An overview of the work carried out by the Technical Advisory Committee on plague

DURING August, 1994 the village Mamla of Beed district of Maharashtra State experienced unusually heavy flea nuisance and ratfalls. Subsequently, a number of cases clinically resembling bubonic plague were reported from Mamla and nearby villages of Beed district. Making a presumptive diagnosis of bubonic plague outbreak and based on the totality of ecological, clinical and serological evidence, the health machinery of the State Government with the support of the Central Government took prompt containment measures.

During September, 1994, government hospitals and private clinics in the City of Surat in the neighbouring State of Gujarat reported an increasing number of patients with a highly fatal illness resembling acute pneumonia. Based on clinical, laboratory and radiological findings a presumptive diagnosis of an outbreak of pneumonic plague was made. The State Government, again with the active support of the Central Government, rapidly instituted containment measures.

The establishment of the Technical Advisory Committee (TAC) on plague, its terms of reference and pattern of working are described in the prologue.

It was plague after all – the evidence

The organism

DRDE, Gwalior had succeeded in obtaining pure cultures of *Y. pestis* in 11 out of 82 samples processed by them from patients suspected of pneumonic plague from Surat. They also succeeded in obtaining 6 *Y. pestis* isolates from different batches of trapped rodents from Beed within a year of the outbreak and another single rodent isolate from Surat City from among 17 animals in August, 1995. Confirmation and characterization of *Y. pestis* was done according to standard procedures such as gross characteristics, colony morphology, staining, biochemical tests, fluorescent antibody to F1 antigen, specific bacteriophage lysis, counter-immunoelectrophoresis and SDS–PAGE analysis. H. V. Batra *et al.* describe (**page 787**) this work in the paper 'Isolation and identification of *Yersinia pestis* responsible for the recent plague outbreaks in India'.

Confirmation that a case or an outbreak is one of plague rests on cultural isolation of *Y. pestis* from clinical specimens obtained from patients with clinical and epidemiological features compatible with plague. The isolates should show lysis by specific bacteriophage and a biochemical profile indicative of *Y. pestis*. This has now been accomplished. The long gap in processing specimens from the time of their collection and attempted culture with consequent deterioration, and the heavy overgrowth of contaminating organisms made culture isolation a difficult process and may explain the low yield of positive cultures for *Y. pestis* (11 positive out of 82 pneumonic patients of Surat city). It is of interest that 9 out of 11 positive cultures were from patients in the early days of the outbreak, presumably before widespread antibiotic use in the community.

Plague can also be confirmed through the passive haemagglutination (PHA) test by demonstrating a fourfold difference in serum antibody titre between specimens taken at appropriate intervals (minimum of 2 weeks) and by demonstrating their specificity for the F1 antigen of *Y. pestis* as determined by haemagglutination inhibition (HI). The serological investigations in Beed and Surat outbreaks were handicapped by lack of availability of paired serum samples at appropriate intervals. Acute phase sera were generally not available. Serological evidence of plague in this study rests largely on high antibody titres in a majority of the serum samples (85.2%) taken from patients in Surat convalescing 2 to 3 months following illness. It is important to note, however, that in all cases, the specificity of passive haemagglutination test was ensured by simultaneously performing a haemagglutation inhibition (HI) test. These results, taken together with specific sero-reactivity to the F1 antigen in humans and dogs in Beed and Surat, reported by the WHO International team provide further confirmatory evidence that the outbreaks in Beed and Surat were due to *Y. pestis* infection. The serological work is presented in this series in the paper entitled 'Passive haemagglutination tests for *Y. pestis* infection in Surat pneumonic patients' by G. S. Agarwal and H. V. Batra (**page 792**).

Tissue pathology

Small tissue samples made available to the TAC from 7 autopsies from the New Civil Hospital were examined histopathologically. While showing no consistent pattern of pathology, there was a variable picture consisting of some cases showing intra-alveolar oedema, haemorrhage and a pneumonic process in the lungs of varying sever-

ity. *Y. pestis* could not be demonstrated in these tissue sections.

Molecular characterization of *Y. pestis* isolates

Molecular characterization of bacterial isolates from pneumonic plague patients in Surat and from rodents and fleas in Beed and Surat together with PCR analysis of tissues obtained at autopsy of 7 patients who died of pneumonic plague, provided a valuable dimension to the study of the Indian plague outbreaks of 1994. These aspects are discussed (**page 794**) in the paper entitled 'The 1994 plague epidemic of India: Molecular diagnosis and characterization of *Yersinia pestis* isolates from Surat and Beed' by S. K. Panda and coworkers in this series.

PCR analysis of autopsy tissues from the 7 patients who died of pneumonic plague, using primers for *Y. pestis*-specific *pla* and *f*1 genes revealed that 5 were positive for *f*1 gene and 2 for the *pla* gene. The results provided unequivocal evidence of tissue invasion by *Y. pestis* and a further confirmation of the Surat outbreak being plague. This is perhaps the first time that *in situ* demonstration of *Y. pestis* in historical material has been attempted successfully.

All the 18 bacterial isolates (11 from human cases in Surat and 7 from rodents in Beed and Surat) were positive for the presence of *pla* and *f*1 genes by PCR.

Ribotyping has proved to be an extremely useful tool for molecular typing of *Y. pestis* isolates. Upon ribotyping, the Indian isolates were identical to one another, whether derived from patients with pneumonic plague or from rodents of Beed and Surat but differed from all the *Y. pestis* strains examined so far by Elizabeth Carniel of the Pasteur Institute, Paris at the WHO Reference Centre on *Yersinia*. Although different from the 16 previously known ribotypes, it does not follow that the Surat strain is new and of recent origin since only 85 of the approximately 6000 *Y. pestis* isolates known to exist in *Y. pestis* collections worldwide have so far been ribotyped. It is noteworthy that Surat and Beed seem to harbour *Y. pestis* of the same ribotype.

Pulsed field gel electrophoresis confirmed that all the three plasmids of *Y. pestis* of molecular weights 7.5, 75 and 110 kb were present in the Surat isolates. This implies the presence of virulence-associated genes and also the conservation of F1 antigen as demonstrated by immunoblotting, giving confidence to the F1-based serology for diagnostic and epidemiological work.

With regard to the presence of an extra protein band in the 25 kd region of Surat strains, initially observed in joint studies carried out at the WHO International Reference Centre on Plague at Fort Collins, Colorado, USA, it is not clear what significance should be attached to this protein band.

What does all this mean? The molecular identity of *Y. pestis* isolates from human cases of pneumonic plague in Surat and of 6 *Y. pestis* isolates from different batches of trapped rodents from Beed within a year of the outbreak and a single rodent isolate from Surat suggest that that isolates were clonal in origin; the possibility is also raised of an epidemiological linkage among them. The organisms that were obtained from both locations might have been derived from pre-existing ones in each of the areas. The possibility that the organisms might have travelled through the movement of rodents and humans cannot be ruled out, given the fact that frequent movement of rodents and their household effects are known to take place between Beed and Surat.

The molecular identity of the isolates from human cases in Surat and of the isolates from environmental samples (rodents) from Beed and Surat plus the fact that 5 out of 7 flea samples collected during the outbreak period were positive for the *f*1 gene of *Y. pestis* suggest that plague was endemic in natural foci in these areas, possibly generating human outbreaks consequent upon major changes in human ecology. Two papers presented in this series entitled 'Ecology of flea-transmitted zoonotic infection in village Mamla, District Beed' (**page 800**) and 'Observations on urban ecology of Surat and bubonic plague transmission in the city' (**page 803**) by V. K. Saxena and T. Verghese throw interesting light on this problem. All in all, TAC was of the opinion that the evidences obtained so far do not indicate that the isolates of *Y. pestis* from the plague outbreaks in India 1994 were the product of genetic manipulation and introduction from outside, a popular theory in the media at that time. Admittedly, there are many gaps in our knowledge of the Indian plague outbreaks of 1994 – bubonic plague in Beed and pneumonic plague in Surat and what connection there may be between the two – which only a continued and active programme of research can fill.

Epidemiological and ecological aspects

As far as the eco-epidemiological background to the outbreak of suspected bubonic plague in the Beed district of Maharashtra is concerned, village Mamla was the index village and the cascade of evidence is as follows, as constructed by the Directorate of Health Services of Maharashtra state:

September, 1993: Earthquake in the neighbouring district of Latur.
September 1993 to July, 1994: Abandoned homes, stored food grains left behind, exponential growth of rat population.
5 August, 1994 onwards: Unusual flea nuisance in Mamla, followed by rat falls in the locality.
26 August, 1994 onwards: Cases with fever and lymphadenopathy started to appear and were reported at the

nearest Primary Health Centre in Kuppa in the area, mass antibiotic prophylaxis and insecticide spraying started on 29 August.

8 September, 1994 onwards: Seropositivity for plague first reported, rising in frequency later, containment measures continued.

20 October, 1994: No new cases reported.

25 October, 1994: WHO declared that the outbreak had been contained.

With regard to the Surat outbreak, during the first week of September, 1994, the monsoon was unusually heavy, a record in the past 25 years; flood waters entered the city and remained stagnant for 5 days; a large number of dead animals were found after the floods receded. Then came the Ganapati festival with close intermingling of huge crowds of people. On 19 September, 1994, an illness characterized by fever, cough, haemophysis, dyspnoea and pulmonary infiltrates on X-ray, in young adults, not responding to penicillin and attended by high initial mortality broke out in Surat city. A suspected clinical diagnosis of pneumonic plague was made by the physicians at the New Civil Hospital, Surat. The patients responded well to tetracycline, aminoglycosides and chloramphenicol. Case fatality rates came down rapidly after the high levels in the first few days. Blood specimens obtained from patients with suspected pneumonic plague revealed sero-positivity to the F1 antigen of *Y. pestis* in 146 out of 1027 samples in a study by the NICD. Reference had already been made to the study by DRDE, Gwalior, wherein 85.2% of serum samples from patients convalescing from what clinically looked like pneumonic plague were positive for antibodies to F1 antigen. Some evidence exists of a clustering of cases and death; taking of antibiotics had a beneficial effect in preventing deaths. The State Health and Civic authorities responded rapidly by instituting active case detection and treatment, providing accurate information to physicians and public on early detection, case management, antibiotic prophylaxis and treatment. 500,000 persons left Surat city in panic to other places in Gujarat, to Bombay and to various destinations in the country, but in none of the urban centres of Bombay, Calcutta and Madras was there any confirmation of plague transmission. The one patient in Delhi who had an enlarged painful lymph node in the right groin with a four-fold rise in titre after haemogglutination inhibition actually travelled to Delhi from rural Maharashtra. No imported plague was reported in any other country.

The lessons learnt

Surveillance of plague is weak in India and needs to be considerably strengthened and expanded. Prompt action to identify the causative agent in a suspected outbreak is of the utmost importance and capabilities for identification of plague bacillus as the aetiological agent need strengthening. State Public Health Laboratories are in need of considerable upgrading of skills to make prompt and accurate diagnosis of plague and other infectious disease agents. Paradoxically, while there is expertise in the country for the study of microbes at sub-cellular and molecular levels, diagnostic capability for the isolation and characterization of common infectious agents at the peripheral levels of the health care system is weak. As a result, a vulnerability in India's health care system relating to rapid diagnosis of infectious disease outbreaks exists. There is little preparedness in the provision of Quality Laboratory Services for accurate confirmation of clinical diagnosis. Current plague experiences revealed that rapid mobilization of institutions and experts around the country in time could have helped in the prompt identification of the causative organism. Proper interaction with the media and the public and seeking their assistance are of critical importance. With the information technology available today, much of the surprise and panic among the national and international communities could have been avoided.

Future prevention and control of plague

Surveillance and response should be regarded as the foundation of infectious disease control, be it plague or any other. India has the potential of being well-served in this regard because of the extensive health infrastructure built over the past few decades. This structure is, however, somewhat handicapped by inadequate interaction between the various levels of care and the network of laboratories existent in the country. Prompt backup support of laboratory services of quality in determining the aetiological agent expeditiously must be ensured in future strategies. A national surveillance and response system in India for the control and prevention of infectious diseases is an urgent necessity. TAC has recommended the following steps:

1. Organize a national debate on the strengths and weaknesses of the existing surveillance system and draw up priorities and build on existing strengths.
2. This should lead to the formation of a National Apex Committee on Surveillance and Response for the Control of Plague consisting of appropriate experts from different disciplines and government and non-government sectors. The committee should be headed by an epidemiologist of eminence.
3. Epidemic preparedness should be integral to the control strategy.
4. The formation of a multi-disciplinary action group would be valuable. The group may consist of an epidemiologist, a clinician, a microbiologist and relevant public health and civil authorities. The group will provide guidelines for case definition,

case management, preventive action and information and communication. The National Reference Centre for plague, which in this case, is the NICD, needs to be strengthened in its microbiological and molecular genetic capabilities. There could be different national reference centres for different diseases depending upon expertise availability.

5. A network of laboratories throughout the country must be identified for providing support to the diagnosis of infectious diseases. The surveillance must include surveillance of emerging drug resistance of plague and other pathogens.
6. NICD and the network should be involved in a continuous process of training and re-training of professionals and technicians in the investigation of plague outbreaks and in establishing aetiology.
7. The capacity for continuous surveillance of animals, vectors and human populations for plague within the overall infectious disease surveillance system has to be developed and maintained. The state and district health services should be the focal points of this system with assistance from the NICD and the national laboratory network.
8. The capacity to respond to plague outbreaks in humans and plague epizootics must be ensured through the development of epidemiologic, ecologic and laboratory investigative capacity.
9. An automated reporting system extending from health centres and laboratories to public health action programmes would be of value.
10. The TAC makes a strong plea for building a well-trained cadre of epidemiologists in the country and in this, medical colleges, schools of public health (where are they?) and specialized institutions devoted to the study of infectious diseases under the ICMR and the NICD will have to play an important role.
11. The value of continuous interaction of the national networks with international networks under WHO and others is inestimable and must be maintained.