Serological Studies on Human Plague in Southern Africa

PART I. PLAGUE ANTIBODY LEVELS IN A POPULATION DURING A QUIESCENT AND A SUBSEQUENT ACTIVE PERIOD IN AN ENDEMIC REGION

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SUMMARY

Yersinia pestis fraction I passive haemagglutination studies on human sera obtained randomly during a plague epidemic showed a 10-fold increase in occurrence of antibodies compared with the results obtained on sera from the same population 11 months earlier during a quiescent phase.

The pre-epidemic population antibody rate was lower than that in a comparable population tested simultaneously in another endemic region, where a recrudescence of plague was not experienced during the following year.

The human plague epidemic was preceded and accompanied by a rodent die-off. A serological survey of the small mammals 2 years prior to the human survey also showed that Y. pestis was present in the environment. Otomys unisulcatus was indicated as being an important rodent in the plague cycle in the area.


A visit by Dr K. F. Meyer of the George Williams Hooper Foundation, San Francisco, to Ovamboland, South West Africa, during the plague epidemic of 1963 stimulated a series of serological studies in a number of endemic areas of southern Africa.

The first human survey was conducted in August 1966, when sera were collected from 548 inhabitants of the Uitenhage district of the Cape Province in the Republic of South Africa. This region, situated between latitude 33° and 34° south and longitude 25° and 26° east, adjoins the coast of the Indian Ocean, has a mild temperate climate and is a known endemic plague area. Prior to this survey the last human outbreak had occurred in the plague-year 1959/1960. The concept of a plague-year taken from one midwinter (1 July) to the next (30 June) was proposed by Davis, since the incidence of plague in man in southern Africa is highest in the summer. During April 1964 a serological survey of plague in small mammals was carried out in the Uitenhage district as part of an extensive survey in southern Africa.

In July 1967 a minor plague epidemic occurred in the same area. Several deaths occurred, the cause of which was suspected to be plague, but this was not confirmed. One male Xhosa, aged 29 years, was admitted to hospital and Yersinia pestis was isolated from inguinal bubo material. Plague haemagglutination tests proved to be positive on three successive occasions. At the time of the outbreak, sera were collected from persons living in the affected area and tested for the presence of plague antibodies. A comprehensive rodent survey was carried out simultaneously.

MATERIALS AND METHODS

In 1966 blood was collected randomly from villagers, labourers on farms and citrus plantations, and from schoolchildren. The survey population consisted partly of Xhosa and partly of Cape Coloureds. The blood samples were centrifuged in the field and the separated sera were stored in a mobile deep-freeze unit.

Yersinia pestis fraction I antigen for the sensitisation of sheep erythrocytes was prepared according to the method of Baker et al., in this Institute. The sensitisation of erythrocytes with a fraction I B concentration of 25 µg/ml was performed according to the method described by Chen and Meyer, with minor modifications, viz.: (a) a 5% suspension of erythrocytes was used instead of a 2.5% suspension during the tanning and sensitisation processes; (b) a 0.35% working suspension of erythrocytes was employed in the microtitre technique of haemagglutination instead of a 0.25% suspension. For the microtitre technique the plates with V-shaped wells were found to give the best results.

It was noted that not all sheep are suitable sources of erythrocytes, and the selection of the animal is a critical factor determining the success or failure of the plague haemagglutination procedure. After trial and error, cells from a single sheep proved highly satisfactory and the use of this animal has yielded consistently good results.

In our experience unpreserved tanned and sensitised erythrocytes can be stored at refrigerator temperature in...
RESULTS

A total of 548 sera was collected in the Uitenhage district in August 1966: 26 sera (4.7%) showed the presence of Y. pestis haemagglutinating antibodies, while 11 (2.0%) had titres of 1:16 or higher. These results are represented in Fig. 1. The highest titre obtained in the survey was 1:32.

The pre-epidemic survey of 1966 in the Uitenhage district was immediately followed by a similar survey conducted in another endemic region situated in the Transkei. This is an inland area between latitude 32° and 30° south and longitude 27° and 28° east. The survey comprised 526 human sera, the great majority of which came from Xhosa. Plague antibodies were present in 50 sera (9.5%), while 27 sera (5.1%) yielded titres of 1:16 or higher. The highest titre was 1:64. The over-all results are represented in Fig. 3.

Table I shows the results of plague haemagglutination tests on 233 small mammals collected during April 1964 in the Uitenhage district.

During the epidemic of 1967 a total of 126 people, including 1 bacteriologically confirmed plague patient and 4 contacts, was serologically tested. In this instance, 59 sera (46.8%) showed the presence of plague antibodies — 18 people (14.3%) having titres of 1:16 or higher. The highest titre was 1:128. The patient showed a rising titre to a maximum of 1:512. The over-all results are represented in Fig. 2.

The absence of normal rodent activity had been noted shortly before the occurrence of human plague. A further 63 rodents of various species were collected for culture and serological tests but Y. pestis was not isolated from these animals. Pooled serum from 5 Otomys unisulcatus yielded plague antibodies to a titre of 1:4, while a titre of 1:8 was obtained from the serum of an unidentified rodent. The others gave negative results, but the pooling of certain sera may have resulted in artificial negative results owing to dilution.
Of great interest is the incidence of plague antibodies in a population during an outbreak of human plague compared with that which prevailed 11 months earlier towards the end of an 8-year quiescent period in respect of plague activity. Other workers have conducted similar serological surveys in endemic regions in other parts of the world, and it is generally agreed that subclinical plague infections occur to a far greater extent than was appreciated before the introduction of serological techniques in this field.

Such inapparent infections are indicated by the occurrence of *Y. pestis* fraction I haemagglutinating antibodies in apparently healthy people residing in endemic areas. The rates of antibody occurrence obtained in the present study are summarised in Table II. A titre of 1:16 is regarded by most workers as being of diagnostic importance. For this reason the results of these surveys have been presented in two categories, viz. presence of antibodies at all levels and that a diagnostic levels. (The possible occurrence of false positives and the interpretation of low haemagglutination titres will be discussed in a subsequent article.) A prominent feature was the 10-fold increase in plague antibody rate of the Uitenhage population when active plague occurred. Concurrently, the diagnostic antibody rate underwent a 7-fold increase.

Although there was only one confirmed symptomatic plague patient during the 1967 epidemic, we have reason to suspect that a thorough retrospective inquiry among the serologically reacting people might have revealed a certain number of subjects with symptoms indicative of clinical plague of mild or moderate degree. A much more intensive investigation and follow-up study carried out during the 1968 plague epidemic in Lesotho revealed a significant number of people who, although at first thought to have had asymptomatic plague infections, gave retrospective histories which left little doubt that they had
TABLE II. PLAGUE ANTIBODY RESPONSE IN HUMAN POPULATIONS OF ENDEMIC REGIONS DURING QUIESCENT AND ACTIVE PERIODS

<table>
<thead>
<tr>
<th>Region</th>
<th>% population with antibodies</th>
<th>% population with titres of 1:16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uitenhage 1966 (quiescent)</td>
<td>4.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Transkei 1966 (quiescent)</td>
<td>9.5</td>
<td>5.1</td>
</tr>
<tr>
<td>Uitenhage 1967 (active)</td>
<td>46.8</td>
<td>14.3</td>
</tr>
</tbody>
</table>

suffered from clinical plague. Extreme care was taken in that study not to ask leading questions.

Another noteworthy feature of the present study becomes apparent on comparison of the results obtained in Uitenhage and in the Transkei in 1966. The percentage of population with plague antibodies in Uitenhage (4.7%) is lower than that in the Transkei (9.5%). It is in the former endemic community, where the antibody rate suggests a comparatively lower resistance, that a recrudescence of plague occurred in the following year, while the Transkei remained quiescent. A low population immunity rate may reasonably be assumed to facilitate an outbreak of the disease. Further data are required before one can postulate a quantitative 'danger zone' of low population antibody rates in plague-endemic regions.

That enzootic plague was present in the Uitenhage district prior to the human outbreak was also revealed by the small-mammal survey, in which 10 of 233 sera (4.7%) had diagnostic titres. The serological results and the isolation of Y. pestis from Otomys unisulcatus indicate that this rodent plays a significant role in the plague cycle of the Uitenhage region.

A portion of this work was included in a thesis by M. I. which was approved for the degree of Doctor of Medicine by the University of the Witwatersrand, Johannesburg.

REFERENCES