Original Articles

CLINICAL FEATURES AND LABORATORY DIAGNOSIS OF XK OR MITE-BORNE TYPHUS AS OBSERVED IN 102 CASES IN THE BARRACKPORE AREA

By K. V. KRISHNAN
R. O. A. SMITH
P. N. BOSE
K. N. NEOGY
B. K. GHOSH ROY
and
M. GHOSH

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WITHIN the last ten years several reports of the occurrence of typhus fevers of all three serological varieties have been published from almost all the provinces of India. XK typhus (mite-borne) appears to be common in most places, X19 typhus (murine, flea-borne) coming second, X2 typhus (the true nature of which is not yet clearly understood) coming third and X19 typhus (louse-borne) coming last. Although the total mortality caused by typhus fevers is not very high the morbidity caused by them is increasing rapidly. While a few years ago these fevers were not considered to be of much public health importance in India, to-day they are undoubtedly as important as the enteric group of fevers in the areas where they occur.

Between 1946 and 1948, 102 cases were diagnosed as typhus in Barrackpore by the Enquiry into Typhus in Bengal at the All-India Institute of Hygiene and Public Health, Calcutta. This number represents merely a fraction of the total number of cases occurring in this area. 72 per cent of these cases were XK typhus, 19 per cent X19 (murine) and 9 per cent X2. In 64 cases of this series the diagnosis was based on (*i*) clinical findings, (*ii*) Weil-Felix reaction, (*iii*) isolation of rickettsia by animal inoculation, and (*iv*) study of pathological lesions caused in experimental animals such as guinea-pigs and mice. In the other 38 the diagnosis was based on clinical findings and a suggestive Weil-Felix reaction; no animal inoculations were done in this group for confirming diagnosis. The clinical and laboratory data collected with respect to all these cases have been analysed and the results are presented in this article. Although accounts of the clinical features of typhus cases are found in many publications the relative importance of serological tests and animal inoculation in the diagnosis of typhus fevers has not been sufficiently stressed. Furthermore, the average practitioner is not yet fully aware that typhus cases are occurring in large numbers among the civilian population in endemic areas and that many of these cases are still being erroneously diagnosed as para-typhoid, dengue, influenza or classified as P.U.O.s, because reliance is placed on clinical findings and laboratory aid is seldom resorted to. Since a sure diagnosis of typhus can only be made in the laboratory and too much reliance cannot be placed on the variable clinical findings (except in the severe forms) it is hoped that this article on the clinical and laboratory diagnosis of typhus fevers in general and of XK typhus in particular will be of interest.

Clinical diagnosis

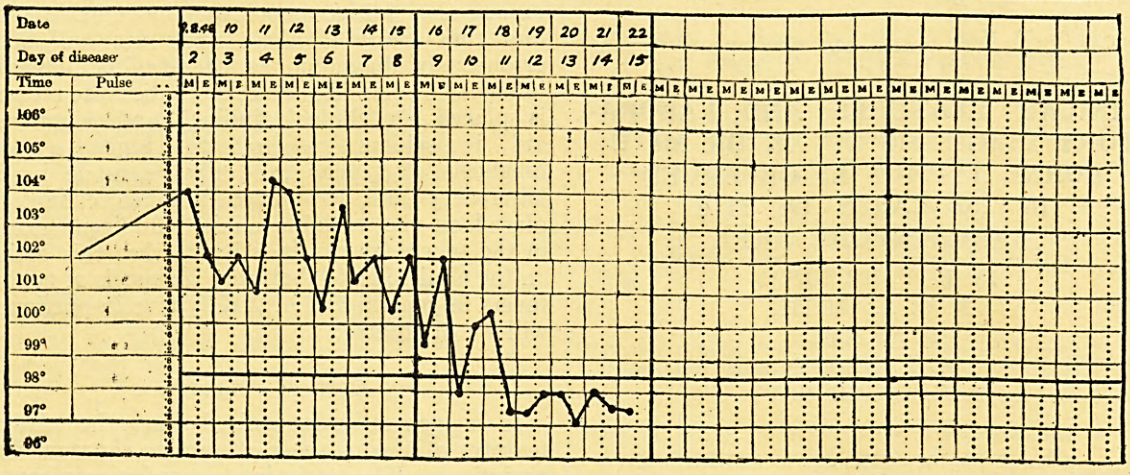
It is generally recognized that the clinical features of typhus cases are apt to show marked variations. The variations may be ascribed to differences in the causal agent, mode of transmission and immunity status of the population affected. From a study of the clinical features of cases in the Calcutta-Barrackpore area it is found that typhus cases can be divided clinically into two types ' mild ' and ' severe '. In this area 80 per cent of cases belong to the mild and 20 per cent to the severe type. Due to the infrequent occurrence of several of the characteristic symptoms in the majority of mild cases, it was found impossible to diagnose them on clinical evidence alone. Laboratory aid had to be sought to obtain a diagnosis in these cases. In the severe cases and in a small percentage of mild cases (*i.e.* in all about 40 per cent of cases) the symptoms were generally characteristic and a provisional diagnosis of typhus could be made. But laboratory aid had to be sought for determining the type of typhus. Clinically, it was not possible to distinguish between the three types of typhus (XK, X19 murine and X2). The differences in the clinical features were not clear cut. In a few of the cases in which some characteristic symptom or other such as eschar or petechial rash was present, one could hazard a guess as to the type but this needed to be confirmed by laboratory tests.

The clinical features of XK typhus which is the predominating type in the Barrackpore area are presented below :

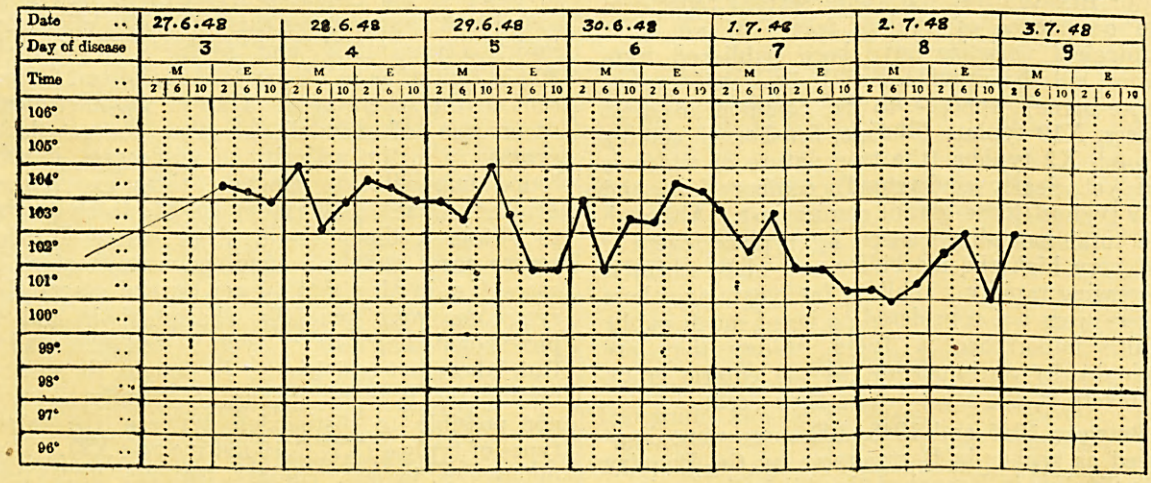
The *onset* of fever in the majority of cases was sudden. In mild cases the temperature went up to 101°F. and 102°F. and occasionally to 103°F. In the severe cases it rose higher and in some reached 105°F. In 60 per cent of the cases fever was remittent in type and in 40 per cent it was continuous. The total duration of fever varied. In the mild type it lasted about 10 to 14 days with an average of 12 days, whereas in the severe type it ranged from 14 to 22 days with an average of 18 days. The termination was either by crisis or by lysis; both were equally frequent (*vide* charts).

CHARTS.

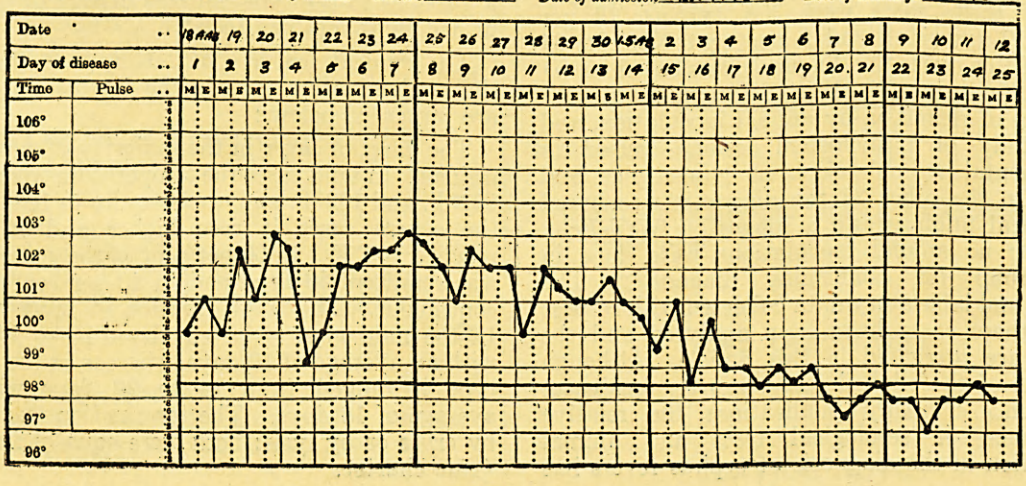
Name Gorakh-nath. Age 35 Disease TYPHUS Date of admission 9.8.48. Date of discharge 22.8.48



Name Bhagawandin Age 45 Disease TYPHUS Date of admission 27.6.48 Date of discharge Expired at 3.20 A.M. on 3.7.48.



Name Dr. Smith Age 56 Disease TYPHUS Date of admission 21.4.48 Date of discharge 12.5.48



Headache was complained of by all patients. In the mild type it was present at the commencement of illness and disappeared a few days afterwards. In the severe type it persisted for a much longer period and was complained of even in the second week of illness. In 30 per cent of all cases it was very severe, persistent and annoying. This feature is of diagnostic significance.

Mental dullness was present in all severe cases and in about 40 per cent of mild ones. It was most marked about the end of the first week of illness and persisted for some days. Patients had a vague and sleepy look and there was much delay in comprehending questions and in replying to them. This was very characteristic.

Delirium of a low muttering type was present in 21 per cent of the severe type only.

Sleeplessness was a characteristic feature of most cases. 41 per cent of the mild type and all cases belonging to the severe type complained of this. It was persistent and troublesome in the severe cases and almost the first thing the patient requested the doctor was for 'some medicine to sleep'.

Deafness was another feature frequently observed. It was noticed in 57 per cent of severe cases and in 18 per cent of mild cases. It was most marked during the second week of illness and cleared up quickly after the temperature dropped to normal.

Congestion of the eyes was an early and constant symptom. In the mild cases it lasted for a few days and in the severe cases it persisted till the middle of the second week. This is a valuable diagnostic sign. In about 50 per cent of the severe cases ptosis of the lids was noticed imparting the sleepy look.

Giddiness was complained of by many patients. In 80 per cent of severe cases and in 15 per cent of mild cases it was marked and troublesome.

Lymphadenitis was noticed in 65 per cent of the cases. It was more frequently seen in the severe cases and less frequently in mild ones. The glands of the neck were most often involved; next in frequency were the axillary glands and lastly the inguinal glands. The glands were shotty and tender. Tenderness was very marked in some cases.

Splenic enlargement was noticed in 26 per cent of cases. The spleen was just palpable in most of these. In two, the enlargement was 2 fingers below the costal margin.

Rash was noted in 75 per cent of severe cases and 7 per cent of mild ones. It appeared on or about the 6th day of illness, was macular in type and distributed mainly on the forehead, arms and legs. It invariably disappeared within a day or two of its appearance. The percentages mentioned would appear very low but in view of the fact that rashes are not easily detected in dark-skinned persons and also that many cases were seen by us late in the course of illness, the figures do not represent a correct estimate of the incidence of rash. Our belief is that if cases are seen early and one is on the look out for rash it will be found in the majority of cases.

Eschar was seen in 5 of the severe cases. None of the mild cases showed an eschar. In two it was a raised papule about the size of a split pea with a red areola round it and in the other three it was a black necrotic ulcer of the same size with a red areola about one inch in diameter. Lymphangitis was present and the glands draining the area were enlarged and tender. The situation of the eschar in 3 cases was the shoulder girdle (in two it was in front and in one at the back); in the fourth case it was on the genitals; and in the fifth on the finger.

Constipation was complained of by the majority of cases and diarrhœa occurred in 6 per cent of cases.

Tongue was found coated and dry in most of the severe cases. In the mild cases it was normal. The coating of the tongue seen in the severe cases of typhus was quite unlike that seen in typhoid. This feature at times helps to distinguish typhus from typhoid cases.

Pain in the muscles was invariably complained of by all patients. Groups of muscles in different parts of the body were affected. The joints were not involved. This feature was helpful in differentiating typhus from dengue cases. In all severe cases and in 32 per cent of mild cases muscular pain was intense and prostrating.

Lung symptoms were noticed in 50 per cent of the severe cases. Congestion of lung was noticed in the second week and crepitations could be heard on auscultation. There was some cough. These symptoms cleared quickly after the temperature came down to normal.

Blood pressure was lowered in the severe cases towards the middle of the second week.

Pulse rate was usually slow in comparison to the temperature at the commencement of illness in all cases. In the severe cases towards the end of the second week it was inclined to be feeble and rapid.

Leucocyte count did not show any significant change in the cases studied.

Urine in the majority of severe cases showed traces of albumin. In the mild cases it was normal.

Case fatality in this series was only 3 per cent, *i.e.* 14 per cent of severe and nil in mild cases. Death occurred in these cases about the middle of the second week.

From the data presented above it will be seen that the majority of mild cases of XK typhus do not show any characteristic symptom by which one could diagnose the disease with certainty on clinical grounds. Most cases complain only of fever, headache and pain in the body. When congestion of the eyes, mental

dullness, sleeplessness and glandular enlargement are present typhus could be suspected. If rash and eschar occur the diagnosis is almost certain. Mild cases have been mistaken for dengue, para-typhoid and influenza. From dengue, typhus cases can be differentiated by the longer duration of fever, absence of saddle-back temperature, absence of joint pains and presence of asthenia disproportionate to the mildness of the symptoms. From influenza, they can be distinguished by the absence of coryza and by the longer duration of fever and characteristic temperature chart. From para-typhoid, it is often difficult to differentiate. Absence of abdominal symptoms such as diarrhœa and tympanites, and presence of congestion of eyes, may help in diagnosis. But the diagnosis must be confirmed in the laboratory.

The clinical features of the severe cases are generally very characteristic and it is easy to diagnose them as typhus on clinical grounds with a fair degree of accuracy. The congestion of the eyes, the dull and sleepy look, the extreme asthenia, the slow pulse, the rash, eschar and glandular enlargement when present are all very typical. When one or two typical cases have been seen, the clinical picture presented by the typhus cases cannot be easily mistaken for any other disease except typhoid. But the presence of the characteristic furred tongue and abdominal symptoms in typhoid and the presence of eschar and glandular enlargement in typhus help in differentiation. As stated above, the type of typhus can only be ascertained by laboratory procedures.

Laboratory diagnosis

In the laboratory, the diagnosis of typhus is established by (*i*) Weil-Felix reaction using 'O' suspensions of proteus X19, XK and X2, (*ii*) complement-fixation test using specific rickettsial antigens, and (*iii*) animal inoculation (using mice and/or guinea-pigs) for demonstrating the presence of rickettsiæ, and associated pathological lesions, such as the Neill-Mooser reaction, or ascites or enlarged spleen. In the present investigation, as suitable rickettsial antigens for the complement-fixation test were not available only the Weil-Felix reaction and animal inoculations were conducted. Diagnosis of typhus was made when positive Weil-Felix agglutination in titre at or above the prescribed diagnostic level was obtained and when rickettsia and characteristic lesions could be demonstrated in experimentally inoculated animals.

Weil-Felix reaction

For the Weil-Felix reaction, suspensions of OX19, OXK and OX2 were used in all cases. These suspensions were prepared and standardized in the laboratory according to the method of Bridges (1944) and compared before use with standard suspensions of OX19, OXK and OX2 obtained from the Military Laboratory, Poona.

Of the cases diagnosed as typhus, 72 per cent proved to be XK, 19 per cent to be X19 (murine) and 9 per cent to be X2. No difficulty was experienced in diagnosing X19 typhus by the Weil-Felix test. The majority of these cases gave agglutination in high titres. A titre of 1 in 500 to 1 in 1,250 for OX19 was obtained in 70 per cent and in 30 per cent the titre ranged from 1 in 250 to 1 in 500. In some of these cases male guinea-pigs were inoculated with crushed blood clot or washed blood cells but the typical Neill-Mooser reaction was not elicited.

The Weil-Felix tests performed on cases of XK typhus revealed that agglutination in high titres is not as frequently obtained in XK typhus as in X19 typhus. Whereas in every case of X19 typhus an increase in titre to or above the diagnostic level for OX19 was obtained, in only 65 per cent of XK typhus were definitely positive Weil-Felix reactions for OXK found. Furthermore, in only 10 per cent of these was the OXK titre over 1 in 500 and among these in a very few only was the highest titre of 1 in 1,250 obtained. In 90 per cent of the positive group the OXK titre ranged from 125 to 250. The diagnostic level in most cases was usually reached by about the 10th day of illness; in some it was not reached until after the crisis or during early convalescence.

In 35 per cent of XK typhus proved by animal inoculation the Weil-Felix reaction did not give a significant OXK titre even on repeated examinations. In 20 per cent the highest titre obtained was only 1 in 50, in 5 per cent it was 1 in 25 and in 10 per cent it was negative. In some of these cases the titre after rising to 1 in 25 or 1 in 50 either remained at that level or came down to a lower level instead of increasing. This feature was noticed in cases belonging to the severe as well as the mild types. Had animal inoculation not been resorted to in these cases, a definite diagnosis would not have been possible.

Of the fever cases seen in the course of this investigation, 231 were non-typhus cases which had been diagnosed as enteric, kala-azar, malaria, influenza or pneumonia. Sera from such cases examined by the Weil-Felix reaction showed that in nearly 30 per cent a positive agglutination in low titres ranging from 1 in 25 to 1 in 50 was present; 68 reacted with OXK, 43 with OX2 and 28 with OX19. Since the highest agglutinin titre in non-typhus cases was 1 in 50, the diagnostic titre for XK typhus was fixed at 1 in 125 or over. But since it was found that 35 per cent of confirmed XK typhus cases also gave low titres, doubtful cases were not rejected straightway as non-typhus (unless an alternative diagnosis had been made and confirmed) but were investigated further by animal inoculation. If the Weil-Felix reaction only is used, 35 per cent of XK typhus cases are likely to be missed. By resorting to animal inoculation in doubtful and sero-negative cases practically every case of XK typhus could be detected.

In this series 7 cases were diagnosed as X2 typhus by the Weil-Felix reaction. The agglutination titre obtained for OX2 in these cases varied from 1 in 125 to 1 in 250. Sera of these cases also agglutinated OXK and OX19 to a lesser degree (1 in 25 or 1 in 50).

Workers in other parts of India have also reported cases of X2 typhus but what form of typhus these X2 cases truly represent is not yet clear. Their diagnosis on serological grounds has generally proved a bit difficult and animal inoculation has not been very helpful. This type needs to be properly investigated and its ætiology and epidemiology correctly determined in India.

Animal inoculation

For demonstrating the presence of rickettsiæ in the blood of patients and for determining the type of typhus, animal experimentation is essential. For these purposes, guinea-pigs, white rats, white mice, monkeys and rabbits have been used; but guinea-pigs and white mice have proved most satisfactory. For diagnosis of murine typhus male guinea-pigs are the best and for diagnosis of XK typhus white mice are preferable. For X2 both guinea-pigs and white mice have been tried, while for some X2 strains, guinea-pigs have proved more satisfactory than mice, for others, neither of the animals have proved suitable. By the use of guinea-pigs and mice the following results may be expected in the different forms of typhus fevers :—

	Guinea-pig				White mouse			
	Fever	Characteristic reaction	Death	Rickettsia	Illness	Characteristic reaction	Death	Rickettsia
Louse-borne ..	+++	Nodes in brain	— (+ in some cases).	++	—	*Nil.* Inapparent infection.	—	+
Flea-borne ..	+++	Neill-Mooser scrotal reaction.	—	+++	+	Lung infection on intra-nasal inoculation.	—	++ Tunica
Tick-borne R.M.S.F.	+++	Scrotal swelling necrosis.	+++	+++	—	Inapparent infection. On intra-nasal infection lung involved.	—	+ ++
Other types, X2	Not yet investigated							
Mite-borne ..	+	Ascites	— in a few +	++	+++	Ascites, spleen enlarged.	++	+++

In the course of the present investigation guinea-pigs and white mice (Haffkine strain) were chiefly used. Blood samples of 66 cases were subjected to animal inoculation. The material inoculated was either crushed blood clot in saline or washed blood cells of the patient. Washed blood cells proved definitely superior and yielded a much higher percentage of positives. In the early part of the investigation blood clot was used but later on it was given up and only washed cells were used. Injections into animals were given intraperitoneally. The dose for mice was, washed blood cells from 1 cc. of blood suspended in saline and for guinea-pigs, washed cells from 5 cc. of blood.

In XK cases animal inoculation was resorted to chiefly for diagnosis of clinically positive and serologically doubtful cases and for clinically doubtful and serologically negative cases. It was found that in 71 per cent of the first and in 10 per cent of the second group rickettsia could be demonstrated in the endothelial cells from peritoneal scrapings after staining with Giemsa stain. When washed blood cells of XK cases were injected into white mice the animals usually became ill between the 6th and 12th days and in a very few instances somewhat later. On sacrificing these animals about the 8th day rickettsiæ could be demonstrated in the peritoneal scrapings in all positive cases. Samples of blood collected from patients in the early and late stages of the disease (3rd to the 16th day of their illness) gave positive results. In all positive animals the pathological lesions met with were ascites and splenic enlargement; the former was very characteristic of XK strains and by no other strain studied was it caused. In a few animals the ascitic fluid was found to be sticky, purulent and blood tinged. Animals not sacrificed in time usually died. Death occurred in most animals about the 10th or 12th day after inoculation. In some it took place later and in a few no death occurred.

Guinea-pigs inoculated with washed blood cells from XK cases ordinarily showed a rise in temperature about the 10th day which lasted for about 2 days and then subsided. When sacrificed during the febrile period ascites and enlargement of spleen were found. No scrotal

reaction was seen in any of them. Rickettsiæ were found in scrapings from the tunica. These strains however could not be maintained by guinea-pig passage; the virulence seemed to die out on or after the 3rd passage in all strains isolated in Bengal so far. On this account mice were preferred and several strains have now been maintained to the 50th passage and above in mice.

In two cases giving highest agglutination with OX2 animal inoculation was done and rickettsia isolated. Both mice and guinea-pigs were used. In the first case the strain behaved in mice and guinea-pigs like an XK strain and caused ascites and enlarged spleen with presence of rickettsia in peritoneal scrapings. This strain was assumed to be one of XK and not of X2 on this account. The other strain behaved in an entirely different manner. On first inoculation into a guinea-pig and a mouse negative results were obtained. But when the spleen and brain emulsion of the mouse was injected intraperitoneally into another mouse, the second mouse showed rickettsiæ on the 10th day and inoculation of infective material from the second mouse proved infective to guinea-pigs also. In the guinea-pig in which it is now being maintained it is causing a characteristic infection with fever and enlarged spleen only; the strain causes no ascites or scrotal reaction and the inoculated animals do not die. Scrapings from tunica of infected animals invariably show rickettsia. Mice do not appear to be as good as guinea-pigs for maintaining this strain. This strain may be considered to be an X2 strain of this area. This will need further investigation. Morphologically also this strain of rickettsia showed some differences from the XK strain. While the XK rickettsiæ look like minute cocco bacilli and are found in plenty, the X2 rickettsiæ are diploid in appearance, look relatively larger and occur in fewer numbers. They are also intra-cytoplasmic.

It will be seen from the data presented that pathogenicity tests are valuable adjuncts to clinical and serological diagnosis and should be done as a routine in sero-negative and doubtful cases. The importance of animal inoculation cannot be over-emphasized if a correct diagnosis of all typhus cases is to be made. In India where the medical statistics has still a big heading entitled ' Pyrexias of unknown origin ' it is very important to resort to all available laboratory aid in order to reduce the number under this heading.

Complement-fixation test

In 1941, Bengtson, and Bengtson and Topping demonstrated that with an antigen prepared from yolk sac cultures of rickettsia, complement-fixation test could be done on the sera of typhus patients and that the test gives more specific results than the Weil-Felix reaction. In the present investigation, due to the non-availability of suitable rickettsial antigens, the complement-fixation test could not be done. But since it was found that in the diagnosis of XK and X19 typhus the Weil-Felix reaction and animal experimentation give very satisfactory results, the need for the complement-fixation test was not felt much. But in the diagnosis of X2 cases in which clear-cut results were not obtained either with the Weil-Felix reaction or with animal experimentation, it was felt that complement-fixation test would be more helpful. From the experience of workers in other countries, this view receives much support. However, for the performance of the test, antigen prepared from local strains of rickettsia would be highly desirable. Local rickettsial strains will have to be isolated, grown in yolk sacs of chick-embryo and antigen suspensions prepared from the culture material. Attempts are being made to do this but X2 cases are few in this area and occur generally in the spring. During last spring only 7 cases were seen and from only one case was a strain isolated. No attempt has yet been made to grow this in the yolk sac. Until this is done, no further information on the value of complement fixation in the diagnosis of typhus can be given. The problem is an important one, specially as there are several foci of X2 typhus in India.

Summary and conclusion

From a study of 102 cases of typhus fever occurring in the Barrackpore area among the civilian population the following conclusions have been drawn :—

That 80 per cent of typhus cases in this area belong to the mild and 20 per cent to the severe type. The total case fatality rate is 3 per cent.

That it is not possible to make a diagnosis of typhus on clinical grounds alone except in about 40 per cent of cases and that in the other 60 per cent laboratory aid has to be sought for establishing diagnosis.

That it is not generally possible to differentiate between X19, X2 and XK cases on the basis of the clinical findings due to the similarity of the characteristic symptoms.

That in 65 per cent of XK cases a sure diagnosis of typhus can be made on the basis of the Weil-Felix reaction. In 25 per cent of cases the agglutinin titre is below the diagnostic level and in 10 per cent negative results are obtained even on repeated examinations.

That the doubtful and sero-negative XK cases can be diagnosed readily by demonstrating rickettsiæ in stained preparations of peritoneal scrapings obtained from white mice inoculated intraperitoneally with washed blood cells of patients and sacrificed about the 10th day after inoculation. That the pathological features in these animals are also quite characteristic—ascites being invariably present.

That for the diagnosis of X19 cases the Weil-Felix reaction is highly satisfactory.

That for the diagnosis of X2 cases the Weil-Felix reaction is not always dependable and animal inoculation is not also of much value. It is possible that the complement-fixation test will be found more helpful, but it has not been tried with local strains.

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THE BREEDING AND MAINTENANCE OF *TROMBICULA DELIENSIS* IN THE LABORATORY FOR EXPERIMENTAL PURPOSES

By K. V. KRISHNAN
R. O. A. SMITH
P. N. BOSE
K. N. NEOGY
B. K. GHOSH ROY
and
M. GHOSH

(From the Bengal Typhus Enquiry at the All-India Institute of Hygiene and Public Health, Calcutta, partly financed by the I. R. F. A.)

THE interest in the life history of the mite *Trombicula deliensis* lies in the fact that it is suspected to be one of the vectors of typhus of the XK serological group in India and Malaya. Before the last great war, fevers of the typhus group were known to exist in certain limited areas in India and the Far East, but the distribution of troops over wide areas in the East during the second world war revealed the widespread nature of the endemic foci of typhus of this type. Bengal which was supposed to be free of such a disease was soon shown to possess many endemic foci, some immediately around Calcutta. Investigations in these foci revealed that rats were infected with *Rickettsia orientalis* the causal agent of XK typhus and that they were also heavily parasitized by larvæ of *T. deliensis* the suspected vector. This gave us an opportunity to find out if *T. deliensis* was the carrier of *R. orientalis* in this area. In that connection we had to breed *T. deliensis* in the laboratory. Before one can obtain certain evidence of the rôle of this mite transmission, it is necessary to breed the mite in captivity so that laboratory-bred larval mites will be available in sufficient numbers for use in transmission experiments. This had not been done till now.

Nagayo *et al.* (1917) were the first to attempt the breeding of Trombiculids. They claimed to have obtained a few adults by feeding nymphs on melon, potato, etc. Our efforts to breed *T. deliensis* using such material as food proved fruitless. We found that the nymphs which were easily obtained were able to find some nutriment from silt. Enriching the silt with bacteria and protozoa did not secure the desired result. At this stage our attention was directed to an article by Wharton and Carver (1946) in which they described the development of a species of Neoschongastia by feeding the nymphs on the eggs of insects. Mosquito and sandfly eggs were used by us to feed the last few nymphs remaining at the close of the 1947 season, and it was soon apparent that this food was suitable for nymphs and adults of *T. deliensis* as well.

T. deliensis is most prevalent around Calcutta, just after the first rains in June. It can be found in adequate numbers on the ears of rats up to November after which the numbers diminish very rapidly. As soon as sufficient numbers of *T. deliensis* larvæ were obtained, the breeding of these mites was begun, *i.e.* about the middle of June 1948, and the following method of breeding was practised with satisfactory results :—

Wild rats collected from rural areas were etherized and those found to be harbouring larval mites in their ears were placed in small wirenetting cages suspended over a dish containing water. Larval mites as they engorged dropped off and fell into the water. On the surface of water the mites could easily be detected with a lens and picked up with a fine brush. The engorged mites were transferred to tubes 3 inches by 1 inch containing about an inch or more of fine moist sterilized sand and closed with well fitting straight sided corks covered with a piece of white cotton cloth. Provided the sand was sufficiently moist, the majority passed into the first resting or nymphochrysalis stage in from a few hours to two days. The nymphochrysalis stage lasted for 7 or 8 days after which the nymphs hatched out. As soon as nymphs were found in a tube, some mosquito eggs were added; the easiest obtainable being culex egg rafts, and these were broken up before delivery into the tube. Nymphs require a fair degree of moisture for satisfactory development. When newly emerged they are cream coloured and very active. Nymphs as well as adults avoid light and always seek the shelter of any irregularity on the surface of the sand. The making of a few trenches or holes in the sand of the tubes is beneficial, as the mites hide in these places and do not endeavour to escape from the tube to such an extent as when there are no such shelters for them to hide in. Despite this, practically from every tube a certain number are lost due to being crushed between the sides of the tube and the cork.

The nymphal stage lasts from 7 to 14 or more days. Though food and moisture are equally available some seem to develop very much slower than others. It is not unusual to see the 3 stages of nymph, imagochrysalis and adult in the same tube which was charged with a batch of engorged larval mites collected on the same day. As the nymphs feed and develop they assume a distinct