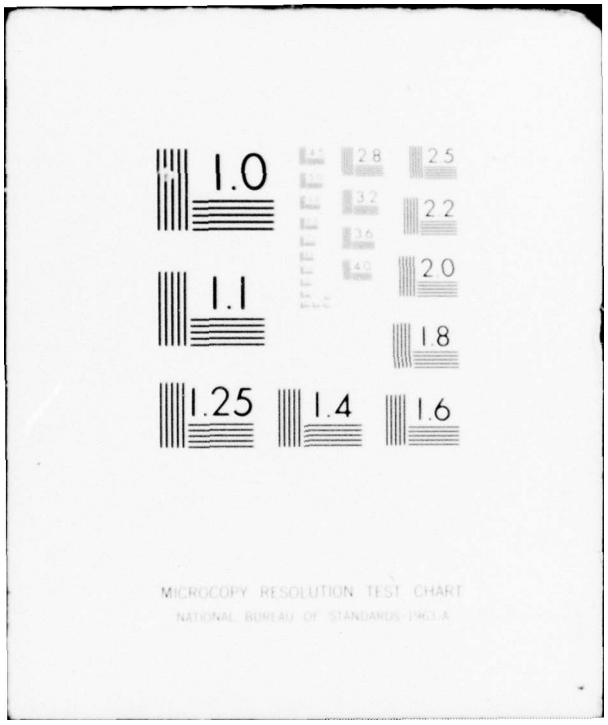
AD-A048 172 ISRAEL INST FOR BIOLOGICAL RESEARCH NESS ZIONA F/G 6/5
AN EPIDEMIOLOGIC AND IMMUNOLOGIC STUDY OF BOUTONNEUSE FEVER IN --ETC(U)
DEC 76 DA-ERO-75-G-041
UNCLASSIFIED NL

| OF |
AD
A048172

END
DATE FILMED
2-78
DDC


1.0
1.1
1.25
1.4
1.6
2.8
2.5
3.2
2.2
3.6
2.0
4.0
1.8
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

AD

AN EPIDEMIOLOGIC AND IMMUNOLOGIC STUDY OF BOUTONNEUSE FEVER IN ISRAEL

FINAL TECHNICAL REPORT

by

Marcus A. Klingberg, M.D.
Robert A. Goldwasser, Ph.D.
Tiberio A. Swartz, M.D.
Wanda Klingberg,Ph.D.
Yonel Steiman,M.Sc.

December 1976

EUROPEAN RESEARCH OFFICE
United States Army
London,England

GRANT NUMBER DA-ERO-75-G-041

Israel Institute for Biological Research
and Tel-Aviv University Sackler School of Medicine
Ness-Ziona, Israel

Approved for Public Release; distribution unlimited

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER (6) and

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
AN EPIDEMIOLOGIC & IMMUNOLOGIC STUDY OF BOUTONNEUSE FEVER IN ISRAEL.

5. TYPE OF REPORT & PERIOD COVERED (9)
FINAL TECHNICAL REPORT. MAR 75— DEC 76,

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s) (10)
MARCUS A. KLINGBERG, ROBERT A. GOLDWASSER, TIBERIO A. SWARTZ, WANDA KLINGBERG, YONEL STEIMAN

8. CONTRACT OR GRANT NUMBER(s) (15)
DAERO-75-G-041

9. PERFORMING ORGANIZATION NAME AND ADDRESS
ISRAEL INSTITUTE FOR BIOLOGICAL RESEARCH, NESS-ZIONA, ISRAEL

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS (17) (12)
6.11.02A-1T161102B71D-00-259

11. CONTROLLING OFFICE NAME AND ADDRESS (11)
U.S. ARMY R&S GROUP (EUROPE)
BOX 65, FPO NEW YORK, 09510

12. REPORT DATE
DECEMBER 1976

13. NUMBER OF PAGES
65

14. MONITORING AGENCY NAME & ADDRESS(If different from Controlling Office)
(12) 68p.

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

APPROVED FOR PUBLIC RELEASE

DISTRIBUTION UNLIMITED

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
(U) SPOTTED FEVER
(U) RICKETTSIAE
(U) TYPHUS, EPIDEMIC
(U) TYPHUS, MURINE

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
SEE OVER

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

189070

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

25 (U) Sera from suspected cases of rickettsioses were tested by fluorescent antibody and complement fixation. The former method revealed more cases of past and present infection than the latter.

Follow-up bleedings from cases of murine typhus and spotted fever indicated that IgM antibodies against rickettsiae may persist at low levels for six months or longer.

Human sera were found to contain a non-antibody inhibitor of reverse passive hemagglutination. However, immune sera could be titrated by measuring the difference in agglutinating titer of antigen diluted in constant concentrations of the immune serum and of normal serum.

Reverse passive hemagglutination can be used to differentiate between epidemic and murine typhus rickettsiae.

A strain of spotted fever was isolated from the blood of a child just before death with disseminated intravascular coagulation. The strain was found to be antigenically similar to five of the six previously isolated in Israel.

AD

AN EPIDEMIOLOGIC AND IMMUNOLOGIC STUDY OF BOUTONNEUSE FEVER IN ISRAEL

FINAL TECHNICAL REPORT

by

Marcus A. Klingberg, M.D.
Robert A. Goldwasser, Ph.D.
Tiberio A. Swartz, M.D.
Wanda Klingberg, Ph.D.
Yonel Steiman, M.Sc.

December 1976

EUROPEAN RESEARCH OFFICE
United States Army
London, England

GRANT NUMBER DA-ERO-75-G-041

Israel Institute for Biological Research
and Tel-Aviv University Sackler School of Medicine
Ness-Ziona, Israel

TABLE OF CONTENTS

SUMMARY

The study was undertaken to continue investigations on the epidemiology of spotted fever in Israel and the serological and biological properties of locally isolated strains. Furthermore, it was undertaken to continue the development of the reverse passive hemagglutination technique as a new serological method for the diagnosis of rickettsial infections.

Sera from suspected cases of rickettsioses were tested by fluorescent antibody and complement fixation. The former method revealed more cases of past and present infection than the latter.

Follow-up bleedings from cases of murine typhus and spotted fever indicated that IgM antibodies against rickettsiae may persist at low levels for six months or longer.

Human sera were found to contain a non-antibody inhibitor of reverse passive hemagglutination. However, immune sera could be titrated by measuring the difference in agglutinating titer of antigen diluted in constant concentrations of the immune serum and of normal serum.

Reverse passive hemagglutination can be used to differentiate between epidemic and murine typhus rickettsiae.

A strain of spotted fever was isolated from the blood of a child just before death with disseminated intravascular coagulation. The strain was found to be antigenically similar to five of the six previously isolated in Israel.

BACKGROUND

The first description of human infections with rickettsiae of the spotted fever group in Israel was published in 1949 (1). At that time, and during the following two decades the disease was thought to be confined to a few definitely defined endemic foci in the coastal area. However, investigations started by this laboratory in 1971 revealed that human cases of infection occurred throughout the country and the incidence of disease was higher than it was previously thought to be.

During the course of these studies six strains of spotted fever rickettsiae were isolated, five of which were antigenically similar and one which differed from the others. All six strains were found to differ antigenically from the taxonomically defined strains available in this laboratory.

Sera from suspected cases of rickettsioses and from cases diagnosed as pyrexias of unknown origin were tested by fluorescent antibody and complement fixation. The former method revealed more cases of past and present infections than did complement fixation and revealed current rickettsioses among the previously undiagnosed cases.

Methods were developed for performing reverse passive hemagglutination (RPH) and reverse passive hemagglutination inhibition (RPHI) tests for the detection and titration of antibodies to rickettsiae in immune and convalescent sera. These preliminary studies showed that the new methods had some advantages as well as disadvantages when compared with other methods available for similar purposes.

The aim of the study

The present study was undertaken in March 1975 with the aim to continue studies on the epidemiology of spotted fever in Israel and the serological and biological properties of the strains of spotted fever isolated during the period of this grant and of previous contracts.

The scope of work during period of this grant was defined as follows:

1) Continued development of the reverse passive hemagglutination technique in the direction of a new serological method for diagnosis of rickettsial infections.

2) Serologic diagnosis of suspected rickettsioses in humans, using various serologic techniques and comparison of the results.

3) Attempts at the isolation of new strains of spotted fever rickettsiae and their characterization by comparison with taxonomically known strains and with other strains already isolated locally.

4) Continued efforts to use gas chromatography for identification of rickettsiae and early diagnosis of infection.

MATERIALS AND METHODS

Diagnostic Serology

Except as otherwise indicated, the materials and procedures for collecting and handling specimens, rickettsial strains, propagation of rickettsiae, preparation of antigens, antisera, fluorescent antibody (FA) methods, complement fixation (CF) techniques, reverse passive hemagglutination (RPH) and reverse passive hemagglutination inhibition (RPHI) procedures, etc. were as previously described (2-5).

As in previous years, bloods or sera from human cases of suspected rickettsioses were received from hospitals serving various areas from the Upper Galilee in the North to the Negev, the South of Israel. In addition, samples were submitted to us from the A. Felix Public Health Laboratory of the Ministry of Health as well as from District Health Offices of the Ministry of Health in Hadera, Netanya, Ashdod, Rehovot and Beersheba.

All sera were tested by CF against soluble group specific spotted fever and typhus antigens by the standard LBCF method and by FA against spotted fever (SF) and murine typhus (MT) rickettsiae. In the FA tests, staining was carried out with anti-human globulins conjugate as well as specific anti-human IgM globulins in duplicate smears. When cross reactions appeared, i.e., staining was positive for both SF and MT, the methods and criteria used for determining the etiologic agent of infection were as described in a previous report (4).

Some difficulties were encountered with some of the fluorescent conjugates used in this laboratory. Some lots of anti-human immunoglobulins gave weakly positive reactions in the staining of SF in controls following the use of human negative sera or saline. It was found that this could be prevented by diluting the anti-human conjugate in soluble group specific SF antigen such as used in the CF test.

In testing for specific IgM antibodies in acute phase sera of suspected rickettsioses, it was found that certain lots of anti-IgM fluorescein conjugates obtained commercially did not give satisfactory results.

It was therefore decided to use a three layer method such as described in an earlier report (3).

Following the serum to be tested, rabbit anti-human IgM serum, appropriately diluted (1:100 - 1:200) was layered on the slide and this was followed by fluorescein labelled caprine anti-rabbit globulin. The rabbit anti-human IgM serum obtained commercially, was found to react slightly with human IgG. In order to prevent staining as a result of this unwanted reaction, the anti-IgM serum was diluted before use in a 1% solution of human purified IgG. The caprine anti-rabbit globulin conjugate also showed some reaction with human immuno-globulins and was diluted before use in a 1% solution of pooled human immunoglobulin in order to prevent "non specific" staining.

Column chromatography

Two types of columns were used in this work: brushite (6) and cellulosic. Brushite was prepared by allowing equal volumes of 0.5 M Na_2HPO_4 and 0.5 M $CaCl_2$ to drip slowly into a container with continuous mixing. The precipitate which formed was washed four times in four volumes of distilled water and finally suspended in 0.001 M phosphate buffer at pH 7.2. This supension of brushite could be stored in the cold up to approximately 10 days, after which it was necessary to prepare fresh.

DEAE (diethylaminoethyl) cellulose and CM (carboxymethyl) cellulose were obtained commercially (Whatman DE11, CM11) and the columns were prepared as described elsewhere (7).

Serological Survey

For purpose of the serological survey two areas were selected: Nazareth in the Northern area of Israel and Naan, a Kibbutz a few miles South of Rehovot. Ninety-one sera were taken from inhabitants of Nazareth and its surroundings and sent to this laboratory by the District Health Office. In Naan 83 bloods taken from 79 individuals were submitted for testing.

Environmental Survey

One hundred and two rats (R. norvegicus and R. rattus) were trapped and bled in the course of investigations on leptospirosis in Israel. Samples of these bloods were submitted to this laboratory for testing for antibodies to MF and SF.

RESULTS

Serological data on suspected rickettsioses in humans

The serological results of FA and CF tests are presented in Table 1. The total number of bloods or sera received in this laboratory from February 1975 - December 1976 was 685 from 552 cases of suspected rickettsioses, 264 of which occurred during 1975 and 288 during 1976.

The sera were tested by both FA and CF using antigens derived from rickettsiae of the spotted fever group and from rickettsiae of murine typhus. In the table are listed all the sera from those cases of which one or more sera gave positive results in the CF and/or FA tests. Under the heading of "town" are listed the cities or settlements in which the corresponding individual had his permanent home, when such information was available. In those cases in which the habitation was unknown, the referring hospital or clinic is given.

Diagnosis of current infection on the basis of the CF test was made on the appearance of a four-fold or greater rise in titer in sequential bleedings. In the case of the FA test, more than minimal staining of specific IgM was used as an indicator of current infection when no increase of titer was noted or only one blood was obtained from the patient.

In some cases of serological crossing it was not possible to differentiate between spotted fever or murine typhus infection on the basis of brightness of staining. Frequently however, in such cases, the complement fixation test showed far less cross reaction and it was therefore possible to make a differential diagnosis on the basis of the two tests.

A summary of the results shown in Table 1 is presented in Table 2. In this table it can be seen that of a total of 685 sera from 552 individuals, 372 sera from 293 cases showed the presence of antibodies to MT and/or SF according to the FA test. Of six hundred fifty-nine sera from 523 cases tested by CF, 254 sera from 219 cases were positive for MT and/or SF. As in previous years many more positive for spotted fever were found using immunofluorescence than using CF tests, and more sera showed cross reaction between MT and SF by FA than by CF.

The FA test permitted serological diagnosis of current infection with SF in 223 individuals and with MT in 49, whereas only 66 cases of SF and 8 of MT could be diagnosed by CF as current infection. The difference between the total number of current infections diagnosed by the two methods was partly due to the fact that in only 138 cases out of the 552 were two or more

bloods received from the same individual. In these 138 cases, CF showed a significant rise in titer for SF in 73 cases and for MT in 8, whereas in the same sera FA indicated current infection with SF, based on IgM staining, in 117 cases and with MT in 9 cases.

A further comparison of the results using the two types of test is summarized in Table 3. In this table are included all the sera of the suspected cases of rickettsioses which were tested by both methods, excluding those which showed anti-complementary activity. In this table it can be seen that in tests for SF, the immunofluorescence methods picked up many more positives than did the CF tests - 341 as compared to 210. However, the CF test also picked up positives missed by FA.

In the case of the murine typhus sera, the overall agreement in the results of the two tests was better than in the case of SF, but here too, each method picked up positives missed by the other. However, neither method showed any advantage over the other, each revealing an approximately equal number of positives missed by the other test.

During the course of this work, the question arose as to what extent could the presence of specific IgM be considered as proof of current infection with the corresponding rickettsiae. Efforts were therefore made to obtain bloods from known cases of rickettsioses at times of up to six months or longer after the primary infection. These bloods were tested by FA for the continuing presence - or disappearance of specific IgM. The results are presented in Table 4. Here it may be seen that in three out of four bleedings taken up to 17 weeks following the first bleeding or onset of disease, specific IgM staining was still relatively strong. In the six bloods collected during periods ranging from 18 to 27 weeks after the first bleeding, one blood still showed the presence of small amounts of specific IgM.

Isolation of Spotted Fever Rickettsiae

During the period of this report a fatal case of spotted fever occurred in a child in the town of Kfar-Saba, about five miles North of Tel-Aviv. On September 29, 1975, this child and her sister became ill and both children were hospitalized on October 2nd. On the next day, one of the children expired with disseminated intravascular coagulation. Just before death, some blood was taken and sent to this laboratory for isolation of rickettsiae.

One ml. of plasma was injected intraperitoneally into each of two adult male guinea pigs. After 72 hours both animals showed fever and orchitis. One animal was sacrificed and a 50% suspension was made of the blood, testes and spleen in sucrose phosphate (8). Aliquots of this material were injected into the yolk sacs of 5 day embryonate hen eggs. The embryos died on the 6th - 7th day following inoculation. Smears were prepared from the yolk sacs and FA

staining showed the presence of spotted fever group rickettsiae. The above isolate was labelled 2607 and was tested for antigenic similarity to the other strains previously isolated locally using FA methods described in a previous report (2). Strain 2607 proved to be antigenically similar to five of the six strains isolated previously S-484, G-212, T-162, T-193, T-487) (2-4).

Environmental Survey

During the period of this report 102 rats (R. norvegicus and R. rattus) were captured and bled. The sera were tested by FA only. Twenty rats were found positive for SF and seven for MT. The rats found positive for spotted fever were collected in the following regions: Ashdod (5 positive/12), Beishan valley (1/25), Sharon district (8/41) and Nazareth (6/24). The seven rats positive for murine typhus were all captured in Nazareth, and thus 13 out of the 24 rats collected in this area showed evidence of rickettsial infection.

The overall rate of infection of the rats captured of the two species, was higher than in previous years (2-4) and reached approximately 20%.

Some clinical and epidemiological observations on spotted fever which occurred in 1975-1976.

The onset of disease by month in the 223 cases in which the diagnosis of spotted fever was confirmed in our laboratory is presented in Table 5. In 1975 no cases were found until April. From April through August the incidence by month remained fairly constant. In September there was a sharp rise in the number of cases which then decreased to the usual level of a few isolated cases in December. In 1976 the picture was somewhat different; there was no sharp peak in the number of cases, a rise in incidence began in June, continued through August and then fell off gradually through October ending with a sharp drop with the onset of colder weather.

The distribution of cases by sex and geographical area are presented in Table 6. Approximately 40% of the cases appeared in the Sharon area(Hadera, Netanya)and an equal number south of the Sharon. This indicates a definite rise in the proportion of the cases appearing in the southern part of the country especially in the vicinity of Beersheba.

In 279 cases of spotted fever infections of which 123 were laboratory confirmed, additional epidemiological and clinical investigations were carried out. The results of these investigations are summarized in Table 7-13.

The overall distribution of cases by sex(M/F)was 1.5 as compared with 1.6 in 1974 (5), 1.3 in 1973 (4) and 2.4 in 1972 (5). As in previous years, the highest M/F ratio was in the 5-14 age group (2:1) as may be seen in Table 7. Here it may also be seen that 71% of the cases appeared in the 0-14 age group with the majority of these cases appearing in ages 5-14. Forty-five (16%) of the cases appeared in the 15-45 age group and 33 (12%) in patients over 45 years old.

In 90% of the cases (249 out of 279) animals were known to be in the environs prior to onset of disease. Information concerning the animal species and numbers in the environment is given in Table 8(a) and 8(b). In the majority of the cases (70%) dogs were present, and cats were present in the environs of about one-half the cases (52%). In over 60% of the cases, two or more animals were to be found in the surroundings. In Table 9 are presented the data obtained concerning the type of contact of the 279 cases of spotted fever with animals.

Information concerning the maximal temperatures, distribution and types of rash, as well as some other clinical signs reported among the 279 selected cases of spotted fever are summarized in Tables 10-13.

Serological Survey

During 1974 a case of laboratory-confirmed spotted fever occurring in Nazareth was brought to our attention in view of the fact that the attending physician described the case as "classical Boutonneuse fever" including the primary lesion or eschar. Unfortunately we learned of the case too late to make attempts at isolation of the infecting organism. As this was the first case of spotted fever in Nazareth from which serum was sent to our laboratory, the aid of the District Health Office was sought in order to carry out a sero-epidemiologic survey of human rickettsial infections in Nazareth. The District Office agreed to cooperate and submitted 91 sera from Nazareth and its surroundings. The sera were tested by FA and CF tests. The results are shown in Table 14.

An additional sero-epidemiologic survey was carried out in Kibbutz Naan, a communal settlement a few miles south of Rehovot. In this settlement, during the past few years, a high number of undiagnosed mild febrile illness has occurred. The results of this survey are also included in Table 14.

As in previous surveys, FA revealed many more positives for spotted fever than did the CF test. Nazareth proved to be an endemic area for spotted fever showing a 25% rate of infection, whereas Naan seemed to be an hyperendemic area with 64.5% of the individuals tested showing signs of previous infection.

Reverse Passive Hemagglutination (RPH) and Reverse Passive Hemagglutination Inhibition (RPHI)

A. Improvements in conditions for RPH and RPHI

1) Preparation of erythrocytes for conjugation: The method used for fixing the erythrocytes with glutaraldehyde was described in an earlier report (5). However, it was found that on prolonged storage at 4°C the glutaraldehyde fixed cells darkened and eventually become unsuitable for conjugation with antibody globulins. This deterioration in the properties of the fixed RBC could be prevented by storage in the deep freezer. After testing several possibilities it was found that optimal results were obtained when the 50% suspension of glutaraldehyde fixed RBC in 0.005 M NaCl was stored first at 4°C for 3-4 days then transferred to -70°C and stored frozen until used. When thawed the RBC suspension showed clumping but the aggregates were easily dispersed by sonication for about 10 seconds without signs of hemolysis.

2) Storage of conjugated erythrocytes: Following conjugation of RBC with antibody globulins the conjugates were frozen and stored at -70°C. On thawing it was necessary to sonicate the suspensions to break up clumps before carrying out RPH or RPHI tests. It was found that the addition of 5% glycerine to the PBS-bovine albumin solution in which the conjugated RBC were suspended would prevent the formation of such aggregates and the need for sonication after thawing. Such suspensions could be frozen and thawed several times with no signs of clumping or change in agglutinability in the presence of the corresponding antigen.

3) Preparation of sera for the RPHI test: Most sera used in the RPHI test were found to contain agglutinins either against the chicken RBC or the globulin used in the preparation of the conjugated erythrocytes or both. It was found that acetone precipitated liver-tissue powders prepared similarly to those prepared for the absorption of fluorescein labelled antisera (9) could be used to remove these agglutinins. Thus chicken-liver powder could be used to remove agglutinins against the chicken erythrocytes and rabbit liver powder to remove the antibodies against the rabbit globulin moiety of the conjugated erythrocytes.

Tissue powders were stored in the deep freezer and retained their activity even after storage of over two years.

One tenth ml. serum was diluted 1:4 in saline and absorbed with 40 mg tissue powder for 1-2 hours at room temperature. Following centrifugation the supernatant fluid was removed and used.

The same treatment described above was used for globulins which were to be conjugated to chicken erythrocytes. When such globulins contained agglutinins against chick RBC the conjugates would show non specific agglutination and it was therefore necessary to absorb the globulins either with chicken liver powder or chicken erythrocytes to remove all anti-chicken antibodies.

4) Diluents for the RPH and RPHI tests: In earlier work, phosphate buffered saline containing 1% bovine serum albumin was used as a diluent for RPH and RPHI tests. In some microtiter plates however, the coated RBC did not settle clearly in the negative controls, making difficult the determination of end points in antigen or antibody titrations. It was thought that changes in the diluent used might improve the test and after testing several variations, the diluent which gave the best results was found to be: physiological saline with the addition of 2% dextrose, 2% normal rabbit serum and 0.08% sodium azide as a preservative. Some batches of normal rabbit sera were found to contain agglutinins against chicken RBC. These sera were absorbed using one volume of 50% chicken RBC suspension per volume normal rabbit serum or 100 mg chicken liver powder/ml rabbit serum. The normal sera were frozen in small aliquots so that any one aliquot of serum was frozen and thawed once only before use. The diluent could be stored at $4^{\circ}C$ for several weeks without deterioration.

5) The RPHI test: In carrying out RPHI tests the period of incubation of antiserum-antigen mixtures was shortened to one hour at $37^{\circ}C$ instead of overnight in the refrigerator as described earlier (5) before adding conjugated RBC. This was found to give more reproducible results and did not result in any loss in the sensitivity of the test.

B. Results of RPHI tests

RPHI tests were carried out with 24 human sera which had previously been shown to be positive for murine typhus by CF and/or FA tests and ten human known negative sera. All known negative sera were negative by RPHI at a dilution of 1:8, the lowest dilution used in the test. All the sera which were positive by FA were also positive by RPHI, including three which were negative by CF. It was therefore concluded that a RPHI titer of 1:8 in tests for murine typhus should be considered as positive.

In the case of RPHI tests for spotted fever, it was reported earlier (5) that known human negative sera gave positive titers ranging from

1:16 - 1:64. Later work however has shown that if care were taken to use a full four to eight hemagglutinating units of antigen, the known sera did not give titers of over 1:16. Therefore titers of 1:32 were considered as positive and 1:16 or less was considered to be negative.

RPHI tests were performed on paired sera from 25 cases of spotted fever infections which had been found positive by CF and/or FA. In 13 cases a rise in titer was detected by all three tests. In nine cases the FA and RPHI tests were positive, whereas the CF test was negative. In seven of these cases both FA and RPHI detected rises in titer. In one case a rise in titer was detected by RPHI and CF but not by FA and in two cases antibodies were detected in all three tests, but no rise in titer was found in any test.

Two hundred thirty-one of the sera submitted to us from cases of suspected rickettsioses were tested by RPHI, using murine typhus and spotted fever soluble antigens, in addition to the usual CF and FA tests. Of the 231 sera, 30 were positive by CF for murine typhus, 49 by FA, and 58 by RPHI. Eighty-six were positive by CF for spotted fever, 205 by FA and 157 by RPHI.

A partial analysis of these results is shown in Table 15. In the case of tests for murine typhus, RPHI and FA each gave negative results with many sera which were positive according to the other test. Thus, the RPHI test was negative in 40% of the FA positive sera and the FA test was negative in approximately 50% of the RPHI positive sera. In tests for spotted fever, the RPHI and FA tests were in better agreement. The FA test picked up 95% of the RPHI positive sera and RPHI was positive in 73% of the FA positive sera. The overall agreement was 73%.

In view of the discrepencies noted in the results of the different tests, it was decided to investigate the possiblity that they were due, at least in part, to the presence in sera of some component which blocks the reaction between the rickettsial antigens and the conjugated erythrocytes. To test for this component, *Kenya tick typhus soluble antigen* was titrated against chicken RBC conjugated with rabbit anti-Indian tick typhus immunoglobulins, in the presence of various dilutions of normal human serum. This serum was obtained from a blood bank and was tested before use for the absence of antibodies to rickettsiae by FA and CF tests. The results of the block titration as shown in Table 16 indicate that the antigen titer is strongly influenced by the concentration of serum used in the test. Thus, there was at least a 16 fold difference in antigen titer in the presence of normal serum at dilutions 1:4 - 1:16 and at serum dilution 1:128 - 1:256. This might explain the fact that sera from normal individuals were found to

give positive RPHI titers at a 1:16 dilution, since four hemagglutinating units of antigens as determined in a diluent containing 2% normal rabbit serum would have no hemagglutinating activity in a 1:16 dilution of normal human serum.

In order to eliminate some of the problems which arose in performing hemagglutination inhibition tests by varying the serum dilution and keeping the agglutinating antigen constant, the reverse procedure was devised, i.e., keeping the serum, which was to be tested, constant and varying the concentration of antigen. The titer of a given serum could then be expressed as the relative amount of antigen whose hemagglutinating activity could be blocked by a given serum concentration as compared with the same concentration of normal serum. An example of such a test is presented in Table 17. When mixed with a 1:10 dilution of convalescent serum No. 2608, Kenya tick typhus antigen agglutinated chicken RBC conjugated with rabbit anti-Indian tick typhus immunoglobulins at an antigen dilution of 1:8, but not at higher dilutions. The same antigen mixed with 1:10 normal human serum agglutinated the same RBC conjugate up to a dilution of 1:512. Thus, serum No. 2608 reduced the antigen titer under these circumstances 64 fold or by six two-fold dilution. For the purposes of this report the number of two-fold dilutions, by which the antigen titer is reduced in a 1:10 serum dilution, will be called the index (RPHI index) of the serum, in this case six. In the same Table, the effect of dilutions of the serum on the RPHI index is presented. Dilution of the serum caused a decrease in the index but the relationship was not one to one. A 64 fold dilution of the serum brought the index down from six, to three.

The RPHI index method was tested for its potential usefulness for diagnostic purposes in spotted fever as compared with FA and CF tests. For this purpose 15 known negative Norwegian sera (4) were used, as well as 33 single sera and 32 paired sera from 16 cases of suspected rickettsioses. Results of a few selected sera are illustrated in Table 18.

The RPHI indexes of the Norwegian sera ranged from 2 to -2. (A negative index number was assigned to a serum when the soluble antigen gave a higher titer in a 1:10 dilution of that serum than in a 1:10 dilution of the selected standard negative serum), and therefore any serum which showed a RPHI index of three or greater was considered as a positive serum.

The overall agreement between FA and the RPHI indexes was 85% and each method was positive in 86% of the sera found positive by the other. In the single sera, the RPHI indexes were positive in 18 out of 44, FA in 15 and CF in 12. In the paired sera, FA showed a rise in titer in 13 of the 16 patients' sera as did the RPHI indexes, whereas CF tests showed a rise in titer in only 7 of the 16 patients. Some of the discrepancies between the

FA results and RPHI indexes were apparently due to the fact that some the sera were from cases of murine typhus and the FA method showed more serological crossing between MT and SF than did RPHI or CF tests.

The RPHI index method was also tested for its potential usefulness for diagnostic purposes in murine typhus as compared with the other tests. A total of 67 sera, some taken at random and some selected, but all derived from suspected rickettsioses were used in this experiment, in addition to 10 known negative Norwegian sera. The serum obtained from a blood bank, which served as the standard negative serum for the determination of RPHI indexes for spotted fever was also used in these tests as the standard negative serum. The RPHI indexes of the Norwegian sera ranged from 2 to -1 and therefore any RPHI index of 3 or greater was considered as positive. The overall agreement here was far better than in the RPHI tests described earlier for MT, and reached 91%. The RPHI index method was positive in all those sera which were positive by FA, but was also positive in 6 sera negative by FA. In three of these sera, the CF test was also positive.

C. The use of RPHI for the differentiation of rickettsial species

Glutaraldehyde fixed chicken RBC conjugated with hyper-immunized rabbit anti-murine and anti-epidemic typhus immunoglobulins were tested against soluble and somatic antigens of both epidemic and murine typhus rickettsiae. The results of such tests are presented in Table 19. The anti MT conjugate reacted well with the various antigens, whereas the anti ET conjugate reacted well only with ET antigens. In only two out of six ET antigens did the anti MT conjugate give significantly lower titers than did the anti ET conjugate with the same antigens. Thus, it would seem that the two conjugates could be used to differentiate between murine and epidemic typhus rickettsiae.

In view of the results described above, RPHI tests were made to determine whether this method could be used to differentiate between murine and epidemic typhus infections. For this purpose sera were used from guinea pigs and rabbits which had been infected with yolk sac suspensions of viable epidemic and murine typhus rickettsiae. The RPHI method used was as described earlier (5), e.g., determination of the highest dilution of serum which would inhibit agglutination using four hemagglutinating units of antigen. The results of this experiment are shown in Table 20. Here the sera showed considerable degrees of specificity but in reverse manner to what was noted using conjugated RBC against antigen preparations. In these cases the epidemic typhus antisera failed to differentiate between murine and epidemic typhus anti-

gens, whereas murine typhus antisera showed considerable differences in titer against the antigens derived from the two species.

Experiments similar to the above were also performed using glutaraldehyde fixed chicken RBC conjugated with immunoglobulins derived from rabbits hyperimmunized against R. rickettsi, R. conori, R. siberica and several local isolates. None of these conjugates differentiated satisfactorily among the antigens either soluble or somatic derived from the various species of spotted fever rickettsiae. As it was thought that these antigens might be mixtures of groups specific and species specific antigens, attempts were made to separate the different antigens with the aid of column chromatography. Calcium phosphate (brushite) columns were used as well as ion excharge columns (DEAE cellulose and carboxymethyl cellulose).

Using brushite columns, several spotted fever antigens were found to adsorb strongly to the brushite in 0.005 M NaH_2PO_4/K_2HPO_4 pH 7.2 and to elute in buffer using the same salts and pH but at a concentration of 0.2 M. The antigens that eluted at this phosphate concentration were unchanged in specificity and the RPH and RPHI methods did not differentiate well among them.

Using the ion exchange columns, antigens were eluted under conditions of constant pH, but with increasing concentrations of NaCl. When the various fractions were tested using RPH for determining antigen content, several well defined peaks were noted. However, here too, no differences in antigenic specificity were found in the eluted material as compared with the antigens before being chromatographed.

DISCUSSION

During the six years in which this laboratory has been engaged in investigations of spotted fever in Israel, 2,003 bloods were received from 1,579 cases as "suspected rickettsioses", i.e., the clinical diagnosis was either spotted fever or murine typhus. Of the 1,579 cases, 702 were confirmed as current infections with rickettsiae, 501 spotted fever and 201 murine typhus. Thus, the physicians were able to make the correct diagnosis on clinical grounds in only 44% of the cases.

From 1972 to 1976, the number od cases of murine typhus diagnosed by this laboratory decreased from 70 in 1972 to 18 in 1976. Spotted fever infections, on the other hand, have not shown any tendency to decrease in number, but on the contrary may be on the increase. Sixty-eight cases were diagnosed as current spotted fever in 1972, 122 in 1973, 67 in 1974, 123 in 1975 and 99 in 1976.

As in previous years, the FA test proved to be more sensitive in picking up past and present infections with rickettsiae. Thus, the FA test permitted serological diagnosis of current infection with SF in 223 cases and with MT in 49, whereas only 73 cases of SF and 8 of MT could be diagnosed as current infections using the CF test. The differences in the results using the two tests were partly due to the fact that in only 138 out of the 552 cases of suspected rickettsioses were paired sera submitted for testing. Since current infection can be diagnosed using the CF test only by showing significant changes in titer in sequential bleedings, only paired sera are useful for this purpose. On the other hand, specific IgM is known to appear as the result of primary infection and then tends to disappear in a relatively short time thereafter. The presence of IgM is therefore indicative of current infection, even when only one serum sample is available. However, as was shown in the present report, low levels of specific IgM can be detected 6 months after primary infection. Therefore it is necessary to be cautious in making a definite diagnosis of current infection on the basis of only low levels of specific IgM.

As described in earlier reports (2-5), spotted fever infections showed a seasonal pattern, reaching maximal incidence in late summer. With the onset of cold weather in November, the number of cases drop off rather sharply.

During the period covered by this report, there was some change in the geographical distribution of spotted fever in Israel. The proportion of cases in the Sharon area dropped to somewhat less than 40%. Of the remaining cases approximately 66% appeared south of the Sharon area, as in years previous to 1974 when 68% appeared north of the Sharon (5).

The distribution of cases by sex (M/F) continued to be greater than unity, especially in the 5-14 age group (M/F = 2:1) where the majority of cases occurred. This probably was due to the closer association of males of this age group with pet animals, particularly dogs.

The close association of cases of spotted fever with household animals was also shown. Out of 279 cases of spotted fever infections, 123 of which were laboratory confirmed, 249 were known to have contact with animals, mostly dogs, and in over 60% of these cases two or more animals were present in the immediate environs.

During the period of this report, for the purpose of an environmental survey, rats were tested for antibodies to rickettsiae. Twenty percent of these rats were found to be positive for SF, indicating a continuing rise over that found in previous years. It is difficult as yet to determine whether these rats may serve as sources of human infection, however they may perhaps serve as sentinel animals, indicating a rise in the "pool" of infected animals in nature which may serve as reservoirs of infection.

In a previous report (5) we described the beginnings of the development of reverse passive hemagglutination (RPH) and hemagglutination inhibition (RPHI) tests suitable for use with rickettsial antigens and antisera. Subsequently improvements were made in the conditions for these tests in the reagents used and in the carrying out of the tests themselves.

Two hundred thirty sera from cases of suspected rickettsioses were tested by RPHI and the results compared with findings in the CF and FA tests. More sera were found positive to MT by RPHI than by either CF or FA. In the case of spotted fever positive sera, RPHI picked up more positives than CF tests, but fewer than FA. When the results were analysed, the agreement found between FA and RPHI was not good in MT and only fair in SF.

In the case of SF, it was found that normal human serum contains some factor which blocks the agglutination of conjugated erythrocytes by corresponding antigens, and the amount of antigen blocked is proportional to the serum concentration. In order to measure the amount of antibody in the presence of the blocking agent it was decided to carry out the RPHI test using a constant concentration of serum and varying the concentration of antigen. The serum concentration was arbitrarily selected as 1:1o. In each test a known normal serum was taken as a reference serum and the antigen titered in the presence of the normal serum diluted 1:10 and in the presence of the serum to be tested, also diluted 1:10.

The number of two-fold dilutions in which a significant difference in agglutination of the conjugated RBC could be seen between the two sera was called the RPHI index of the serum being tested.

The RPHI index method was tested for its potential usefulness as a diagnostic tool and the results seem promising. The agreement between this method and FA, in the relatively few sera tested, was good both in cases of murine typhus and spotted fever.

The use of RPH and RPHI for the differentiation of rickettsial species proved disappointing in the case of SF, but seems promising for the differentiation of murine and epidemic typhus rickettsiae or antisera.

In the case of known rabbit and guinea pig antisera it was found possible to differentiate among the sera resulting from the immunization or infection of the animals with the two different rickettsiae. Unfortunately, known positive human epidemic typhus antisera were not available to us so that we were unable to test this method with human sera.

Attempts were made to separate various antigenic components from spotted fever antigens, using brushite anionic and cationic ion-exchange columns, in the hope that some of these components would be species specific rather than group specific. Results here too were disappointing. Species specific antigens were not detected using RPH or RPHI techniques, but only group specific antigens.

CONCLUSIONS

1. Fluorescent antibody methods revealed 293 past and present rickettsioses in sera from 552 suspected cases. Of these, 223 were shown to be current spotted fever and 49 current endemic typhus.

2. On the basis of complement fixation tests using paired sera, current infections with spotted fever were confirmed in 73 cases and with murine typhus in eight. In the same sera, immunofluorescent staining of IgM detected 117 cases of current spotted fever and nine cases of current murine typhus.

3. Specific IgM antibodies against rickettsiae following primary infection, may persist at low levels for six months or longer indicating the need for caution in diagnosing current infections on the basis of the presence of only minimal amount of specific IgM.

4. Normal human serum contains some factor which inhibits agglutination, by homologous antigen, of glutaraldehyde fixed chicken erythrocytes conjugated with anti-spotted fever immunoglobulin. The amount of antigen inhibited is proportionate to the concentration of serum present.

5. It was found possible to titrate the level of antibodies against rickettsiae by making serial dilutions of antigen in the presence of constant concentrations of test sera, and adding glutaraldehyde fixed chicken erythrocytes conjugated with homologous immunoglobulin. The same procedure was followed using normal human serum. The antibody titer or RPHI index of the unknown serum was then expressed as the difference in the amount (or number of antigen dilutions) inhibited by the known negative serum and the unknown serum.

6. The RPHI index method was tested with selected animal immune sera and humane acute and convalescent sera and found to give results in good agreement with fluorescent antibody methods.

7. Reverse passive hemagglutination, using erythrocytes conjugated with serum globulins from animals immunized against murine and epidemic typhus differentiated between antigens prepared from the two species of rickettsiae.

8. One isolation of spotted fever rickettsiae was made from blood taken from a child just prior to death with disseminated intravascular coagulation. The strain proved to be antigenically similar to five of the previous isolates (T-162, T-193, G-212, S-484, T-487) but differed from one previous isolate (T-174).

REFERENCES

1. Valero, A. Rocky Mountain Spotted Fever in Palestine. Harefuah 36: 99-101 (1949)(in Hebrew).

2. Klingberg, M.A., Goldwasser, R.A., Swartz, T.A., Klingberg, W. and Steiman, Y. An Epidemiologic and Immunologic Study of Boutonneuse Fever in Israel. Final Technical Report. Contract No. DAJA37-71-C-0852 (1971).

3. Klingberg, M.A., Goldwasser, R.A., Swartz, T.A., Klingberg, W. and Steiman, Y. An Epidemiologic and Immunologic Study of Boutonneuse Fever in Israel. Final Technical Report. Contract No. DAJA31-72-C-1814 (1973).

4. Klingberg, M.A., Goldwasser, R.A., Swartz, T.A., Klingberg, W. and Steiman, Y. An Epidemiologic and Immunologic Study of Boutonneuse Fever in Israel. Final Technical Report. Contract No. DAJA37-73-C-3126 (1974).

5. Klingberg, M.A., Goldwasser, R.A., Swartz, T.A., Klingberg, W. and Steiman, Y. An Epidemiologic and Immunologic Study of Beoutonneuse Fever in Israel. Final Technical Report. Contract No. DAJA37-74-C-2959 (1975).

6. Tiselius, A., Hjertén, S. and Levin, Ö. Protein Chromatography and Calcium Phosphate Columns, Arch. Biochem. Biophys. 65, 132-155 (1956).

7. Petersen, E.A. Cellulosic Ion Exchangers in: Laboratory Techniques in Biochemistry and Molecular Biology. Vol. 2. (T.S. Work and E. Work, eds), North Holland Publishing Co., Amsterdam pp. 225-396 (1970).

8. Bovarnik, M.R., Miller, J.C. and Snyder, J.C. The Influence of Certain Salts, Amino Acids, Sugars and Proteins on the Stability of Rickettsiae. J. Bacteriol. 59, 509-522 (1950).

9. Coons, A.H., Leduc, E.H. and Connolly, J.M. Studies on Antibody Production. I. A Method for the Histochemical Demonstration of Specific Antibody and its Application to a Study of the Hyper-Immune Rabbit. J. Exper. Med. 102, 49-60 (1955).

10. Topping, N.H. and Shepard, C.C. The Preparation of Antigens from Yolk Sacs Infected with Rickettsiae. Pub. Hlth Rep. 61, 701-707 (1946).

Table 1

SERA POSITIVE FOR SPOTTED FEVER AND/OR MURINE TYPHUS - SEROLOGICAL RESULTS
(1.3.75 - 31.12.76)

No.	Name	Town	CF[1]		FA[2]		IgM[3]	
			SF[4]	MT[5]	SF	MT	SF	MT
2226	M.D.	Rehovot	-	-	-	-	-	-
	M.D.$_2$	"	-	-	≥40	-	40	-
2253	G.H.	Nazareth	-	≥8	-	>40	ND	>40
2257	V.O.	"	-	-	40	-	-	ND
2259	S.A.	"	-	-	40	-	-	ND
2260	O.Z.	"	-	-	40	40	-	-
2261	A.M.	"	-	-	40	-	-	ND
2349	D.D.	Haifa	16	-	≥40	-	≥40	-
2350	B.N.	Beersheba	-	-	-	-	-	-
	B.N.$_2$	"	≥64	8	≥40	-	≥40	-
2366	N.S.	Naharia	16	-	40	-	40	-
2369	B.N.	Kfar-Saba	160	-	40	-	40	ND

[1]CF - Complement Fixation Test.
[2]FA - Indirect staining with fluorescent antibody against human gamma globulins.
[3]IgM - Indirect staining with fluorescent antibody against IgM gamma globulins.
[4]SF - Spotted fever.
[5]MT - Murine typhus.
[6] - Subscripts indicate successive bleedings.
\- - Indicates <4 in the CF test or <40 in the FA test.
ND - Not done.

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2369	B.N.$_2$	Kfar-Saba	160	-	≥ 40	-	40	ND
2372	P.B.	" "	-	-	-	-	ND	ND
	P.B.$_2$	" "	20	-	40	-	40	ND
2373	M.T.	Haifa	-	160	-	≥ 40	-	≥40
2374	B.Z.R.	Tel Hashomer Hosp.	-	160	-	> 40	ND	≥40
2375	H.M.	" " "	-	320	-	> 40	ND	≥ 40
2376	F.B.	Tel-Aviv	10	-	-	40	ND	40
	F.B.$_2$	Tel-Aviv	20	80	40	40	40	40
2377	S.O.	Kfar-Saba	5	-	-	-	ND	ND
	S.O.$_2$	" "	10	-	-	-	ND	ND
	S.O.$_3$	" "	40	-	40	-	-	ND
2380	K.Z.	Haifa	-	160	≥40	≥ 40	40	≥40
2381	N.H.	"	20	-	≥ 40	-	40	ND
2382	L.S.	Netanya	40	-	40	-	-	-
2383	H.M.	Haifa	-	80	-	-	-	-
2384	E.E.	Gaza	-	320	-	-	-	-
2385	A.H.	Haifa	-	160	40	≥ 40	-	40
2386	I.G.	Rehovot	-	320	-	≥ 40	-	≥ 40
2387	T.M.	Tel-Hashomer Hosp.	-	160	-	≥ 40	-	≥ 40
2388	N.I.	Tel-Aviv	-	-	-	-	-	-
	N.I.$_2$	" "	-	-	-	≥ 40	-	≥40

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2390	G.M.	Haifa	-	-	40	-	40	-
2391	B.H.A.	Haifa	≥64	-	40	-	40	-
2420	K.B.	Haifa	-	≥64	-	>40	-	≥40
2421	P.N.	"	32	-	40	-	40	-
2454	H.G.	Netanya	16	-	40	-	40	-
2456	M.O.	Meir Hospital	-	-	≥40	-	40	-
2457	B.N.	Kfar-Yona	4	-	40	-	-	-
	$B.N._2$	" "	128	-	≥40	-	40	-
2462	F.F.	Zahalon Hosp. Tel-Aviv	-	-	-	-	ND	ND
	$F.F._2$	" " " "	≥64	-	-	-	ND	ND
2463	L.I.	Netanya	64	-	≥40	-	40	ND
2474	S.H.	Asaf Harofe Hospital	-	8	-	40	ND	40
	$S.H._2$	" " "	32	64	-	≥40	ND	≥40
2475	A.S.	Beersheba	-	-	-	-	-	-
	$A.S._2$	"	32	-	≥40	-	40	-
2476	D.A.	Beersheba	-	-	-	-	-	-
	$D.A._2$	"	32	-	40	-	40	-
2477	C.A.	Rehovot	-	≥64	40	160	40	≥40
2496	G.H.	Kiriat Haim	≥64	≥64	≥40	40	≥40	40
2498	H.R.	Haifa	64	-	40	-	40	-
2499	P.Z.	"	64	-	40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2502	S.R.	Haifa	-	-	-	-	-	-
	$S.R._2$	"	256	-	>40	-	40	-
2503	A.G.	Asaf Harofe Hospital	-	-	40	-	40	-
2504	B.I.B.	Tel-Aviv	-	-	-	≥40	-	40
	$B.I._2$	" "	ND	ND	-	>40	-	≥40
2506	D.D.	Tel-Mond	-	-	40	-	-	-
	$D.D._2$	" "	64	16	≥40	-	40	-
2510	K.G.	Ein Vered	-	-	-	-	-	-
	$K.G._2$	" "	8	-	≥40	-	40	-
2514	D.L.	Netanya	32	-	40	-	40	-
2515	A.E.	Kfar-Saba	-	-	40	-	-	-
	$A.E._2$	" "	-	-	≥40	-	40	-
2516	I.A.	Haifa	32	32	40	-	40	-
2518	S.S.	Kiriat Haim	-	-	≥40	-	≥40	-
2519	S.L.	" "	32	64	-	40	-	-
2524	S.O.	Kfar-Yona	-	-	40	-	-	-
2532	N.R.	Tel-Aviv	-	256	40	>40	40	≥40
2533	B.N.	Hadassah Hosp. Tel-Aviv	-	-	-	-	-	-
	$B.N._2$	" " " "	-	32	>40	-	>40	-
2534	G.A.	Ramat Hasharon	-	-	-	-	-	-
	$G.A._2$	" "	256	-	≥40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2535	I.G.	Kfar Yona	-	-	40	-	40	-
	$I.G._2$	" "	-	-	≥40	-	40	-
2537	O.M.	Rehovot	≥256	-	>40	-	40	-
	$O.M._2$	"	≥256	-	>40	-	40	-
2538	L.A.	Rehovot	-	-	-	-	-	-
	$L.A._2$	"	-	-	≥40	-	40	-
2539	K.E.	Netanya	-	-	≥40	-	40	-
	$K.E._2$	"	8	4	40	40	40	-
2540	T.E.	"	-	-	40	-	40	-
	$T.E._2$	"	-	4	40	-	-	-
2541	F.I.	"	-	16	40	-	-	-
2544	D.N.	Netanya	128	-	>40	-	≥40	-
	$D.N._2$	"	128	-	ND	ND	ND	ND
2546	C.O.	"	32	-	≥40	-	>40	-
2547	M.A.	Haifa	ND	ND	≥40	-	≥40	-
	$M.A._2$	"	ND	ND	>40	-	40	-
2548	L.A.	"	-	-	≥40	-	≥40	-
	$L.A._2$	"	ND	ND	>40	40	40	-
2549	P.M.	"	ND	-	>40	≥40	40	-
	$P.M._2$	"	≥256	-	≥40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2550	B.B.I.	Haifa	128	-	≥40	-	40	-
	$B.B._2$	"	128	-	≥40	40	40	-
2551	R.R.	"	-	-	≥40	-	40	-
	$R.R._2$	"	8	-	≥40	-	40	-
2552	R.I.	"	8	-	40	-	-	-
	$R.I._2$	"	16	-	≥40	-	≥40	-
2553	B.D.S.	"	-	-	40	-	40	-
	$B.D._2$	"	256	-	≥40	-	40	-
2555	H.M.	Rehovot	4	-	-	-	40	-
	$H.M._2$	"	32	-	≥40	-	≥40	-
2556	M.E.	Meir Hospital	-	-	≥40	-	40	-
2557	R.D.	" "	-	-	-	-	-	-
	$R.D._2$	" "	-	-	≥40	-	≥40	-
2558	P.A.	" "	64	16	40	-	40	-
2559	S.O.	" "	-	-	40	-	-	-
	$S.O._2$	" "	64	-	≥40	40	≥40	40
2560	G.M.	" "	-	-	40	-	40	-
2561	A.E.	Ashkelon	-	-	40	-	40	-
2562	C.I.	Rehovot	-	-	-	-	-	-
	$C.I._2$	"	32	-	≥40	40	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2563	V.E.	Beersheba	8	-	-	-	-	-
	V.E.$_2$	"	32	-	≥ 40	-	≥ 40	-
2564	S.U.	Jerusalem	64	16	> 40	40	≥ 40	40
	S.U.$_2$	"	64	32	> 40	40	≥ 40	40
	S.U.$_3$	"	16	8	≥ 40	ND	40	ND
2565	A.S.	Rehovot	16	8	40	-	40	-
	A.S.$_2$	"	16	8	40	-	40	-
2567	M.E.	Beersheba	-	-	-	-	-	-
	M.E.$_2$	"	256	-	> 40	-	40	-
2568	E.S.	Meir Hospital	-	-	≥ 40	-	40	-
2569	T.C.	" "	-	-	40	-	40	-
	T.C.$_2$	" "	8	-	≥ 40	-	≥ 40	-
2570	S.I.	" "	16	-	40	40	40	-
2571	G.G.	" "	8	-	40	-	40	-
	G.G.$_2$	" "	64	-	≥ 40	-	40	-
2572	Z.I.	" "	-	-	40	-	40	-
	Z.I.$_2$	" "	-	-	40	-	40	-
2573	H.I.	" "	-	-	40	-	40	-
	H.I.$_2$	" "	-	-	≥ 40	-	≥ 40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2574	B.D.I.	Meir Hospital	-	-	40	-	40	-
	$B.D._2$	" "	32	-	≥ 40	-	≥ 40	-
2575	K.H.	" "	-	-	40	-	40	-
	$K.H._2$	" "	32	-	≥ 40	40	≥ 40	40
2576	H.I.	" "	-	-	40	-	-	-
2577	M.S.	" "	-	-	40	-	40	-
2578	A.R.	" "	-	-	40	-	40	-
2579	S.M.	" "	8	-	≥ 40	-	≥ 40	-
2580	S.S.	" "	-	-	> 40	-	40	-
	$S.S._2$	" "	32	-	≥ 40	-	≥ 40	-
2581	H.B.	" "	64	-	≥ 40	-	40	-
	$H.B._2$	" "	ND	ND	> 40	-	≥ 40	-
2582	O.G.	Jerusalem	8	-	40	-	40	-
2583	S.A.	"	-	-	40	-	40	-
2584	V.E.	Beersheba	-	-	40	-	40	-
2585	B.D.	Rehovot	-	64	40	> 40	40	≥ 40
	$B.D._2$	Rehovot	4	64	≥ 40	> 40	40	≥ 40
2586	A.M.	Haifa	64	-	> 40	-	40	-
2587	R.A.	"	AC	AC	> 40	> 40	40	40
2588	V.A.	"	16	-	> 40	-	40	-
2589	C.S.	"	-	≥ 64	40	>40	-	40

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2590	P.S.	Haifa	8	4	40	-	-	-
2591	M.B.I.	Haifa	≥64	-	≥40	-	40	-
2592	M.I.	Assaf Harofe Hospital	-	-	40	≥40	40	40
2593	V.M.	" " "	-	32	40	≥40	40	40
2594	S.M.	" " "	-	32	40	≥40	-	≥40
2595	M.M.	Tel-Aviv	-	-	≥40	40	40	-
	$M.M._2$	" "	-	-	≥40	40	40	-
	$M.M._3$	" "	-	-	≥40	40	40	-
	$M.M._4$	" "	16	-	≥40	-	40	-
2597	V.O.	Netanya	-	-	-	-	40	-
	$V.O._2$	"	16	-	≥40	40	40	-
2599	O.O.	Pardesia	8	-	-	-	-	-
	$O.O._2$	"	64	-	>40	-	40	-
2600	R.M.	Meir Hospital	32	-	≥40	40	40	-
2601	A.I.	Kfar Saba	-	-	40	-	-	-
	$A.I._2$	" "	8	-	>40	-	≥40	-
2602	E.R.	Netanya	-	-	40	-	40	-
	$E.R._2$	"	16	-	≥40	40	40	-
2603	M.Z.	Haifa	-	-	-	-	-	-
	$M.Z._2$	"	128	8	>40	-	≥40	-
2605	A.A.	"	-	-	40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2608	M.D.	Tel-Aviv	256	-	> 40	40	40	ND
2609	Z.B.	Rehovot	-	-	40	-	-	-
	$Z.B._2$	"	16	-	≥ 40	-	≥ 40	-
	$Z.B._3$	"	ND	ND	> 40	-	40	-
2610	A.O.	Jerusalem	≥ 64	-	≥ 40	-	40	-
2611	E.R.	Jerusalem	-	-	≥ 40	-	40	-
2612	S.U.	Beersheba	128	-	≥ 40	40	40	-
2613	R.I.	"	-	-	≥ 40	40	40	-
2614	O.R.	Pardesia	-	-	40	-	-	-
	$O.R._2$	"	64	-	> 40	-	≥ 40	-
2615	R.Z.	Meir Hospital	4	-	40	-	-	-
2616	S.R.	Herzlia	-	-	≥ 40	-	-	-
	$S.R._2$	"	≥ 256	-	> 40	-	≥ 40	-
2617	H.S.	Netanya	-	-	-	-	-	-
	$H.S._2$	"	64	-	≥ 40	-	≥ 40	-
2618	A.S.	Kfar-Saba	-	-	40	40	-	-
	$A.S._2$	" "	≥ 256	-	> 40	-	≥ 40	-
	$A.S._3$	" "	ND	ND	> 40	-	-	-
2619	H.A.	Netanya	-	-	> 40	-	40	-
2620	C.I.	Herzlia	-	-	40	-	-	-
	$C.I._2$	"	≥ 256	-	> 40	-	≥ 40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2621	M.I.	Kfar Yona	-	-	40	-	-	-
	M.I.$_2$	" "	32	-	40	-	40	-
2622	S.A.	Rehovot	-	-	≥40	-	40	-
2623	B.M.	Tel-Aviv	-	-	40	-	-	ND
	B.M.$_2$	" "	8	-	40	ND	40	ND
2626	H.M.	Haifa	128	-	≥40	>40	≥40	≥40
	H.M.$_2$	"	-	4	≥40	-	-	-
2627	R.	Beersheba	16	4	40	-	40	-
2629	O.S.	Hadera	AC	AC	≥40	≥40	40	40
	O.S.$_2$	"	≥64	≥256	≥40	>40	≥40	≥40
	O.S.$_3$	"	64	64	≥40	>40	40	≥40
2630	J.A.	Nazareth	64	-	≥40	-	≥40	-
2632	R.R.	Ramla	≥128	-	>40	-	≥40	-
	R.R.$_2$	"	≥64	-	>40	-	-	-
2633	S.S.	Kadima	-	-	≥40	-	40	-
2635	I.D.	Netanya	128	-	>40	-	≥40	-
2636	Z.I.	"	16	-	≥40	-	40	-
2637	S.D.	Kfar Saba	32	-	>40	-	≥40	-
	S.D.$_2$	" "	≥64	-	>40	-	-	-
2638	S.A.	Kfar Saba	8	-	≥40	-	40	-

TABLE 1 (*Cont.*)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2639	A.D.	Netanya	16	-	≥ 40	-	≥ 40	-
2640	B.I.	Rehovot	-	≥ 256	≥ 40	>40	≥40	≥ 40
	$B.I._2$	"	8	≥256	≥40	> 40	40	≥ 40
	$B.I._3$	"	8	≥256	≥40	> 40	40	≥ 40
2541	Z.R.	"	-	≥ 256	≥ 40	> 40	≥ 40	≥ 40
2642	S.A.	Kfar Saba	-	-	≥ 40	-	40	-
2643	A.I.	" "	16	-	≥ 40	-	40	-
2644	S.D.	" "	32	-	≥ 40	-	≥ 40	-
2645	S.I.	" "	16	-	> 40	-	40	-
2646	A.S.	Kfar Yona	-	-	≥ 40	-	40	-
2647	M.M.	" "	256	-	> 40	-	≥ 40	-
2648	L.S.	" "	-	128	≥ 40	> 40	≥ 40	≥ 40
2649	S.S.	" "	-	-	40	-	40	-
2650	L.R.	Jerusalem	128	-	> 40	-	≥ 40	-
2652	S.R.	Rehovot	-	-	40	-	40	-
	$S.R._2$	"	-	-	≥ 40	40	40	-
2653	V.A.	Beersheba	-	-	40	-	-	-
	$V.A._2$	"	64	-	≥40	-	≥ 40	-
2654	V.H.	"	-	-	40	-	40	-
	$V.H._2$	"	32	16	40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2657	S.S.	Rehovot	-	32	≥40	>40	40	≥40
2658	V.O.	Beersheba	-	-	40	-	-	-
	$V.O._2$	"	64	-	≥40	-	40	-
2659	B.S.Z.	Rehovot	AC	AC	>40	>40	≥40	≥40
2660	C.M.	"	64	≥128	>40	>40	≥40	40
	$C.M._2$	"	-	≥128	40	>40	40	≥40
2663	S.R.	Tel-Aviv	-	≥128	≥40	>40	40	≥40
2664	K.S.	" "	-	-	40	-	40	-
2665	B.I.R.	" "	-	≥128	>40	>40	≥40	≥40
2666	B.A.D.	Kfar Saba	-	-	>40	-	40	-
	$B.A._2$	" "	64	-	>40	-	≥40	-
2667	A.S.	Netanya	-	-	≥40	-	40	-
2668	H.R.	Kfar Saba	16	-	40	40	40	-
2669	H.O.	Netanya	-	-	≥40	-	40	-
	$H.O._2$	"	64	4	40	-	≥40	-
2672	B.A.	Haifa	32	-	>40	-	40	-
2673	B.A.	"	-	-	40	-	-	-
2676	I.G.	Herzlia	-	-	40	-	-	-
	$I.G._2$	"	128	-	>40	40	≥40	-
2677	G.E.	Jerusalem	-	-	≥40	-	40	-
	$G.E._2$	"	-	-	≥40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2680	C.I.	Tel-Aviv	-	-	40	≥ 40	40	≥ 40
2726	T.M.	Rehovot	-	32	-	> 40	-	40
2760	T.O.	Tel-Aviv	-	-	≥ 40	-	40	-
2761	B.R.	Ashkelon	-	≥ 64	≥ 40	> 40	≥ 40	≥ 40
2770	G.M.	Beersheba	≥ 32	-	-	-	-	-
2851	M.L.	Ashdod	≥ 16	-	40	-	40	-
2853	C.E.	Jerusalem	-	-	≥ 40	-	≥ 40	-
2854	S.R.	"	≥ 16	-	≥ 40	-	≥ 40	-
2859	Z.M.	Rehovot	32	≥ 256	40	> 40	≥ 40	≥ 40
2860	A.S.	Beersheba	-	-	40	-	40	-
2867	P.S.	Haifa	-	≥ 256	≥ 40	> 40	≥ 40	≥ 40
2868	H.A.	"	-	16	-	-	-	-
2873	A.Z.	Beersheba	-	-	-	-	-	-
	$A.Z._2$	Beersheba	8	-	≥ 40	-	≥ 40	-
2881	M.I.	Beersheba	64	-	> 40	-	≥ 40	-
	$M.I._2$	"	64	-	> 40	-	≥ 40	-
2883	Z.N.	Netanya	16	-	-	-	-	-
2884	R.R.	Kfar Yona	-	-	-	-	-	-
	$R.R._2$	" "	8	-	≥ 40	-	≥ 40	-
2885	S.S.	" "	-	-	-	-	-	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
	$S.S._2$	Kfar Yona	-	-	≥ 40	-	≥ 40	-
2887	I.I.	Netanya	-	-	>40	40	>40	≥ 40
2893	K.S.	"	-	-	-	-	-	-
	$K.S._2$	"	-	-	≥ 40	-	≥ 40	-
2895	Z.O.	Beersheba	8	-	≥ 40	-	≥ 40	-
	$Z.O._2$	"	4	-	≥ 40	-	>40	-
2896	E.I.	Beersheba	-	-	-	-	-	-
	$E.I._2$	"	≥ 32	-	>40	-	≥ 40	-
2900	T.A.	Netanya	4	-	≥ 40	-	40	-
2901	H.H.	"	8	8	≥ 40	-	≥ 40	-
2902	S.M.	"	-	-	≥ 40	-	≥ 40	-
2903	Z.A.	"	16	8	≥ 40	-	40	-
2904	Z.S.	Tel-Mond	32	128	> 40	≥ 40	≥ 40	40
2905	D.S.	Kfar Saba	-	-	-	-	-	-
	$D.S._2$	" "	-	-	> 40	-	> 40	-
2907	K.E.	Haifa	-	-	> 40	-	≥ 40	-
2908	E.R.	"	-	-	≥ 40	-	≥ 40	-
2909	S.I.	Beersheba	-	-	40	-	40	-
	$S.I._2$	"	-	-	≥ 40	-	≥ 40	-
2910	A.A.	Tel-Aviv	-	-	40	-	40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
	A.A.$_2$	Tel-Aviv	-	-	>40	-	>40	-
2911	A.A.	" "	-	-	-	-	-	-
	A.A.$_2$	" "	≥ 32	-	≥40	-	40	-
2913	M.S.	Tel-Mond	-	-	-	-	-	-
	M.S.$_2$	" "	-	-	≥40	-	≥40	-
2915	B.I.	Tel-Aviv	-	-	40	-	-	-
	B.I.$_2$	" "	-	-	≥40	-	≥40	-
2916	R.M.		-	-	40	>40	40	≥40
2918	T.D.	Beersheba	-	-	-	-	-	-
	T.D.$_2$	"	ND	ND	>40	-	≥40	-
2920	B.E.M.	"	-	-	>40	>40	≥40	40
2921	F.R.	Tel-Aviv	-	-	-	-	-	-
	F.R.$_2$	" "	-	-	>40	-	≥40	-
2928	A.S.	" "	-	-	40	>40	-	≥40
2930	G.G.	Netanya	-	-	-	-	-	-
	G.G.$_2$	"	32	-	≥40	-	≥40	-
2935	I.G.	Kfar Yona	-	-	40	-	-	-
	I.G.$_2$	" "	64	-	≥40	-	≥40	-
2936	B.I.	Hadera	-	-	-	-	ND	ND
	B.I.$_2$	"	≥128	-	≥40	-	40	ND

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2937	A.P.	Taibe	8	-	≥ 40	-	≥ 40	-
2938	S.H.	Kfar Saba	-	-	≥ 40	-	-	-
	$S.H._2$	" "	≥ 128	-	>40	-	≥ 40	ND
2939	S.N.	Tel-Mond	-	-	40	-	-	-
	$S.N._2$	" "	≥ 128	-	>40	-	≥ 40	ND
2940	O.G.	Kfar Saba	-	-	-	-	-	-
	$O.G._2$	" "	≥ 128	-	≥ 40	-	≥ 40	ND
2944	G.O.	Tel-Aviv	-	-	40	-	-	-
	$G.O._2$	" "	≥ 128	-	≥ 40	-	≥ 40	ND
2945	M.R.	" "	≥ 128	-	-	-	-	-
	$M.R._2$	" "	≥ 128	≥ 64	≥ 40	-	≥ 40	ND
2946	B.M.	Hadera	≥ 128	-	-	-	-	-
2953	S.M.	Tel-Aviv	8	-	40	-	-	-
	$S.M._2$	" "	8	-	≥ 40	-	40	-
2954	K.S.	Haifa	≥ 128	≥ 64	>40	>40	≥ 40	-
2955	H.A.	"	-	-	≥ 40	-	≥ 40	ND
2956	A.R.	"	≥ 128	-	>40	-	≥ 40	ND
2957	N.S.	"	≥ 128	-	≥ 40	-	≥ 40	ND
2958	K.R.	Tel-Aviv	-	64	40	>40	40	≥ 40
2960	A.I.	Rehovot	-	-	40	>40	40	≥ 40

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2961	B.S.	Rehovot	-	≥ 64	40	> 40	40	≥ 40
	$B.S._2$	"	≥ 64	≥ 64	≥ 40	> 40	40	≥ 40
2963	H.A.	Jerusalem	≥ 128	8	≥ 40	-	≥ 40	ND
2964	H.A.	"	-	-	-	-	-	ND
	$H.A._2$	"	16	-	≥ 40	-	-	ND
2966	C.M.	Netanya	-	-	-	-	-	-
	$C.M._2$	"	-	-	40	-	≥ 40	-
2967	P.M.	Hadera	-	≥ 64	40	40	≥ 40	-
2968	L.H.	Magdiel	-	-	-	-	-	-
	$L.H._2$	"	≥ 32	-	> 40	40	≥ 40	≥ 40
2969	G.I.	Meir Hospital	≥ 128	-	≥ 40	-	≥ 40	ND
2972	M.M.	" "	16	8	-	-	-	ND
	$M.M._2$	" "	16	8	> 40	-	≥ 40	ND
2973	S.I.	" "	≥ 128	-	> 40	-	40	ND
	$S.I._2$	" "	8	-	≥ 40	-	40	ND
2974	Z.M.	Rehovot	-	-	40	-	ND	ND
	$Z.M._2$	"	≥ 32	-	≥ 40	-	≥ 40	-
2975	K.B.	"	-	-	-	-	ND	-
	$K.B._2$	"	≥ 32	-	> 40	-	≥ 40	ND

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2976	P.G.	Kfar Yona	-	16	-	>40	-	-
	P.G.$_2$	" "	≥128	64	≥40	>40	≥40	-
2977	T.I.	Kadima	-	-	-	-	-	-
	T.I.$_2$	"	16	-	≥40	-	≥40	-
2978	S.T.	Jerusalem	-	-	-	-	-	-
	S.T.$_2$	"	≥32	-	>40	-	≥40	-
2979	T.A.	Rehovot	-	-	-	-	ND	ND
	T.A.$_2$	"	-	-	≥40	-	≥40	-
2981	O.M.	Beersheba	-	-	-	-	-	-
	O.M.$_2$	"	≥32	16	>40	-	40	-
2982	H.R.	Kaplan Hospital	8	-	40	-	-	-
	H.R.$_2$	" "	≥32	-	-	-	ND	ND
	H.R.$_3$	" "	ND	ND	≥40	-	-	-
2983	L.H.	Haifa	-	-	≥40	-	≥40	-
2984	I.D.	Kiriat Motzkin	16	-	>40	-	≥40	-
2985	E.E.	Haifa	≥32	8	>40	-	40	-
2987	A.M.	Hadera	4	-	40	≥40	≥40	≥40
2988	A.A.	"	8	≥32	-	-	-	-
2989	A.I.	"	-	-	-	-	-	ND
	A.I.$_2$	"	-	-	40	-	≥40	ND

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
2993	B.M.	Netanya	-	-	-	-	ND	ND
	$B.M._2$	"	≥ 32	-	> 40	-	≥40	-
2994	H.D.	Meir Hospital	-	-	-	-	ND	ND
	$H.D._2$	" "	-	≥ 32	40	>40	-	40
2995	F.D.	" "	≥ 32	-	≥40	-	-	ND
2997	B.Z.	Tel-Aviv	-	-	-	-	-	-
	$B.Z._2$	" "	-	-	≥ 40	-	≥ 40	-
2998	A.M.	Beersheba	≥32	-	>40	-	≥ 40	-
2999	D.I.	"	8	-	-	-	-	-
3000	C.N.	"	-	-	-	-	-	-
	$C.N._2$	"	-	-	≥ 40	-	≥ 40	-
3002	B.K.	Tel-Aviv	-	-	≥ 40	-	40	-
3003	B.R.	Beersheba	4	-	≥ 40	-	40	-
3004	F.S.	"	16	-	≥ 40	-	40	-
3005	B.M.	"	16	-	≥40	-	≥40	-
3006	A.A.	Tel-Aviv	128	-	>40	-	≥40	ND
	$A.A._2$	" "	128	-	≥40	-	≥40	-
3007	H.Z.	Beersheba	≥32	-	>40	-	≥40	ND
3009	H.I.	Tel-Aviv	-	8	40	≥40	40	40
3014	R.R.	Kfar-Vitkin	-	-	-	-	ND	ND

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
	R.R.$_2$	Kfar-Vitkin	16	-	≥ 40	-	≥ 40	-
3015	C.T.	Tel-Aviv	≥ 32	-	> 40	-	40	-
3016	B.M.	Raanana	≥ 32	-	> 40	-	≥ 40	-
3017	B.A.	"	≥ 32	-	> 40	-	≥ 40	-
3019	B.B.	Tel-Aviv	-	-	40	-	40	-
	B.B.$_2$	" "	≥ 128	-	≥ 40	-	40	-
3021	B.D.	Kfar Yona	-	-	-	-	ND	ND
	B.D.$_2$	" "	-	-	≥ 40	-	≥ 40	-
3022	A.E.	" "	-	-	-	-	ND	ND
	A.E.$_2$	" "	16	-	≥ 40	-	> 40	-
3023	G.B.	Ramataim	-	-	-	-	ND	ND
	G.B.$_2$	"	ND	ND	> 40	-	≥ 40	-
3025	S.O.	Tel-Aviv	-	≥ 128	40	> 40	≥ 40	≥ 40
	S.O.$_2$	" "	-	≥ 128	40	> 40	40	40
3026	M.I.	" "	≥ 32	-	≥ 40	-	≥ 40	ND
3029	V.E.O.	Hadera	8	4	-	-	ND	ND
	V.E.$_2$	"	16	4	≥ 40	-	≥ 40	-
3030	K.B.	Tel-Aviv	16	≥ 32	≥ 40	> 40	40	≥ 40
3034	S.A.	" "	-	-	40	-	ND	ND
	S.A.$_2$	" "	-	-	≥ 40	-	≥ 40	-

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
3036	I.O.	Netanya	-	-	-	-	ND	ND
	$I.O._2$	"	≥ 32	-	>40	-	≥40	-
3037	I.A.	"	-	-	-	-	-	-
	$I.A._2$		-	-	≥ 40	-	≥ 40	-
3039	S.Z.	Meir Hospital	4	-	≥ 40	-	-	-
3040	R.Z.	Rehovot	≥ 32	-	40	-	-	-
3041	S.S.	Tel-Aviv	8	≥ 32	-	> 40	ND	≥ 40
3042	K.S.	Petah-Tikva	-	4	-	> 40	ND	≥ 40
3044	B.O.	Tel-Aviv	-	-	-	-	ND	ND
	$B.O._2$	" "	-	-	≥ 40	-	≥ 40	-
3046	B.O.V.	" "	-	≥ 32	-	> 40	ND	≥ 40
3050	P.N.	Beersheba	≥ 32	-	≥ 40	-	40	ND
3052	A.E.	Haifa	≥ 32	-	> 40	-	40	ND
3053	P.I.	"	8	4	≥ 40	-	≥ 40	ND
3054	K.S.	"	-	≥ 32	≥ 40	>40	40	-
3055	R.S.	"	≥ 32	-	>40	-	>40	ND
3059	T.E.	Tel-Aviv	-	64	-	-	ND	ND
	$T.E._2$	" "	-	64	40	≥ 40	-	≥ 40
3064	S.D.	Kfar Saba	-	-	-	-	ND	ND
	$S.D._2$	" "	-	-	40	-	≥ 40	ND

TABLE 1 (Cont.)

No.	Name	Town	CF		FA		IgM	
			SF	MT	SF	MT	SF	MT
3069	N.E.	Tel-Aviv	-	-	-	-	ND	ND
	$N.E._2$	" "	-	-	-	-	≥40	ND
	$N.E._3$	" "	-	-	40	-	≥40	ND
3070	P.I.	Beersheba	-	-	-	-	ND	ND
	$P.I._2$	"	≥32	-	>40	-	-	-
3071	M.A.	Beersheba	≥32	-	>40	-	≥40	-
3072	M.E.	"	-	-	>40	-	≥40	-
3073	F.H.	"	8	8	≥40	-	40	-
3076	H.M.	Hadera	≥128	32	>40	>40	-	-
3080	G.A.	Tel-Aviv	-	-	-	-	ND	ND
	$G.A._2$	" "	-	-	≥40	-	≥40	ND
3082	S.G.	Kfar Saba	-	4	-	≥40	ND	≥40
3083	N.R.	Beersheba	-	8	-	>40	ND	≥40
3115	S.N.	Beilinson Hospital	≥32	-	-	-	-	-
3117	K.Z.	Tel-Aviv	-	-	>40	-	40	-
	$K.Z._2$	" "	≥32	-	>40	-	≥40	-

Table 2

HUMAN SERA SUBMITTED FOR SEROLOGICAL DIAGNOSIS OF RICKETTSIAL INFECTIONS TESTED BY FLUORESCENT ANTIBODY (FA) AND COMPLEMENT FIXATION (CF)

Test	Sera tested	Positive sera[1]	Sera positive for			AC	Current Infections[2] (No. of Cases)	
			SF only	MT only	SF + MT		SF	MT
FA	685 (552)[3]	372 (293)	273 (214)	21 (17)	78 (62)		223	49
CF	659 (523)	254 (219)	160 (146)	49 (39)	45 (34)	3	73	8

1. In the CF test, a titer of ≥ 4 was considered positive; in the FA test, a titer of ≥ 40 was considered positive.

2. In the case of the FA test, diagnosis was based on a rise in serological titer in successive bleedings, or the presence of specific IgM antibodies. When antibodies were present to both SF and MT, the diagnosis was made according to the higher titer.

   In the CF test, the diagnosis was based on a rise of at least four-fold in titer in successive bleedings. Two or more sera were received from 142 patients only.

3. Numbers in parentheses indicate the number of individuals from whom corresponding sera were derived.

Table 3

COMPARISON OF THE RESULTS OF FLUORESCENT ANTIBODY (FA) AND COMPLEMENT FIXATION (CF) TESTS IN HUMAN SERA TESTED AGAINST SPOTTED FEVER (SF) AND MURINE TYPHUS (MT) RICKETTSIAE

Test	Sera tested for SF		Sera tested for MT	
	FA+ [1]	FA-	FA+	FA-
CF+ [2]	191	19	61	32
CF-	150	296	34	529

1. In the FA test a titer of ≥ 40 was considered as positive.

2. In the CF test a titer of ≥ 4 was considered as positive.

Table 4

PERSISTENCE OF SPECIFIC IgM IN SERA OF PATIENTS FOLLOWING PRIMARY INFECTION WITH SPOTTED FEVER (SF) OR MURINE TYPHUS (MT)

Case No.	Date of Bleeding	CF[1]		FA[2]			
		SF	MT	SF		MT	
				Ig[3]	IgM[4]	Ig	IgM
2564	12. 9.75	64	16	3	2	±	±
	18. 9.75	64	32	3	2	±	±
	21. 3.76	16	-	+	±	ND	ND
2603	23. 9.75	-	-	-	-	-	-
	28.10.75	128	4	3	+	-	-
	2. 2.76	16	-	3	-	-	-
2609	6.10.75	-	-	±	-	-	-
	16.10.75	16	-	2	+	-	-
	1. 4.76	ND	ND	3	-	-	-
2618	7.10.75	-	-	±	-	±	-
	17.10.75	≥256	-	3	+	-	-
	9. 4.76	ND	ND	3	-	-	-
2626	27. 9.75	-	128	2	+	3	+
	2. 2.75	4	-	+	-	-	-
2629	20.10.75	-	≥256	+	+	2	+
	23.10.75	-	≥256	2	2	3	2
	3. 2.76	AC	AC	2	±	4	2

Table 4 (Cont.)

Case No.	Date of Bleeding	CF[1]		FA[2]			
		SF	MT	SF		MT	
				Ig[3]	IgM[4]	Ig	IgM
2632	27.10.75	128	-	4	+	-	-
	22. 2.76	≥64	-	4	-	-	-
2637	14.10.75	32	-	3	+	-	-
	7. 4.76	≥64	-	4	-	-	-
2640	28. 7.75	-	≥256	2	2	4	2
	23.10.75	8	≥256	3	±	3	2
2660	10.11.75	64	≥128	3-4	2	4	+
	22. 2.76	-	≥128	+	±	4	2+

1. Complement fixation test.

2. Immunofluorescent staining.

3. Immunofluorescent staining of immunoglobulins.

4. Immunofluorescent staining of IgM immunoglobulins. Immunofluorescent staining was carried out as a 1:40 dilution of the patients' serum. The brightness of staining was evaluated as ± - 4+.

Table 5

MONTH OF ONSET OF 223 CASES* OF SPOTTED FEVER INFECTIONS IN HUMANS

January 1975 - December 1976

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Total Cases
1975	0	0	0	10	7	7	12	13	41	21	10	3	124
1976	1	1	0	2	2	10	20	26	19	16	2	0	99

* Cases confirmed in the Ness-Ziona laboratory.

Table 6

DISTRIBUTION OF 223 CASES* OF SPOTTED FEVER INFECTION IN HUMANS BY SEX AND GEOGRAPHIC AREA, 1975 - 1976

Geographic area	Total	Sex	
		Male	Female
Naharya	1	1	0
Haifa	44	22	22
Hadera	16	8	8
Netanya	72	49	23
Tel-Aviv	48	30	18
Jerusalem	10	5	5
Beersheba	32	19	13
Total	223	134	89

* Cases confirmed in the Ness-Ziona laboratory.

Table 7

DISTRIBUTION OF 279 CASES* OF SPOTTED FEVER INFECTION BY AGE AND SEX, 1975 - 1976

Age / Sex	0-4	5-14	15-44	45+	Unknown	Total
Male	40	85	23	19	-	167
Female	30	43	22	14	3	112
Total	70	128	45	33	3	279

* Epidemiologically investigated (123 laboratory confirmed).

Table 8

ANIMAL PRESENT IN THE ENVIRONMENT OF 279 CASES* OF SPOTTED FEVER INFECTION
1975 - 1976

(a)

Kind of animals	No. of Cases
Dogs	195
Cats	144
Horses, donkeys	65
Sheep, goats	32
Cattle	19
Rodents	34
Hedgehogs	20
Poultry	8
Camel	5
None or unknown	30

* Epidemiologically investigated (123 laboratory confirmed).

(b)

No. of animals [1]	No. of cases
None	30
One	79
Two	94
Three or more	76

1. Number of animals of one or more species in the household.

Table 9

TYPE OF CONTACT OF THE 279 CASES* OF SPOTTED FEVER INFECTION WITH ANIMALS, 1975 - 1976

Type of contact	No. of cases
Frequent close contact	75
Sporadic	55
At play	49
Not defined or unknown	100
Total	279

* Epidemiologically investigated (123 laboratory confirmed).

Table 10

MAXIMAL TEMPERATURE OBSERVED IN 279 CASES* OF SPOTTED FEVER INFECTION, 1975 - 1976

Temperature	No. of cases
$\leq 38.9^{\circ}C$	10
$39^{\circ} - 39.9^{\circ}C$	71
$40^{\circ} - 40.9^{\circ}C$	143
$\geq 41^{\circ}C$	31
Unknown	24
Total	279

*Epidemiologically investigated (123 laboratory confirmed).

Table 11

SOME CLINICAL SIGNS REPORTED IN 279 CASES* OF SPOTTED FEVER INFECTION, 1975 - 1976

Clinical signs	No. of cases
Lymphadenopathy -	
Generalized	16
Localized	43
Bleeding	4
Hepatosplenomegaly or Splenomegaly only	10
Jaundice	2
Neurological Signs	6
Others	4

* Epidemiologically investigated (123 laboratory confirmed).

Table 12

TYPE OF RASH IN 279 CASES * OF SPOTTED FEVER INFECTION, 1975 - 1976

Type of rash	No. of cases
Maculopapular	139
Hemorrhagic	31
Unknown	109
Total	279

* Epidemiologically investigated (123 laboratory confirmed).

Table 13

DISTRIBUTION OF RASH IN 279 CASES* OF SPOTTED FEVER INFECTION, 1975 - 1976

Distribution of rash		No. of cases
Generalized		180
Localized -	upper + lower limbs	41
	upper limbs + abdomen	5
	lower limbs	8
	lower limbs + abdomen	2
	abdomen + upper and lower limbs	4
	abdomen, back, chest	4
	lower + upper limbs + back + chest	11
Other		3
Unknown		21
Total		279

* Epidemiologically investigated (123 laboratory confirmed).

Table 14

SEROLOGICAL SURVEY FOR SPOTTED FEVER (SF) AND MURINE TYPHUS (MT)

Place	Number of sera tested	Number of indiv-iduals	Number of sera positive for SF		Number of sera positive for MT	
			CF [1]	FA [2]	CF	FA
Nazareth	91	91	3	23	3	7
Naan	83	79	7	51	0	4

1. Complement fixation. Titer ≥ 4 were considered as positive.

2. Immunofluorescence tests. Titer ≥ 40 was considered as positive.

Table 15

COMPARISON OF THE RESULTS OF FLUORESCENT ANTIBODY (FA) AND REVERSE PASSIVE HEMAGGLUTINATION INHIBITION (RPHI) TESTS IN HUMAN SERA TESTED FOR SPOTTED FEVER (SF) AND MURINE TYPHUS (MT)

Test	Sera tested for SF		Sera tested for MT	
	RPHI+ [1]	RPHI-	RPHI+ [2]	RPHI-
FA+ [3]	150	55	30	19
FA-	7	19	28	154

1. In the RPHI test for spotted fever, a titer of ≥ 32 was considered as positive.

2. In the RPHI test for murine typhus, a titer of ≥ 8 was considered as positive.

3. In the FA test a titer of ≥ 40 was considered as positive.

Table 16

TITRATION OF KENYA TICK TYPHUS ANTIGEN [1] BY REVERSE PASSIVE HEMAGGLUTINATION IN THE PRESENCE OF VARIOUS DILUTIONS OF NORMAL HUMAN SERUM

Normal Human Serum Dilution	Antigen dilution 1:											
	16	32	64	128	256	512	1024	2048	4096	8192	C.C. [2]	C.C. [2]
1:2	4 [3]	4	4	4	4	4	4	4	4	4	4	4
1:4	4	4	4	4	3	2	-	-	-	-	-	-
1:8	4	4	4	4	3	+	-	-	-	-	-	-
1:16	4	4	4	4	4	2	-	-	-	-	-	-
1:32	4	4	4	4	4	4	4	-	-	-	-	-
1:64	4	4	4	4	4	4	4	-	-	-	-	-
1:128	4	4	4	4	4	4	4	4	2	-	-	-
1:256	4	4	4	4	4	4	4	4	3	ND	-	-

1. Method I antigen (10).

2. Cell control - Conjugated chicken erythrocytes plus the corresponding dilution of normal human serum.

3. The degree of hemagglutination was read as + to 4+.

The diluent used in the test was saline plus 2% dextrose. The conjugate was glutaraldehyde fixed chicken RBC conjugated with rabbit anti-Indian tick typhus immune globulin. The antigen serum mixtures were incubated at 37°C one hour before addition of conjugate. The plates were then allowed to stand at room temperature until read.

Table 17

TITRATION OF KENYA TICK TYPHUS ANTIGEN [1] BY REVERSE PASSIVE HEMAGGLUTINATION IN THE PRESENCE OF NORMAL HUMAN SERUM AND VARIOUS DILUTIONS OF CONVALESCENT HUMAN SERUM

	Serum Dilution	Antigen dilution 1:								
		8	16	32	64	128	256	512	1024	2048
Normal Human Serum	1:10	4 [2]	4	4	4	4	4	2+	-	-
Convalescent Serum # 2608	1:10	3+	-	-	-	-	-	-	-	-
	1:20	-	-	-	-	-	-	-	-	-
	1:40	3+	-	-	-	-	-	-	-	-
	1:80	4	2	-	-	-	-	-	-	-
	1:160	4	4	4	-	-	-	-	-	-
	1:320	4	4	4	3	-	-	-	-	-
	1:640	4	4	4	3	-	-	-	-	-

1. Method I antigen (10).

2. The degree of hemagglutination was graded as + to 4+.

The antigen dilutions were made in saline containing 2% dextrose. The 1:10 dilutions of normal human serum and serum 2608 were made in saline. The following dilutions of 2608 1:20 - 1:60 were made in saline plus normal human serum such that the serum concentration was kept constant at 1:10. The conjugate used was glutaraldehyde-fixed chicken RBC conjugated with rabbit anti-Indian tick typhus immune globulin. The antigen-serum mixtures were incubated at 37°C one hour before addition of conjugate. The plates were then allowed to stand at room temperature until read.

Table 18

REVERSE PASSIVE HEMAGGLUTINATION INDEX IN SELECTED CASES OF SPOTTED FEVER COMPARED WITH IMMUNOFLUORESCENT AND COMPLEMENT FIXATION TESTS

Human Serum No. dil. 1:10	Antigen dilution 1:[1] 32	64	128	256	512	1024	2048	Inhibition Index[2]	FA Results at 1:40	CF Titer
Normal	4[3]	4	4	4	3	2	-	-	-	-
2963	4	3	2	-	-	-	-	4	3+[4]	≥ 128
2968 I	4	4	4	4	-	-	-	2	-	-
II	3	-	-	-	-	-	-	5	3+	▸ 32
2975 I	4	4	4	4	3	2	-	0	-	-
II	4	3	-	-	-	-	-	4	3-4+	▸ 32
2976 I	4	4	4	3	-	-	-	2	-	-
II	-	-	-	-	-	-	-	≥6	2+	▸ 128
2978 I	4	4	4	4	+	-	-	2	-	-
II	4	2	-	-	-	-	-	5	4+	▸ 32

1. The antigen used was Kenya tick typhus antigen Method I (10). Dilutions were made in saline containing 2% dextrose. 0.025 ml serum diluted in saline was added to each well. Plates were incubated at 37°C one hour and the chicken RBC conjugated with rabbit anti-Indian tick typhus immunoglobulin were added. The plates were then allowed to stand at room temperature until read.

2. The inhibition index is considered as the number of two fold dilutions in which a significant difference in hemagglutination can be seen between the given serum and the standard normal serum used.

3. The degree of hemagglutination was graded as + to 4+.

4. These numbers represent brightness of fluorescent staining graded as + to 4+.

Table 19

DIFFERENTIATION OF EPIDEMIC AND ENDEMIC TYPHUS ANTIGENS BY REVERSE PASSIVE HEMAGGLUTINATION

Antigens		Conjugates	
		Anti-MT [1]	Anti-ET [2]
MT Method I [3] Soluble Antigen	1	512 [4]	16
	2	1024	16
	3	256	16
MT Somatic Antigen [5]	1	64	16
	2	512	32
	3	64	16
	4	1024	32
ET Method I [3] Soluble	1	512	256
	2	128	1024
	3	512	256
	4	512	256
	5	512	512
	6	64	256
ET Somatic Antigen	1	64	32
	2	1024	512

1. Glutaraldehyde-fixed chicken RBC conjugated with hyper-immunized rabbit anti-murine typhus immunoglobulin.

2. Glutaraldehyde-fixed chicken RBC conjugated with hyper-immunized rabbit anti-epidemic typhus immunoglobulins.

3. Method I antigen prepared as described in (10).

4. Reciprocal of highest dilution at which partial agglutination could be observed.

5. Somatic antigens prepared as described in (4).

Table 20

DIFFERENTIATION OF EPIDEMIC AND ENDEMIC TYPHUS INFECTIONS BY REVERSE PASSIVE HEMAGGLUTINATION INHIBITION

Source of Serum	Type of Infection	Antigen [1]	
		MT [2]	ET [2]
Guinea pig 1	MT	16 [3]	<8
Guinea pig 2	MT	256	32
Rabbit 1	MT	1024	32
Guinea pig 3	ET	64	64
Guinea pig 4	ET	64	64
Guinea pig 5	ET	64	32
Guinea pig 6	ET	64	64
Rabbit 2	ET	64	32

1. The antigens used in this test were somatic antigens prepared as described in (4).

2. MT, ET - Murine Typhus and Epidemic Typhus.

3. Reciprocal of the highest dilution which inhibited four heamgglutinating units of the designated antigen as measured against its corresponding conjugate.

APPENDIX A

Scientists and Supervisors

M.A. Klingberg, M.D.	- Principal Investigator
R.A. Goldwasser, Ph.D.	- Co-principal Investigator
T.A. Swartz, M.D., M.P.H.	- Co-principal Investigator
Wanda Klingberg, Ph.D.	- Co-principal Investigator
Y. Steiman, M.Sc.	- Research Associate

Other Personnel

Rina Shalev	- Laboratory Technician
Katherin Cohar	- Laboratory Technician
M. Iris	- Field Worker

Cooperating Physicians and Other Professional Workers in the Field

Corina Costin, M.D., M.P.H.	- Deputy Chief Epidemiologist, Division of Epidemiology, Ministry of Health, Jerusalem.
Purer Yona, Ph.D.	- Director, A. Felix Public Health Laboratories, Ministry of Health, Tel-Aviv.
Rachel Zmutski	- A. Felix Public Health Laboratories, Ministry of Health, Tel-Aviv.
M. Lucian, M.D.	- District Epidemiologist, Haifa District, Haifa.
Dalia Rachin, R.N.	- District Epidemiological Nurse, Haifa District, Haifa.