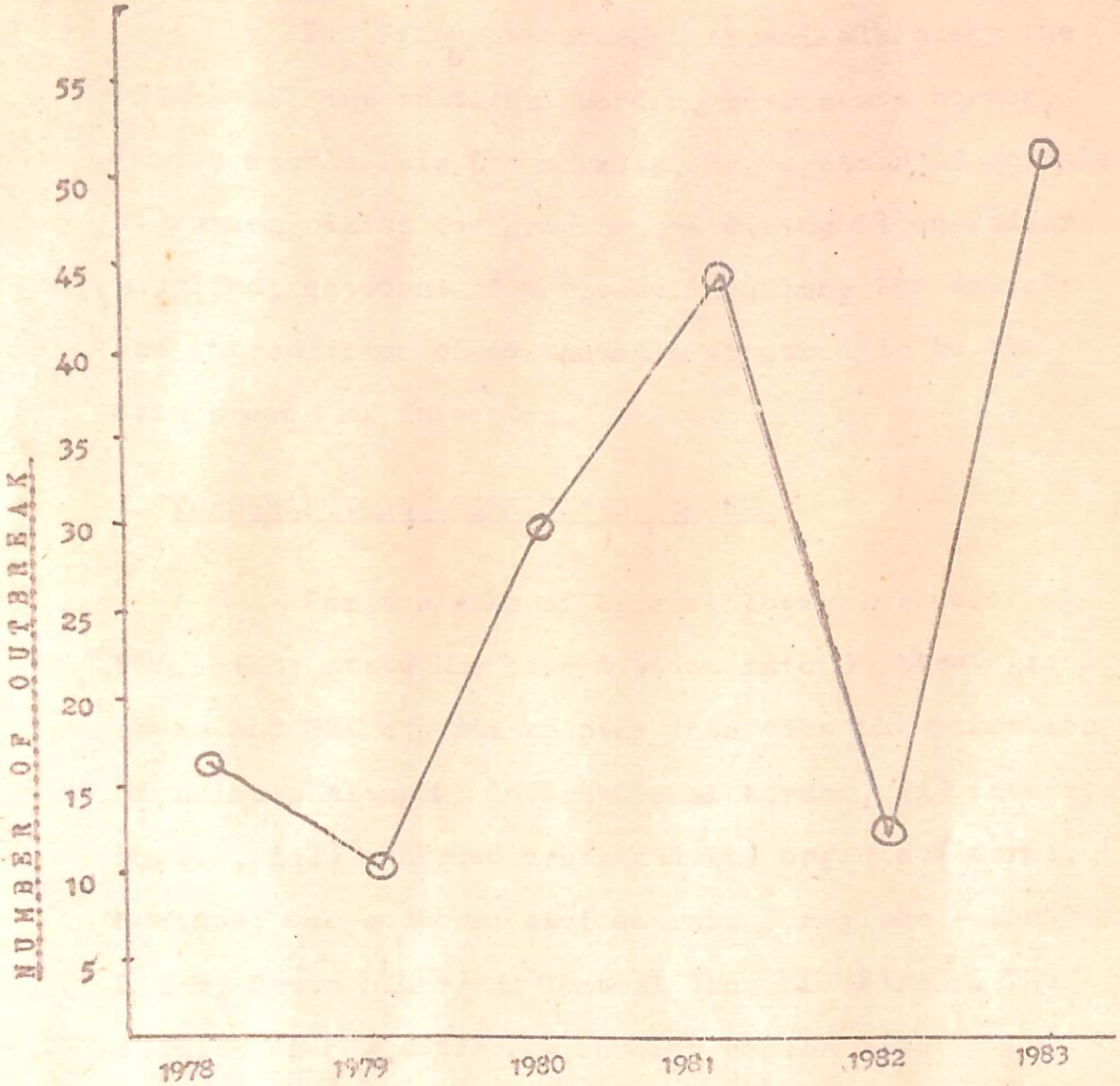
FMD outbreaks were found to be fairly widespread throughout the state of Bihar during the last 3 years (1981 to 1983). A total of 109 outbreaks of this disease were recorded from different districts of the state. Table-16 depicts the incidence of FMD during the years 1981-83. An overall morbidity rate of 7.67% was recorded, while themortality rate in percent was 1.96%. The maximum number of outbreaks was recorded during the year 1983 (53) while only 12 outbreaks were recorded in the year 1981. The morbidity rate in percent was maximum (8.66) during the year 1981 followed by 7.88% in 1983 and 2.01% during 1982. The mortality rate was maximum (8.93%) during the year 1982 followed by low mortality rate of 1.60% and 1.20% during the years 1982 and 1983, respectively. The frequency of outbreaks from 1978-83 has been shown in Graph-3 to depict cyclic pattern of the outbreaks.

An unusual feature observed in three outbreaks during 1982 was the occurrence of haemorrhagic gastroenteritis in FMD affected calves below one year of age. All the animals showing haemorrhagic diarrhoea succumbed to the disease. The stool examination of the animal showing diarrhoea did not show the involvement of any pathogenic bacteria and parasites. Post-mortem examination revealed

Table-16

Incidence of FMD during the years 1981-1983 in Bihar

Sl. No.	Year of outbreak	Total number of outbreak	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Death	Mortality rate (per cent)
1.	1981	45	26,762	2,317	8.66	37	1.60
2.	1982	12	5,595	112	2.01	10	8.93
3.	1983	52	24,785	1,954	7.88	39	1.99
Total :-		109	57,142	4,383	7.67	86	1.96



SEQUENCIES OF FMD OUTBREAK DURING [illegible] - 1983.

(A CYCLIC PATTERN)

Graph - 3

haemorrhagic gastroenteritis as the main gross lesion. The other outbreaks during the period under report showed the typical lesion of FMD.

The frequent movement of animals along the Indo-Nepal international border, interstate border, common cattle fair and markets, cogregation of animals at common places for grazing and during flood, fodder supplied, movement of personnel handling the animals and introduction of new animals appeared to be the main source of infection.

I. TOPOGRAPHICAL DISTRIBUTION OF FMD

For the sake of epidemiological studies of FMD, Bihar state has been divided into 39 strategic areas and 320 cohorts keeping into view the migration of animals along i) international border, ii) interstat border, iii) milkshed areas and iv) organised farms. Besides, the state is divided into 3 regions - North Bihar, South Bihar and Central Gangetic Plains. The details of FMD outbreaks showing region wise distribution during the years 1981-83 has been presented in Table-17. A total of 44 outbreaks could be recorded in North Bihar and South Bihar each while only 21 outbreaks recorded from the Chotanagpur Plateau The morbidity rate in percent was 8.68, 6.77 and 8.19 in North Bihar, South Bihar and Chotanagpur Plateu

Table-17

Topographical incidence of FMD during the year 1981-83 in Bihar

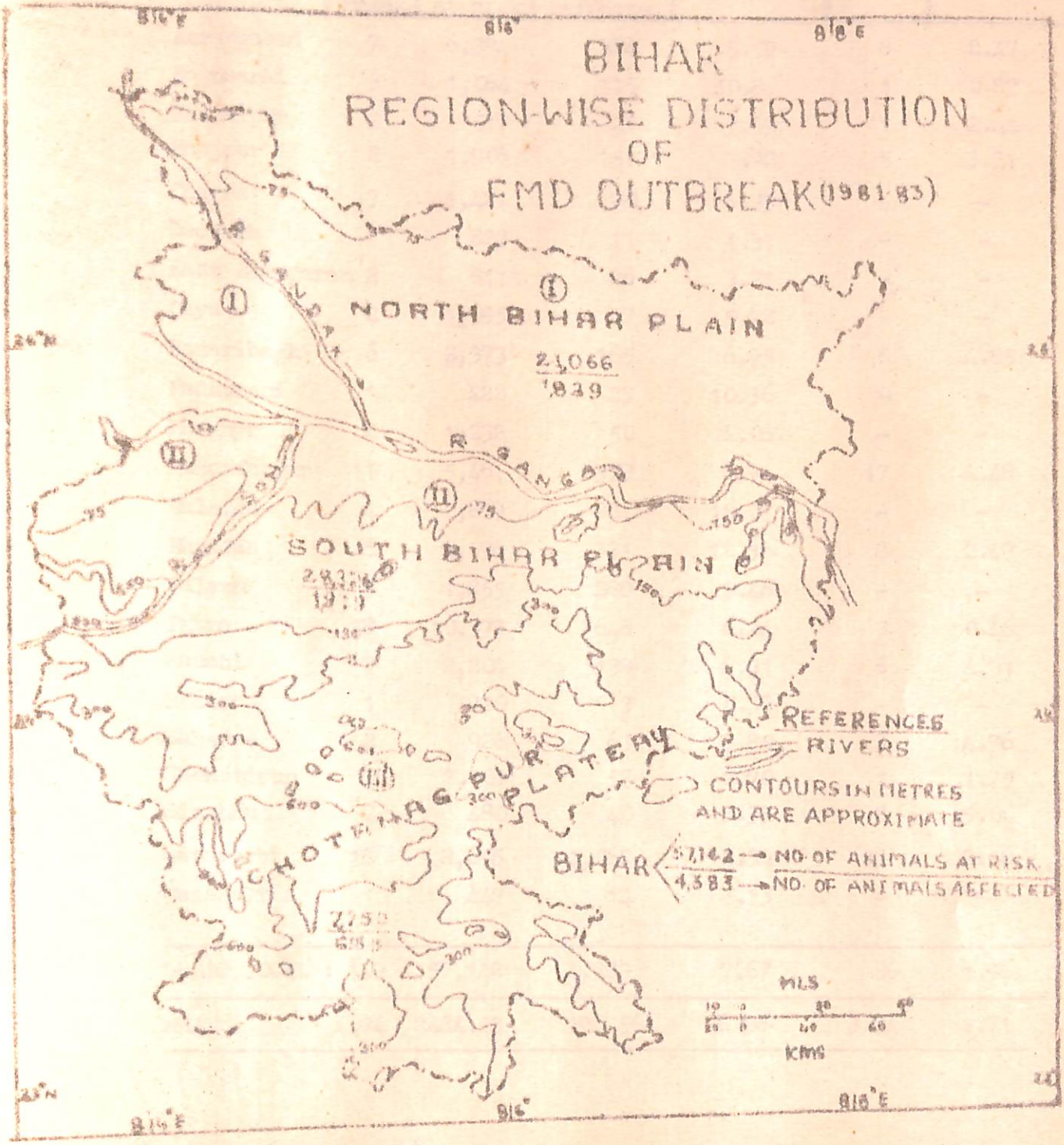
Sl. No.	Region	Number of outbreaks	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Number of death	Mortality rate (per cent)
1.	North Bihar	44	21,066	1,829	8.68	45	2.46
2.	Central Gangetic Plain (South Bihar Plain)	44	28,326	1,919	6.77	25	1.40
3.	Chotanagpur Plateau	21	7,750	635	8.19	16	2.52
Total :-		109	57,142	4,383	7.67	86	1.96

respectively. The statistical analysis revealed that the geographical regions in Bihar state affects the incidence of disease significantly (X^2 = 68.39 P< 0.01). Map-I shows the morbidity pattern in three geographical regions of Bihar.

The district-wise incidence of FMD has been presented in Table-18 and Map-II. The morbidity rate in percent was maximum in Patna district (12.36%) and Sitamarhi (12.34) while lowest morbidity rate in percent was recorded in Rohtas district (0.38). The mortality rate was maximum in Singhbhum where the population density was low. The lowest morbidity rate was observed in Patna district (0.46%).

II. SEASONAL INFLUENCE OF INCIDENCE OF FMD

The temporal distribution of the data revealed seasonal rhythm in the incidence of FMD. It would be obvious from Table-19 that the highest morbidity rate in per cent was during the monsoon (9.97) followed by winter (8.49), summer (6.87) and lowest during post monsoon season (4.96). The mortality rate in percent observed during post-monsoon was 3.03 , winter 2.57, summer 1.74 and 1.52 during the monsoon period.The maximum number of outbreaks occurred during the summer and lowest during

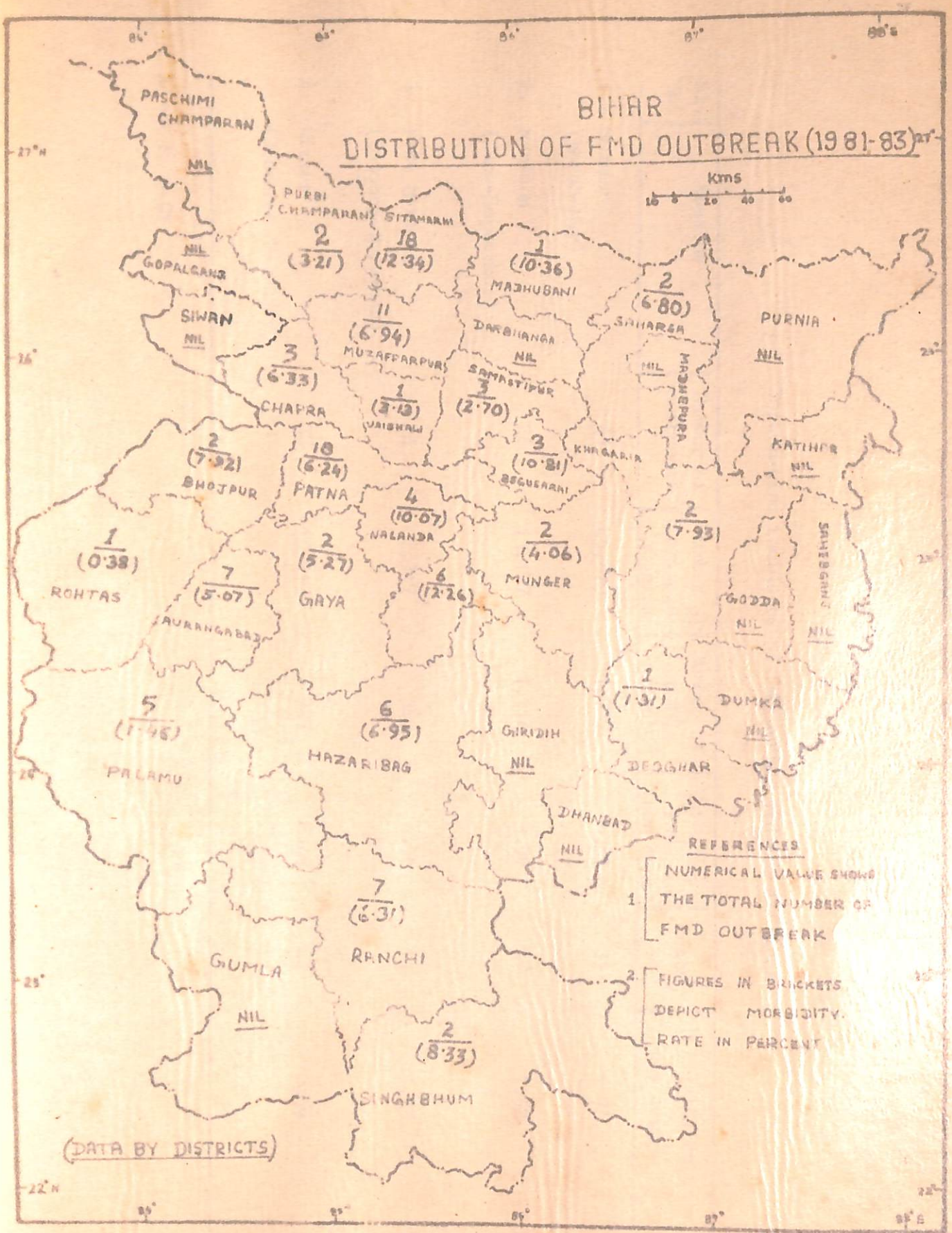

BIHAR
REGION-WISE DISTRIBUTION
OF
FMD OUTBREAK (1981-83)
I
NORTH BIHAR PLAIN
21066
1939
II
SOUTH BIHAR PLAIN
CHOTANAGPUR PLATEAU
R. GANGA
REFERENCES
RIVERS
CONTOURS IN METRES
AND ARE APPROXIMATE
BIHAR
21142 → NO. OF ANIMALS AT RISK
4583 → NO. OF ANIMALS AFFECTED
MLS
KMS

MAP I

Table-18

District-wise incidence of FMD during 1981-83 in Bihar

Districts	No. of Cut-break	No. of animals at risk	No. of animals affected	Morbidity per cent	No. of death	Mortality
Aurangabad	7	6,394	324	5.07	8	2.47
Begusarai	3	1,064	115	10.81	1	0.87
Bhagalpur	2	517	41	7.93	1	2.44
Bhojpur	2	1,906	151	7.92	5	3.31
Chapra	3	1,421	90	6.33	-	-
Deogarh	1	823	11	1.31	-	-
East Champaran	2	811	26	3.21	-	-
Gaya	2	1,695	97	5.72	-	-
Hazaribagh	6	2,373	165	6.95	8	4.85
Madhubani	1	222	23	10.36	-	-
Monghyr	2	1,232	50	4.06	-	-
Muzaffarpur	11	5,461	379	6.94	17	4.48
Nalanda	4	2,819	284	10.07	-	-
Nawadah	6	2,596	321	12.36	8	2.49
Palamu	5	1,855	280	1.46	-	-
Patna	18	10,377	648	6.24	3	0.46
Ranchi	7	2,204	139	6.31	6	4.31
Rohtas	1	790	3	0.38	-	-
Saharsa	2	926	63	6.80	3	4.76
Samastipur	3	2,109	57	2.70	1	1.75
Singbhum	2	480	40	8.33	2	5.00
Sitamarhi	18	8,605	1,062	12.34	23	2.16
Vaishali	1	447	14	3.13	-	-
GRAND TOTAL :	109	57,142	4,383	7.67	86	1.96
MEAN	4.74	2484.43	190.56	6.38	3.74	1.71


BIHAR
DISTRIBUTION OF FMD OUTBREAK (1981-83)
Kms
0 20 40 60
PASCHIMI CHAMPARAN
NIL
PURBI CHAMPARAN
2 (3·21)
SITAMARHI
18 (12·34)
1 (10·36)
MADHUBANI
GOPALGANJ
NIL
SIWAN
NIL
11 (6·94)
MUZAFFARPUR
DARBHANGA
NIL
2 (6·80)
SAHARSA
PURNIA
NIL
3 (6·33)
CHAPRA
1 (3·13)
VAISHALI
3 (2·70)
SAMASTIPUR
MADHEPURA
NIL
2 (7·92)
BHOJPUR
18 (6·24)
PATNA
3 (10·81)
BEGUSARAI
KHAGARIA
KATIHAR
NIL
4 (10·07)
NALANDA
2 (7·93)
1 (0·38)
ROHTAS
2 (5·27)
7 (5·07)
GAYA
AURANGABAD
6 (12·26)
2 (4·06)
MUNGER
GODDA
NIL
SHEIKHPURA
NIL
1 (1·31)
DUMKA
NIL
5 (1·46)
PALAMU
6 (6·95)
HAZARIBAG
GIRIDIH
NIL
DEOGHAR
DHANBAD
NIL
REFERENCES
1 NUMERICAL VALUE SHOWS THE TOTAL NUMBER OF FMD OUTBREAK
2 FIGURES IN BRACKETS DEPICT MORBIDITY RATE IN PERCENT
7 (6·31)
RANCHI
GUMLA
NIL
2 (8·33)
SINGHBHUM
(DATA BY DISTRICTS)

MAP - II

the post monsoon. The statistical studies reveals that the seasons significantly influence the incidence of FMD in this state ($X^2 = 201.15$, $P < 0.01$).

The month-wise incidence of FMD has been presented in Table-20. It is evident that maximum number of outbreaks occurred in the month of December (21) followed by April (17), March (14), June (15), November (12), May (10) , February and August (5 each), July and September (3 each) and January and October (2 each). The morbidity rate in percent varied from 13.93 to 2.21 with a mean of 6.75 . Comparative data on the effect of seasons on FMD outbreak during 1981-83 has been depicted in Graph-4.

III. SPECIES-WISE INCIDENCE OF FMD

Table-21 depicts the species-wise distribution of FMD outbreaks during the years 1981-83. The maximum number of cattle (26,627) were at risk of which 3,854 went down with the disease followed by buffaloes (13,903/506) and goats (9585/23) while sheep and pigs could not pickup the infection and so no outbreak could be recorded. The morbidity rate in percent was maximum in cattle (14.47) followed by buffaloes (3.64) and goats (0.24). The mortality rate in percent was maximum in goat (4.35) followed by cattle (2.05) and

Table-20

Month-wise incidence of FMD during 1981-83 in Bihar

Sl. No.	Months	Number of outbreaks	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Number of death	Mortality rate (per cent)
1.	January	2	543	12	2.21	-	-
2.	February	5	2,022	120	5.93	8	6.66
3.	March	14	7,718	519	6.72	8	1.54
4.	April	17	7,609	903	11.87	13	1.44
5.	May	10	10,552	357	3.38	10	2.80
6.	June	15	6,755	941	13.93	14	1.49
7.	July	3	1,907	162	8.49	2	1.23
8.	August	5	2,611	158	6.05	3	1.90
9.	September	3	1,904	53	2.78	1	1.89
10.	October	2	1,306	61	4.67	-	-
11.	November	12	5,867	302	5.15	11	3.64
12.	December	21	8,348	796	9.52	16	2.01
Total :-		109	57.142	4,383	7.67	86	1.96
Mean		9.08	4761.83	365.25	6.75	7.17	2.05

Graph - 4.

MONTH-WISE INCIDENCE OF FMD (1981 - 83)

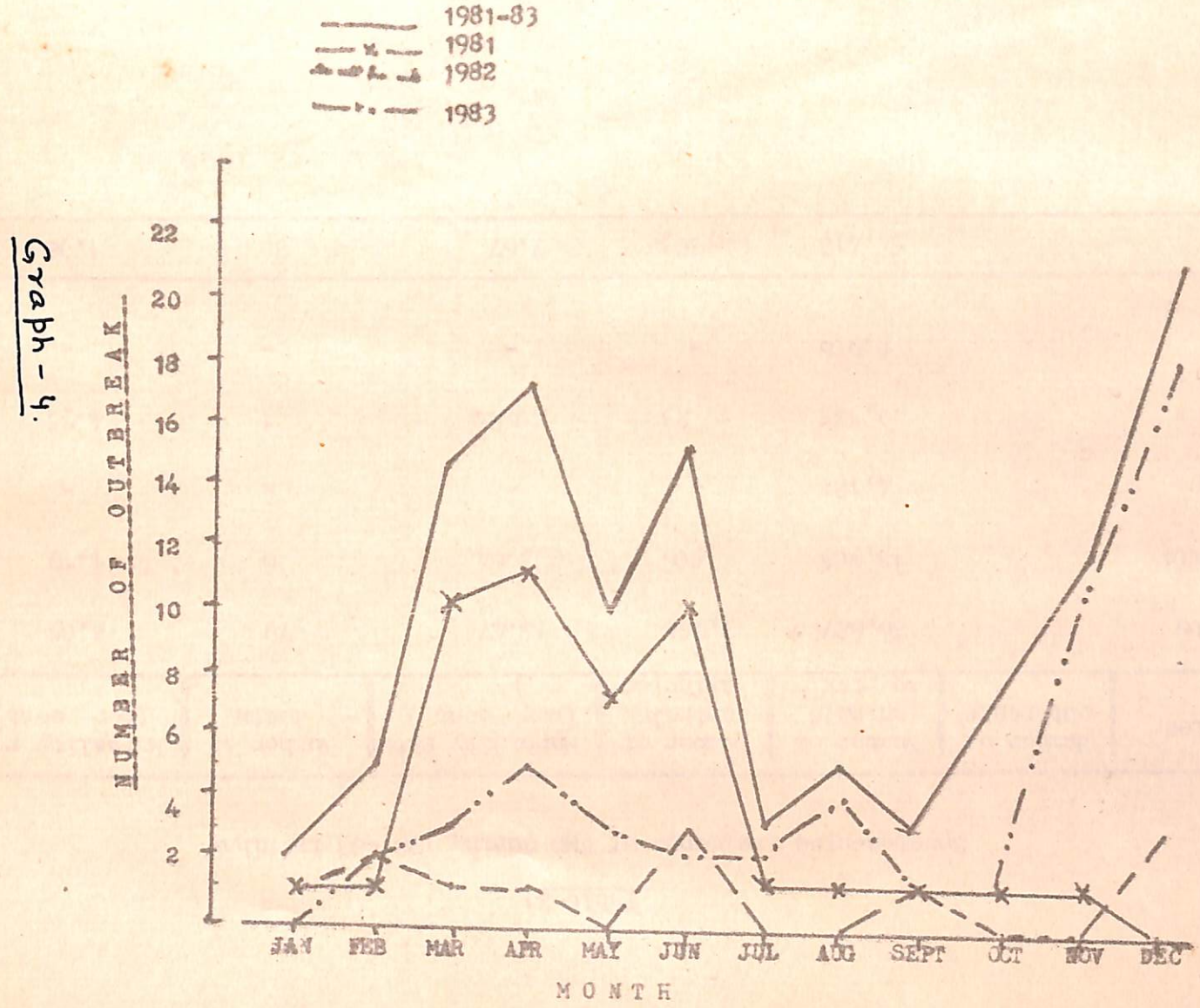


Table-21

Species-wise incidence of FMD during 1981-83 in Bihar

Sl. No.	Species	Number of outbreaks	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Number of death	Mortality rate (per cent)
1.	Cattle		26,627	3,854	14.47	79	2.05
2.	Buffalo		13,903	506	3.64	6	1.19
3.	Sheep		4,121	-	-	-	-
4.	Goat		9,585	23	0.24	1	4.35
5.	Pig		2,906	-	-	-	-
Total :-			54,412	4,383	7.67	86	1.96

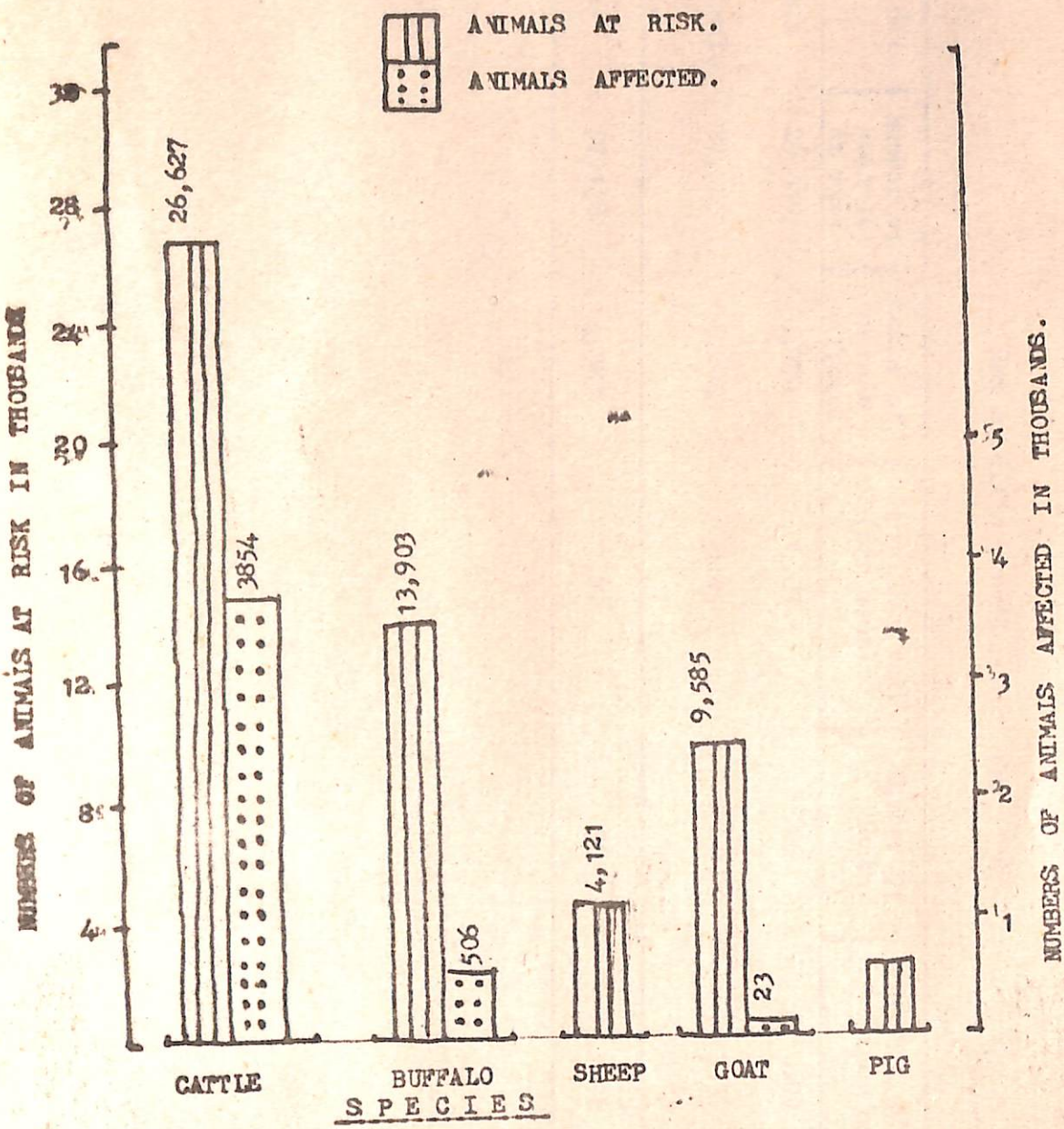
buffaloes (1.19). Statistical significantly influence the incidence of FMD ($P < 0.01$) . Species wise morbidity pattern has been showin Graph-5.

IV. SEX SPECIFIC PREVALENCE OF FMD

Altogether 29,519 male animals were at risk of which 1,779 animals went down with the disease with an morbidity rate of 6.03%. Among females altogether 27,623 animals were at risk of which only 2,604 animals could pickup the disease with the morbidity rate of 9.43. Table-22 depicts the sex specific prevalence of FMD during the period under report. The statistical analysis of these data revealed that the sex of animals significantly affects the incidence of disease ($P < 0.01$).

The sex specific prevalence of FMD among different species of animals has been presented in Table-23. The female cattle was found to be more susceptible to the disease than male, morbidity rate being 22.80% in females and 9.96% in males. Among buffaloes and goats, the morbidity rate was more in females (4.25% and 0.31%) than in males (1.93% and 0.18%). In all 53 deaths were reported in females cattle with mortality rate of 3.98%, whereas in male cattle only 26 deaths were recorded, the mortality rate being 1.03% only.

SPECIES-WISE MORBIDITY PATTERN OF FMD



Graph - 5

Table-22

Sex specific prevalence of FMD (1981-83)

Sl. No.	Sex	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Number of death	Mortality rate (per cent)
1.	Male	29,519	1,779	6.03	26	0.99
2.	Female	27,623	2,604	9.43	60	3.37
Total :-		57,142	4,383	7.67	86	1.96

Table-23

Sex specific prevalence of FMD during 1981-83 in Bihar

Sl. No.	Species	Sex	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Number of deaths	Mortality rate (per cent)
1.	Cattle	Male	17,050	1,699	9.96	26	1.03
		Female	9,577	2,155	22.50	53	3.98
2.	Buffaloes	Male	3,668	71	01.93	-	-
		Female	10,235	435	04.25	6	1.38
3.	Sheep	Male	2,159	-	-	-	-
		Female	1,962	-	-	-	-
4.	Goats	Male	5,117	9	0.18	-	-
		Female	4,468	14	0.31	1	7.14
5.	Pig	Male	1,525	-	-	-	-
		Female	1,381	-	-	-	-
Total :-			57,142	4,383	7.67	86	1.96

The mortality rate among females buffaloes and goats was 1.38% and 7.14%, respectively whereas no death could be recorded among male buffaloes and goats.

V. AGE SPECIFIC INCIDENCE OF FMD

Age-wise epidemiological data revealed that almost all age groups of animals were affected with FMD during the years, 1981-83 (Table-24). The morbidity rate was lowest (1.12%) in animals below 6 months of age in which 97 animals out of 8,618 went down with the disease. The morbidity rate in percent among the animals between 6 months oto 12 months of age was 4.61. Maximum number of animals affected with the disease were above one year of age in which group the morbidity rate was highest (10.15%). Graph-6 depicts the morbidity pattern of FMD among different age groups of animals.

The mortality rate was highest (52.57%) among animals below 6 months of age and lowest (0.21%) in the animals above one year of age. The statistical analysis of the epidemiological data revealed that the age of animals significantly influences the incidence of the disease ($P < 0.01$).

VI. TYPOGRAPHICAL STUDIES

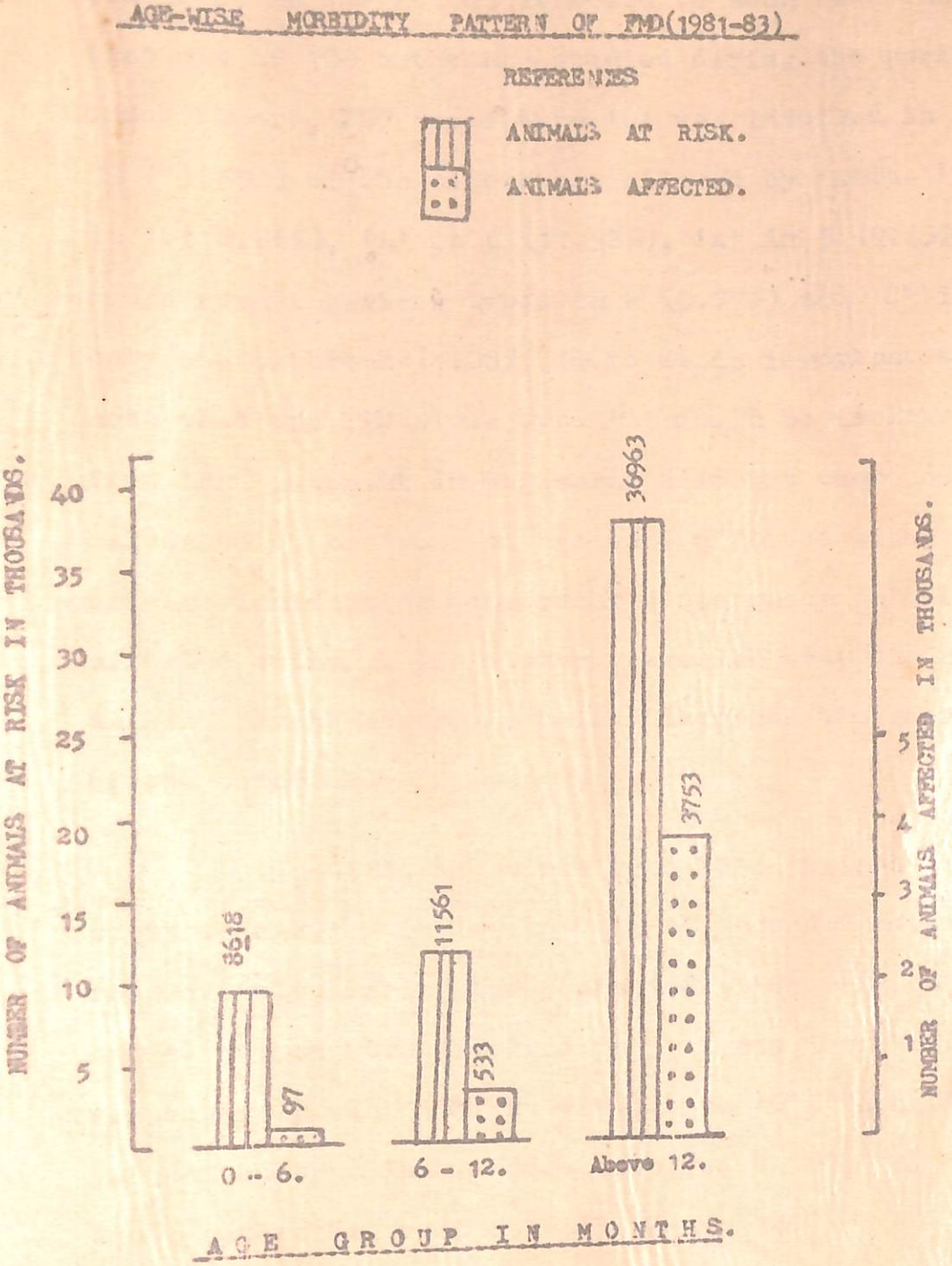
The typographical studies of FMD in the

Table-24

Age specific prevalence of FMD

Sl. No.	Age group in months	Number of animals at risk	Number of animals affected	Morbidity rate (per cent)	Number of death	Mortality rate (per cent)
1.	0 - 6	8,618	97	1.12	51	52.57
2.	6 -12	11,561	533	4.61	27	5.06
3.	Above 12	36,963	3,753	10.15	8	0.21
Total :-		57,142	4,383	7.67	86	1.96

AGE-WISE MORBIDITY PATTERN OF FMD(1981-83)



Graph - 6

state of Bihar during the years 1981-83 revealed that FMD virus types 'O' and 'Asia-1' were more prevalent in the state. It would be seen from Table -25 that out of 109 outbreaks studied during the period under report, FMD virus type 'O' was involved in 39 (73.58%) of the outbreaks followed by 'Asia-1' in 10(18.86%), 'A' in 6 (11.32%), 'A' in 5 (9.43%), cross reacting virus types in 2 (3.77%) and 'C' in only one outbreak (1.88%). Here it is important to note that the FMD virus type 'C' could be isolated from lone outbreak in Begusarai district where only calves below one year of age were affected with the disease manifesting haemorrhagic diarrhoea in all FMD affected animals. The history revealed that the new animals purchased from outside Bihar was the source of the outbreak.

In 1981, FMD virus type 'O', 'Asia-1' and subtype 'A-22' were involved in 17, 6 and 2 outbreaks respectively. During the years 1982, 'O', 'Asia-1' and 'C' types were involved in 5, 2 and 1 outbreaks, respectively. In 1983 FMD virus type 'O', 'Asia-1', 'A' and subtype 'A-22' were involved in 17, 10, 5 and 6 outbreaks, respectively (Graph-7).

(i) <u>District-wise Distribution of FMD Virus types</u>

The distribution of different types

Table-25

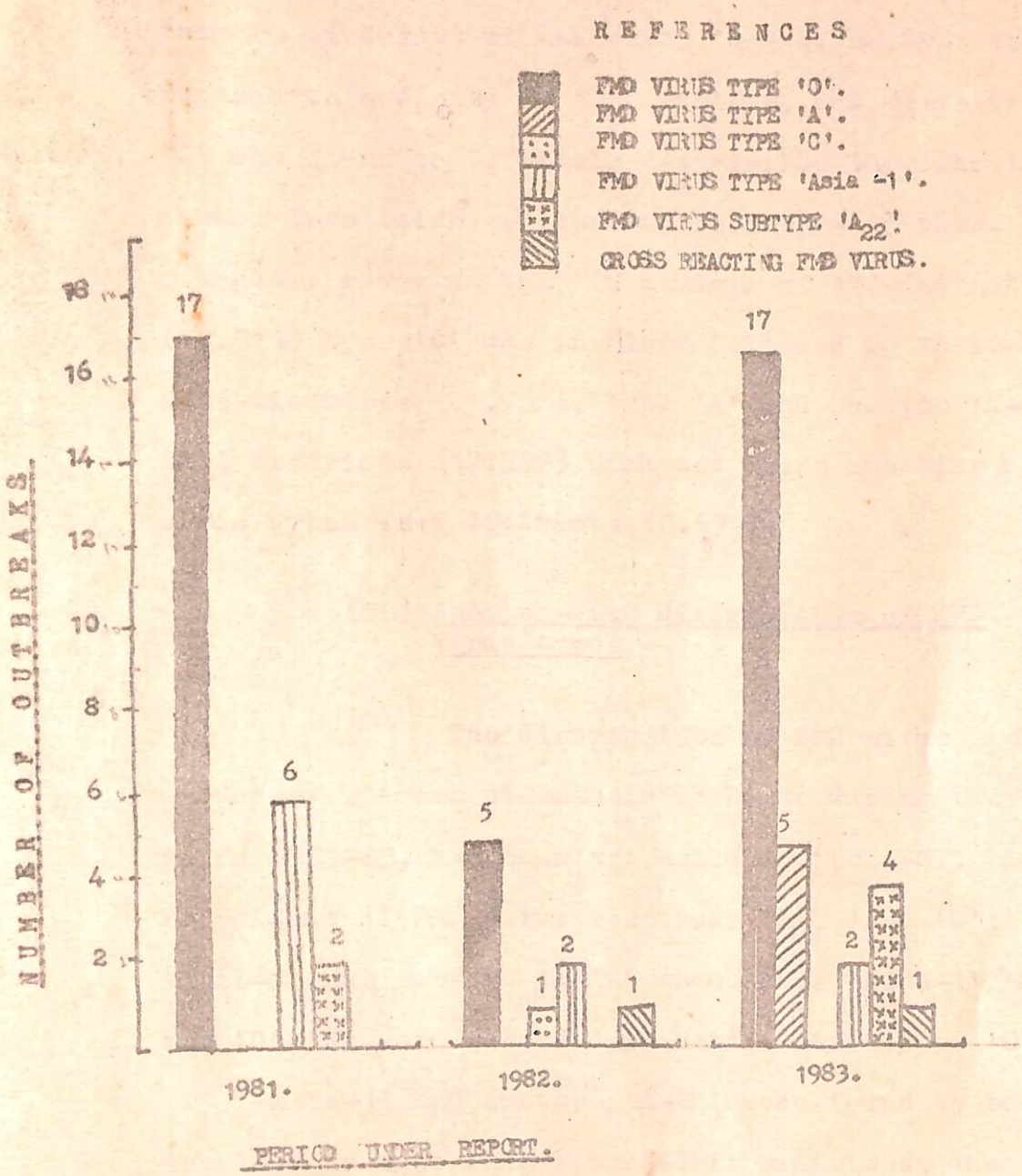
Distribution of FMD virus types in Bihar during 1981-83

Sl.	Year	FMD virus types recovered					
		'O'	'A'	'C'	'Asia-1'	'A-22'	'Cross reacting'
1.	1981	17	-	-	6	2	-
2.	1982	5	-	1	2	-	1*
3.	1983	17	5	-	2	4	1**
Total :-		39	5	1	10	6	2

* : FMD virus type 'Asia-1' cross reacting with 'O'

**: FMD virus type 'O' cross reacting with 'A'.

PREVALENT FMD VIRUS TYPES DURING THE YEAR 1981 - 83.



Graph: 7

of FMD virus prevalent in different districts of Bihar state during 1981-83 has been shown in Table-26 and Map-III. Altogether 109 outbreaks were recorded from 23 districts of the state and virus type could be ascertained only in 17 districts. FMD virus type 'O' was found to be widely distributed throughout the state. Involvement of various serotypes in these outbreaks revealed that in maximum of 17 district (73.91%) type 'O' was involved followed by 'Asia-1' in 5 districts (21.73%), type 'A' and subtype 'A-22' in 4 districts (17.39%) each and cross reacting FMD virus types in 2 districts (8.69%).

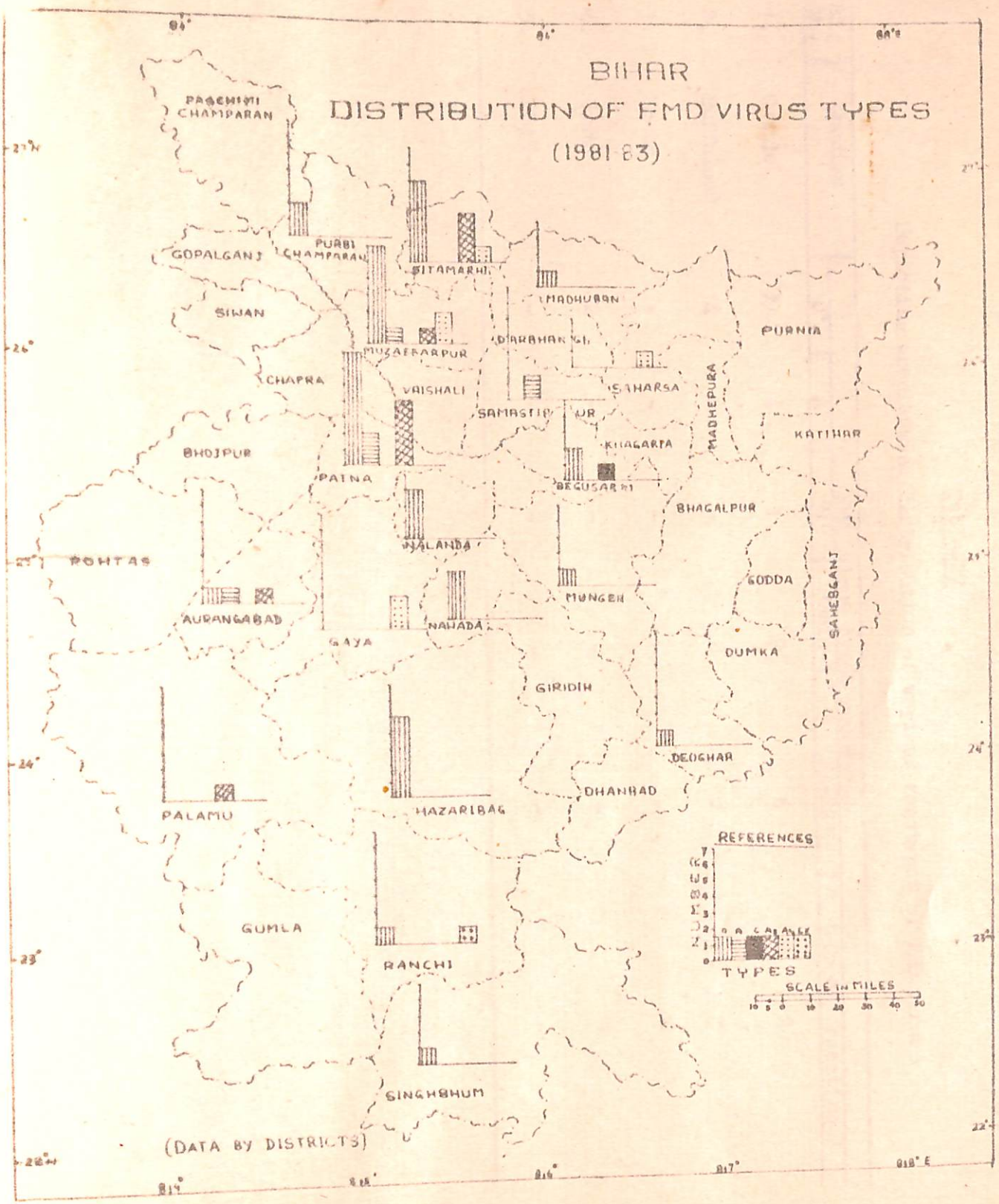
(ii) Species-wise Distribution of FMD Virus types

The distribution of FMD virus among different species of animals in Bihar during the years 1981-83, has been presented in Table-27. Prevalence of FMD virus serotypes 'O', 'A', 'C', 'Asia-1' and subtype 'A-22' as well as 'Asia-1'/'O' and 'O'/'A' were recorded in cattle while type 'O' 'A', 'Asia-1' and subtype 'A-22' were found to be involved in outbreaks recorded in buffaloes. The two outbreaks recorded in goats revealed the involvement of FMD virus type 'O'.

Table-26

Districtwise distribution of FMD virus types

Sl. No.	District	Types of FMD virus recovered					
		O	A	C	Asia-1	A_{22}	Cross reacting
1.	Aurangabad	1	1	-	1	-	-
2.	Begusarai	2	-	1	-	-	-
3.	Bhagalpur	-	-	-	-	-	-
4.	Bhojpur	-	-	-	-	-	-
5.	Chapra	-	-	-	-	-	-
6.	Deogarh	1	-	-	-	-	-
7.	East Champaran	2	-	-	-	-	-
8.	Gaya	-	-	-	-	2	-
9.	Hazaribagh	5	-	-	-	-	-
10.	Madhubani	1	-	-	-	-	-
11.	Monger	1	-	-	-	-	-
12.	Muzaffarpur	6	1	-	1	2	1
13.	Nalanda	3	-	-	-	-	-
14.	Nawadah	3	-	-	-	-	-
15.	Palamu	-	-	-	1	-	-
16.	Patna	7	2	-	4	-	-
17.	Ranchi	1	-	-	-	-	1
18.	Rohtas	-	-	-	-	-	-
19.	Saharsha	-	-	-	-	1	-
20.	Samastipur	-	1	-	-	-	-
21.	Singhbhum	1	-	-	-	-	-
22.	Sitamarhi	5	-	-	3	1	-
23.	Vaishali	-	-	-	-	-	-
	Total:-	39	5	1	10	6	2


BIHAR
DISTRIBUTION OF FMD VIRUS TYPES
(1981-83)
PASCHIMI CHAMPARAN
PURBI CHAMPARAN
GOPALGANJ
SIWAN
SITAMARHI
MADHUBAN
DARBHANGA
PURNIA
MUZAFFARPUR
CHAPRA
VAISHALI
SAHARSA
SAMASTIPUR
MADHEPURA
KATIHAR
KHAGARIA
BHOJPUR
PATNA
BEGUSARAI
BHAGALPUR
NALANDA
ROHTAS
GODDA
MUNGER
SAHEBGANJ
AURANGABAD
NAWADA
GAYA
DUMKA
GIRIDIH
DEOGHAR
DHANBAD
PALAMU
HAZARIBAG
REFERENCES
TYPES
GUMLA
RANCHI
SCALE IN MILES
SINGHBHUM
(DATA BY DISTRICTS)

MAP III

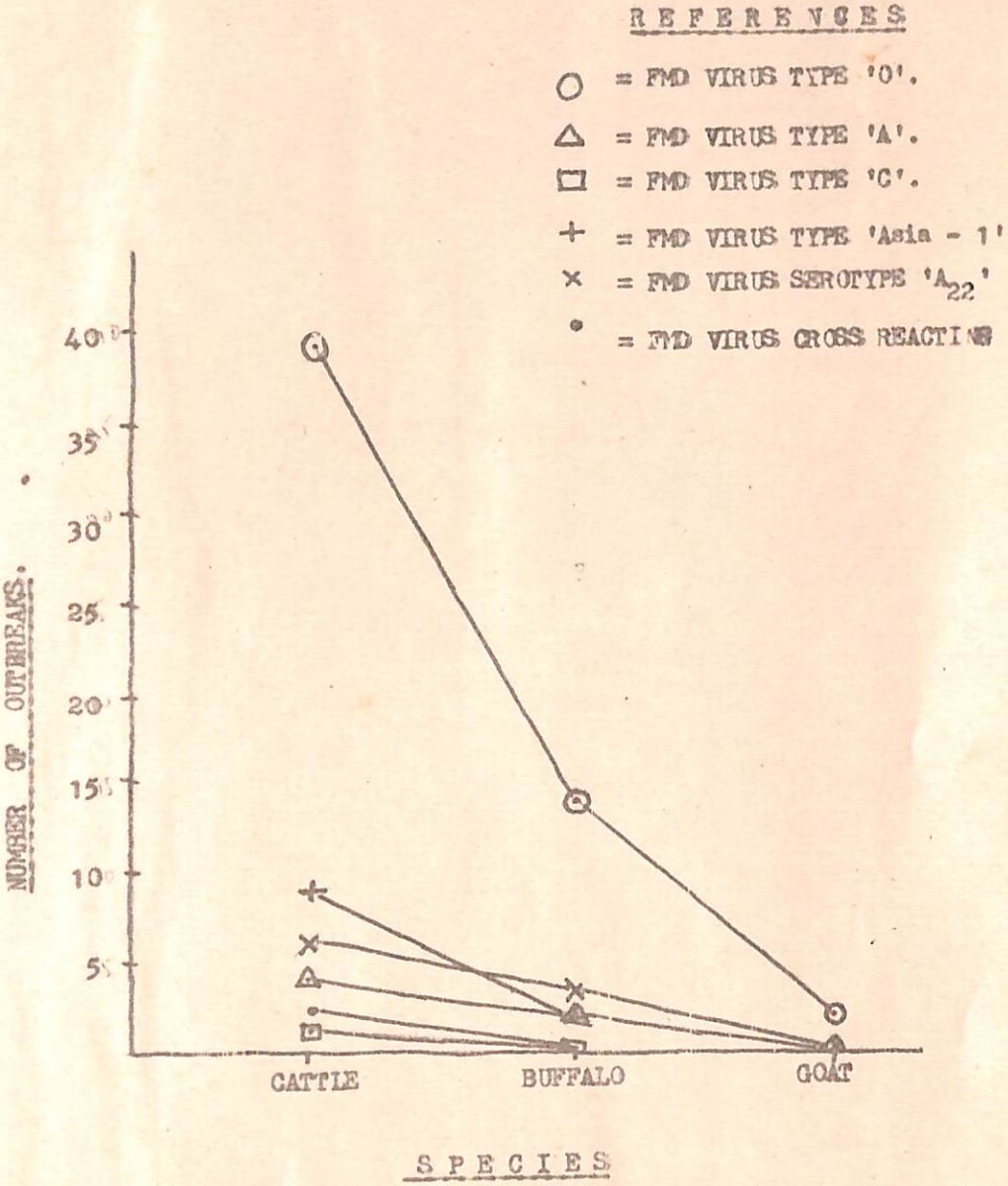
Table-27

Depicting specieswise distribution of various serotypes of FMD virus

Sl. No.	Species	FMD virus types recovered					
		'O'	'A'	'C'	'Asia-1'	'A-22'	' Cross reacting '
1.	Cattle	39	4	1	9	6	2
2.	Buffaloes	14	2	-	2	3	-
3.	Sheep	-	-	-	-	-	-
4.	Goat	2	-	-	-	-	-
5.	Pig	-	-	-	-	-	-

It would be obvious from Graph-8 that the FMD virus types 'O', 'A', 'C', 'Asia-1' and subtype 'A-22' were involved in 39, 4, 1, 9 and 6 outbreaks, respectively in cattle. Besides involvement of cross reacting type 'Asia-1'/'O' and 'O'/'A' was revealed in two outbreaks. In buffaloes FMD virus type 'O' was involved in 14 outbreaks followed by 'A-22' in 3 and type 'A' and 'Asia-1' in 2 outbreaks each. In goats both the outbreaks revealed the involvement of FMD virus type 'O'. Pigs and sheep could not pickup the disease.

SPECIES - WISE DISTRIBUTION OF FMD VIRUS TYPES



Graph: 8

D I S C U S S I O N

It has now been established that quick and timely detection of virus serotypes is essential for an effective control of FMD specially in endemic areas like India. Various serological tests have been employed for the detection and typing of FMD virus. For the quick, timely, accurate and reliable results, it is essential that the serological tests to be employed should offer definitive results in majority of cases (a) on the original test samples without further passage of virus, (b) should be sensitive and capable of detecting the specific antigen even in small quantities and (c) should be affected by low grade of contamination by heterotypic virus.

The results of the present investigation indicates that out of 131 samples, only 53 (40.46%) revealed the presence of FMD virus on the original test samples, while the majority of 78 (59.54%) samples were declared negative on account of samples being anticomplementary, or containing low quantity of virus or no virus at all. To assess the comparative efficacy of suckling mouse and tissue culture system in recovery of the residual virus from the original samples on the one hand, and to ascertain the relative efficiency of MCFT, SNT and ELISA test in detecting and typing of FMD virus , on the other hand all 78 MCFT negative samples were passaged through suckling

mice and tissue culture simultaneously and the
preparations were subjected to MCFT, SNT and ELISA
for serotyping. Out of 78 smples of mouse passaged
44(56.41%) samples yielded the virus which could be
serotyped by the MCFT while the remaining 34 (43.59%)
samples were declared negative. It is interesting to
note that out of 78 MCFT negative samples passaged
three times in cell culture (BHK-21 clone 13), 54(69.23%)
samples produced CPE and the virus materials could be
serotyped using SNT . Of the remaining 24 samples,
20 proved non viable while 4 samples although produced
CPE in lower dilutions but did not show any evidence
of significant neutralization with virus specific
antiserum and as such were also treated as negative.
To assess further the efficacy of ELISA as compared
to MCFT and SNT, the same 78 MCFT negative samples
were tested by ELISA. It is worthwhile to mention that
the largest number of samples, 58(74.35%) could be
typed by this test and only 20 samples remained untyped.
ELISA was found to be sensitive for original materials,
mouse passaged as well as tissue culture passaged
virus materials and at the same time it was found to
be more simple, reliable, accurate and less time
consuming for the quick detection of FMD virus. The
details of discussion on each aspect has been presented
on the following pages.

The perusal of the results of serotyping by MCFT (Table-8) of original samples makes it amply clear only 53 samples (40.46%) could be typed, while majority of 78 (59.54%) samples remained untyped. The present findings is neither alarming nor suprising rather it merely shows the extent upto which MCFT can detect the virus in the original samples. Our finding is more or less in agreement with that of Rweyemamu et al. (1978) . The variation in the percentage of results might be due to the fact that in the present investigation only 131 samples were compared whereas the result of Rweyemamu et al. (1978) was based on 603 samples.

The tongue epithelium appeared to be the most suitable materials for typing of FMD virus by MCFT because 59.64% of the tongue materials could be typed whereas only 29.09% of feet epithelium, 18.18% of gum materials and 16.66% of plate materials could be typed. None of the two eye samples could be typed by MCFT. Korn (1957) reported the detection of FMD virus from oral mucus membrane in 17.07% of the samples. In the present study altogether 50% of the oral materials yielded the presence of FMD virus. This difference is probably due to the fact that Korn isolated the virus during the incubation period of the disease.

It is pertinent to mention here that in the present study out of 78 MCFT negative samples, 41(31.29%) samples proved to be anticomplementary and 37(28.24%) samples proved to be negative. The present findings slightly differs from the Rweyemamu et al. (1978) who reported 17% samples with anticomplementary effect and 22% proved negative. Again, such differences are bound to occur in biological studies since conditions remain variable at different places and from laboratory to laboratory. Such variations are also positive as no single important factor has been detected which exert the anticomplementary effect.

MCFT, of course, was simple and easy to conduct for serotyping but was less efficient in detecting the virus from samples having lower concentration of virus. Forman (1974) opined that the error in microplate test was quite acceptible for routine differentiation of field strains of FMD virus if the test were sufficiently replicated. Darbyshire et al. (1972) compared the macro and micro technique for CFT and found the result to be comparable. The micro technique was considered to be economical with reagents and capable of similar accuracy.

In the present study out of 131 samples, 5 proved to be of cross reacting types ('Asia-1'/'O'). Sarma et al. (1977) studied some of the cross reacting isolates primarily with CFT, SNT and plaque assay in calf kidney cell culture and described cross reactions due to a simple mixture of two or more types of virus which appears to be most convincing.

During the present study the amount of complement and haemolytic system fixed was 3 units and 4 MHD/ml respectively. Rai (1980) employed 2.5 units of complement and 4 MHD/ml of haemolysin with satisfactory result. Rao et al. (1983) modified MCFT for typing of FMD virus in which 25/μl each of antiserum, antigen, complement and haemolytic system were used making a total of 0.1 ml of the reagents.

The possible causes of samples remaining untyped in this study could be attributed to two factors, either there might have been low concentration of virus in the original samples in capable to make enough antigen-antibody complex needed to fix complement or there might have been complete absence of the virus in the sample. Accordingly, assuming above hypothesis such samples (78) were passaged through unweaned mice to get the residual virus replicated. After subjecting the samples to the

maximum of three passages in suckling mice, carcass antigens, the mice were serotyped by MCFT which was able to type 44 (56.41%) out of 78 samples. This indicates that the MCFT failed to detect the FMD virus in the majority of the original samples because of the presence of lower amount of the virus which got concentrated after repeated mouse passage. The usefullness of suckling mice for the propagation of FMD virus have been reported by several workers (Skinner, 1951; Giraud & Giaccio, 1952; Kotsche, 1955; Graves and Poppension, 1960). However, serotyping by mCFT of only original materials is risky because it may give false negative result thus leading to unrestricted spread of the disease. Therefore passage through mice, or certain other living system might be considered necessary specially in case of sample having low concentration of virus. Skinner (1952) also reported the increase in virus titre after mouse passage.

The affected mice showed typical symptoms of FMD infectious i.e., paralysis of muscles of hind quarter and respiratory distress followed by death after 24 hours and within 48 to 72 hours. This type of clinical symptoms are in agreement with Skinner (1951) and Sehgal (1969).

Altogether 34 (43.59%) of the 78 mouse passaged samples showed either atypical symptoms (15) or no clinical symptoms (19) and could not be typed by MCFT and as such were treated as negative. The number of samples exhibiting atypical symptoms were rather found to the increasing from 4 in the first passage to 13 during the second passage and 15 in third passage. Even after 3 passages in mice, these samples gave negative results in the MCFT. Tewari et al. (1980) encountered 20% of the samples showing irregular mortality or other atypical symptoms in mice and suggested passage of such erratic samples in bovine kidney cell culture to improve the rate of virus recovery.

The third portion of 78 samples declared negative by direct MCFT, were processed for passage through tissue culture system (BHK-21). After cell culture passage, 54 of the 78 samples showed typical CPE in BHK-21 cells while the rest 24 samples did not show any CPE (Table- 11). This findings clearly indicates that cell culture passage is superior than mice for propagation of antigenic materials as 10 more samples produced clearcut CPE as compared to the mouse passage. Capstick et al. (1962), Mowat and Chapman (1962), Telling and Elsworth (1965) also

reported susceptibility of BHK-21 cells to the FMD virus and found it suitable for propagation of the virus. Clarke and Spier (1980) observed the variation in the susceptibility of BHK-21 population to three strains of FMD virus. Again, Clarke and Spier (1983) reported that the reduced ability of apthovirus to replicate in BHK-21 cell line was due to restriction occurring during synthesis and processing of virus macromolecules.

Superiority of bovine kidney cell culture over mice for recovery of virus has been reported by various other workers (Patty et al. 1965; Cottral et al. 1966; Tewari et al.,1980). In the present investigation it was observed that virus could be recovered from 29.41% of the samples which were either non viable or were giving irregular mortality in unweaned mice. Tewari et al. (1980) recovered FMD virus from 20% samples which were either non viable or giving irregular mortality. The differences of susceptibility of cell lines might be sequelae to use of different types of cell culture since BHK 21 cell line was used in the present study while Tewari (1980) used bovine kidney cell culture.

Superiority of other cell culture systems over mice has also been reported by various other

workers. Chapman and Ramshaw (1971) reported that susceptibility of IB-rS-2 and BHK-21 cells was higher than that observed in unweaned mice. However, in the present study IB-RS-2 cell line was not found to give encouraging result as even on third passage in this cell line virus materials took more than 48 hours to exhibit CPE. Further studies may be required to see the suitability of IB-RS-2 for the recovery of FMD virus from field samples. Susceptibility of calf thyroid (CT) monolayer culture to the FMD virus was studied by Snowdon (1966) and higher titre was obtained in CT cultures than pig or baby hamsters kidney cultures or unweaned mice and cattle tongue epithelium. Mackowiak and Land (1958) observed that titration in porcine kidney cell culture was more sensitive, quicker and accurate than in mice. Thus, depending upon the facilities available in the laboratory BHK 21 or other cell cultures systems may be tried as an alternative to the unweaned mice because it is more susceptible, accurate and less costly.

The BHK 21 cell cultures adapted virus preparations were subjected to serum neutralization test. Consequently, 10 more samples were found to be positive which were declared negative by MCFT even after mouse passage. This 10 samples included 9 tongue epithelium and one feet epithelium samples.

It indicates that tongue epithelium samples are more susceptible to the BHK 21 cell culture passage. Rweyemamu et al. (1978) also observed that in 89% cases MNT resulted in positive typing whereas only in 43% cases positive typing was obtained by CFT.

Heterotypic reactions have been observed in neutralization test (Cottral,1972). These only accounted for a small proportion of the total samples. In our case it accounted for only 2.9% of the samples in the form of cross reaction. It was further demonstrated by Rweyemamu et al. (1978) that minimal heterotypic virus contaminations interfered with specific typing in MNT. It was found that SNT was relatively simple to carry out, was economical and did not require accurately standardized reagents.

The neutralization test was found to be more strain specific than CFT (Rweyemamu et al.,1975 and 1977). Rai (1980 b) also made a comparative study of MCFT and micro neutralization test in subtyping of FMD virus and found the SNT to be more sensitive than CFT.

The superiority of SNT over CFT may be explained on the basis that whereas CFT failed to detect the samples either on account of samples

being anticomplementary or containing low titre of virus, SNT is not affected by the anticomplementary factor. However, SNT has got one demerit because the field samples can not be used directly, only the tissue culture adapted samples can be subjected to SNT. It is , therfore, logical to suggest that SNT can not be employed as routine practice for the typing of FMD virus because it is more time consuming than CFT. The samples which failed to yeild FMD virus by CFT ought to be tried by SNT before declaring it negative.

The samples adapted to tissue culture alongwith original samples and mouse passage samples were subject to ELISA test. The result (Table-13) revealed that out of 78 samples tested as high as 58 (74.35%) samples were typed. The ELISA test was sensitive enough to detect the presence of virus in 14 samples declared negative by MCFT even after mouse passage and 4 samples found to be negative SNT after tissue culture passage. The ELISA test prove to be more sensitive than both CFT and SNT. Crowther and Abu-Elzein (1979) also found ELISA to be 50-100 times more sensitive than CFT. Superiority of ELISA in the present study is evident from the fact that out of 78 samples, 58 samples were found to be positive in

ELISA whereas only 54 could be typed by SNT and only 44 by CFT.

Rai et al. (1981 a,b) also applied ELISA successfully for typing and subtyping of FMD virus. Perrin et al. (1982) also applied ELISA in FMD research and observed that purified apthovirus antigen gave better result than crude antigen.

The incidence of FMD was wide spread throughout the state of Bihar during the year 1981-83. The highest (52) number of outbreaks occurred in 1983 and lowest (12) in 1982. This indicates that the incidence of FMD in Bihar has got certain trend

The region wise distribution of FMD outbreaks indicates a positive correlation between the incidence of FMD and livestock density. Highest incidence was reported from the areas at high density of animal population. The 44 (40.37%) outbreaks were recorded in North Bihar and South Bihar Plains each which have high population density, and only 21 (16.34%) could be recorded from Chotanagpur Plateau (Table-17) which has comparatively low population density. This observation is in complete agreement with the observation of Hugh-Jones (1972) for an epidemic in England. However, Sharma et al. (1981) reported a negative correlation between incidence of FMD and regional

livestock density in Uttar Pradesh. They also described that probably in our ecosystem, animal movement might be more important in determining the frequency of FMD than the livestock density. The mortality was highest (45) in North Bihar. This might be attributed to the high incidence of FMD among the animals below one year of age. In this region, FMD affected animals also exhibited haemorrhagic gastroenteritis in three of the outbreaks. All the cattle below one year of age affected with FMD showed the symptom of bloody diarrhoea. The possibility of bacterial or parasitic involvement were ruled out by stool examination. The atypical form of the disease has also been noticed in buffaloes (Hajela and Sharma,1978). A further detailed study of these isolates is required to ascertain its behaviour as compared with the standard virus.

The temporal distribution of data revealed a seasonal rythm in the incidence of FMD in Bihar. The disease frequency increased from March onwards reached the peak in April-May (Table-20). Another peak was during November and December. The period of increased frequency correspondance to the time of greatest animals movement. The reasons for livestock movement were varied which included seasonal migration of animals, cattle fair, floods, agricultural operations and delivery of farm products to the markets. The

dessimination of the disease by movement of men, vehicle and air has also been reported by Sellers and Forman (1973). Ahuja et al. (1981) also confirmed the enhancement of spread of disease of cattle fairs, common grazing, seasonal migration and movement of technical personel.

De Mello (1946) reported the spreading of the disease more rapidly in summer than in winter. Huq and Khan (1963) reported the prevalence of FMD in Pakistan between the month of December to April. Our observation partially agrees with that of Gajpati and D'Souza (1968) who reported the prevalence of FMD in the month of November to February. The seasonal pattern of our findings also corroborates to the findings of Chakrabarty et al. (1979) in Assam and Plotnikov et al. (1972) who reported two major peaks of disease incidence, one in autumn-winter and other in summer.

The highest morbidity rate (13.93%) was recorded in the month of June and lowest (2.21%) in the month of January.

Stratification of morbidity pattern in affected livestock reveals the highest attack rate among cattle (14.47%) followed by those among buffaloes (3.04%) and goats (0.24%). Sharma et al. (1981) observed

highest morbidity in buffaloes which is contrary to the present findings. Our findings appears to be in agreement with Rai and Ahuza (1978) who also reported highest morbidity rate in cattle. The mortality rate was highest in goats (4.35%). The apparently low incidence of FMD in goats may be attributed to a combination of factors including sub-clinical infection (Burrows, 1968; Gibbs et al.,1978; Sharma,1978) mild clinical sign of short duration (Faleoner,1972; Uppal et al..1972; Plotnikov, 1974) and low infection rate in this species.

Sex-wise distribution of epidemiological data (Table-22) revealed that the sex of animals significantly ($P < 0.01$) affected the incidence of disease. The females were more susceptible than male. Morbidity rate was more (9.43%) in females than in male (6.03%). The mortality rate was also higher (3.37%) in females than in male (0.99%). Prasad & Singh (1981) also reported higher incidence in females. They also recorded high incidence in milking animals, animals under gestation, heifers between 1-2 years .

The statistical analysis of epidemiological data on age wise distribution of incidence of FMD (Table-24) revealed that age significantly ($P < 0.01$) affects the incidence of FMD. The animals upto 6 months

of age were less susceptible to the disease than animals between 6 to 12 months of age, the morbidity percentage being 1.12 and 4.61 respectively. These observation suggests the effectiveness of maternal immunity provided by endemically exposed dams to their calves.This contention is supported by observation recorded in Russia as well as India (Khukhorov et al.,1973 and Sharma et al.,1981). However further specific studies are required to define the vertical immune relationship between the dam and its calf in the FMD ecosystem in this state.

The typographical studies of FMD in the state of Bihar revealed FMD virus type 'O' and 'Asia-1' to be more prevalent in the state . Besides FMD virus type 'C', 'A' and subtype 'A-22' were also recorded in few of the outbreaks. It is interesting to note that type 'C', 'A' and subtype 'A-22' was found in very limited outbreaks and the history of the outbreaks revealed that some new animals were brought in those village from outside the state. The animals might have been carrier of FMD virus type 'C' or A or 'A-22' which was precipitated by stress condition due to long transportation. Prasad and Singh(1981) also observed acceleration of morbidity rate on account of long transportation.

The highest incidence of FMD outbreaks due to virus type 'O' may be attributed to the fast spreading nature as well as immunogenicity of the etiological agents. Similarly spreading of FMD outbreaks due to virus type 'C' may be accounted to the restrictive nature of the virus as also its strong immunogenicity. However, it would be desirable to conduct detail study to ascertain the above contention.

FMD virus type 'O' was prevalent in most of the districts followed by 'Asia-1', subtype 'A-22' and type 'A'. In the district like Sitamarhi and Muzaffarpur more than one type of FMD virus were prevalent. This may be explained on the fact that large scale movement of animals took place alongwith international border with Nepal.

The speciswise distribution of FMD virus revealed that all the four serotypes and subtype 'A-22' are prevalent in cattle . Buffaloes did not experienced the outbreaks involving FMD virus type 'C'. Goats were affected with only 'O' type of FMD virus. The prevalence of FMD type 'O' in all the susceptible animals indicated a comparatively wide host spectrum of 'O' type of FMD virus (Sharma et al.,1981).

S U M M A R Y

A toal of 131 samples consisting of feet epithelium (49), tongue epithelium (54), gum tissue (11), palate tissue (6) and eye lesions (2) from the FMD affected animals under village condition as well as 6 feet and 3 tongue epithelial samples collected from affected animals kept under farm condition were processed for FMD virus typing. The serological tests used in the present study for FMD virus typing included MCFT, SNT and ELISA. Comparative efficacy of suckling mouse and BHK-21 cell lines were studied for the recovery of FMD virus from the field samples.

All the 131 original samples were subjected to MCFT for FMD virus typing using 3 units of complement and 4 MHD/ml of haemolysin. The result of MCFT revealed the presence of FMD virus in 53 (40.46%) of the samples, while the remaining 78 (59.54%) samples were found negative. The negative samples consisted of both showing anticomplementary effect (31.29%) and samples containing low amount of virus (28.24%). MCFT, thus did not prove to be a very sensitive test for detection of FMD virus in field samples. Cross reactions as well as anti-complementary effects of antigen as encountered during the test were other disadvantages.

All the 78 MCFT negative samples were passaged thrice through suckling mice, of which 44 samples were

found to be positive for FMD virus while 34 samples failed to yield FMD virus even after three consecutive passages in mice. These negative samples included the samples showing either atypical symptoms in inoculated mice or no symptoms at all.

All the 78 MCFT negative samples were passaged through BHK-21 cell line of which 54(69.23%) produced CPE and were found to be positive for FMD virus by SNT. It took 18-24 hours to produce CPE in BHK-21 cell line. Two to three passages of virus materials were required to give clearcut CPE. Thus, BHK-21 cell line proved superior to suckling mice for the recovery of FMD virus from the field samples. The SNT though very sensitive was found to be more time consuming and cumbersome as it required tissue culture adaptation of field virus prior to the test.

A part of the original materials of 78 MCFT negative samples were subjected to ELISA out of which 58(74.35%) samples yielded the presence of FMD virus. ELISA was found to be far more sensitive and reliable than CFT. It remained unaffected by anticomplementary effect of antigens and did not show any cross reaction. An added advantages with this test was that the charged ELISA plates could

be stored at 4°C for longer period and could be used for FMD virus typing from field samples expecting the results within 5-6 hours.

The effect of different epidemiological factors like topography, season, sex, species and age on the incidence of disease was also studied during the present investigation. The statistical analysis of the data on topographical distribution of FMD outbreaks revealed that the geographical regions in Bihar influenced the incidence the disease significantly. High frequency of the outbreaks (44) was recorded in North Bihar and South Bihar Plains while only 21 outbreaks were recorded from the Chotanagpur Plateau.

The data on seasonal distribution of the incidence of FMD revealed that the maximum number of outbreaks occurred during summer season (41) followed by winter (28), monsoon (26) and minimum in post monsoon (14). The morbidity was however, highest in the monsoon (9.97%) followed by winter (8.47%), summer (6.87%) and post monsoon (4.96%). The statistical analysis of data revealed that the season significantly influences the incidence of the disease.

Cattle was found to be most susceptible species to the FMD with the highest morbidity rate (14.47%) followed by buffaloes (3.64) and lowest in goats (0.24%). The statistical analysis of data revealed that species also influence the incidence of FMD. The lower incidence in goats and absence of outbreak in sheep during the period was attributed to mild clinical symptoms or subclinical infection which usually remained unnoticed.

Further analysis of the epidemiological data revealed that age of the animal signficantly affected the incidence of FMD. The animals upto 6 months of age were less susceptible to the disease than animals, between 6-12 months or more. The role of maternal immunity in affording protection against FMD to younger animals below 6 months of age were also discussed.

The influence of sex on susceptibility to FMD virus were found to be statistically significant. Females were more susceptible than males. The factors associated with higher incidence of the disease in females have been discussed.

The typographical study revealed that FMD virus type 'O' was most prevalent in Bihar followed by 'Asia-1', 'A-22' & 'A', while FMD virus type 'C' was

involved in only one outbreak. The FMD virus type 'O' appeared to be most wide spread while type 'C' most restrictive in nature.

The species-wise distribution of FMD virus revealed that all four serotypes and subtype 'A-22' were prevalent in cattle. Goats were affected with 'O' types of FMD virus while in buffaloes FMD virus type 'O','A' and subtype 'A-22' were involved. The prevalence of FMD virus in the susceptible animals indicated a comparatively wide host spectrum of type 'O' virus.

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