Passive haemagglutination tests for *Y. pestis* infection in Surat pneumonic patients

G. S. Agarwal and H. V. Batra

Defence Research and Development Establishment, Jhansi Road, Gwalior 474 002, India

Passive haemagglutination tests, utilizing a highly purified, specific Fraction 1 antigen of *Yersinia pestis* have been employed to detect antibodies in the sera of pneumonic patients of Surat city. The high antibody titres observed in a majority of sera samples (85.2%) from convalescent patients 2 to 3 months following the outbreak are indicative of *Y. pestis* infection.

SEROLOGICAL responses against the *Y. pestis*-specific Fraction 1 (F1) antigen have been successfully employed for detection of plague in serosurveillance studies. Even at the time of outbreaks, serological tests have their merits in providing confirmatory evidences of plague infection particularly so when the bacterial isolations have been neglected. Passive haemagglutination/passive haemagglutination inhibition tests (PHA/PHAI) and complement fixation test employing the highly purified, specific Fraction-1 antigen of *Y. pestis* are commonly used to detect antibodies in plague[1–3]. Plate- and dot-enzyme linked immunosorbent assays (ELISAs) for detection of antibodies to F1 and a monoclonal antibody based ELISA for F1 antigen detection have been described[4–6]. The WHO recommended tests are PHA and PHAI and these are reported to have a high degree of specificity and sensitivity for detection of anti F1 antibodies[7].

The serum samples from human patients, rodents and other animals following suspected bubonic plague in district Beed and suspected pneumonic plague outbreaks in Surat city were tested at various intervals by several institutions namely, the National Institute of Communicable Diseases (NICD), The National Institute of Virology (NIV) and WHO. The present work reports the antibody test results on serum samples collected from convalescent patients from the outbreak-affected areas.

Materials and methods

Serum samples

A batch of 27 convalescent serum samples from patients with suspected pneumonic plague from Surat were collected in the last week of December, 1994, two to three months after the outbreak. Another batch of 102 serum samples were collected from Surat city during the month of March, 1995.

Eighteen serum samples collected from Beed district of Maharashtra in the month of April, 1995 and 13 serum samples during July, 1995 were also tested. These human serum samples were from the general population of that area.

A group of 55 human serum samples of pyrexia cases (pyrexia of unknown origin, PUO) negative for typhoid and malaria infections collected prior to the suspected plague outbreaks from a non-affected area were also tested simultaneously.

PHA and PHAI tests

The sera were examined for antibody to the F1 antigen using the PHA and PHAI microtitration procedures recommended by WHO for the serodiagnosis of plague[8,9]. Briefly, serum samples were heat inactivated at 56°C for 30 minutes and then 50 µl of packed, washed sheep red blood cells (SRBC) added to every 0.5 ml of test serum. They were adsorbed for 60 minutes at room temperature and then centrifuged at 1500 rpm for 10 minutes. For PHA test, 25 µl of PHA diluent (1% normal rabbit serum in normal saline) was added to all wells of 'U'-shaped microtitration plates. For PHAI test, the diluent used had 0.2 mg/ml F1 antigen in PHA diluent. In the next step, 25 µl of test serum was added to the first well of microtitration plate and serially diluted. A volume of 25 µl of appropriately diluted F1 sensitized SRBC was added to all the wells. Plates were covered and incubated at room temperature for 6 h and then overnight at 4°C.

Results

The wells were examined for flocculent, lattice agglutination. Negative wells showed a sharp-edged button or a defined circle. End-point titres were determined by taking the last well showing complete agglutination. Final titres were determined by subtracting PHAI titres from PHA titres of individual sera samples. Among the first batch of 27 samples from suspected convalescent plague patients from Surat, 23 showed titres of 1:16 or more

Table 1. PHA and PHAI titres of tested human sera samples

Place	Period of collection	Total tested	Number with titres								Total positive (%)
			Nil	1:4	1:8	1:16	1:32	1:64	1:128	1:256	
Surat	Dec. '94	27	–	–	4	2	12	7	2	–	23 (85.2)
Surat	March '95	102	77	6	10	2	3	2	1	1	9 (08.8)
Beed	April '95	18	12	3	3	–	–	–	–	–	–
Beed	July '95	13	11	1	1	–	–	–	–	–	–
Gwalior	July '94	55	52	–	1	1	1	–	–	–	2 (03.6)

(Table 1). Of the 102 sera samples collected from Surat in the second batch, only 9 showed positive titres. Human serum samples collected 8 to 11 months after the outbreak in district Beed were found to be negative.

Two of the 55 sera samples of PUO cases from non-affected area were also positive with titres of 1:16 and 1:32, respectively.

Discussion

Confirmation of a clinical diagnosis of plague is based chiefly on isolation of *Y. pestis* and/or demonstration of four-fold rise of antibody titres at paired sera testing. It is well established that *Y. pestis* antibody is more readily detected than isolation and identification of *Y. pestis*.

Confirmation of plague by serology, particularly in pneumonic cases, is retrospective to the outcome for the patient as most fatalities occur within a week of infection, whereas, antibodies generally appear in significant titres about one to two weeks following the infection. Therefore, antibody detection from patients during the period of acute illness is of limited value. Serology on convalescent patients, two weeks to few months after infection is more suggestive of plague occurrence. A serologic titre of 1:10 or more in the absence of known recent plague vaccine history is considered positive for plague antibodies. A four-fold rise in titre between two time distanced samples (greater than two weeks apart) is considered confirmatory[8]. Since acute phase serum samples collected during the outbreak were not available for testing, it was not possible to analyse results of paired sera testing. Serological results on blood samples collected from convalescent patients 2–3 months following the pneumonic outbreak were, therefore, analysed independently. A majority of cases (23 out of 27) showed high levels of positive titres (>1:16 to >1:256). Such a large proportion of convalescent cases showing positive PHA and PHAI titres against specific F1 antigen of *Y. pestis* is highly suggestive of plague in pneumonic patients of Surat city. Only two of the 55 sera samples from the non-affected area had positive titres. Detection of these two positive cases from among the PUO cases from the non-affected area and that too, prior to the appearance of the outbreak is enigmatic. Could it be due to the presence of *Y. pestis* infection in an inapparent form in that region or a cross-reactivity with other disease-causing agents? This question needs further study.

Serum samples collected 5–6 months after the outbreak showed a low percentage of positive titres. A progressively decreasing number of positive serological reactions within a period of several months is indicative of effective control of the outbreak[7].

Human serum samples from district Beed were all found negative for antibodies to F1 antigen. Since the sample collection from the individuals was after 8–11 months of suspected plague outbreak in that area, probably the antibody titres by this time had disappeared. Moreover, these samples were from general population and more likely to be the contact individuals rather than the patients who suffered with suspected bubonic plague illness.

1. Chen, T. H. and Meyer, K. F., *J. Immunol.*, 1954, **72**, 282–298.
2. Chen, T. H. and Meyer, K. F., *J. Immunol.*, 1955, **74**, 501–507.
3. Bhagavan, N. V., Chen, T. H. and Meyer, K. F., *Proc. Soc. Exp. Biol. NY*, 1956, **91**, 353–356.
4. Williams, J. E., Arntzen, L., Robinson, D. M., Cavanaugh, D. C. and Isaacson, M., *Bull. WHO*, 1982, **60**, 777–781.
5. Williams, J. E., Gentry, M. K., Braden, C. A., Tyndal, G. L., Altieri, P. L., Berman, S. and Robinson, D. M., *Bull. WHO*, 1988, **66**, 77–82.
6. Williams, J. E., Gentry, M. K., Braden, C. A., Leister, F. and Yolken, R. H., *Bull. WHO*, 1984, **62**, 463–466.
7. Cavanaugh, D. C., Thorpe, B. D., Bushman, J. B., Nicholes, P. S. and Rust, J. H. Jr., *Bull. WHO*, 1965, **32**, 197–203.
8. Bahmanyar, M. and Cavanaugh, D. C., *Plague Manual*, World Health Organization, Geneva, 1976.
9. *WHO Tech. Rep.*, Series. No. 447, WHO Expert Committee on Plague, 4th edition, 1970.

ACKNOWLEDGEMENTS. We are obliged to Prof. V. Ramalingaswami, Chairman and Dr T. Verghese, Member Secretary, Technical Advisory Committee on Plague, Govt. of India for providing us the opportunity and the required samples/reagents to carry out this work. We thank Dr R. V. Swamy, Director and Shri K. M. Rao, Joint Director, DRDE, Gwalior for providing all the necessary facilities for this work. We sincerely thank Dr Veena Mittal, Dr Gautam Biswas and Dr A. K. Khera of NICD for providing serum samples from outbreak areas.