KYASANUR FOREST DISEASE — AN INFECTION OF MAN BY A VIRUS OF THE RSS COMPLEX IN INDIA

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Reprinted from the PROCEEDINGS OF THE SIXTH INTERNATIONAL CONGRESSES ON TROPICAL MEDICINE AND MALARIA — Volume V, September 5-13, 1958

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TELFORD H. WORK [1]

Kyasanur Forest Disease was recognized and described as a new arthropod-borne Group B virus disease of man and monkeys in the tropics as a result of investigations initiated in March and April, 1957 (Work and Trapido, 1957). Aside from being an infection causing serious illness with extensive morbidity and substantial mortality in the epidemic area, the disease has virological and epidemiological features of considerable significance. These are: 1) the etiological agent is a new member of the Russian spring-summer (RSS) virus complex; 2) it is the first human disease in the tropics to be attributed to a virus of the RSS complex; 3) viral damage to the central nervous system of man has not been observed whereas hemorrhagic complications occur in the more serious cases; 4) it is the first tick-borne virus disease of man recognized in India; 5) it is the second arbor virus infection of serious consequence to man known to involve wild forest monkeys; and 6) it appears to be a recent introduction of the virus into the locality in Shimoga District of Mysore State (Fig. 1) where the disease has been recognized as new in the human inhabitants and monkeys during the past three spring and summer seasons (1955-1958).

Preliminary reports (Work *et al.*, 1957; Laxmana Rao, 1958) of the clinical course of what was originally thought by local practitioners and public health authorities to be an enteric fever (Seshagiri Rao, 1957; Narasimha Murthy, 1958) have proved to be a comprehensive description of the disease. History of forest exposure,

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Proc. 6th Int. Congr. Trop. Med. and Malar., 5: 180-196, 1959

often where monkey mortality has been observed, three to eight days before onset is almost invariable. The patients usually deny tick bites prior to onset, although they are acquainted with ticks and their attachment to man at other times. The onset is sudden at any time of day and is accompanied by either fever or severe headache, the one soon followed by the other. Marked inflammation of the scleral

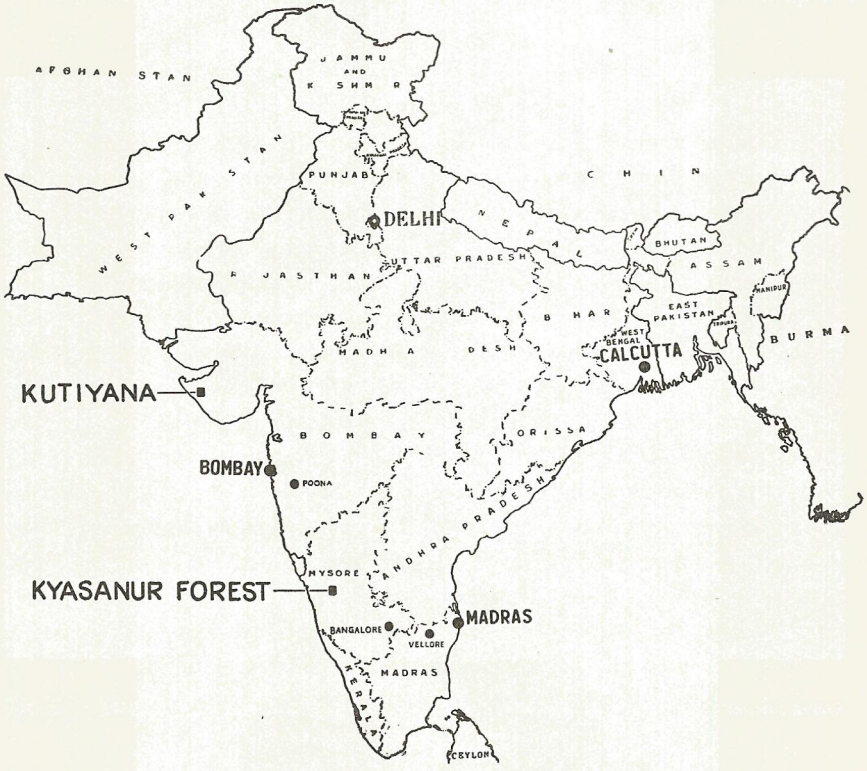

Fig. 1

and palpebral conjunctivae develops in most patients soon after onset and continues for the duration of fever, which lasts from 5 to 14 days (Fig. 2). Severe pain extends over the back, particularly in the lumbar region, and into the extremities (Fig. 3). The neck often becomes stiff and painful and the patient suffers severe prostration followed by lassitude, drowsiness, mental confusion, and, rarely, coma a few days after onset. Coma is an ominous sign.

About the third day of illness vomiting or diarrhoea or both may develop and continue for one or two days to more than a week. It is at this time that hemorrhagic signs, such as bleeding gums, epistaxis, hemoptysis, hematemesis, tarry stools, and frank blood in the stools,

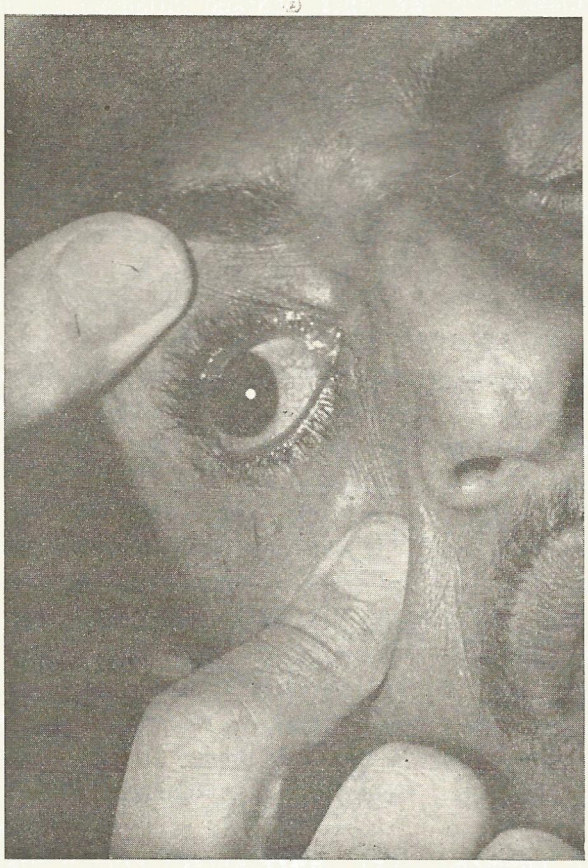

Fig. 2 — Inflammation of the conjunctivae is marked during the febrile phase of Kyasanur Foreste Disease

appear in more serious cases. The hemorrhagic tendency may be prolonged for several weeks. Rales and crepitations, often the result of oozing of blood into the alveoli, may be heard in the lungs at this stage. These are a precursor to pneumonia and demand immediate

treatment with antibiotics and intravenous fluids. A papulo-vesicular eruption on the soft palate appears in many patients by the third day and is an important diagnostic sign (Fig. 4).

Abdominal examination usually yields negative physical signs, except for an occasional palpable spleen and tenderness in any or all quadrants when there is severe diarrhoea or evidence of hemorrhage in the gastro-intestinal tract. No skin erythema, petechiae, or eruption

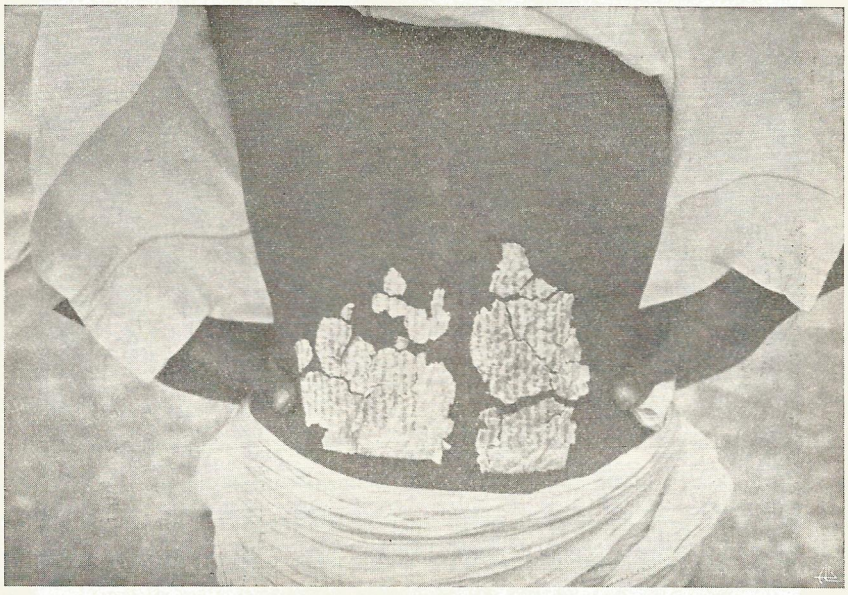

Fig. 3 — Using eggwhite as adhesive, application of newspaper to most painful areas indicates severest pain is in the lumbar region

has been observed, but there are sometimes red macules and papules in the axillae which may be sites of recent tick attachment.

Aside from frequent stiff neck, occasional positive Kernig's sign, and drowsiness, no abnormality attributable to involvement of the nervous system has been noted. Extremity reflexes are active and equal bilaterally, and the cranial nerves are intact. The neck stiffness may be due to crampy pain in the muscles, since no abnormal cellular or chemical alterations have been seen in cerebrospinal fluid.

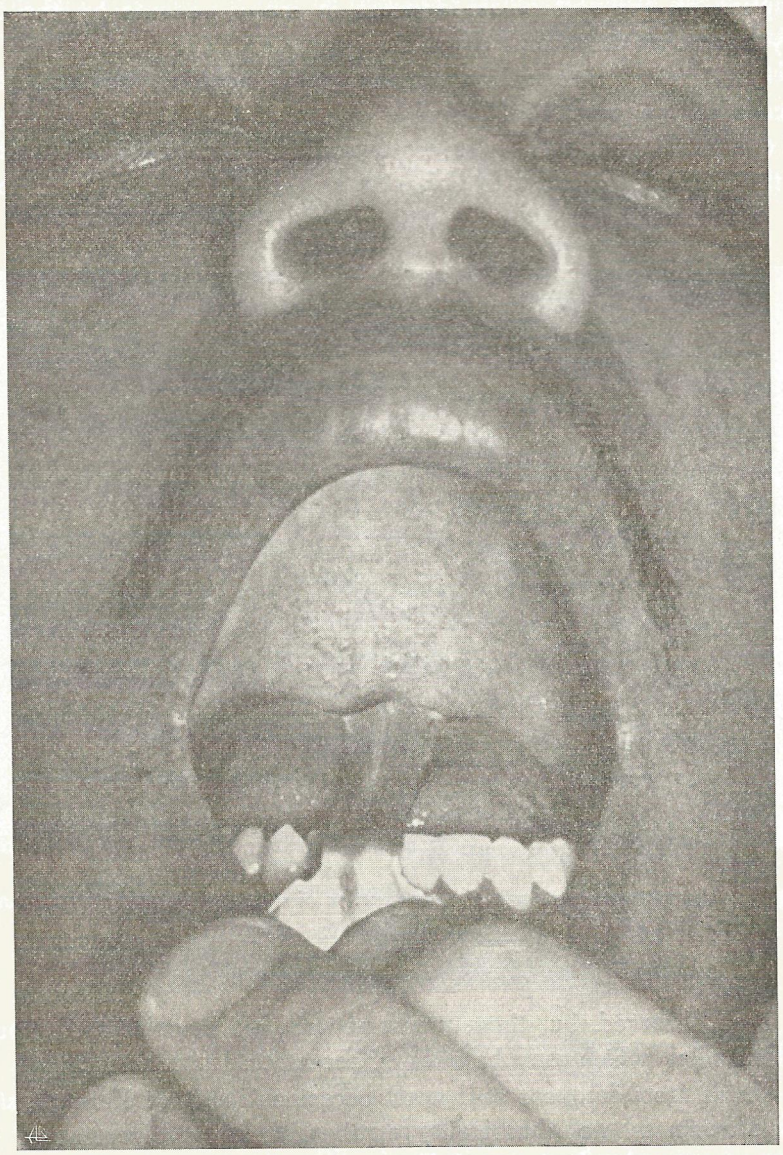

Fig. 4 — The papulo-vesicular eruption on the soft palate seen in some patients is an important diagnostic sign

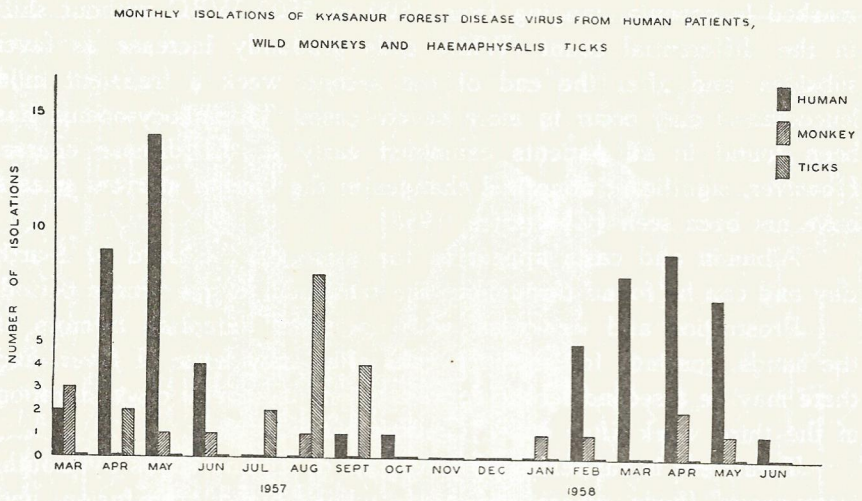

Fig. 5

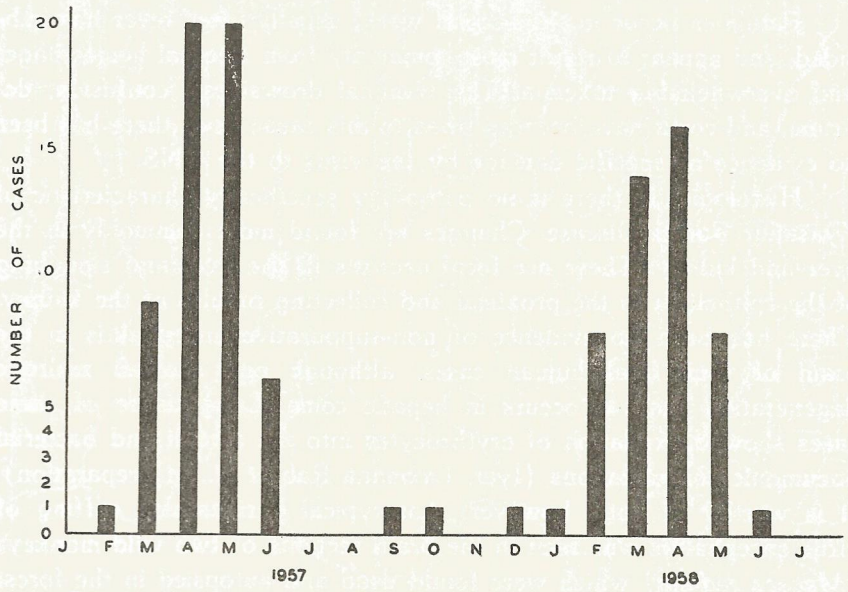

Fig. 6 — Occurrence by month of laboratory proved cases of Kyasanur Forest Disease

Blood examination, on the other hand, invariably reveals a marked leucopenia, ranging from 1500 to 3500 WBC without shift in the differential count. White cells gradually increase as fever subsides, and after the end of the second week a transient mild leucocytosis may occur in more severe cases. Thrombocytopenia has been found in all patients examined early in the disease course. However, significant abnormal changes in the sternal marrow smears have not been seen (Chatterjea, 1958).

Albumin and casts appear in the urine on the third or fourth day and can be found throughout the remainder of the febrile period.

Prostration and weakness, with occasional intention tremors of the hands, continue for several weeks after subsidence of fever, and there may be a second febrile episode of one to several days' duration in the third week after onset. Convalescence is slow.

Treatment is supportive and consists of forcing fluids by mouth, intravenous fluids where indicated, whole blood transfusion and plasma in cases with profuse hemorrhage or shock, high protein diet, and vitamins. The pain suffered early in the course may be somewhat alleviated by analgesics if the patient can tolerate them.

Fatalities occur in the second week, usually after fever has subsided, and appear to result most commonly from internal hemorrhages and overwhelming toxemia. The terminal drowsiness, confusion, delirium, and coma have been ascribed to this cause since there has been no evidence of specific damage by the virus to the CNS.

Histologically there is no pathology specifically characteristic of Kyasanur Forest Disease. Changes are found most commonly in the liver and kidney. These are focal necrosis in the liver and sloughing of the epithelium in the proximal and collecting tubules of the kidney. There has been no evidence of non-suppurative encephalitis in the brain of four fatal human cases, although one showed neurone degeneration such as occurs in hepatic coma. Lung tissue of three cases showed exudation of erythrocytes into the alveoli and bacterial pneumonic consolidations (Iyer, Laxmana Rao *et al.*, in preparation). It is worthy of note, however, that typical perivascular cuffing of virus encephalitis was seen in the brain sections of two wild monkeys *(Macaca radiata)* which were found dead and autopsied in the forest of the epidemic area and which yielded virus (Iyer, Work *et al.*, in preparation).

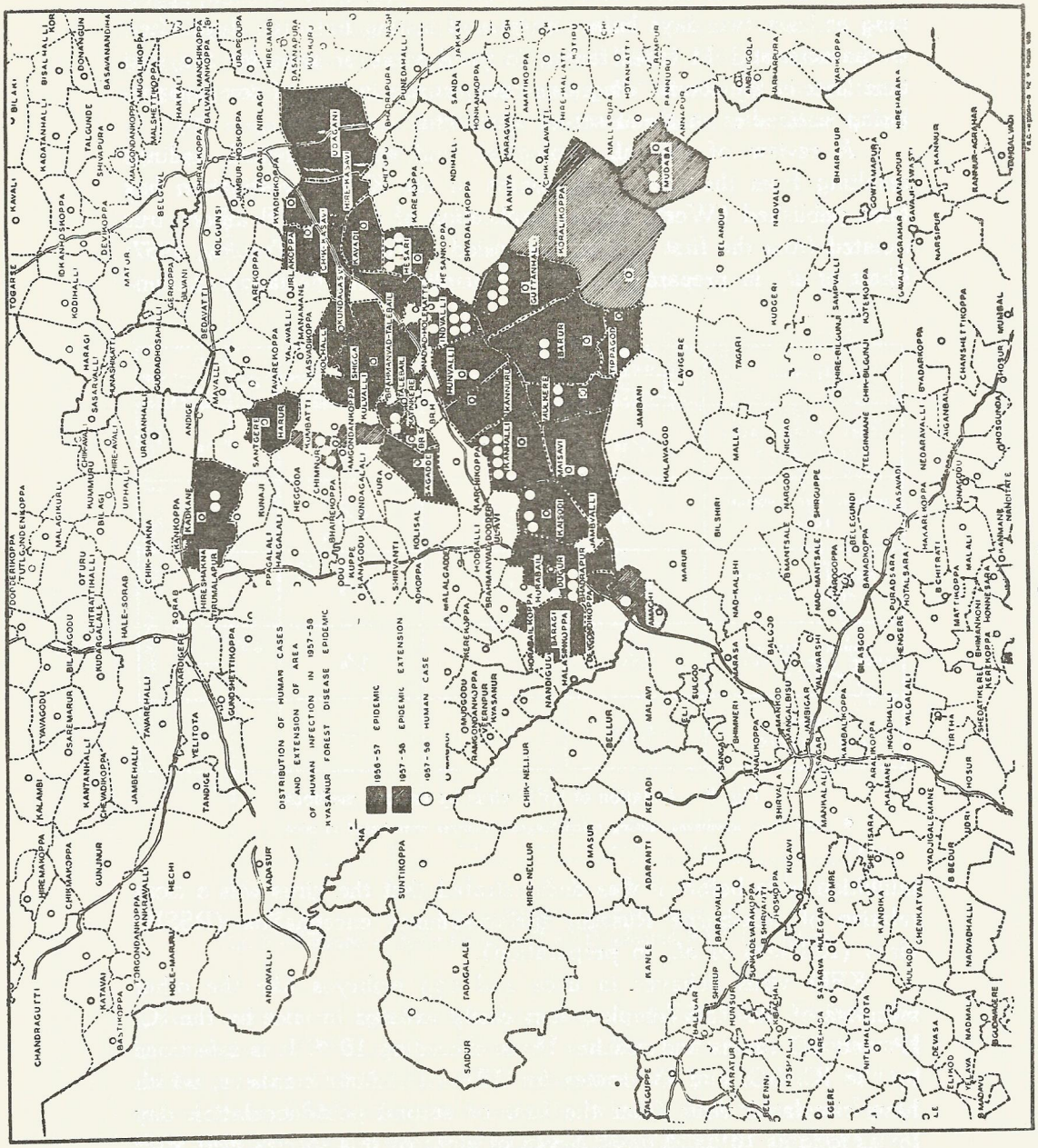
DISTRIBUTION OF HUMAN CASES
AND EXTENSION OF AREA
OF HUMAN INFECTION IN 1957-58
KYASANUR FOREST DISEASE EPIDEMIC
1956-57 EPIDEMIC
1957-58 EPIDEMIC EXTENSION
1957-58 HUMAN CASE

Fig. 7

Diagnosis of suspected cases is most expeditiously accomplished by isolation of the virus from blood, since a prolonged viremia, beginning at least two days before onset and lasting until ten days after, is characteristic. In cases failing to yield virus or seen for the first time later in the course, diagnosis rests on rise in titer of complement-fixing antibodies in serial serum specimens.

A review of clinical, virological, and epidemiological findings resulting from the first nine months of the KFD investigation has been published (Work, 1958). Implication of a Group B arbor virus isolated from the first monkey autopsied (Work and Trapido, 1957; Bhatt *et al.*, in preparation) was accomplished by hemagglutination-

SPECIMEN	INFANT MICE +VE / NO. TESTED	CETC +VE / NO. TESTED	MKTC +VE / NO. TESTED	HKTC +VE / NO. TESTED
ACUTE HUMAN SERA FROM FIELD	1/36	—	0/36	0/19
KNOWN +VE HUMAN SERA	4/4	3/4	0/4	1/4
MONKEY TISSUES FROM FIELD	5/5	3/5 ●	2/5 ●	—
MONKEY TISSUES FROM LAB. INFECTED MONKEYS	13/13	—	4/8	—
TICKS LAB INFECTED	2/5	2/5	—	—

Fig. 8 — Isolation of KFD virus by various methods

● Only those specimens causing cytopathogenic changes were tested in mice

inhibition tests before it was demonstrated that the virus was a close relative of tick-borne Russian spring-summer encephalitis (RSSE) virus (Kulkarni *et al.*, in preparation).

KFD virus behaves in mice and egg embryos like the other members of the RSS complex. It is easily isolated in mice by the IC, IP, and SC routes and reaches titers exceeding 10^{-8}. It is infectious by the IC, SC, and IV routes for *Macaca radiata* monkeys, which have circulated virus from the first or second post-inoculation day for as long as 10 to 14 days. Seven of eight of this species died after

the experimental infection by IV inoculation (Work *et al.*, in preparation).

Mice and monkeys vaccinated SC with formalin vaccines prepared by Buescher *et al.* with RSSE and P9605 KFD viruses show a distinct difference in protection against IV challenge inoculations of KFD virus (Buescher, 1958). In adult mice there is at least a log

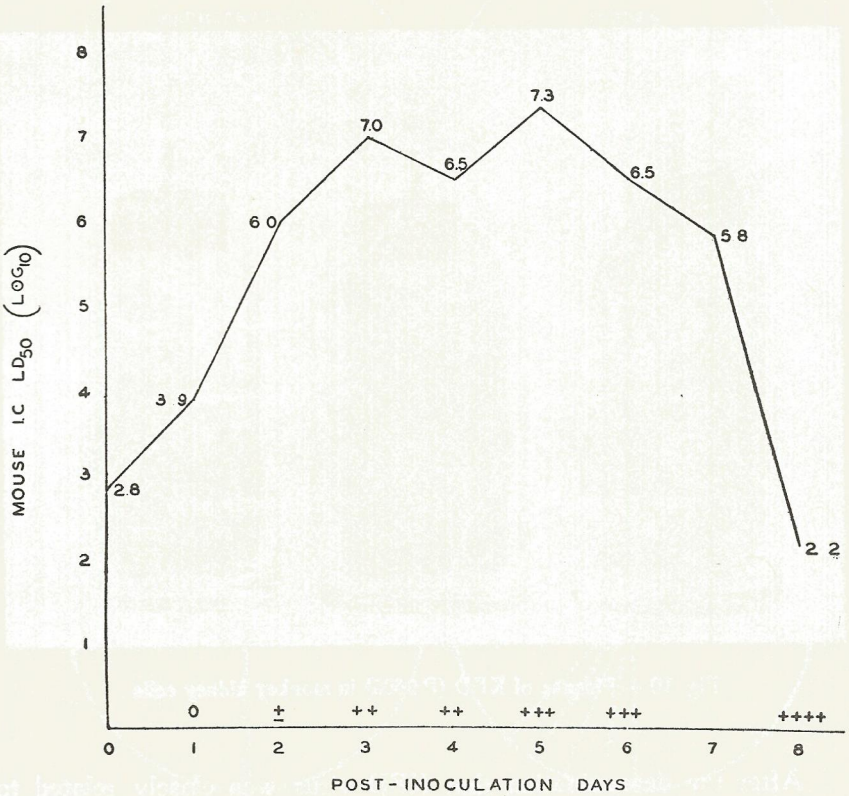

Fig. 9 — Results of daily titration of KFD infected MKTC medium

greater protection against the homologous KFD than against the heterologous RSSE strain. With *M. radiata* monkeys, intravenous challenge with 32 000 LD_{50} KFD virus resulted in the death of two out of three RSSE-vaccinated animals, which circulated virus for as long as seven days, while none of the KFD-vaccinated monkeys died

and only one circulated virus, at barely detectable level for only four days (Work *et al.*, in preparation).

This indication of a distinctive antigenic difference of KFD from RSSE virus has been borne out by similar results in mice and guinea-pigs obtained by Buescher and Shah in Washington. KFD virus has been cultivated and adapted in cultures of hamster and monkey kidney cells. Details of this will be discussed by Dr. Bhatt (Discussion).

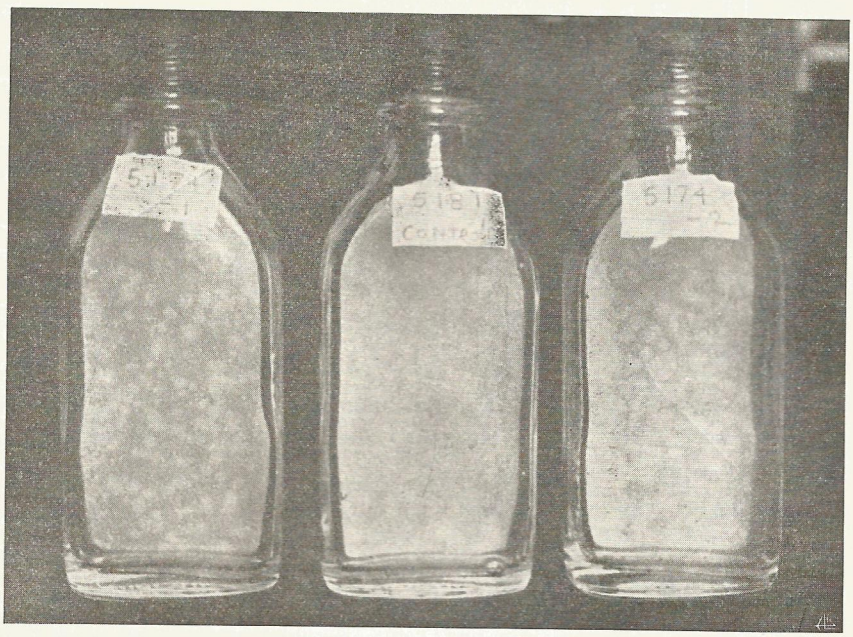

Fig. 10 — Plaques of KFD (P 9605) in monkey kidney cells

After the determination that KFD virus was closely related to RSSE and the finding of ticks attached to three of the first four healthy monkeys collected in the epidemic area in April, 1957, our investigations shifted toward a tick-borne virus etiology (Trapido and Work, to be published). KFD virus was isolated from three pools of mixed species of *Haemaphysalis* larvae collected by cotton flannel drags of the infected forest floor in April, 1957. Virus was also isolated ten times from *Haemaphysalis spinigera*, once from *H. tur-*

turis, and once from *H. papuana* during subsequent months (Trapido *et al.*, 1959).

Of the seven species of small mammals trapped in the infected area — *Rattus rattus wroughtoni, R. r. rufescens, R. blanfordi, Funambulus tristriatus, Tatera indica, Mus booduga,* and *Suncus murinus* — almost 6 percent of *R. r. wroughtoni* and 35 percent of the three-striped palm squirrel, *F. tristriatus,* had specific neutralizing antibodies in their sera. As animals of the same species collected outside the epidemic area failed to give any positive sera, it appears that the

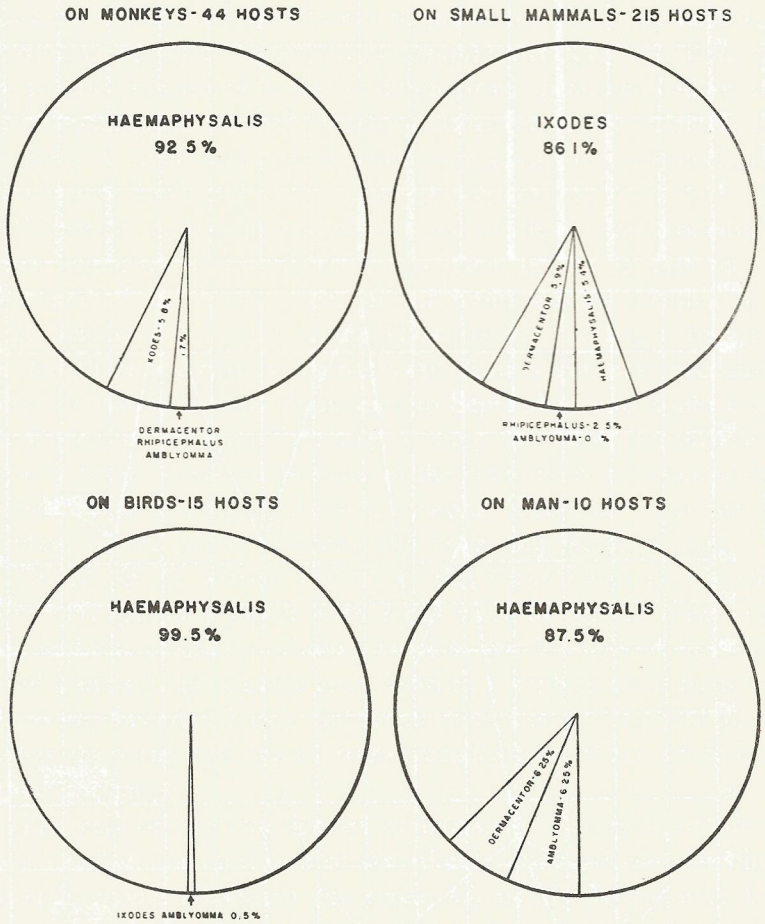

Fig. 11 — Ticks of all stages on indicated hosts

rodents sustained the infection in the wild. Sick or dead rodents have not been recovered in the area, but no burrows have yet been exposed to determine whether they contain dead animals or adult ticks.

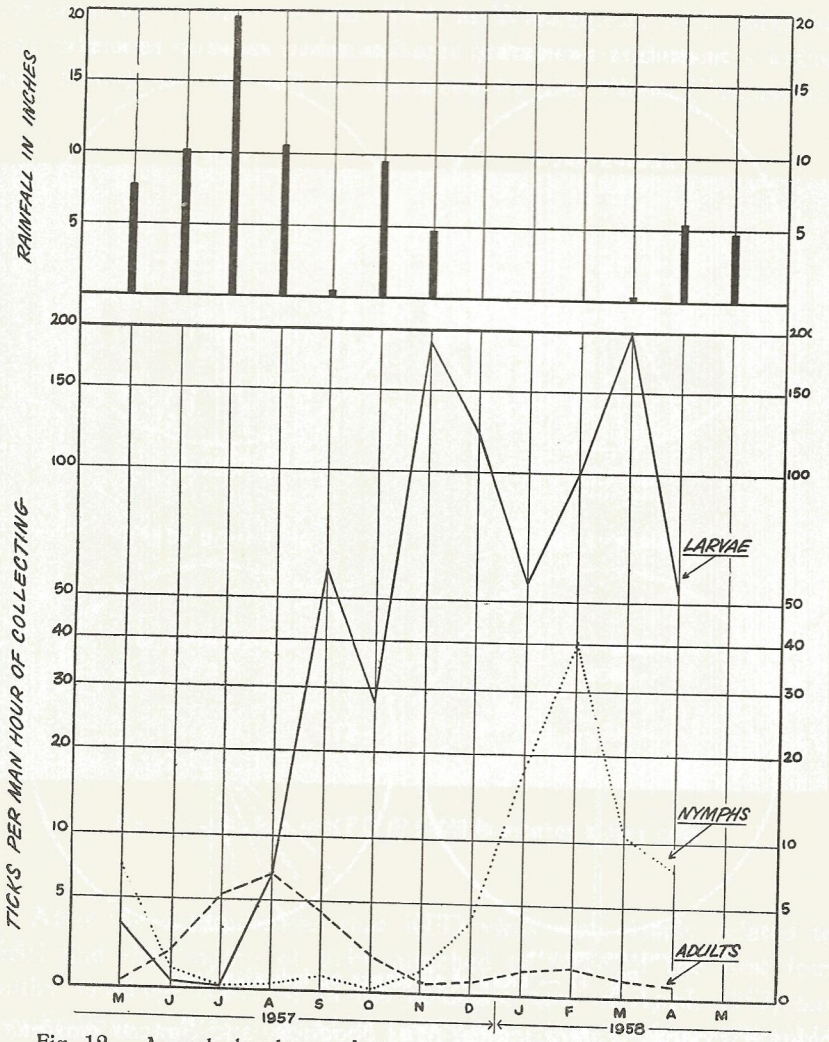

Fig. 12 — Annual abundance of *Haemaphysalis* ticks in Kyasanur forest (dragging and hand picking on vegetation)

Species of *Ixodes* ticks were found most commonly on these small animals, although the highest yield of *Haemaphysalis* species was

taken from the tree-climbing squirrel, *F. tristriatus*. This evidence involves small forest mammals as potential disseminators of the virus and certainly as hosts of the tick species from which virus has been isolated. Sera of cattle native to the infected area also contain neutralizing antibodies to KFD virus while sera collected from cattle grazed in adjacent uninfected areas were negative for KFD antibodies.

All the implicated species of *Haemaphysalis* ticks have been taken from various species of birds — jungle fowl, peacock, Indian robin, and white-throated ground thrush — collected in the infected forest area. Specific neutralizing antibodies have been demonstrated in sera of the jungle fowl and golden-backed woodpecker.

Persistent and intensive collections of ticks from rodents and primate hosts, as well as forest drags, have produced data on cyclic appearance of different stages of different species. Details of this will be discussed by Dr. Trapido (Discussion). Suffice it to mention here that there are two cycles per year. Adults are most predominant during the monsoon rains, from June to September, and again increase from December through February. During the latter period they are relatively fewer, however, because of the continuous presence of larvae followed by increasing numbers of nymphs. These observations differ according to the genus involved and collecting methods used.

Dr. M. G. R. Varma (1958) of the Virus Research Centre has infected *H. spinigera* larvae with KFD virus by letting them feed on white leghorn chicks circulating virus in the blood and has demonstrated trans-stadial passage from larva to nymph to adult by infecting one- to six-day-old chicks. Sufficient time has not elapsed to show whether the virus has passed transovarially from these adult females to their progeny.

The role of the two species of monkeys, *Presbytis entellus* and *Macaca radiata*, beyond that of remarkably good sentinels, is obscure. Although people inhabiting the forest adamantly denied ever having seen or heard of monkeys being found dead in the forest prior to December, 1955, dead monkeys have been found in 1957 and 1958 in an area covering about 600 square miles. The human epidemic area is only about 100 square miles in extent. No monkeys outside this area have yielded virus from organs obtained at autopsy. Yet many show histological changes consistent with those found in both the monkeys of the epidemic area from which virus has been recovered

and the monkeys that died from laboratory induced infections (Work *et al.*, in preparation).

Extensive collection of 133 monkeys in and adjacent to the epizootic area has failed to produce any with specific antibodies from outside the more restricted human epidemic area. Ticks have been taken from many of these monkeys, but the largest yield has been from the animals collected within the epidemic area. It remains to be determined, therefore, whether the wild monkeys, which are numerous, act as a virus reservoir or do no more than provide possible long-distance transport of infected ticks or circulating virus.

When an analysis of KFD isolations from human patients, wild monkeys, and *Haemaphysalis* ticks is charted (Fig. 5) it is apparent that virus was prevalent and constantly present and that the presently recognized affected area is permanently infected. Failure to isolate the virus in November and December, 1957, was probably due to subsidence of collecting activity in the infected area during that period.

With these preliminary data and observations in hand, the opportunity to study in more detail another epidemic year of Kyasanur Forest Disease dating from cessation of the annual monsoon rains at the end of August, 1957, has provided considerable definitive information and raised additional questions (Work *et al.*, 1959).

The number of human cases that occurred in the 1957-1958 epidemic was smaller but nevertheless significant (Fig. 6). One hundred and eighty-one cases were officially reported from January through the end of July, 1958. The mortality was approximately 3 percent. It was possible to confirm the diagnosis by laboratory methods in a larger percentage of reported cases. Actually 51 of 181 were proved in the laboratory, compared to 56 of 466 in the 1956-1957 epidemic.

Human cases continued to occur in the villages affected last year. Children under 15 years of age were affected in larger proportion and numbers this year.

Distribution of the 1957-1958 cases on a map (Fig. 7) shows that the infection spread slightly to the east and west, with no evidence of extension north or south. No human cases were reported from the monkey epizootic area, outside the contiguous epidemic area.

In conclusion, it is interesting to note that KFD is the second arthropod-borne virus infection causing serious disease in man to be elucidated in India since 1952, the first being Japanese B encephalitis which was identified in southeastern India in 1955 (Work and Shah, 1956). The existence of both of these viruses in India was suspected from the results of Smithburn's neutralization tests on survey sera collected by Kerr and Gatne in 1952 (Smithburn *et al.*, 1954; Kerr and Gatne, 1954). Neutralizing antibodies to RSSE virus were found in sera collected in Kutiyana, an inland town on an arid western plain of Saurashtra, the peninsular land mass which extends into the Arabian Sea north of Bombay (Fig. 1). This finding has been confirmed by collections made in Saurashtra in January, 1958, but further epidemiological investigations in the area have not yet been initiated.

The Russian spring-summer virus disease problem, which in the past two decades has increased in complexity and grown in extent from the Pacific to the Atlantic, now extends to the Indian ocean to become the immediate arthropod-borne virus disease problem of greatest geographical range.

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DR. P. N. BHATT [1] (India) — It will be appropriate to present our experience with Kyasanur Forest Disease virus in tissue culture of monkey kidney (MKTC), hamster kidney (HKTC), and chick embryo (CETC) cells.

Initial efforts were directed toward isolating virus from organs of the first dead monkey from Shimoga District of Mysore State. It was possible to isolate KFD virus from serum, spleen, and liver of this monkey in chick embryo and/or monkey kidney cells. Subsequently, attempts were made to isolate virus from various sources in tissue culture. The results are presented in Fig. 8. As the figure makes clear, KFD virus can be isolated in tissue culture systems. Experiments are being done to compare the sensitivity of infant mouse and chick embryo cells for isolation of this virus from various specimens (Fig. 8).

Cellular changes produced in MKTC during early passages consisted of rounding of the cells without any particular pattern and without all the cells being affected. After further passages clear cytopathogenic changes were observed two to five days after inoculation. There were also areas of retraction in the monolayer cells. By about 10 to 12 days after inoculation there was complete destruction of cells.

A more or less similar pattern of adaptation was seen with CETC. At times, non-specific changes complicated the picture. The incubation period varied from three to seven days. The virus behaved similarly in HKTC except that the incubation period was 36 to 72 hours. Two strains of KFD virus — one from a human being and one from a monkey — are being passed in tissue culture, the former in MKTC and HKTC, the latter in CETC, MKTC, and HKTC. No significant change in adult mouse pathogenicity has been noticed up to 60th passages.

Studies are being conducted to determine the growth curve of KFD virus in tissue culture. This information will ultimately help in preparation of TC antigens. Fig. 9 illustrates the results of one such experiment in *Macaca radiata* kidney cells. It has been observed that the MKTC cell sheet is completely destroyed by KFD virus in 10 to 12 days when Earle's medium with lactalbumin hydrolysate and calf

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serum is used. This destruction was not observed when Hanks' medium was employed with lactalbumin hydrolysate and calf serum.

Attempts to neutralize the virus in tissue cultures with specific immune sera have been encouraging, and further tests are planned.

We have obtained plaques in MKTC with P9605 strain (Fig. 10) and attempts are under way to obtain CF or HA antigen from TC, using P9605 or W372 strain of virus.

DR. H. TRAPIDO[1] (India) — In comment on the paper by Dr. T. H. Work on Kyasanur Forest Disease, I might present briefly a summary of the salient entomological data that have been gathered. In Table 1 is given a list of the ticks found in the KFD epizootic area. Present are ticks of the genera *Ixodes, Haemaphysalis, Rhipicephalus, Boophilus, Dermacentor, Amblyomma,* and *Aponomma. Haemaphysalis* is the genus with the greatest number of species represented, 10 having been identified thus far. During 1957 KFD virus was isolated from ticks 16 times, in each instance from *Haemaphysalis.* The first two

TABLE 1 — *List of tick species found in the KFD epizootic area*

Ixodes:	*Rhipicephalus:*
ceylonensis (?)	*sanguineus*
kerri	*haemaphysaloides*
Haemaphysalis:	*Boophilus:*
formosensis	*microplus*
papuana	*Dermacentor:*
turturis	*auratus*
bispinosa	*Amblyomma:*
cuspidata	*integrum*
aculeata	*testudinarium*
spinigera	*Aponomma:*
wellingtoni	*laeve*
minuta	
cornigera	
sp. indeter.	

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isolations were from mixed pools of larval and nymphal *Haemaphysalis* which could not be specifically identified. The remaining 14 isolations were from adults, both males and females — 12 from *H. spinigera* and one each from *H. papuana* and *H. turturis*. No isolations were made from *Ixodes* ticks, although large numbers of these were processed.

Table 2 lists the ticks taken on monkeys. It can be seen from this table that by far the commonest ticks ectoparasitic on monkeys are larvae and nymphs of *Haemaphysalis*. Moreover, the two species of *Haemaphysalis* that were taken on monkeys as adults and could be specifically identified *(spinigera* and *turturis)* are ones from which virus has been isolated.

While authentic records of ticks attached to man are not easily obtained in the KFD area, the bulk of the records collected relate to

TABLE 2 — *List of ticks taken on monkeys*

Tick	Stage	Percentage of monkeys infested	Average per infested animal
Ixodes sp.	Larvae	18	7.1
Ixodes kerri	Adults	2	1.0
Ixodes ceylonensis (?)	Adults	2	1.0
Haemaphysalis spp.	Larvae	59	33.0
Haemaphysalis spp.	Nymphs	61	2.7
Haemaphysalis turturis	Adults	9	2.0
Haemaphysalis spinigera	Adults	2	1.0
Rhipicephalus sp.	Nymphs	2	1.0
Dermacentor sp.	Larvae	7	2.0
Dermacentor sp.	Nymphs	5	1.5
Amblyomma sp.	Larvae	9	1.5
Amblyomma sp.	Nymphs	2	1.0

Haemaphysalis (see Table 3). Now that nymphs of this genus can be identified to species, all those found on man are known to be *spinigera*, the species from which virus has most often been isolated. It is of interest that thus far no *Ixodes* have been taken attached to man. These host-tick relationships are graphically summarized in Fig. 11. We see from this that *Haemaphysalis* ticks predominate on

monkeys, birds, and man, while *Ixodes* are most common on small mammals.

Fig. 12 illustrates the population fluctuations observed in the genus *Haemaphysalis* during the first year of study in relation to rainfall. As several different species are involved, these data should be considered with reserve. It is apparent, however, that the immature stages are abundant during the dry time of year and the adults most common during the monsoon.

TABLE 3 — *List of ticks taken attached to man*

Tick	Stage	Number taken
Haemaphysalis sp.	Larvae	21
Haemaphysalis spinigera	Nymphs	7
Dermacentor sp.	Larvae	1
Dermacentor sp.	Nymphs	1
Amblyomma sp.	Nymphs	2
Total		32

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