

2. Title: Pasteurella pestis Infection in Humans.

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#### OBJECTIVES

- A. To determine if and to what extent inapparent Pasteurella pestis infection occurs in humans.
- B. To measure some of the physiological parameters in plague patients.

#### A. Inapparent Human Plague

##### DESCRIPTION

Observations from earlier studies have indicated that many individuals residing in plague endemic areas have demonstrated reasonably high levels of antibody without previous plague immunization and without clinical disease. A recent study on plague patients and their contacts afforded the opportunity to obtain sera and immunization data on a group of 28 bacteriologically confirmed bubonic plague patients.

In addition, the institution of a mass immunization campaign by the Ministry of Health gave us the opportunity to bleed 81 male volunteers aged 13 to 18 years from a private Catholic school, 82 volunteers from the staff of the Provincial Hospital, and 62 volunteers from a Public Health training group sponsored by the Red Cross during the month of January 1969. All were residents of Nha Trang in more or less high risk areas of the city. All indicated they would submit to a second bleeding early in May so that sera obtained could again be examined for antibody levels. Questionnaires were filled out by these volunteers at the first bleeding indicating age, sex, address, immunization status, time in residence at Nha Trang, etc. Questionnaires will again be utilized at the second bleeding requesting information on interim immunization, recent disease, overt plague within the household, etc.

##### PROGRESS

The immunization status of the patients studied is presented in Table 1. Only one patient, a 12 year old female who denied immunization, demonstrated antibody to P. pestis on admission. One contact, her mother age 51 demonstrated antibody while the other contact her father age 50, showed no antibody. No one in the family had ever received plague vaccine, or for that matter immunization of any kind. Of the immunized individuals, 10 had received Pasteur vaccine within 4 mos of their disease, and all had been immunized at least two months prior to disease.

Table 2 lists the immunization status of contacts of the plague patients studied. It is important to note that the number of individuals who claimed to have received plague immunization had the same proportion of reactive sera as did the group who denied ever having plague immunization. This suggests the failure of the vaccine to elicit an antibody response and suggests that subclinical or inapparent infection with P. pestis is responsible.

Table 3 presents the age distribution of the contacts from whom sera were obtained for serological testing. Since it appears that males are more reluctant to be bled only about 1/3 of the 68 listed individuals who provided sera were males, although of all contacts queried approximately 50% were males. It appears that, even with the few numbers in each age group, there is no appreciable difference in sero-reactivity and age.

Attempts to obtain a second serum on these contacts one month later were quite unsuccessful and only 12 followup sera were obtained. Of these 12 sera 6 demonstrated high levels of antibody while the initial sera were void of antibody. No plague vaccine had been administered to these individuals nor did they experience any illness during the interim between bleedings. Two of these individuals were from a single household from which P. pestis was demonstrated in fleas obtained from animals trapped in the house. One of the two had been immunized 5 months prior. One of the remaining four who showed a positive serology in the second serum had been immunized 3 months prior, two denied immunization and the other individual had been hospitalized with overt plague 5 months earlier and had never received immunization.

A convalescent serum specimen was obtained 4 weeks later from 5 of the plague patients whose acute sera were non-reactive. All five of these convalescent sera demonstrated antibody titers in excess of 1:32.

Information accumulated from compiling the data obtained from the initial questionnaire of subjects bled in January 1969 indicated that 107 of the group had received plague immunization within the last 6 months during the mass immunization program established by the Ministry of Health; 23 had never received vaccine and the remaining 66 had been immunized more than six months ago or were not certain. The age range of the group was from 10 to 54 years with the majority being males between age 14 and 20. That this was a fairly stable population is indicated by the fact that 50% of the group had lived at the same residence for 3 to 12 years, 25% less than 3 years and 25% more than 12 years. Of the group 50% had 6-10 individuals in the household, 25% had less than 6, 25% had more than 10. Only two of the group reported a case of plague among one of the household members within the past year. Results of testing for plague antibody revealed only a single positive serum although 107 individuals had received plague vaccine within the previous 6 months. Subsequent serological testing on the second sera is expected to indicate the amount of inapparent plague occurring among this group.

#### B. Observations on Plague Patients

##### DESCRIPTION

Sera were obtained from a series of 23 bubonic plague patients confirmed by either positive bubo aspirate or blood culture. Chest films of all patients revealed no pneumonic involvement. Severity of disease ranged from patients who were clinically slightly ill and almost afebrile to those who were very ill with temperatures of 103-104°F.

A concurrent study on plague therapy was simultaneously being conducted with these patients during this study, therefore no control could be exercised over the drugs which individual patients received. Therapy consisted of either I.V. streptomycin in 5% D/W or a combination of I.V. streptomycin in 5% D/W along with oral sulfadiazine.

Conditions in the Province hospitals of Vietnam are almost uniformly poor. Consequently, patients are hesitant to be admitted and in turn will not remain hospitalized for any period of time following subjective improvement in their clinical condition. The short period of observation of these patients made accurate judgments of clinical response to therapy quite difficult. No deaths occurred.

Blood was drawn upon admission for SGOT, BUN, and serum glucose levels. Spinal fluid was obtained from eight of the patients. Spinal fluid pressures, microscopic findings, culture, CSF protein and glucose values were determined. EKG's were done on 9 of the patients. No second blood or CSF specimens could be obtained from these patients for comparative studies since patients usually departed from the hospital at night when no staff were on duty.

## PROGRESS

### 1. Blood urea nitrogen

The BUN ranged from 9.0 to 32.4 with a mean value of 16.47. It would appear from this limited study that renal function is probably not impaired to any significant degree in bubonic plague. In all patients with a BUN greater than 20 mg%, blood had been obtained prior to the time I.V. therapy was initiated. Mild dehydration may have contributed to the slightly elevated BUN in some of these patients.

### 2. Serum glutamic oxalotransaminase (SGOT)

SGOT's ranged from 12.4 to 210 Sigma units. One patient had an SGOT above 59 units (210). The mean value was 41.43 units, with a median of 33.0 units which is within the normal range. The patient whose SGOT was 210 units was a 13 y/o boy. No explanation for this elevation can be given.

Patients who had been on streptomycin therapy greater than 24 hours tended to have slightly elevated SGOT values. Whether or not lysis of the plague bacillus occurs, with subsequent release of an endotoxin which affects liver function, is a question which needs further investigation. A larger series of patients is needed and other liver function studies over a longer period of time following initial symptoms of bubonic plague must be done before definite conclusions can be drawn.

### 3. Serum glucose

Serum glucose (Somogyi) was determined on 20 patients, the range obtained being 26 to 171 mg%, with a mean of 74.12 mg%. Five of these twenty patients had serum glucose levels of less than 40 mg%. Interestingly, two of these 5 patients were on I.V. dextrose. Of 8 patients on I.V. dextrose, one had a serum glucose level greater than 95 mg% (171 mg%). This poses the question as to whether laboratory error was significant, or if liver or pancreatic function is altered by bacterial toxin. Again, further investigation of glucose metabolism is needed before definite conclusions can be made.

### 4. Cerebrospinal fluid

Having observed several hundred cases of bubonic plague over a period of years it has been our impression that a fairly significant number of patients exhibit meningeal signs, i.e., nuchal rigidity or positive Kernig's sign. CSF studies were obtained on 8 of the 23 patients in this series, each of which exhibited one or both of the above symptoms. CSF protein ranged from 5—20 mg% with a mean of 13.4 mg%. No patient had a CSF protein value above normal. CSF glucose ranged from 50—96 mg% with a mean value of 68.7 mg%. Two patients had CSF glucose levels of 95 mg%. All other CSF glucose levels were within normal range, even though most of these patients were on I.V. dextrose.

One CSF specimen grew out P. pestis on culture from the third tube of the tap. The CSF was clear with no evidence of fresh blood. This was in an 11 y/o boy from whom a positive P. pestis blood culture was obtained. The positive CSF culture may have been due to bloody contamination. This patient recovered nicely without prolonged neurological symptoms. It would appear that meningeal irritation exists in a fair number of patients with bubonic plague. Is this due to a release of bacterial toxin or to actual P. pestis meningitis? Further investigation is needed to elucidate this matter.

### 5. Electrocardiograms

Seven of nine patients on whom EKG's were run had abnormal tracings. One of the patients with an abnormal tracing was a 49 y/o male. All others were 25 y/o or less. S—T elevation appeared to be the most common abnormality, however, this is not an uncommon finding in the younger patient up to age 15 yrs. We anticipate doing a series of EKG's on normal Vietnamese of this age group, hoping to learn how commonly this phenomenon occurs. Interestingly, tracings on mice which have been injected with lysed plague bacilli show S—T elevation also. All patients on whom tracings were done in this study had been on I.V. streptomycin therapy long enough to have produced lysis of the plague bacilli. Does the lysed plague bacillus produce a cardiomyopathy in the human? More work is needed in this area.

### 6. Hemograms

Hemograms, consisting of total white cell count, hematocrit, total platelet count and differential count, were done on 21 patients with confirmed bubonic plague. As expected, total white counts were often elevated, ranging from 7,100 to 30,500. Differential counts revealed the usual left shift concomitant with a bacterial infection. Hematocrits were surprisingly high for the Vietnamese populations. Again, this may have been subsequent to mild dehydration in febrile patients. Platelets were found adequate in all patients, which eliminates the likelihood of thrombocytopenia being responsible for the typical hemorrhagic symptoms found in patients with terminal bubonic plague. Studies on clotting mechanisms are indicated to elucidate the actual cause of the hemorrhagic symptoms exhibited in late stages of untreated bubonic plague.

Table 1  
Nha Trang Patient Immunization

	Immunized	Not Immunized
Sero—negative	13	14
Sero—positive	0	1
% Positive	0	6.7 %

Table 2  
Nha Trang Contacts

	Immunized	Not Immunized
Sero negative	18	39
Sero positive	6	11
% Positive	25 %	22 %

Table 3  
Age distribution versus serology (Contacts)

Age Group	No. tested	No. Positive	% positive
0—9	10	3	30 %
10—10	23	4	18 %
20—29	8	1	13 %
30—39	18	4	22 %
40—49	7	2	28 %
50—59	4	1	25 %
> 60	4	2	50 %
Total	68	17	25 %