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AN EPIDEMIOLOGIC AND IMMUNOLOGIC STUDY OF BOUTONNEUSE FEVER IN ISRAEL

FINAL TECHNICAL REPORT

by

Marcus A. Klingberg, M.D.
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December 1971

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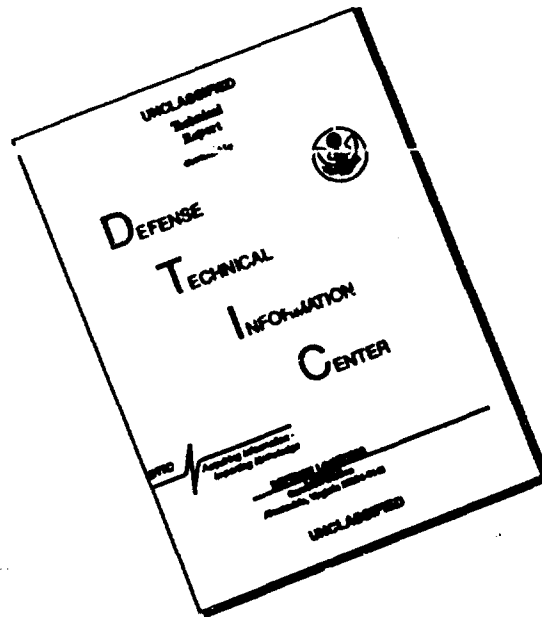
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13. ABSTRACT The study is directed towards identification and investigation of the etiologic agent responsible for a rickettsial infection resembling boutonneuse fever which occurs in certain endemic foci in Israel. The frequency of occurrence of antibodies to spotted fever among inhabitants of the Ashdod area was determined using fluorescent antibodies. It averaged 10.5% among the persons in one of the subdistricts studied. Diagnostic serology of clinical rickettsioses indicated that spotted fever infections are more widespread in Israel than previously thought. Antibodies to spotted fever rickettsiae were found in a high percentage of dogs from the areas in which the clinical disease appears. The fluorescent antibody test was found to be more sensitive than complement fixation in that it picked up more reactors and gave higher serological titers. Three isolates of spotted fever rickettsiae, two from ticks and one from human blood, were made and shown to be antigenically identical. These strains differed in their antigenic composition from the Malish strain and the Indian tick typhus strain of <i>R. conori</i> . The Indian tick typhus strain and the Malish strain were not serologically identical. Key words: Rickettsia; Boutonneuse fever; Spotted fever; Israel rickettsial infections; Israel tick typhus.			

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SUMMARY

The study was undertaken in order to investigate and to determine the etiological agent responsible for the infection resembling boutonneuse fever and to study the mode of transmission of the disease occurring in certain endemic foci in Israel.

Fluorescent antibody methods were used to determine the frequency of individuals with antibodies to spotted fever in the Ashdod area. Low to moderate endemicity of the infection was found in the subdistricts studied.

Diagnostic serology carried out on the bloods of clinical rickettsioses submitted to this laboratory, indicated that infections with spotted fever rickettsiae are more widespread in Israel than previously thought.

Fluorescent antibody methods were found to be more sensitive than complement fixation tests in the detection of positive reactors as well as in detecting a rise in titer in the case of spotted fever infections. The staining of specific IgM antibodies was used to determine current infection when consecutive bleedings were not available, or the first bleeding was taken too late to find a rise in titer on subsequent bleeding.

Three strains of spotted fever rickettsiae were isolated from the blood of a clinical case and from dog ticks. They were found to be identical as tested by F.A. methods but differed from the strains of *R. conori* available to this laboratory at the present time.

BACKGROUND

The spotted fever group of rickettsioses comprises: Rocky Mountain Spotted Fever, rickettsialpox and the tick-borne typhus fever rickettsioses. The tick-borne typhus fever rickettsioses include the diseases variously known as: Fievre boutonneuse, Marseilles fever, South African tick bite fever, Kenya tick typhus, Indian tick typhus, North Asian tick-borne rickettsiosis and Queensland tick typhus (1,2).

In contrast with Rocky Mountain Spotted Fever (2,3) the tick-borne typhus fevers are mild to moderate non-fatal illnesses. They are caused by rickettsiae closely related antigenically to each other and to *Rickettsia rickettsi*. As the name of the group indicates the agents are transmitted by the bites of Ixodid ticks (2).

It is known from previous studies that the Mediterranean basin is a zone where various diseases described collectively as boutonneuse fever are distributed (4,5). The disease is also widespread in the Near Eastern countries and it occurs as well in the whole area surrounding the Black Sea including the South Coast of the Crimea in the U.S.S.R. (6). Recently it was also described in West Pakistan (7). This zoonotic infection which is caused by *Rickettsia conori* is believed to be usually transmitted to man from dogs, which are considered as the usual reservoir, by the common dog tick *Rhipicephalus sanguineus*, which is the dominant vector. Occasionally, however, other ticks have also been incriminated as vectors, and other vertebrates (bovine, ovine, rodents) have also been suspected as possible reservoirs (8).

The disease, in the common form, clinically resembles epidemic typhus and clinicians (well acquainted with the disease), are able to diagnose boutonneuse fever only on the basis of epidemiological data and on the initial local lesion or eschar which is present in most cases at the onset of fever. This lesion is a small button like ulcer with a black necrotic center and a surrounding red erythematous area which appears at the site of the transmitting tick bite (2).

The first cases of the disease in Israel suspected to be boutonneuse fever on clinical grounds were recorded in the Haifa bay area 23 years ago (9). About 15 years ago in an agriculture settlement, Kfar-Vitkin, near the coast about 20 miles north of Tel-Aviv appeared additional cases of the disease which was diagnosed as boutonneuse fever. The diagnosis was based on the clinical aspect - an exanthem similar to that of spotted fevers and a rise of complement-fixing (C.F.) antibody titer against C.F. group-specific antigen derived from *R. rickettsi*. In the period following the first cases, the infection spread slowly to neighbouring settlements.

Approximately three years ago, a new focus of infection appeared in the Ashdod area, also in the coastal region but about 20 miles south of Tel-Aviv. Since that time it appears that the disease has a tendency to spread to other areas of the country.

Clinically, the disease as it appears in Israel differs from the common description of fievre boutonneuse in the literature as well as of other members of the tick-borne typhus group. The disease in Israel is usually milder and of shorter duration than generally known, and the local lesion or typical black spot or "tache noire" at the site of the tick bite is constantly absent.

The aim of the study

The present study was undertaken in September 1970, with the aim to investigate and to determine the etiological agent responsible for the infections resembling boutonneuse fever and to study the mode of transmission of the disease occurring in certain endemic foci in Israel.

The scope of work during the first year of study was defined as follows:

1. Collecting specimens in the field (bloods from men and animals) for the purpose of carrying out a sero-epidemiologic survey of human, wild and domestic animal populations;
2. An entomologic survey with the collection of ticks and other arthropods for future isolation work;
3. Preparation of immunological reagents and their standardization.

In addition to the planned work the following investigations were carried out:

- (a) Serologic diagnosis of suspected rickettsioses in humans, using the fluorescent antibody (F.A.) methods and C.F. techniques;
- (b) Isolation of rickettsiae and attempts at their identification;
- (c) Serologic investigations, using the F.A. method.

MATERIALS AND METHODS

Serologic survey

A sample of 85 families, from the Ashtrom area of the Ashdod subdistrict, including 420 individuals, obtained by random sampling, was included in the study. This represents about 15 percent of the total number of families living in this area, where the presence of spotted fever infection had been previously documented.

Personal data on each of the members of the above families were recorded on a family recording form, at the time when blood was drawn from the individuals

Concomitantly, personal data as well as blood samples were obtained from 68 individuals belonging to 24 families, as well as from another 98 individuals from the "Vav" area of the same subdistrict where the presence of spotted fever infection had been previously suspected.

Finally, 91 individuals from a third area of the Ashdod subdistrict, thought until now to be free of spotted fever infection, were also introduced in the study and blood collected from them.

Collection and handling of blood specimens

Blood specimens were collected aseptically from the vein; as soon as the blood clot formed the specimens were placed on ice and transferred to the laboratory on the same day. The serum was separated by two centrifugations in a refrigerated centrifuge and stored immediately in a deep freezer at -70°C .

Environmental survey

Simultaneously, an environmental investigation was conducted in the above areas of the Ashdod subdistrict, which resulted in trapping of 90 rodents (58 - rats; 11 - meriones; 1 - gerbil; 20 - house mice), and 9 hedgehogs. Also blood samples from four dogs were collected.

Ticks identified as *Rhipicephalus sanguineus* and *Borophilus annulatus* were also collected from rodents, dogs and hedgehogs.

Rickettsial strains

Except for local isolates most of the rickettsial strains used in this work were obtained from Dr. B.L. Elisberg of the Walter Reed Army Institute of Research. These strains were: *R. mooseri* (Wilmington - passage history unknown), *R. rickettsi* E8 (Sheila Smith), *R. akari* E30 (MK), *R. conori* E38 (Malish), *R. conori* E10 (Indian tick typhus), *R. siberica* E18 (246), *R. australis* E120 (Phillips). Two additional strains of *R. conori* isolated in North Africa were recently obtained from Dr. M. Capponi of the Pasteur Institute, Paris, and work has just begun with these rickettsiae. These strains were labelled A38 and R42. Their passage history is unknown.

Propagation of rickettsiae

The various strains of rickettsiae were grown by inoculation into the yolk sacs of 6-7 day old embryonate hen eggs. Eggs, in which death of the embryo occurred during 72 hours following inoculation were discarded. Following this period eggs were candled twice daily and the yolk sacs harvested individually after embryo death. Smears were made from each yolk sac for evaluation of rickettsial growth. Several yolk sacs from each egg passage which showed abundant rickettsiae were preserved as seed stock.

Preparation of rickettsial antigens

Soluble antigens were prepared from each of the above mentioned strains by Method I of Topping and Shepard (10). Embryonate chicken eggs 6-7 days old were inoculated via the yolk sac with an appropriate dilution of 20 percent infected yolk sac suspension. Dilutions were made in Sucrose-Phosphate-Glutamate solution (11) (S.P.G.) such that embryo death occurred on the fifth and sixth day after inoculation. Following death of the embryo, yolk sacs were harvested and stored individually at -70°C until used. Smears were made from each yolk sac prior to storage for evaluation of rickettsial growth. Smears were stained with fluorescein conjugated serum and examined under a fluorescence microscope. Yolk sacs showing the best rickettsial growth were thawed, pooled and emulsified in .013M phosphate buffered saline pH 7.0 to make a 20 percent suspension. The suspensions were shaken up in two volumes of cold anesthetic ether, and allowed to stand overnight in separatory funnels in the cold room. The aqueous phase was removed, excess ether driven off with the use of nitrogen gas and the preparation clarified by centrifugation at 10,000 G. The supernatant was used in the complement fixation test. When necessary, such antigens were concentrated approximately 5-fold by dialysis against polyethylene-glycol. Occasionally a precipitate formed which was removed by high speed centrifugation. Antigens prepared in this manner gave titers of 1:16 - 1:32 in the complement fixation test using microtiter equipment and following the LBCF procedure (12).

For fluorescent antibody inhibition (F.A.I.) tests (13), Method I antigens prepared by ether extraction of infected yolk sacs, as described above, were used but without clarifying by centrifugation or concentration by dialysis. In order to improve the performance of some of these antigens for the F.A.I. tests 3-4 volumes of Method I antigen were centrifuged at 10,000 G for 30 minutes and the pellets resuspended in one volume of non-centrifuged Method I antigen. The resulting turbid suspensions were then treated by sonication in the MSE 100 watt Ultrasonic Disintegrator for several minutes until a significant reduction in turbidity took place.

Preparation of antisera

Guinea pigs and rabbits were injected intraperitoneally with 0.5 ml of 10 percent yolk sac suspensions of the spotted fever rickettsiae. The temperatures of the guinea pigs were measured daily and the presence or absence of orchitis was noted until the disappearance of signs of infection. One month after infection the animals were bled by cardiac puncture and the serums titrated by indirect F.A. against the corresponding strains of rickettsiae. Animals showing unsatisfactory titers received booster injections of 1 ml of the corresponding Method I antigens and were again bled after ten days.

Complement-fixation test

A standard micro complement-fixation test technique was used according to the LBCF method (Lab Branch CF) modified by Dr. Charles C. Shepard of the Center of Disease Control, Public Health Service, Atlanta, Ga.

The titrations of hemolysin, complement and antigen were performed in the standard volume of 1.0 ml and measured spectrophotometrically. The hemolysin was determined by the plateau method. Complement was determined as 50 percent unit, and five 50 percent units were used in the test.

The micro LBCF test was performed using the microtiter system. Sera were inactivated for 30 minutes at 56°C. Two-fold dilutions were prepared in disposable microtiter plates with 0.025 ml loops from 1:2 through 1:64 but occasionally to 1:512 and 0.025 ml containing the optimal quantity of antigen were added to each dilution. For each serum dilution in the test, a serum-C' control was added. Controls of a tigen and complement with 5C'H₅₀, 2.5C'H₅₀ and 1.25C'H₅₀, reference serum and RBC were included. After overnight incubation in the refrigerator (4°C), 0.025 sensitized red blood cells were added and the plates were transferred to a 37°C incubator. After 30 minutes the plates were removed, centrifuged and read by comparison with a 30 percent color standard.

A rise in titer of four-fold or greater in two consecutive bleedings was considered as proof of current infection. When no such rise in titer was found or when only one serum was obtained, a titer of 1:4 or greater was considered as indicative of exposure to the corresponding agent, but not of current infection.

Fluorescent antibody procedures

For the direct staining of *R. mooseri* and *R. prowazeki*, fluorescein conjugated hyperimmune rabbit serum was used from rabbits immunized with *R. prowazeki*. For the direct staining of all rickettsiae of the spotted fever group, fluorescein conjugated immune human serum prepared from a convalescent case of Rocky Mountain Spotted Fever was used (14). This conjugate was prepared in 1957 and proved to be still highly satisfactory for the above purpose.

For indirect staining, appropriate anti-serum-globulins antisera were prepared and conjugated as described elsewhere (13,15). Anti-human-gamma-globulin fluorescein conjugate obtained from the Baltimore Biological Laboratories was also used in some of these tests. F.A.I. tests were performed by diluting sera to be tested in the appropriate Method I antigens prepared as described above, followed by incubation at room temperature for one hour. The mixtures were then layered over yolk sac smears of rickettsiae and incubated for 30 minutes in a wet chamber at 37°C. The slides were washed for 10 minutes in phosphate buffered saline, dried briefly and stained with the corresponding conjugated anti-serum-globulin antiserum.

Animal sera collected in the field, for which no anti-serum globulin antiserum was available were tested with the complement staining method (15).

Normal unheated guinea pig sera, stored at -70°C until used were tested for the presence of antibodies to the rickettsial agents by the indirect staining procedure, using fluorescein conjugated rabbit anti-guinea pig serum-globulin antisera. Those guinea pig sera which showed no staining at a 1:10 dilution were used for complement staining.

In this procedure, the guinea pig serum was diluted 1:20 in phosphate buffered saline and this mixture used as the diluent for the sera to be tested. The serum complement mixtures were then layered over yolk sac smears of rickettsiae and incubated for 30 minutes in a wet chamber at 37°C. The slides were washed for 10 minutes in phosphate buffered saline, dried briefly and stained with the fluorescein conjugated rabbit anti-guinea pig serum-globulin antiserum.

In order to determine the specificity of the indirect and complement staining procedures, 20 sera collected in this laboratory from individuals aged 6 months to 1 year without history of suspected rickettsial infection were titrated against rickettsial smears prepared from yolk sacs infected with *R. prowazeki*, *R. mooseri*, *R. rickettsi*, *R. akari* and *R. conori*. In several cases, doubtful staining was noted at a 1:10 dilution of the sera, but none were positive at 1:40. The latter dilution was therefore taken as the lowest dilution indicating a significant antibody titer.

For staining of human IgM antibodies, an anti-human IgM conjugate was used obtained from "Miles Yeda", Rehovot.

RESULTS

Serologic studies

The results of the serologic study of the above three population groups are presented in Table 1.

These data point to a clear cut difference among the three groups with respect to the percent of sera containing antibody to spotted fever: 10.5 percent positive in the Ashdod area group, in contrast to 1.5 and 3.3 percent in the groups from the other two areas.

The age distribution of individuals with antibody to spotted fever varied. In the 0 - 4 age group 14.3 percent were positive; lower values were found in the 5 - 9 and 10 - 14 age groups (8.9 and 8.4 percent, respectively) followed by an increase in percent of positives in the 15+ age group (17.2 percent). These variations were not found to be statistically significant.

Environmental studies

Antibodies to spotted fever were present in the blood of the four tested dogs and one gerbil among the fifteen animals which have been tested to date from the Ashdod area (Table 2).

Serologic data on suspected rickettsioses in humans

The serological results of F.A. and C.F. tests are presented in Table 3. The total number of sera received during the period of the contract was 107 from a total of 74 clinical cases. It may be seen that most of the sera sent to this laboratory for diagnostic serology came from the coastal strip from Haifa in the North, to Ashkelon in the South. Most but not all cases were tested by F.A. and C.F. for both spotted fever (SF) and murine typhus (MT) antibodies. In many cases, sera were sent for retrospective testing i.e. bleedings were carried out up to several months after the onset of clinical disease. In such cases, no attempt was made to determine the presence of IgM antibodies. In the case of acute or early convalescent sera, these tests were performed, and in several instances permitted the diagnosis of current infection when a second bleeding was not available or when the first bleeding was too late to show a rise in titer on subsequent bleedings. When the acute or early convalescent serum showed a positive titer by the F.A. test but was negative for IgM, this was not considered to be a current infection of murine typhus or spotted fever.

In 49 out of the 74 cases laboratory data indicated the possibility of spotted fever infection. Their distribution by sex, age and geographical area is illustrated in Table 4.

Figure 1 points to the geographical spread of the above spotted fever suspected cases.

Some complementary epidemiologic and clinical data concerning selected cases are presented in Table 5.

A summary of the results shown in Table 3 are presented in Tables 6a and 6b. In these tables it may be seen that the F.A. method picked up more positive reactions than did the C.F. test. Furthermore the number of sera showing positive reactions to both SF and MT was higher than could be expected, especially in the F.A. test, if one were to assume that these were the results of infection with both types of organisms either simultaneously or consecutively.

The results are further compared in Table 7. Both in tests of murine typhus and spotted fever, the F.A. tests gave more positive reactions than did the C.F. tests, and this difference is seen especially in the case of spotted fever reactions.

The number of individuals who showed a rise in titer during the course of successive bleedings by the two tests is shown in Table 8. In the case of spotted fever infections, the F.A. method showed a rise in titer in 11 cases and the C.F. tests in seven. These findings were reversed in the case of murine typhus infections where the C.F. tests showed a rise in titer in more cases (six cases) than did the F.A. tests (four cases).

In addition to the human sera tested, in several instances bleedings were made from animals trapped in the field in the immediate area where the cases occurred, ectoparasites were collected and bleedings were made from dogs belonging to the families involved or their neighbours. Not all of these materials have as yet been worked up but out of 18 dog bloods collected, 16 have been tested for antibodies to spotted fever rickettsiae by the F.A. complement staining technique and/or by the C.F. test. Of the 16 tested 12 proved to give positive reactions, indicating a high rate of infection (the results are summarized in Table 9).

Isolation of rickettsiae and attempts at their identification

At the end of February 1971 a case of fever with papulo-hemorrhagic rash appeared in Rishpon, an agricultural settlement approximately five miles north of Tel-Aviv. The patient (case No. 161 - Table 4), Mrs. V.G. 41 years of age, stated that she did not recall having been bitten by a tick. However, the household dog slept at night in the kitchen and Mrs. V. recalled that in the morning she sometimes noticed ticks in the kitchen sweepings. On March 31 blood was taken from the patient, and six ticks collected from the dog. The C.F. reaction was positive at 1:8 with spotted fever antigen and indirect F.A. staining was positive to 1:40 against smears of *R. conori*, using conjugates directed either against human gamma-globulins or IgM gamma-globulins. Three months later IgM antibody staining had disappeared but the usual indirect F.A. staining gave a titer of 1:640 whereas the C.F. test remained positive to 1:8. Blood taken from Mrs. V.'s dog on the same date proved to be positive by C.F. and by F.A. staining using the complement staining technique.

The ticks from the dog - all of which were *R. sanguineus* - were washed in 70% alcohol followed by several washings in sterile saline and then triturated in 6 ml S.P.G. containing 100 units penicillin + 50γ streptomycin/ml. 0.5 ml of the tick tissue suspension were injected intraperitoneally into each of two adult male guinea pigs and 0.5 ml injected into each yolk sac of eight 6-day embryonate hen eggs.

No febrile reaction was noted in the guinea pigs nor any other signs of overt disease. After 28 days the animals were bled and tested for antibodies to *R. conori* by F.A. and to group specific spotted fever antigen by C.F. One guinea pig gave a positive reaction by both tests.

In the embryonate eggs injected with the tick suspensions death of the embryos appeared on the third, fourth and fifth day following injection. Smears taken from the yolk sacs at that time and stained with Giesma stain and with F.A. failed to reveal any rickettsiae. Nevertheless, the yolk sac of each egg was triturated in S.P.G. to make a 10% suspension and passaged to five additional embryonate eggs. Six of the eight passaged yolk sacs proved to be contaminated on this passage. Following injection of the other two yolk sacs, embryo death appeared on the fifth and sixth day. Smears prepared from these second passage yolk sacs showed positive F.A. staining of spotted fever rickettsiae.

Additional passages were made and fourth passage material was injected intraperitoneally into adult male guinea pigs for testing of its pathogenicity for these animals and for preparing antisera. All of the guinea pigs showed elevated temperatures of 40°C or higher by 72 hours and scrotal reactions. Two of the guinea pigs were sacrificed after 48 hours of fever and smears made from scrapings of the tunica vaginalis revealed intracytoplasmic rickettsiae.

Soluble antigens were prepared from fourth passage yolk sac material and of later passages by Method I. When necessary, the antigens were concentrated five-fold by dialysis in the cold against concentrated solutions of polyethylene glycol.

Serums from the guinea pigs infected with the isolate described above were serially diluted in normal yolk sac extract and tested by indirect F.A. staining against smears of each of the known strains of spotted fever rickettsiae, as well as against smears of yolk sacs infected with the isolate. The results of one of these sera shown in Table 10 clearly indicate a significant difference in the serological response to the isolate and to *R. australis*, *R. rickettsi* and *R. axari* with less clearly defined differences to *R. siberica* and the strains of *R. conori*.

In order to determine whether the variations in staining of the various rickettsiae were significant, cross inhibition tests were carried out similar to those described for the differentiation of the rickettsiae of murine and epidemic typhus (13). The serum to be tested was serially diluted in homologous and heterologous rickettsial antigens and in normal yolk sac antigen and the mixtures then used for indirect F.A. staining of the smears of the various rickettsiae. The results of some of these tests are shown in Tables 11 and 12.

When the results shown in Table 11 are compared with those in Table 10, one may see that when the antigens used as diluents were heterologous to the antiserum, F.A. staining by the antiserum to the local isolate was strongly inhibited against those rickettsiae homologous to the antigen used as the diluent, but not to the rickettsiae homologous to the serum. On the contrary, when the diluent antigen was homologous to the rickettsiae used for producing the antiserum, F.A. staining of both homologous and heterologous rickettsiae was strongly inhibited. Tables 12 and 13 show essentially the same results but the sera used were against *R. siberica* and strains of *R. conori*.

In Table 13 it may be noted that some antigenic difference seems to exist between the Malish strain of *R. conori* and the Indian tick typhus (ITT) strain. The Malish antigens strongly inhibited the staining of both ITT and Fievre Boutonneuse (FB) rickettsiae by ITT antiserum whereas ITT antigen showed much less activity in the inhibition of staining of FB rickettsiae by FB antiserum. This might indicate that the Malish strain contains all the antigenic components of the ITT strain as well as some antigenic component lacking in ITT.

An additional isolate was made in this laboratory from *R. sanguineus* taken from a dog belonging to S.M. (case No. 69 in Table 3). These ticks were treated in the same manner as described above for the previous isolate. In this case however, both guinea pigs showed elevated temperatures by the third and fourth day and both developed orchitis. Rickettsiae were also found in the yolk sac smears on the first passage and these rickettsiae were also shown to belong to the spotted fever group by F.A. staining. For purposes of identification this strain has been labelled T-193.

A third isolate was made locally from a blood taken from case No. 55 in Table 3. This isolation was done in the A. Felix Public Health Laboratory of the Ministry of Health which received an early acute phase bleeding from this case. The isolation was made by the intraperitoneal injection of male guinea pigs. A piece of tunica vaginalis from one of the guinea pigs was sent to this laboratory for purposes of comparison of the isolate with those made previously in our laboratory from ticks. The tissue was triturated in S.P.G. and passaged in six day old embryonate hen eggs. This isolate will be referred to as isolate No. 212.

Antigens of isolates T-193 and 212 and their corresponding antisera were prepared as described in Materials and Methods and used in the F.A.I. test in comparison with local isolate ISTT in order to determine their identity or lack of identity with the latter isolate.

The results of these tests are presented in Table 14. According to these results the three isolates are identical to each other, at least from the point of view of their antigenic composition.

Similar comparisons of North African strain R. 42 and A38 with the local isolates and with the known strains of *R. conori* have been begun but the results are not yet available.

On the basis of the results indicated in the above tables, it may be concluded that the rickettsiae isolated from the dog ticks and from the human blood differ antigenically from the other representative strains of spotted fever in our hands and may constitute a new member of this group. It is tentatively proposed to name this organism *Rickettsia israelis*, and the disease caused in man Israel tick typhus (ISTT). According to the results obtained so far, the antigenic structure seems to be closer to *R. conori* and *R. siberica* than to *R. rickettsi*, *R. akari* or *R. australis*.

Preparation of washed somatic rickettsial antigens for the purpose of micro-agglutination and specific complement-fixation tests

Attempts were made to produce somatic rickettsial antigens for micro-agglutination tests. Several methods were used for concentrating and purifying rickettsial suspension but none as yet have yielded preparations which gave satisfactory reproducible titers in the microagglutination test using known high titer serums.

In the case of *R. prowazeki* and *R. mooseri*, the particulate antigens prepared gave satisfactory results in the C.F. test and differentiated known epidemic typhus and murine typhus sera. The particulate antigens prepared in similar fashion from various strains of the spotted fever group of rickettsiae were all highly anticomplementary and could not be used for C.F. tests. Attempts are being continued to produce suitable antigens of this type both for the microagglutination and C.F. tests.

DISCUSSION

The frequency of individuals with antibody to spotted fever found in the Ashdod area, in contrast to the very low frequency in the individuals from the other two nearby areas, suggests the existence of a moderate epidemicity of this infection in the Ashdod area.

It seems reasonable to assume that the antibodies to spotted fever rickettsiae among the younger age groups appeared as a result of infection in Israel. On the other hand some of the positive results in the case of the adolescents and adults, most of them immigrants from the North African countries, may have been due to exposure to spotted fever infection prior to their immigrating into Israel.

It has become clear that infections with spotted fever rickettsiae are much more widespread than previously thought. Serums taken from cases diagnosed as spotted fever on clinical grounds as well as several serums from cases diagnosed as endemic typhus have been shown to give positive serological reactions with spotted fever antigens mainly when tested with indirect F.A. staining procedures. Most of the cases were from the coastal region from Haifa in the North to Ashdod in the South, but one was found farther inland in the Jerusalem area. Serological diagnosis of current infection was made on the basis of a rise in titer as detected by C.F. or F.A. when acute and convalescent sera were available, or by F.A. staining of specific IgM gamma-globulins when only the convalescent serum could be obtained. For this purpose fluorescein labelled caprine anti-IgM human gamma-globulin was used. The F.A. method proved to be more sensitive than the C.F. tests, and frequently proved to be positive, or to detect a rise in titer when the C.F. tests failed to do so. IgM antibodies were found to appear within a week after onset of symptoms and persisted for approximately two months.

The age distribution of cases correlates with the data collected from the serologic survey and points to a higher risk of exposure in the lower age groups.

The appearance of antibodies to both spotted fever and murine typhus rickettsiae in a large proportion of the positive reacting sera, though not necessarily in equal titer, is difficult to explain in view of the fact that these rickettsiae do not possess common antigen. Furthermore in several cases IgM antibodies were also present to both types of rickettsiae, although again

not necessarily in equal titer. The presence of IgM antibodies to a given microorganism is usually considered as indicative of current, or very recent infection and they do not generally reappear in case of recrudescence, reinfection or in the recall phenomenon. It would however be unreasonable, on epidemiologic grounds, to assume that the cases in which IgM antibodies appeared to both murine typhus and spotted fever were cases of simultaneous or sequential infection.

Three isolates have been made of rickettsiae of the spotted fever group, two from ticks taken from dogs associated with human cases of clinical rickettsiosis and one from the blood of a human case. The three isolates have been shown to be identical in antigenic composition, according to F.A. tests, but different from the two known strains of *R. conori* in our laboratory.

The fact that a rickettsia of the spotted fever group differing from the others hitherto described is present in Israel may be one explanation for the clinical and epidemiologic differences noted in the human disease in this country as compared to classical boutonneuse fever, i.e. relatively mild disease, lack of the eschar and the absence of history of tick bite.

It is of interest to note that the strains of *R. conori* i.e. the Malish strain and the ITT strain also showed some differences in antigenic make-up when tested by the F.A. method. Antigens prepared from the Malish strain strongly inhibited the reaction of the corresponding antiserum as well as the ITT antiserum with both rickettsiae whereas the antigen prepared from ITT failed to inhibit staining of the Malish strain by its homologous antiserum. This would seem to show that at least one of the antigens present in the Malish strain of rickettsiae is missing in the ITT strain. This might indicate that the various *R. conori* strains are not completely homologous in the composition of their somatic antigens and that the F.A. method might be useful in identifying the various strains appearing in different parts of the world as well as in the study of their epidemiology.

CONCLUSIONS

1. Spotted fever infections were found to be more widespread in Israel than previously thought.
2. Antibodies to spotted fever rickettsiae were found in a high percentage of dogs tested from the areas in which the clinical disease appears.
3. The F.A. test was found to be more sensitive than the C.F. test in that it picked-up more reactors and gave higher serological titers.
4. Three isolates of spotted fever rickettsiae, two from tick and one from a human blood were made and shown to be antigenically identical. These strains differed in their antigenic composition from the Malish strain and the Indian tick typhus strain of *R. conori*.
5. The Indian tick typhus strain and the Malish strain were shown to be not antigenically identical.

RECOMMENDATIONS

The findings presented can be considered only as preliminary, and not all the material has as yet been worked up. It is necessary to complete the tests on the material collected so far. It would be highly desirable to continue to collect additional materials in the field from other areas, e.g. bloods and ectoparasites for further attempts at isolation and serological studies.

It is further recommended that additional strains of *R. conori* be obtained from other laboratories for purposes of comparison with each other and the local isolates.

Additional attempts should be made to prepare antigens suitable for specific C.F. tests and/or microagglutination and to compare results using these tests with those obtained with the F.A. methods.

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Table 1

Distribution of Antibodies* Against Spotted Fever (SF) and Murine Typhus (MT) in Three Subdistricts of Ashdod

A G E	Ashtrom Area					Vav Area					Control Group					
	No. of blood samples	No. tested	Positive		No. of blood samples	No. tested	Positive		No. of blood samples	No. tested	Positive		No. of blood samples	No. tested	Positive	
			SF	MT			SF	MT			SF	MT				
Total	420	325	34	10.5	19	5.8	1	1.5	2	2.9	91	3	3.3	8	8.8	
0- 4	26	21	3	14.3	0	0.0	0	0.0	0	0.0						
5- 9	109	79	7	8.9	1	1.3	0	0.0	0	0.0	51	2	3.9	4	7.8	
10-14	194	167	14	8.4	10	6.0	1	10.0	0	0.0						
15-24	13	7	2	28.6	0	0.0	0	0.0	0	0.0	5	0	0.0	0	0.0	
25-44	49	32	5	12.9	4	12.5	0	0.0	2	7.4	34	1	2.9	4	11.8	
45-64	22	15	3	20.0	4	26.6					1	0	0.0	0	0.0	
65+	5	4	0	0.0	0	0.0										
Unknown	2	0														

* Tested by indirect staining with Fluorescent antibody

Table 2

Animals Trapped in the Ashdod Area and Tested by F.A.*

Animals	Trapped	Tested by F.A.	Positive for Spotted Fever
Dogs	4	4	4
Rats	58	9	0
Mice	20	-	-
Hedgehogs	9	1	0
Meriones	11	-	-
Gerbils	1	1	1

* F.A. - Fluorescent antibody staining, using the complement staining technique

Table 3
Serums Received for Diagnostic Serology 12.8.70-12.8.71

No.	Name	District	C.F. ¹		F.A. ²		IgM ³	
			SF ⁴	MT ⁵	SF	MT	SF	MT
1	A.A.	K. Chaim	1:64	-	≥1:40	-		
2	L.N.	K. Chaim	1:16	-	1:40	-		
3	D.G.	K. Chaim	1:16	-	≥1:40	-		
4	A.P.	K. Chaim	-	-	≥1:40	-		
5	A.Z.	K. Chaim	1: 8	-	1:40	-		
6	H.I.	K. Chaim	-	-	1:40	-		
7	S.R.	K. Chaim	-	-	≥1:40	-		
8	P.A.	K. Chaim	-	-	1:40	-		
9	K.A.	K. Chaim	-	-	1:40	-		
10	S.M.	K. Chaim	-	-	-	-		
11	I.S.	K. Chaim	-	-	≥1:40	-		
12	I.A.	K. Chaim	-	-	≥1:40	-		
13	B.D.	K. Chaim	-	-	1:40	-		
14	P.N.	Ashdod	1:64	-	1:640	-	≥1:40	
	P.N.* ₂	Ashdod	128-256	-	1:2560	-	≥1:40	
15	M.A.	Yavne	-	-	-	-		
16	A.A.	Yavne	-	≥1:64	≥1:40	≥1:40		
	A.A.* ₂	Yavne	-	≥1:64	-	≥1:40		
17	M.D.	Kibutz Nahshon	-	≥1:64	-	≥1:40		
18	S.A.	Rehovot	-	1:512	-	≥1:40		
19	Z.Z.	Akron-Rehovot	-	1:32	-	≥1:40		
20	A.M.	Rehovot	-	1:512	-	≥1:40		
21	G.A.	Akron-Rehovot	-	1:32	1:40	≥1:40		
22	B.G.M	Rishon-Le-Zion	1:8	-	1:40	-	1:40	

1 C.F. - Complement fixation test

2 F.A. - Indirect staining with F.A. against human gamma-globulins

3 IgM - Indirect staining with F.A. against human IgM gamma-globulins

4 SF - Spotted fever

5 MT - Murine typhus

* Subscripts indicate successive bleeding

- Indicates <4 in the C.F. test or <1:40 in the F.A. test

Blank spaces in the results columns indicate that the test was not done

Table 3 (Cont.)

No.	Name	District	C.F.		F.A.		IgM	
			SF	MT	SF	MT	SF	MT
23	I.Z.	Ramle				≥1:40		
24	S.R.	Rishon-Le-Zion	1:8		1:40		1:40	
	S.R. ₂	Rishon-Le-Zion	1:16		1:40		-	
25	A.H.	Ramle	-	-	-	-		
	A.H. ₂	Ramle	-	-	-	-		
26	T.R.	Ramle	-	-	-	-		
	T.R. ₂	Ramle	-	-	-	-		
27	I.M.	Rehovot	1:8	≥1:256	-	≥1:40		
28	K.M.	Jerusalem	1:4	-	1:40	1:40	1:40	1:40
	K.M. ₂	Jerusalem	1:64	1:64	1:640	≥1:640	1:160	≥1:640
	K.M. ₃	Jerusalem			1:160		1:10	1:10
29	Z.I.	Ramle	-	-	-	-		
	Z.I. ₂	Ramle	-	-	-	-		
30	A.A.	Ramle	-	≥1:512	1:40	≥1:10240	1:40	≥1:10240
	A.A. ₂	Ramle	-	≥1:512	1:40	≥1:10240	1:40	≥1:10240
31	I.M.	Tel-Aviv		1:256		1:2560		
	I.M. ₂	Tel-Aviv		1:64	-	≥1:40		
32	V.G.	Rishpon	1:8		1:40		1:40	
	V.G. ₂	Rishpon	1:8		≥1:640		-	
33	V.I.	Rishpon	-	1:8	-			
34	H.A.	Ashdod	1:8	-	1:40		-	-
35	H.D.	Ashdod	1:16	-	1:40	-	-	-
36	H.Z.	Ashdod	1:16	-	1:40	-		
37	H.I.	Ashdod	1:16		1:40	-		
38	B.A.	Ramle	-		-	-		
39	F.R.	Ashkelon	-	-	1:40	-	1:40	
	F.R. ₂	Ashkelon	1:16	-	1:160		1:10	
40	G.M.	Ashkelon	≥1:64		≥1:40	-	1:40	
41	F.M.	Ashkelon	-		-			
42	F.S.	Ashkelon	-		1:40			
43	G.E.	Ashkelon	-		-			
44	G.S.	Ashkelon	-		1:40			

Table 3 (Cont.)

No.	Name	District	C.F.		F.A.		IgM	
			SF	MT	SF	MT	SF	MT
45	L.D.	Hofit	-	-	1:40		1:40	
	L.D. ₂	Hofit	-	-	1:2560		≥1:40	
46	S.E.	Hofit	1:8	-	1:640		1:40	
47	B.I.R	Ramle	-	-	-	-		
48	H.R.	Ramle	-	≥1:64	-	≥1:40		1:40
49	T.N.	Rehovot	1:32	-	1:2560	1:40	≥1:40	1:40
	T.N. ₂	Rehovot	1:64	-	1:640	1:40	1:40	1:40
50	L.R.	Akron-Rehovot	-	≥1:64	≥1:40	≥1:40	1:40	1:40
51	T.V.	Rehovot	-	-	-	-		
52	L.R.	Akron-Rehovot	-	-	-	-		
53	M.D.	Ramle	-	-	1:40	-		
54	G.S.	Nof-Yam	≥1:64	-	1:640	1:40	1:40	-
	G.S. ₂	Nof-Yam	≥1:64	-	1:2560	1:40	≥1:40	1:10
55	G.P.	Nof-Yam	1:32	1:16	1:640	1:40	1:40	1:40
	G.P. ₂	Nof-Yam	1:32	1:16	1:640	1:40	≥1:40	1:10
56	V.T.	Ashkelon	-	-	-	-		
	V.T. ₂	Ashkelon	-	-	-	-		
57	H.O.	Kfar Yona	-	-	-	-		
	H.O. ₂	Kfar Yona	-	-	-	-		
	H.O. ₃	Kfar Yona	-	-	1:2560	1:40	≥1:40	1:10
	H.O. ₄	Kfar Yona	-	-	1:2560	1:40	≥1:40	1:10
	H.O. ₅	Kfar Yona	1:16	1:8	1:640	-	1:40	-
	H.O. ₆	Kfar Yona	-	1:4	1:640		1:40	
58	E.I.	Ashdod	-	-	≥1:40	-	≥1:40	
	E.I. ₂	Ashdod	-	-	1:640	-		
59	A.D.	Sderoth	-	-	-	-		
	A.D. ₂	Sderoth	1:64	1:8	1:2560	-	1:160	
60	S.R.	Netanya	-	-	-	-	-	-
	S.R. ₂	Netanya	-	-	1:640	-	≥1:40	-
	S.R. ₃	Netanya	-	-	1:640	-	≥1:40	
	S.R. ₄	Netanya	1:64	-	1:640	-	-	

Table 3 (Cont.)

No.	Name	District	C.F.		F.A.		IgM	
			SF	MT	SF	MT	SF	MT
61	G.Z.	Rehovot	-	≥1:64	≥1:40	≥1:40	-	≥1:40
62	G.S.	Rehovot	-	≥1:64	-	≥1:40	-	≥1:40
63	K.Z.	Kfar-Saba	1:64	-	1:160	-	≥1:40	
	K.Z. ₂	Kfar-Saba	1:64	-	1:2560	-	1:40	
64	S.S.	Sderoth	-	-	1:640	-	≥1:40	
65	T.E.	Sderoth	-	-	1:640		1:40	
66	S.I.	Ashdod	-	-	-	-		
	S.I. ₂	Ashdod	-	-	-	-		
67	B.I.A	Bnei Dror	-	-	-	-		
	B.I.A ₂	Bnei Dror	1:32	1:16	1:2560	-	≥1:40	
68	D.Z.	Kfar Yona	-	1:16	-	-		
	D.Z. ₂	Kfar Yona	1:64	1:32	1:2560	1:160	≥1:40	1:40
	D.Z. ₃	Kfar Yona	1:64	1:16	1:10240	1:640	1:40	1:40
69	S.N.	Hofit	1:16	-	1:640	-	≥1:40	
	S.N. ₂	Hofit	1:32	-	1:640	-	≥1:40	
70	P.M.	Kfar-Saba	1:64	-	1:640	-	1:40	
71	N.D.	Netanya	1:32	-	1:40	-	-	
72	S.M.	Kfar-Saba	1:64	-	1:640	1:40	≥1:40	-
73	N.E.	Kfar-Saba	1:64	-	1:640	-	-	-
74	M.Y.	Hofit	1:16	-	1:160	1:40	-	-

Table 4

Distribution by Sex, Age and Geographical Area
of 49 Suspected Spotted Fever Infections in Humans

Geographic area	Total	S e x			Age group				
		M	F	Unknown	0-14	15-44	45-64	65+	Unknown
Total	49	21	23	5	29	13	2	-	5
Haifa	13	9	4	-	13	-	-	-	-
Sharon	12	5	7	-	7	5	-	-	-
Rehovot	5	2	3	-	2	3	-	-	-
Ashdod-Ashtrom	13	5	8	-	7	4	2	-	-
Jerusalem	1	-	1	-	-	1	-	-	-
Unknown	5	-	-	5	-	-	-	-	5

Table 5

Clinical, Epidemiologic and Laboratory Data on Selected Cases of Spotted Fever

No.	Name	Age	Sex	Area of residence	Date of onset	Clinical Diagnosis	Laboratory results			Epidemiologic data
							1st blood	F.A.* 2nd blood	IgM**	
1	K.M.	22	F	Jerusalem	2.71	Spotted Fever (Hadassah Hospital)	1: 40	1: 640	1:40	Close contact with tick infested dogs.
2	V.G.	42	F	Rishpon	2.71	Spotted Fever	1: 40	≥1: 640	1:40	Close contact with tick infested dogs. Rickettsiae isolated from ticks.
3	L.D.	27	F	Hofit	7.71	Spotted Fever	1: 40	1:2560	1:40	Close contact with tick infested dogs.
4	S.N.	4	M	Hofit	7.71	Spotted Fever	1:640	1: 640	≥1:40	Close contact with tick infested dogs. Rickettsiae isolated from ticks.
5	F.R.	3	F	Ashkelon	7.71	Spotted Fever (Ashkelon Hospital)	1: 40	1: 160	1:40	Contact with tick infested dogs in the immediate environment of the patient.
6	G.P.	63	F	Nof Yam	9.71	Spotted Fever (Kfar-Saba Hospital)	1:640	1: 640	1:40	Contact with tick infested dog. Rickettsiae isolated from blood of the patient.
7	G.S.	67	M	Nof Yam	9.71	Spotted Fever (Kfar-Saba Hospital)	1:640	1:2560	1:40	Husband of case No. 6. Intimate contact with tick infested dog.

* F.A. - Indirect staining with F.A. against human gamma-globulins

** IgM - Indirect staining with F.A. against human IgM gamma-globulins

Table 6a

Human Sera Submitted for Serological Diagnosis of Rickettsial Infections
Tested by Fluorescent Antibody (F.A.) and Complement-Fixation (C.F.)

Test	Murine Typhus		Spotted Fever	
	Number Tested	Number Positive	Number Tested	Number Positive
C.F.	91	27	104	42
F.A.	90	31	105	71

In the C.F. test a titer of $\geq 1:4$ was considered as positive

In the F.A. test a titer of $\geq 1:40$ was considered as positive

Table 6b

Human Sera Tested for Both Spotted Fever (SF)
and Murine Typhus (MT) by Complement Fixation
(C.F.) and Fluorescent Antibody (F.A.)

Test	Number tested	Positive for		
		SF only	MT only	SF and MT
C.F.	89	27	16	9
F.A.	89	38	9	20

In the C.F. test a titer of $\geq 1:4$ was considered as positive

In the F.A. test a titer of $\geq 1:40$ was considered as positive

Table 7

Comparison of the Results of Fluorescent Antibody (F.A.)
and Complement Fixation (C.F.) Tests on Human Sera
Tested against Spotted Fever (SF) and Murine Typhus (MT)
Rickettsiae

Test	Sera tested for SF		Sera tested for MT	
	F.A.+	F.A.-	F.A.+	F.A.-
C.F.+	41	1	21	4
C.F.-	30	33	9	49

In the C.F. test a titer of $\geq 1:4$ was considered as positive

In the F.A. test a titer of $\geq 1:40$ was considered as positive

Table 8

Number of Cases Which Showed a Rise in Titer
During Successive Bleedings

	Spotted Fever		Murine Typhus	
	F.A.*	C.F.**	F.A.*	C.F.**
1st serum positive	8	1	1	0
1st serum negative	3	6	3	6
Total	11	7	4	6

* F.A. - Fluorescent antibody

** C.F. - Complement fixation

Table 9

Animals Trapped in the Areas of Appearance of
Clinical Cases and Serologically* Tested

Animals	Trapped	Serologically tested	Positive for Spotted Fever
Dogs	18	16	12
Rats	13	-	-
Mice	1	-	-
Hedgehogs	5	-	-

* Tested by the F.A. complement staining technique and/or
C.F. test.

Table 10

Titration by Fluorescent Antibody (F.A.) of Antiserum from
Guinea Pig Injected with Rickettsial Isolate¹

Serum	Dilution	S m e a r s						
		ISTT ²	NQTT ³	STT ⁴	RMSF ⁵	RP ⁶	FB ⁷	ITT ⁸
ISTT 28571/6	1 : 10	4+*	3+	4+	4+	4+	4+	4+
	1 : 40	4+	2+	4+	-	3+	3+	4+
	1 : 160	2+	-	+	-	-	+	+
	1 : 640	+	-	-	-	-	-	±

- 1 Titrations were carried out by the indirect F.A. procedure using yolk sac smears of the appropriate spotted fever rickettsiae
 - 2 ISTT - Smears of yolk sacs injected with local isolate
 - 3 NQTT - *R. australis* - Queensland tick typhus
 - 4 STT - *R. siberica* - North Asian tick typhus
 - 5 RMSF - *R. rickettsi* - Rocky Mountain spotted fever
 - 6 RP - *R. akari* - Rickettsial pox
 - 7 FB - *R. conori* - Fievre boutonneuse (Malish strain)
 - 8 ITT - *R. conori* - Indian tick typhus
- * Staining was graded from 4+ - brilliant staining - to ± weak but visible staining

Table 11
Inhibition by Soluble Rickettsial Antigens of Fluorescent Antibody (F.A.)
Staining of Spotted Fever Rickettsiae

Guinea pig serum	Dilution	Diluent: STT*		RMSF*		RP*		ITT*			FB*			ISTT*			NQT	
		Smears	:ISTT	STT	ISTT	ISTT	RMSF	ISTT	RP	ISTT	ITT	FB	ISTT	ITT	FB	ISTT	ITT	NQT
ISTT 28571/6	1 : 10	3-4+	2-3+	4+	-	4+	-	4+	-	4+	3+	2+	3+	2+	3+	2-3+	4+	-
	1 : 40	3+	±	3-4+	-	3+	-	3+	-	3+	±	±	3+	±	+	+	3+	-
	1 : 160	2+	-	2+	-	2+	-	2+	-	2+	-	-	4-2+	-	-	-	2+	-
	1 : 640	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	-

* Method I antigens

Abbreviations as in Table 10

Table 12
Inhibition by Soluble Rickettsial Antigens of Fluorescent Antibody (F.A.)
Staining of Spotted Fever Rickettsiae

Guinea pig serum	Dilution	Diluent:				NYS*			ISTT*			STT*			ITT*	
		Smears :		STT	ISTT	ISTT	ITT	STT	ISTT	ITT	STT	ISTT	ITT	ISTT	ITT	ITT
STT 26371/3	1 : 40			4+	4+			4+	±		2+	-				
	1 : 160			3+	+			3+	-		+	-				
	1 : 640			2+	±			2+	-		-	-				
	1 : 2560			±	-			+	-		-	-				
ITT 16571/A	1 : 10						4+		±	3+				3+	3+	
	1 : 40						4+		-	2-3+				±	2+	
	1 : 160						2+		-	2+				-	±	
	1 : 640						-	+	-	±				-	-	

* Method I antigens. NYS - Normal yolk sac extract.
Other abbreviations as in Table 10

Table 13

Inhibition by Rickettsial Antigens of Fluorescent Antibody (F.A.) Staining of Spotted Fever Rickettsiae

	Dilution	Diluent:			NYS*			FB*			ISTT*			ITT*			
		Smears :			ISTT	FB	ITT	ISTT	FB	ITT	ISTT	FB	ITT	ISTT	FB	ITT	
FB R-261	1 : 10		4+		4+		4+	+		+	+	2+	3+		2+	3+	3+
	1 : 40		3-4+		4+		4+	-		±	±	-	3+		±	2-3+	2+
	1 : 160		2+		3+		3+	-		-	-	-	2+		-	±-2+	-
	1 : 640		±		+		+	-		-	-	-	±		-	±	-
ITT 16S71/C	1 : 10				3+		3+			±-2+	2+					3+	3+
	1 : 40				3+		3+			-	+					-	-
	1 : 160				±-2+		±			-	-						
	1 : 640				±		±			-	-						

* Method I antigens

Abbreviations as in Tables 10 and 12

Table 14
Inhibition by Rickettsial Antigens of Fluorescent Antibody (F.A.) Staining of Spotted Fever Rickettsiae

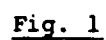
Antiserum	Dilution	Diluent:				NYS*				ISTT*				T-193*				212*			
		Smears :				ISTT	T-193	212		ISTT	T-193	212		ISTT	T-193	212		ISTT	T-193	212	
ISTT 28571/6	1 : 20	4+	4+	4+	4+	4+	4+	4+	2+	2+	3+	2+	3+	3-4+	4+	3+	2+	2+	2+	2+	2+
	1 : 40	4+	4+	4+	4+	4+	4+	4+	2+	2+	2+	2+	2+	2+	2+	2+	2+	+	+	+	+
	1 : 160	3+	3+	3+	3+	3+	3+	3+	±	±	±	±	±	±	-	±	-	-	-	-	-
	1 : 640	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
T-193 151171/2	1 : 20	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	3+	4+	4+	4+	4+	3+	3+	3+	3+	3+
	1 : 40	4+	4+	4+	4+	4+	4+	4+	3+	2+	2+	2+	2+	+	+	+	2+	+	2+	+	+
	1 : 160	2+	2+	2+	2+	2+	2+	2+	±	±	-	-	-	-	-	-	-	-	-	-	-
	1 : 640	±	±	±	±	±	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-
212 G.P.123	1 : 10	3+	3+	3+	3+	3+	3+	3+	4+	4+	4+	3+	4+	4+	4+	4+	4+	4+	4+	4+	3+
	1 : 40	4+	4+	4+	4+	4+	4+	4+	2+	2+	2+	2+	2+	3+	3+	2+	2+	2+	+	+	+
	1 : 160	2+	2+	2+	2+	2+	2+	2+	-	-	-	-	-	±	±	±	±	-	-	-	-
	1 : 640	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-

* Method I antigen

T-193 Local isolate from *R. sanguineus*

212 Local isolate from human blood

Abbreviations as in Tables 10 and 12



APPENDIX A

The following personnel was engaged in the work under this contract:

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. Steinman, M.Sc.	- Research Associate

Other Personnel

Ada Israeli, R.N.	- Public Health Nurse
Esther Potashnik, B.Sc.	- Laboratory Technician
Rina Shalev	- Laboratory Technician
Kalia Grinberg	- Laboratory Technician
M. Iris	- Field Worker

Assistance of Public Health Nurses

Zipora Melamed, R.N.	- Public Health Nurse, District Center, Ashkelon
Shoshana Hammer, R.N.	- Public Health Nurse, Ashdod
Ica Nelson, R.N.	- Public Health Nurse, Ashdod