MAP AND FIGURES
Fig. 1

PERCENTAGE DISTRIBUTION OF VARIOUS STAGES OF *HAEMAPHYSALIS* (ALL SPECIES) ON BIRDS BY MONTHS.

SAMPLE SIZE (Total No. Ticks of all Stages)

- Jan: 788
- Feb: 992
- Mar: 376
- Apr: 256
- May: 133
- Jun: 55
- Jul: 183
- Aug: 140
- Sep: 100
- Oct: 3032
- Nov: 3049
- Dec: 670

PERCENT

- Larvae
- Nymphs
- Adults
Fig. 2

PERCENTAGE DISTRIBUTION OF VARIOUS STAGES OF HAEM. SPINIGERA ON BIRDS BY MONTHS

SAMPLE SIZE (Total No. Ticks of all Stages)


0 10 20 30 40 50 60 70 80 90 100

= LARVAE

= NYMPHS

= ADULTS
Fig. 3

PERCENTAGE DISTRIBUTION OF VARIOUS STAGES OF HAEM. WELLINGTONI ON BIRDS BY MONTHS

SAMPLE SIZE (Total No. Ticks of all Stages)

PERCENT


= LARVAE

= NYMPHS

= ADULTS
Fig. 4

PERCENTAGE DISTRIBUTION OF VARIOUS STAGES OF *HAEM. TURTURIS* ON BIRDS BY MONTHS

![Graph showing percentage distribution of various stages of *Haem. Turturis* on birds by months.](image-url)
Fig. 5

GRAPHIC REPRESENTATION OF SEPARATE AND MIXED INFESTATIONS OF VARIOUS STAGES OF *HAEMAPHYSALIS* (ALL SPECIES) ON BIRDS THROUGHOUT THE YEAR

SIZE OF HOST SAMPLE (NO. OF BIRDS EXAMINED)

PERCENT OF ALL BIRDS EXAMINED POSITIVE FOR INDICATED CATEGORY


- - = LARVAE ONLY
- - = NYMPHS & ADULTS
- = LARVAE & NYMPHS
- - = LARVAE, NYMPHS & ADULTS
- = NYMPHS ONLY
- - = ADULTS ONLY
- - = LARVAE & ADULTS
Fig. 6

GRAPHIC REPRESENTATION OF SEPARATE AND MIXED INFESTATIONS
OF VARIOUS STAGES OF HAEM. SPINIGERA ON BIRDS
THROUGHOUT THE YEAR

SIZE OF HOST SAMPLE
(NO. OF BIRDS EXAMINED)

PERCENT OF ALL BIRDS EXAMINED
POSITIVE FOR INDICATED CATEGORY


= LARVAE ONLY

= LARVAE & NYMPHS

= NYMPHS ONLY

= LARVAE, NYMPHS & ADULTS

= ADULTS ONLY
Fig. 7

GRAPHIC REPRESENTATION OF SEPARATE AND MIXED INFESTATIONS OF VARIOUS STAGES OF HAEM. WELLINGTONI ON BIRDS THROUGHOUT THE YEAR

SIZE OF HOST SAMPLE (NO. OF BIRDS EXAMINED)

PERCENT OF ALL BIRDS EXAMINED POSITIVE FOR INDICATED CATEGORY


= LARVAE ONLY
= NYMPHS & ADULTS
= LARVAE & NYMPHS
= LARVAE, NYMPHS & ADULTS
= NYMPHS ONLY
= ADULTS ONLY
= LARVAE & ADULTS
Fig. 8

GRAPHIC REPRESENTATION OF SEPARATE AND MIXED INFESTATIONS
OF VARIOUS STAGES OF HAEM. TURTURIS ON BIRDS
THROUGHOUT THE YEAR

SIZE OF HOST SAMPLE (NO. OF BIRDS EXAMINED)

PERCENT OF ALL BIRDS EXAMINED POSITIVE FOR INDICATED CATEGORY


- LARVAE ONLY
- NYMPHS & ADULTS
- LARVAE & NYMPHS
- LARVAE, NYMPHS & ADULTS
- NYMPHS ONLY
- ADULTS ONLY
LARVA AND PUPA OF *Aedes (Stegomyia) w-albus* Theobald, 1905 (Diptera-Culicidæ).

BY

P. K. Rajagopalan, M.Sc.

(From the Virus Research Centre, Poona.*

[Received for Publication, November 4, 1955.]

Barraud (1934) in his monograph on the culicine mosquitoes of India stated that the larvæ of *Aedes w-albus* Theobald, were unknown. So far as known to the author descriptions of larvæ and pupæ of *Aedes w-albus* have not yet been published. This paper is meant to provide such descriptions based on the examination of larval and pupal skins which were determined definitely to be those of *Aedes w-albus*.

**Material and methods.**

Several adults of both sexes of *Aedes w-albus* were collected in the gardens in and around Poona, brought alive to the laboratory, identified and released for egg laying in a small mosquito cage (12" × 12" × 15"). Two hollow bamboo cylinders 5" high with an inner diameter of 2" filled with water and a Petri-dish with a moist filter paper were provided for the mosquitoes to lay eggs. The females were allowed to feed on human blood by introducing a hand into the cage and the males were fed with raisins. Seven eggs were laid initially on the wet filter paper but later on some more eggs were laid inside the bamboo pieces containing water. The eggs were transferred to white enameled bowls and the resultant larvæ were fed on yeast powder. The larvæ hatched into pupæ and the pupæ into adults. The discarded larval and pupal skins were mounted and the resulting adults pinned. The larvæ of one batch were individually isolated in numbered specimen vials containing water and the larval skin, pupal skin and the resulting adult of each larva were separately mounted and marked with the same number so that we have for study all the three stages of the same individual.

*The Virus Research Centre, Poona, has been established by the Indian Council of Medical Research with the assistance from and the co-operation of the Division of Medicine and Public Health of the Rockefeller Foundation and the Government of Bombay.*

(481)
Larva and Pupa of Aedes (Stegomyia) W-albus Theobald, 1905.

Larva.

The nomenclature used in the description of the larva is that of Belkin (1950).

Head: (Fig. 1).—Length: 0.73 mm. Width: 0.83 mm.

Fig. 1.—Ventral and dorsal sides of the head capsule. Fig. 4.—Thorax and abdominal segments I to VII. Fig. 6.—8th abdominal segment, siphon and the anal segment.
P. K. Rajagopalan.

Seta O short, stout and spinose, situated lateral to seta 1; seta 1 slightly curved, stout and about four times as long as seta O; seta 2 and 3 absent; seta 4 well developed, small with 8 to 9 branches; seta 5 single, slightly longer than seta 1; seta 6 well developed, bifid from base and about half as long as the antenna; seta 7 small, tridif from base; seta 8 single, long and situated at about two thirds the distance from the anterior end of the frontoclypeal. seta 9 single, long, situated lateral to the frontoclypeal suture, slightly behind the level of seta 8; seta 10 long, bifid from base; seta 11 long, bifid or tridif from base; seta 12 shorter than seta 11, tridif; seta 13 single, long; seta 14 long, bifid from base; seta 15 small, bifid. Mentum (Fig. 2) with eight to ten well developed teeth on either side of the central tooth.

Antenna: (Fig. 3).—Length 0.21 mm. Breadth: 0.03 mm.

Shaft about seven times as long as broad, smooth, not spiculated, of uniform light brown colour, slender and about one third the length of the frontoclypeus. Seta 1 single, delicate and arising a little more than half way from the base; seta 2 long, tapering and about 0.7 the length of the antenna; seta 3 stout, curved slightly, shorter than seta 2; seta 4 stout, tapering and of medium length; seta 5 short, broad and blade like with a hyaline end; seta 6 short, stout and pointed.

Thorax: (Fig. 4).—Length: 1.15 mm. Breadth: 1.53 mm.

Prothorax.—Seta O minute, trifid from base; seta 1, 2 and 3 arise very closely; seta 1 long, bifid or tridif from base; seta 2 single, as long as seta 1; seta 3 single or bifid from base, as long as seta 1; seta 4 bifid or tridif from base, about 0.3 the length of seta 1; seta 5 single, long; seta 6 single, long, minutely barbed; seta 7 bifid or tridif from base, long, minutely barbed; seta 8 small, tridif from base; seta 9 to 12 arise from a common tubercle; seta 9 long, well developed with two branches; seta 10 single, about two-thirds the length of seta 9; seta 11 single, delicate, small; seta 12 single, delicate and about as long as seta 10; seta 14 small, with 3 to 4 branches.

Mesothorax.—Seta 1 small, tridif from base; seta 2 single, small; seta 3 single or bifid, small; seta 4 single or bifid, small; seta 5 single, long, well developed and minutely barbed; seta 6 well developed, long with about 3 to 4 branches, which are minutely barbed; seta 7 single, delicate and about half as long as seta 5; seta 8 long, seck developed with 2 to 3 branches; seta 9 to 12 arise from a common tubercle; seta 9 single or bifid, long; seta 10 single, long; seta 11 reduced, single and delicate; seta 12 single, delicate and about twice as long as seta 11; seta 13 bifid or tridif from base, small; seta 12 small, tridif from base, slightly shorter than seta 13.

Metathorax.—Seta 1 small, tridif from base, and well developed; seta 2 single or bifid, small; seta 3 small with 2 to 3 fine branches; seta 4 single, bifid or tridif from base; seta 5 single, small; seta 6 small, single or bifid from base; seta 7 well developed, long with 3 to 4 stout branches, minutely barbed; seta 8 small, tridif from base; seta 9 to 12 arise from a common strongly minutized metathoracic pleural tubercle (Fig. 5); seta 9 long, well developed, single or bifid from base; seta 10 single or bifid, long and well developed; seta 11 very much reduced, single delicate; seta 12 single, delicate and twice as long as seta 11; seta 13 small, stellate with 3 to 4 branches.
Abdomen: (Fig. 4).—Length of each abdomen segment in relation to its width progressively increased from segment I to segment VII. The measurements of segments I, IV and VII are: Segment I—length 0'46 mm., width 0'46 mm.; Segment IV—length 0'54 mm., width 0'92 mm.; Segment VII—length 0'69 mm., width 0'83 mm.

Segment I.—Seta 1 small, bifid or trifid from base; seta 2 small, bifid or trifid from base; seta 3 single or bifid, small; seta 4 small with 3 to 4 fine branches; seta 5 small with 3 to 4 branches; seta 6 well developed, long, with two to three branches minutely barbed; seta 7 single, long, minutely barbed; seta 8 small, bifid or trifid from base; seta 9 small, stellate with 3 to 4 branches; seta 12 small, single or bifid from base; seta 13 small, single.

Segment II.—Seta 1 small, well developed, trifid from base; seta 2 small, single or bifid; seta 3 small with 3 to 4 fine branches; seta 4 single, small; seta 5 small with 2 to 3 well developed branches; seta 6 long with two to three well developed branches, minutely barbed; seta 7 small with three to four fine branches; seta 8 small, bifid or trifid from base; seta 9 single or bifid, small; seta 10 single, small; seta 11 single, small; seta 12 single, small; seta 13 small, with three to four branches.

Segment III.—Seta 1 well developed, longer than seta 1 in the preceding segments with three fine branches; seta 2 single, small; seta 3 small, single or bifid and seta 4 small, single or bifid; seta 5 well developed, small with three to four branches; seta 6 long, single or with two stout branches, minutely barbed; seta 7 single small; seta 8 small, bifid or trifid from base; seta 9 small with three to four fine branches; seta 10 single, small; seta 11 and 12 single and small; seta 13 stellate with three well developed branches.

Segment IV.—Seta 1 small, trifid from base; seta 2 single, small; seta 3 single, small; seta 4 single, small; seta 5 small, well developed with three branches; seta 6 single, long with barbs; seta 7 single, delicate, small; seta 8 single or bifid, small; seta 9 small, trifid from base; seta 10 single, small; seta 11 and 12 single, small and almost equal in size; seta 13 stellate, well developed, trifid from base.

Segment V.—Seta 1 small, trifid from base; seta 2 single or bifid, small; seta 3 single or bifid, small; seta 4 single, small; seta 5 small, trifid from base; seta 6 single, long; seta 7 single or bifid, small; seta 8 small, bifid or trifid from base; seta 9 small, bifid or trifid from base; seta 10 single, slightly longer than seta 9; seta 11 and 12 single, small and about the same length; seta 13 stellate, small, trifid from base.

Segment VI.—Seta 1 small, trifid from base, well developed; seta 2 single, small; seta 2 single, small; seta 4 single, small; seta 5 small, trifid from base; seta 6 single, long with barbs; seta 7 single, small; seta 8 bifid or trifid from base, small; seta 9 single or bifid from base, small; seta 10 and 11 single, small, about equal in length; seta 12 single, small; seta 13 small, trifid from base.

Segment VII.—Seta 1 trifid from base, well developed and longer than the ones in the preceding segments; seta 2 single, small; seta 3 single or bifid, small; seta 4 single or bifid, small; seta 5 small, trifid from base; seta 6 bifid from base, shorter than seta 6 in the preceding segments; seta 7 small, bifid from base; seta 8 trifid from base, small; seta 9 small, bifid or trifid from base; seta 10 single, small; seta 11 single, small; seta 12 single, small; seta 13 small, trifid from base.
Fig. 2.—Cephalothorax.  Fig. 10.—One of the respiratory trumpets.  Fig. 11.—Metanotum and the abdominal segments of the pupa.
Segment VIII (Fig. 6).—Seta 1 strong, well developed with 3 to 4 branches; seta 2 single or bifid, long; seta 3 stellate, well developed with five to six branches; seta 4 single or bifid, long; seta 5 well developed, long with three to four stout branches.

Comb (Fig. 6) of seven to eight strong teeth, with a fringe of small basal denticles in some of them. The average length of each comb tooth (Fig. 7) is about 0.11 mm. Siphon (Fig. 6): Length of the siphon (0.81 mm.) is less than twice the diameter at base (0.41 mm.) of the siphon. Pecten of 7 to 8 small teeth (Figs. 6 and 8) with 2 to 4 lateral denticles each. Average length of a pecten tooth is about 0.04 mm. Seta 1 (siphonal tuft) is about 1/3 the length of the siphon itself; has 3 to 4 strong, minutely barbed branches; seta 2 single, small; seta 3 single or bifid from base, small; seta 4 single or bifid from base, small; seta 5 single, small; seta 6 single, small; seta 7 single, small; seta 8 small, bifid from base; seta 9 single, small; seta 13 long, well developed, single or bifid.

Anal segment (Fig. 6).—Nearly enclosed in a chitinized ring; seta 1 (lateral saddle hair) bifid, about as long as saddle itself; seta 2, the upper (inner) caudal seta, divided into 2 or 3 long, stout branches; seta 3, the lower (outer) caudal seta, divided into 2 or 3 long stout branches; seta 4 (ventral brush) with 5 to 6 tufts. Papilla long (0.85 mm.) with rounded tips.

Pupa.

The nomenclature used in the description of the chaetotaxy of the pupa is that of Knight and Chamberlain (1948) as revised by Belkin (1952).

Cephalothorax : (Fig. 9).—Respiratory trumpets of moderate length with oblique opening (Fig. 10). Seta 1 single or bifid from base, small; seta 2 single, small; seta 3 bifid from base; seta 4 single or bifid, longer than seta 3; seta 5 bifid from base and as long as seta 4; seta 6 single or bifid from base, slightly shorter than seta 4; seta 7 single or bifid, as long as seta 4; seta 8 bifid from base, about the length of seta 3; seta 9 single and about as long as seta 2.

Metanotum : (Fig. 11).—Seta 10 single, small and stout; seta 11 single, twice as long as seta 10; seta 12 single, about the length of seta 10.

Abdomen : (Fig. 11).

Segment I.—Seta 1 (denticratic tuft) with numerous branches to form a well developed float hair; seta 2 single, small; seta 3 single, delicate and about 4 times the length of seta 2; seta 4 small with four to five fine branches; seta 5 as long as seta 4, trisid from base; seta 6 single, delicate and about the length of seta 4; seta 7 single, small; seta 10 single, about the length of seta 3.

Segment II.—Seta 0 single, minute; seta 1 single, small; seta 2 single, about twice as long as seta 1; seta 3 single, as long as seta 2; seta 4 single, trisid from base; seta 5 single, trisid from base and as long as seta 4; seta 6 single, stout and about as long as seta 10 in segment II; seta 7 single, delicate and slightly shorter than seta 6; seta 10 single, about half as long as seta 6.
Segment III.—Seta O single, minute; seta 1 small, trifid from base; seta 2 single, small; seta 3 single, stout and twice as long as seta 2; seta 4 small, bifid from base; seta 5 small, bifid from base; seta 6 single, well developed and as long as seta 6 in the preceding segment; seta 7 single and small; seta 8 small, bifid from base; seta 10 single, small; seta 12 single, minute; seta 13 single, small.

Segment IV.—Seta O single, minute; seta 1 small, trifid from base; seta 2 single, small; seta 3 single, stout and thrice as long as seta 1; seta 4 single, stout and four times as long as seta 1; seta 5 small, bifid from base; seta 6 single, twice as long as seta 1; seta 7 single, small; seta 8 bifid from base, small; seta 10 single, small and about as long as seta 2; seta 12 single, minute; seta 13 single, small, about as long as seta 10.

Segment V.—Seta O single, minute; seta 1 bifid from base, as long as seta 1 in the preceding segment; seta 2 single, small; seta 3 single, as long as seta 2; seta 4 single, stout and long; seta 5 small, bifid from base; seta 6 bifid from base, as long as seta 4; seta 7 single, as long as seta 1; seta 8 single, as long as seta 2; seta 10 bifid from base, as long as seta 8; seta 12 single, small; seta 13 single, small.

Segment VI.—Seta O single, minute; seta 1 bifid from base, about the same length of seta 1 in segment V; seta 2 single, small; seta 3 single, small; seta 4 single, stout and long; seta 5 single, small; seta 6 bifid from base, about twice as long as seta 1; seta 7 single, about half as long as seta 6; seta 8 small, bifid from base; seta 10 single, small; seta 12 single, minute; seta 13 single, small.

Segment VII.—Seta O single, minute; seta 1 small, bifid from base; seta 2 as long as seta 1; seta 3 single, small; seta 4 single, comparatively reduced; seta 5 single, about the same length as seta 4; seta 6 single, small and very much reduced; seta 7 single, about twice as long as seta 6; seta 8 small, bifid from base; seta 10 single, small; seta 12 single, small; seta 13 single, small.

Segment VIII.—Seta O single, minute; seta 4 single, delicate and as long as seta 7 in segment VII; seta 7 well developed, single, stout with four to five plumose branches.

Paddles.—Oval with rounded ends, with dark midrib, margin of the paddles fringed with numerous small hairs throughout the margin. Seta 7 single, stout and of medium length.

Points of difference in the larval characters of Aedes w-albus and Aedes (Stegomyia) albopictus Skuse.—

The larva of Aedes w-albus very closely resembles that of Aedes albopictus, but the two can be distinguished by the following features:

<table>
<thead>
<tr>
<th>Aedes-w-albus</th>
<th>Aedes albopictus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antennal shaft</td>
<td>...</td>
</tr>
<tr>
<td>Seta 6 on the head capsule</td>
<td>Bifid.</td>
</tr>
<tr>
<td>Comb</td>
<td>...</td>
</tr>
<tr>
<td>Length of the siphon</td>
<td>Less than twice the diameter at base.</td>
</tr>
</tbody>
</table>
Summary.

The chaetotaxy of the larva and pupa of *Aedes variegatus* is described. The major points of difference between the larva of *Aedes variegatus* and *Aedes albopictus* are also mentioned.

The study was carried out under the guidance of Dr. T. Ramachandra Rao, Medical Entomologist, to whom the author offers his thanks. The encouragement and advice given by Dr. J. Austin Kerr, Director, is gratefully acknowledged.

REFERENCES.

BARAUD, P. J. (1934) ... ... 'The Fauna of British India, including Ceylon and Burma. Diptera, V. Family Culicidae'.


ISOLATION OF FIVE STRAINS OF SINDBIS VIRUS IN INDIA.

By


From

THE INDIAN JOURNAL OF MEDICAL RESEARCH

CAMBRIDGE PRINTING WORKS, DELHI.
1960.

ISOLATION OF FIVE STRAINS OF SINDBIS VIRUS IN INDIA.


[Received for publication, September 5, 1959.]

Taylor and Hurlbut (1953) reported the isolation of five similar strains of a virus from culicine mosquitoes collected in the region of Sindbis village, 15 miles north of Cairo, Egypt. The isolations were first made in July and August of 1952 and then in the succeeding summers of 1953 and 1954. The same virus was isolated from the blood of a juvenile crow, Corvus corone sardonius, in 1953 (Work, Hurlbut and Taylor, 1955).

The virus strains were first designated 'Coxsackie-like', because the disease produced in infant mice was characterized by encephalitis and myositis of the skeletal muscles. Subsequent serological and immunological studies showed that the Egyptian virus was a new agent belonging to group A of arthropod-borne viruses (Casals and Brown, 1954) and unrelated to Coxsackie viruses. Taylor et al. (1955) have described the characteristics of this virus and its distribution in the Nile Delta of Egypt. Sindbis virus was isolated in 1954 from culicine mosquitoes collected near Johannesburg, South Africa (Weibren, Kokernot and Smithburn, 1956).

This report deals with five strains of Sindbis virus isolated from birds, mosquitoes and mites collected in widely separated regions of southwestern India. These were encountered during a survey of wild birds, mammals and arthropods for viral agents at the Virus Research Centre (VRC) during 1953.

SOURCE OF VIRUSES.

The first strain (B322/23/24) was obtained from a pool of spleens of three white wagtails, Motacilla alba, collected by shooting on October 13, 1953, at Pashan tank, 7 miles west of Poona, Bombay State. This migratory insectivoros bird breeds in the far north and arrives in Bombay State during the first week of October. A total of 52 birds of this species were tested. The study of migratory birds was confined to wagtails, sandpipers and stints, and a total of 150 of these birds were collected and tested shortly after their arrival in India from their summer breeding grounds. The wagtails showed a 35 per cent infection rate for haemoproteus blood parasites and all three of the birds in the B322/23/24 pool were positive for this parasite.

1. From the Virus Research Centre, Poona (maintained jointly by the Indian Council of Medical Research and The Rockefeller Foundation).
2. Research Officer, Virus Research Centre.
3. Staff Member, The Rockefeller Foundation, Deputy Director, Virus Research Centre, Poona, 1952-1954.
5. Research Assistant (Entomology), Virus Research Centre.
6. Research Assistant (Zoology), Virus Research Centre.
Isolation of Five Strains of Sindbis Virus.

There were two isolations of Sindbis virus from hill mynas, *Gracula religiosa*, from near Cochin, Kerala State. Four young hill mynas were obtained from a bird dealer in Alwaye on December 29, 1953. These were selected from a large collection of hill mynas soon after they were captured in the hill forest east of Alwaye. They were transported by motor car, arriving in Poona on January 3, 1954. Myna B571 was found dead in its cage on the morning of January 6, 1954, and myna B572 died later the same day. Two of the hill mynas remained well. The mynas were protected from insects at night by a fine cloth screen. Sindbis virus was isolated from the spleen tissue of both B571 and B572. The degree of insect exposure of these birds in the forest can be judged by the fact that the blood smear of B571 showed hemoproteus, filaria, and leucocytozoan parasites, and B572 showed hemoproteus, filaria, and trypanosome parasites. The study of resident wild birds was for the most part concerned with nestlings and fledglings of colonial birds collected in Poona district. A total of 61 common myna, *Acridotheres tristis*, 128 common house-crow, *Corvus splendens*, and 23 weaver bird, *Ploceus philippinus*, nestlings or fledglings, were collected in the Poona district during the south-west monsoon period of 1953 and tested for virus. These were negative except for B125 common house-crow. A virus was isolated from the spleen tissue of this crow fledgling. This virus was stored for future study and when passage was attempted a year later the virus was no longer viable. It was characterized by pathogenicity for infant mice and it sometimes produced illness in 3 to 4-week old mice inoculated intracerebrally. The incubation period was 2 to 5 days in infant mice. This virus was not pathogenic for chick embryos. Ranikhet virus was isolated from the parasitic koel cuckoo B12 found in a common house-crow nest on June 12, 1953 (Shah and Johnson, 1959).

The two isolations of Sindbis virus from arthropods were from mosquitoes and mites collected at Devimane ghat field station located on the Sirsi-Kumta road in the tropical evergreen forest of North Kanara district, Mysore State. The mosquito strain of Sindbis virus was obtained from the A1036b pool of culicine mosquitoes (17 C. vishnui, 12 C. khazan, 4 C. mimus, 2 C. bitaeniorhynchus, and 15 C. Mochthogones sp.) collected from outdoor resting places along the Benohli river on December 18, 1953. The mosquitoes were killed and stored immersed in pure glycerol and kept at refrigerator temperature until they were processed on December 29, 1953. The programme of study of mosquitoes of the Poona district has been published (Rao and Rajagopalan, 1957). There were no virus isolations from mosquitoes collected in this district during 1953-1954. The mite strain of Sindbis virus was recovered from the A1036 pool of about 300 *Bdeilomysus burza* mites taken from domestic chickens at the village of Raghisalli on the Sirsi-Kumta road on December 22, 1953. These mites were kept alive at refrigerator temperature until tested on December 28, 1953. Three pools of mites collected from chicken coops at Raghisalli were negative for virus.

*Primary isolation and characteristics of the virus strains.*—The Swiss albino mice used as experimental animals were from the VRC mouse colony established in 1952 from mice imported from the Rockefeller Foundation Virus Laboratories in New York. During the initial period of development of the mouse colony only
young male mice were available for routine use in testing field specimens. Infant mice were used for sub-passage where the immature mice sickened following inoculation with field specimens. The wagtail strain of Sindbis B322/23/24 was established from the brain harvest of one of a group of seven-week old mice, inoculated intracerebrally with this specimen, and killed when it sickened on the fourth post-inoculation day. Second passage in infant mice killed all the mice by the fourth day but did not produce overt disease in older mice. There was no Sindbis virus in storage or in passage at the VRC. The mouse colony was soon large enough so that it was possible to test all field specimens in 2 to 3-day old infant mice and the other four strains of Sindbis virus were isolated in infant mice. All suspensions were made in a diluent of 0.75 per cent bovine albumin in 2.5 c.c. of phosphate saline containing 1,000 units penicillin and 1 mg. streptomycin per c.c. Each mouse received 0.015 c.c. by intracerebral inoculation. The Culex A1033b suspension produced symptoms of slight illness in two infant mice on the 12th post inoculation day. The first passage of B571 myna spleen produced illness in a single infant mouse on the fifth post-inoculation day, and the B572 myna strain was established from two infant mice showing symptoms on the 13th post-inoculation day. The A1036 mite suspension produced illness after a five day incubation period in all of seven infant mice inoculated.

Four of the five strains showed consistent incubation periods and produced a 100 per cent mortality in infant mice in their second and third passage. Minor but significant variations between strains were observed. The incubation period of the wagtail B322/23/24 strain was of about three days as compared to that of about five days for the Culex A1033b strain. The two hill myna strains gave evidence of a 'zone phenomenon', with survival of some of the mice receiving the most concentrated virus suspension, but 100 per cent mortality in those receiving higher dilutions. Recovery of some mice following hind limb paralysis and survival of other mice showing persistent paralysis was characteristic for the hill myna strains. Following a few intracerebral passages, the differences between the strains became less marked. Mite strain A1036 was characterized by erratic mortality and a widely variable incubation period until the tenth intracerebral passage. There was a marked 'zone phenomenon', in the early passages of this strain, similar but more marked than that observed with the hill myna strains.

All strains were pathogenic for infant mice, older mice usually remaining healthy following intracerebral inoculation with the virus. However, the B322/23/24 strain was established from a sick, seven-week old mouse, and virus could be isolated from the brains of apparently healthy adult mice which had been inoculated 4 to 5 days previously with this and other strains of the Sindbis virus.

The pathogenicity of the virus for mice decreased rapidly with increase in age and size. Table I gives the results of the titrations of the A1036 mite strain of Sindbis virus in mice < 1, 1, 2, 3, 4, 7, 14 and 21 days of age. Litters of age 7 days and less were weighed. The < 1 and 1-day old mice behaved similarly, giving comparable virus titres and average survival time (AST). The 2-day old mice, while giving the same titre, showed an increase in AST. In the 3 and 4-day old
Isolation of Five Strains of Sindbis Virus.

**Table I.**

Variation in susceptibility of mice by size and age.

<table>
<thead>
<tr>
<th>Age of litter, days</th>
<th>Range of weight of litters (each litter of 8)</th>
<th>Mean weight of litter</th>
<th>Sindbis virus :</th>
<th>Average survival time (AST) and survival ratio (SR)* 14 day observation period</th>
<th>Virus titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>11-0 – 12-0 G</td>
<td>11-5 G</td>
<td>10^{-1}</td>
<td>10^{-1} 10^{-2} 10^{-3} 10^{-4} 10^{-5} 10^{-6} 10^{-7} 10^{-8} 10^{-9}</td>
<td>10^{4} – 5</td>
</tr>
<tr>
<td>1</td>
<td>12-5 – 13-5 G</td>
<td>12-1 G</td>
<td>2-0</td>
<td>2-4 2-5 4-1 14-0 14-0 14-0 14-0 14-0 14-0</td>
<td>10^{5} – 6</td>
</tr>
<tr>
<td>2</td>
<td>14-0 – 16-0 G</td>
<td>14-7 G</td>
<td>2-5</td>
<td>4-7 5-0 8-0 14-0 14-0 14-0 14-0 14-0 14-0</td>
<td>10^{6} – 7</td>
</tr>
<tr>
<td>3</td>
<td>16-2 – 18-0 G</td>
<td>17-7 G</td>
<td>8-1</td>
<td>7-5 7-5 12-1 14-0 14-0 14-0 14-0 14-0 14-0</td>
<td>10^{7} – 8</td>
</tr>
<tr>
<td>4</td>
<td>20-0 – 23-5 G</td>
<td>22-3 G</td>
<td>12-0</td>
<td>8-1 10-0 12-0 12-0 12-0 12-0 12-0 12-0 12-0</td>
<td>10^{8} – 9</td>
</tr>
<tr>
<td>7</td>
<td>29-0 – 33-5 G</td>
<td>30-0 G</td>
<td>10-7</td>
<td>14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0</td>
<td>10^{9} – 10</td>
</tr>
<tr>
<td>14</td>
<td>Not weighed</td>
<td>2-8</td>
<td>14-0</td>
<td>14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0</td>
<td>&lt;10^{10} – 11</td>
</tr>
<tr>
<td>21</td>
<td>Not weighed</td>
<td>14-0</td>
<td>14-0</td>
<td>14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0 14-0</td>
<td>&lt;10^{10} – 11</td>
</tr>
</tbody>
</table>

* Numerator indicates the number of mice which survived, the denominator the number inoculated.

mice the titre decreased and the AST was even more prolonged. In the 7-day old mice the titre dropped by more than 4-0 log, LD_{50} and none of the 14- and 21-day old mice died following inoculation with the most concentrated virus suspension.

All of the Sindbis strains could be readily propagated in 5 to 7-day old white leghorn chick embryos. Deaths of the embryos were observed at 24 to 72 hours after inoculation into the yolk sac (YS). Infected chick embryo suspensions, when titrated intracerebrally in infant mice, had LD_{50} end-points about one log higher than those obtained by titration of infected infant mouse brains.

The *Culex* A1033b strain, eighth mouse brain passage, was easily adapted to chick embryo tissue culture and produced cytopathic effect in less than 24 hours leading to complete cell destruction in two to three days. The harvested culture fluids contained 6-0 to 7-0 log, CPD_{50} of the virus per c.c. The second tissue culture passage was tested in 48-hour old monolayers from trypsinized cells of 10-day old chick embryos. The preparations were stained and examined four days after inoculation and agar overlay. Circular plaques with clear well-defined margins were seen.

Two of the Sindbis strains, viz., *Culex* A1033b and the B572 myna strain, were tested in 1 to 7-day old chicks by subcutaneous inoculation. Circulating virus was demonstrable consistently on the second day and in a majority of tests of the third and fourth day bleedings. Neutralizing and haemagglutination inhibiting antibodies were present in the 4 to 5-week post inoculation serum specimens.

Rhesus macaque, *Macaca mulatta*, monkeys were inoculated with three of the Sindbis strains, some intracerebrally, others subcutaneously, with 3-4 to 7-3 log, LD_{50}
of the virus. The monkeys did not show febrile response or any other sign of illness. Blood specimens were taken daily for 10 days following inoculation for each of two strains. The wagtail B322/23/24 strain circulated in the blood for the first three days after intracerebral inoculation of 7.1 log. \( \text{LD}_{50} \) of the eight mouse brain passage virus. The blood serum specimens were negative on the monkey receiving a subcutaneous inoculation of 4.3 log. \( \text{LD}_{50} \) of the ninth mouse brain passage of the A1036 mite strain of Sindbis virus. Serum specimens taken 3 to 5 weeks post-inoculation had a high neutralization index.

All the Sindbis strains passed the Seitz EK filter pads without significant loss of titre. Specimens of the virus lyophilized from the frozen state and stored in sealed glass ampoules at refrigerator temperature retained their infectivity.

**Identification of the virus strains.**—The definitive identification of two of the five strains, the A1036 mite strain and the B572 myna strain was done by Dr. Jordi Casals at the Rockefeller Foundation Virus Laboratories in New York. Antigens of the A1036 mite strain and the B572 myna strain were prepared from serum and brain respectively of infected infant mice, and hyperimmune sera were obtained from adult mice after repeated intraperitoneal injections of the virus. The complement fixation and haemagglutination inhibition tests done at the New York laboratory showed that the two Indian strains were completely and reciprocally cross reactive with Sindbis virus isolated in Egypt. Doctor Casals concluded that both of the Indian strains were identical and closely related to or identical with the Sindbis virus from Egypt (Casals, 1965). The other strains were identified by intracerebral cross neutralization tests in infant mice. The results are given in Table II. Strains

**Table II.**

**Cross neutralization tests of the Indian Sindbis virus strains.**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Virus:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wagtail B322/23/24</td>
</tr>
<tr>
<td>B322/23/24</td>
<td>1/1 4-1</td>
</tr>
<tr>
<td>Wagtail 40 Rhesus</td>
<td>1/1 5-5</td>
</tr>
<tr>
<td>A 1033b 6 Rhesus</td>
<td>1/1 3-7</td>
</tr>
<tr>
<td>Mosquito</td>
<td>1/1 3-3</td>
</tr>
<tr>
<td>A 1036 51 Rhesus</td>
<td>1/1 3-4</td>
</tr>
</tbody>
</table>

N.I. = Neutralization index. Inverse of log. \( \text{LD}_{50} \) of virus neutralized.

* With fresh serum 50 per cent in final serum virus mixture.

† With fresh serum 50 per cent in final serum virus mixture.

B571 and B572 were neutralized by all the three heterologous sera, specifically immune to B322/23/24, A1033b and A1036 viruses. These results confirm the similarity of B572 and A1036 and also identify the other three strains as being the same virus. The B571 strain of Sindbis virus was re-isolated by inoculation of the
Isolation of Five Strains of Sindbis Virus.

spleen specimen into chick embryos. This virus was tested in its CE2M4 passage against serum specimens taken prior to and following inoculation with the A1036 strain of Sindbis virus. The post-inoculation serum specimen neutralized > 4.9 log. LD₅₀ of the virus, confirmation that the reisolated strain was identical with the original B571 strain.

Taylor et al. (loc. cit.) have observed a decrease in virus neutralizing capacity of Sindbis immune sera stored over a period of time and restoration of the same by addition of fresh serum. With Sindbis, as with many other arthropod borne viruses, such as Japanese B encephalitis, dengue, Kaysanur forest disease and western equine encephalitis, a labile factor of the fresh serum has been found essential for demonstration of the maximum neutralizing capacity. The fresh serum by itself does not reduce the virus titre and non-immune sera tested with addition of fresh serum do not show evidence of virus neutralization. As described in the subsequent section, virus dilutions for neutralization tests were made routinely in undiluted unheated non-immune monkey serum (NMS). Comparison of duplicate titrations of virus, diluted in fresh serum and in phosphate saline, revealed no significant difference. Table III gives the result of serial tests on an immune serum of a monkey infected in the laboratory. The fresh pre-infection serum of the monkey was titrated against the virus in a neutralization test and was clearly negative. The decrease in neutralizing capacity on storage and its restoration by addition of NMS is clearly seen.

**Table III.**

Decrease in neutralization index of an immune monkey serum collected 20-1-55, after storage at —20°C. and its restoration by addition of fresh unheated normal monkey serum (NMS).

<table>
<thead>
<tr>
<th>Date of test</th>
<th>Inactivation of serum 56°C./1/2 hr.</th>
<th>Dilution of serum.</th>
<th>Neutralization index log. LD₅₀.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-1-55</td>
<td>No</td>
<td>1/1</td>
<td>3.7</td>
</tr>
<tr>
<td>8-7-55</td>
<td>No</td>
<td>1/1</td>
<td>1.2</td>
</tr>
<tr>
<td>8-7-55</td>
<td>Yes</td>
<td>1/1</td>
<td>1.5</td>
</tr>
<tr>
<td>8-7-55</td>
<td>No</td>
<td>1/2 in unheated NMS*</td>
<td>3.6</td>
</tr>
<tr>
<td>8-7-55</td>
<td>Yes</td>
<td>1/2 in unheated NMS*</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*50 per cent fresh serum in final serum virus mixture obtained by diluting virus in 100 per cent NMS.

Serum survey.—Human serum specimens tested in virus neutralization tests (NT) were from the following localities: (a) Small villages situated on the Sirsi-Kumta road, between 16 and 24 miles south-west of Sirsi, in the evergreen forest of North Kanara district, Mysore State, 56 sera; (b) Khadakwasala, a rural area 11 miles south-west of Poona, 43 sera; (c) Poona city, 66 sera; and (d) Jamshedpur, an industrial city in Bihar State, 64 sera. Serum specimens were tested from 57 rhesus monkeys captured in North India, 17 jungle crows, 3 common house-crows and 19 adult mixed breed chickens from Poona. Eight horse serum specimens were tested: 4 from Delhi and 4 from Jammu-Kashmir. In both instances, the
animals had suffered from a paralytic disease said to resemble epidemic equine encephalitis.

The sera were tested undiluted against the 8th to 10th infant mouse brain passage of mite virus A1036. Diluent for the virus was either 0.75 per cent bovine albumin in phosphate saline or undiluted, unheated, non-immune monkey serum (NMS), used within less than 24 hours of its collection. The serum virus mixture was incubated at 37°C in a water bath for two hours and then inoculated intracerebrally into each of a litter of 1 to 2-day old mice. A serum was regarded as positive if it protected 75 per cent or more of the mice and negative if it protected 25 per cent or less. Protection of between 25 per cent and 75 per cent of the mice and/or significantly increased average survival time was considered as satisfactory evidence of partial protection (pp). Positive sera were, as a rule, retested in <1 and 1-day old mice as it was observed that susceptibility of the mice decreased rapidly with increase in age and size and that 3- to 4-day old mice tended to give falsely positive results.

Table IV gives the results of serum virus neutralization tests of human, animal, and bird sera from various regions of India. Of the 164 human sera, 5 neutralized Sindbis virus, an overall rate of about 3 per cent positive. On a regional basis the immunity rate was as follows: 4 in 43 from Khadakwasala (9 per cent); 1 in 55 from Devimane (2 per cent), and none in 66 from Poona. The Khadakwasala positives came from 2 male donors, 14 and 18 years of age, and 2 females, 22 and 60 years of age. The Devimane positive specimen was from a 26-year old female. Of the 76 sera from donors under 15 years of age, 1 was positive and 4 of 88 sera from donors 15 years of age or older were positive. The 64 sera from Jamshedpur were all negative except one which showed partial protection (pp) but these were tested without the addition of fresh serum. Serum specimens were tested from 57 rhesus monkeys, 27 within 3 days of collection, and 30, 2 to 11 months after collection. These were all tested without the addition of fresh serum. All were negative. Of the 8 horses sera collected sometime after a paralytic illness, 1 was positive, 1 partially protective (pp) and 6 negative. One crow serum was positive of 20 collected on the Poona-Khadakwasala road. All 19 of the chicken sera were negative.

**Discussion.**

The discovery of Sindbis virus in India reported in this paper is the first evidence for the presence of a group A arthropod-borne virus in India. The isolation of this virus from a migratory insectivorous bird, the white wagtail, Motacilla alba, is the first reported from a migratory bird. This demonstrates that migratory birds do become infected in nature and could form one of the means for the dispersal of this virus in nature. The recovery of Sindbis virus from hill mynas, Gracula religiosa, collected in the forest of Kerala State and from arthropods collected in the forest of Mysore State shows that the tropical hill forest is either an endemic focus of this virus or a receptive environment for the introduction and establishment of the virus. The isolation of Sindbis virus from mites collected from domestic chickens is the first reported from Bdellonysus bursa mites. This
Isolation of Five Strains of Sindbis Virus.

### TABLE IV.
Results of neutralization tests on human, animal and bird area.

<table>
<thead>
<tr>
<th>Place of collection.</th>
<th>Age.</th>
<th>WITH FRESH SERUM:</th>
<th>WITHOUT ADDITION OF FRESH SERUM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Devimane area (Mysore)</td>
<td>Less than 15</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15 and above</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>2. Poona city and camp (Bombay)</td>
<td>Less than 15</td>
<td>55</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>15 and above</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>3. Khadakwasala (Bombay)</td>
<td>Less than 15</td>
<td>66</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>15 and above</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Grand total</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td>4. Jamshedpur (Bihar)</td>
<td>Under 15</td>
<td>164</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>15 and above</td>
<td>9</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Grand total</td>
<td>55</td>
<td>...</td>
</tr>
</tbody>
</table>

**Animal:**
1. Rhesus monkeys (N. India)

<table>
<thead>
<tr>
<th>Age.</th>
<th>WITH FRESH SERUM:</th>
<th>WITHOUT ADDITION OF FRESH SERUM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult and subadult</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Adult and subadult</td>
<td>30</td>
<td>...</td>
</tr>
</tbody>
</table>

2. Horse Jammu-Kashmir-Delhi

<table>
<thead>
<tr>
<th>Age.</th>
<th>WITH FRESH SERUM:</th>
<th>WITHOUT ADDITION OF FRESH SERUM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

**Bird:**
1. Crows Poona area (Bombay)

<table>
<thead>
<tr>
<th>Age.</th>
<th>WITH FRESH SERUM:</th>
<th>WITHOUT ADDITION OF FRESH SERUM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 juvenile</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>17 adults</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

2. Fowls Poona (Bombay)

<table>
<thead>
<tr>
<th>Age.</th>
<th>WITH FRESH SERUM:</th>
<th>WITHOUT ADDITION OF FRESH SERUM:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>19</td>
<td>0</td>
</tr>
</tbody>
</table>

isolation evidently represents a chance feeding of the mites on a chicken during the viremic phase of the infection. That it was not the tissues of the mites but the blood which contained the virus, is indicated by the failure to isolate the virus from the unfed mites collected in the chicken coops.

In contrast to the experience in Egypt (Taylor et al., loc. cit.) where about 30 per cent of the human sera were positive for Sindbis virus antibodies, human infection appears to be uncommon in India. A moderate immunity rate was observed for the rural village of Khadakwasala, which lies near an artificial lake and adjacent to a range of hills. The low incidence of human immunes in Devimane, where the arthropod strains were isolated, appears paradoxical but the density of culicine mosquitoes necessary for the transfer of the virus from wildlife to man may be too low to produce more than isolated infections in the forest village, while in regions with extensive cultivation of the soil and irrigation practices characterized
by residual pools of water, such as the Nile Delta of Egypt, the mosquito vectors become so abundant that the virus spills over into a variety of aberrant hosts, including man. The rôle of migratory insectivorous bats in the ecology of Sindbis virus remains to be studied. The number of bats in Egypt and India and the migratory nature of some species make this host suspect as a means for the dissemination of the virus.

The present study shows that Sindbis virus is widespread in the avian and arthropod fauna of India. While no clear picture of the ecology of the virus emerges from the data presented, both resident and migratory birds have been found infected and may serve as a means for local and regional dispersal of the virus. It is evident that the virus does infect man but whether or not it causes any illness is not known. Sindbis virus has not been isolated from man so far, nor has it been associated etiologically with any human illness.

**Summary.**

Sindbis virus was isolated from wagtails, *Motacilla alba*, collected in Poona district, Bombay State; from hill mynas, *Gracula religiosa*, collected in Kerala State; from a mixed pool of *Culex* spp., mosquitoes and *Besantonyssus bursa* mites from North Kanara district, Mysore State.

**REFERENCES.**


CASALS, J. (1955) ... ... Personal communication.
KYASANUR FOREST DISEASE.
Part VII.
PATHOLOGICAL FINDINGS IN MONKEYS, PRESBYTIS ENTELLUS AND MACACA RADIATA, FOUND DEAD IN THE FOREST.

By
IYER, C.G.S., WORK, T.H., NARASIMHA MURTHY, D.P., TRAPODO, H., AND RAJAGOPALAN, P.K.

From
THE INDIAN JOURNAL OF MEDICAL RESEARCH

CAMBRIDGE PRINTING WORKS,
DELHI.
1960.
KYASANUR FOREST DISEASE.

Part VII.

PATHOLOGICAL FINDINGS IN MONKEYS, *PRESBYTIS ENTELLUS* AND *MACACA RADIATA*, FOUND DEAD IN THE FOREST.

C.G.S. IYER¹, T.H. WORK², D.P. NARASIMHA MURTHY³, H. TRAPIDO⁴, and P.K. RAJAGOPALAN⁵.

Received for publication, 3rd August, 1959.

During the early months of 1957, the occurrence of a peculiar epizootic fatal to large numbers of wild monkeys was reported from forested areas of Shimoga District, Mysore. Investigations were initiated by the Virus Research Centre of the Indian Council of Medical Research, and it was learnt that this epizootic was concurrent with a more than sporadic occurrence of a febrile illness (sometimes with a fatal outcome) among human beings living in or near the same areas (Work and Trapido, 1957; Work et al., 1957). Viruses of a similar or identical type were isolated from both the monkeys and the diseased human beings. The disease in man was subsequently named Kyasanur Forest Disease, and the etiological agent, Kyasanur Forest Disease (KFD) virus. This virus has been demonstrated to be a member of the Russian Spring Summer complex (Work, 1958).

In the course of the initial investigations made by the Virus Research Centre, material for pathological examination was collected from a number of monkeys found dead in the forest at varying periods of time after death. The pathological changes observed in tissues of these animals and their significance are the subject of this paper.

MATERIALS AND METHODS.

Assorted specimens from 22 monkeys belonging to the two indigenous species affected, *P. entellus* (P.e.), the 'black-faced' langur, and *M. radiata*, (M.r.), the 'red-faced' bonnet macaque, were available for study. Estimates of the probable intervals between death and the performance of autopsy ranged from about 30 minutes to more than 72 hours for individual animals. On every animal as thorough a post-mortem examination as possible was made, and portions of all available viscera and tissues were collected for study. The collections from some

1. Senior Research Officer, Indian Council of Medical Research Neuropathology Unit, Tata Memorial Hospital, Parel, Bombay 12, India.
2. Director, Indian Council of Medical Research, Virus Research Centre, Poona, India, and Staff Member, The Rockefeller Foundation.
3. Medical Officer of Health, Sagar, Mysore State.
4. Deputy Director, Virus Research Centre, and Staff Member, The Rockefeller Foundation.
5. Research Assistant, Virus Research Centre.
Kyasanur Forest Disease.

animals were of necessity incomplete because of the corpses having been exposed in the forest to the depredations of carrion-feeders. All tissues were fixed in 10 per cent formalin before being processed further. Both frozen and paraffin sections, stained as necessary, were employed for histological examination.

Observations.

The 22 animals in the series have been divided into four groups on the basis of the estimated intervals between death and autopsy. Group I consists of animals with estimated periods after death of not more than 6 to 8 hours, and Group IV of animals estimated to have been dead for at least 48 hours, some of them for more than 72 hours, before autopsy. Table I summarizes the number and types of animals in each of the four groups.

Group I.—There were six animals in this group, four M. radiata and two P. entellus. One animal (M.r.) was a presumably normal monkey collected from the Sagar-Sorab Road area. A report in some detail of the histological findings in two representative animals of this group will be followed by a brief general review of the changes in the rest.

Table I.

Number and species of monkeys studied and estimated intervals between death and autopsy.

<table>
<thead>
<tr>
<th>Species</th>
<th>Group I (30 min. - 8 hrs.)</th>
<th>Group II (=12 hrs.)</th>
<th>Group III (=24 hrs.)</th>
<th>Group IV (48 - 72 hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. radiata</td>
<td>4</td>
<td></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>P. entellus</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Monkey I (P.e.—V.R.C. Nos. W 377-390; N.P. 900).—This animal died shortly after being found moribund in a forest near Ulavi. The only remarkable findings upon gross examination of the viscera were clots of blood in the anal sphincter, a moderate swelling and pallor of the renal cortex. The main histological alterations were found in the liver and kidneys.

Liver.—There was general preservation of the lobular architecture. In places there was separation of the liver cords and irregular and discontinuous variability of staining of the liver cell cytoplasm. The nuclei were regular, rounded and either vesicular or deepstaining.

Small vacuoles negative for fat were encountered in the central or midzonal cells. Scattered small, round and regular eosinophilic inclusions in the cytoplasm were also seen (Plate III, fig. 1). A coarsely granular, brown-yellow pigment was found in the cytoplasm of cells in the mid and central zones. This pigment was P.A.S. negative, gave a negative reaction for iron and was rendered slightly prominent by Sudan staining. It was resistant to treatment with acid alcohol or hydrogen peroxide for periods up to 48 hours. Similar pigment was seen in the bodies of hypertrophic Kupffer cells and in focal collections of histiocytes in the parenchyma representing focal necroses (Plate III, fig. 2). There was a marked prominence
**Fig. 1.**
High power view of liver from monkey (NP 900) showing pigment in parenchymal cells and presence of rounded inclusions (indicated by arrows).

**Fig. 2.**
Another area from the liver of the same monkey as Fig. 1 viewed under medium magnification. Note that there are three focal necroses represented by collections of hypertrophic histiocytes in the parenchyme.

**Fig. 3.**
Section of renal cortex (NP 900) seen under medium magnification. Note the fraying of cytoplasmic outlines of the convoluted tubules and the presence of debris in the tubular lumen.
Fig. 4.
Section of renal medulla from monkey (NP 900) showing degenerative alterations in the Henle's tubules and a small recent interstitial hemorrhage.

Fig. 5.
Low power view of section from basal ganglion of monkey (NP A-83) showing histological features of encephalitis.

Fig. 6.
Medium magnification of a segment of Fig. 5 showing the details of nerve cell degeneration and neuronophagia.
FIG. 7.
Kidney of monkey (NP A-61). Note that the outlines of the convoluted tubules are maintained, nuclei appear indistinct in places, and there is desquamation of either cells or debris into the tubular lumina.

FIG. 8.
Section of liver of monkey (A-148) showing pronounced disruption of the liver cords and numerous Councilman bodies (indicated by arrows) lying free in the section.

FIG. 9.
Section of cerebral cortex of monkey (A-148) viewed under medium magnification. Note general prominence of glial nuclei and two nerve cells in the centre of the field undergoing neuronophagia.
of Kupffer cells, some of which appeared huge and some binucleated, with occasional ones in mitosis. The vascular territories were unremarkable and sections stained for reticulum (Gomori's method) disclosed a slight jumbling of the trabecular architecture in the midzones.

**Kidney.**—The capsule was normal. In the cortex the glomeruli appeared fairly well preserved. The convoluted tubules in the cortex appeared indistinct, with fragmentation of the cell outlines, pyknosis of nuclei and desquamation of amorphous debris into the tubular lumen (Plate III, fig. 9). In a number of areas rounded, deep pink, hyaline masses of coagulated material were seen in and between the tubular cells and lying free in the lumina. In the renal medulla the cytoplasm of Henle's tubules was rendered into deep eosinophilic indistinct masses, with pyknotic nuclei detached from the basement membrane and lying free in the lumen (Plate IV, fig. 4). Small seepages of recently exuded blood and rare focal areas of calcification or siderosis or both were the other findings of note. There were no inclusions in the kidney.

Sections of the other organs revealed no particular features. There was depletion of the malpighian follicles and prominence of sinusoids and fittoral cells in the spleen. Brownish-black pigment granules were found in the mucosa of the intestinal villi.

**Brain.**—No remarkable features were found on gross examination except prominence of small blood vessels on the surface. Numerous sections of cerebral cortex, basal ganglia, brainstem and cerebellum revealed patchy outfall of neurons and degenerative alterations in those that remained, without any marked reactive or inflammatory changes. Specifically, there were no histological features of an encephalitis. There were no inclusions in either nerve cells or glia.

**Monkey 2 (M.r.—V.R.C. Nos. W1013-1019; N.P. A-83).**—This monkey was found freshly dead in Kyasanur Forest. The period elapsed between death and autopsy was estimated to have been less than one hour.

Examination of exposed viscera and brain revealed no remarkable features. Histological examination showed rare focal necroses in the liver, with pigmentation in the parenchymal cells similar to that encountered in the previous monkey. Kupffer cells were prominent and there was a moderate mononuclear cell exudate in the portal radicles. Inclusions were not found in the liver.

The kidney appeared much better preserved than in Monkey 1 and presented only mild albuminoïd degeneration of the cortical convoluted tubules. Rare focal necroses, indicated by small interstitial collections of mononuclears and histiocytes, were encountered in the ventricular myocardium. The rest of the viscera revealed no changes from the normal.

**Brain.**—Sections of the various regions of the brain revealed in the cortex and basal ganglia focal areas of neuron degeneration, neuronophagia and formation of clusters of microglia, along with moderate mononuclear cell cufing of blood vessels. These features were consistent with those of a disseminated nonsuppurative encephalitis (Plate IV, figs. 5, 6). There were no inclusions in the brain.
Kyasanur Forest Disease.

Of the four other monkeys in group I, one, a *Macaca radiata*, was a presumably normal animal collected fresh from the forest near Sagar-Sorab Road. There were no remarkable findings in this animal except rare focal necroses in the liver and a few recent hemorrhages in the kidney medulla and the alveoli of the lung.

The remaining three monkeys, two *M. radiata* and one *P. entellus*, exhibited diverse numbers of focal necroses in the liver with, in one case, coagulative necrosis and the presence of Councilman bodies. Prominence of Kupffer cells and of inflammatory cells varied in both the sinusoids and the portal radicles. The histological appearance of the kidney tissues also showed variation, with features in all three simulating albuminoid degeneration of the convoluted tubules. One case showed the presence of recent hemorrhages in the medulla. Scattered focal necroses were found in the ventricular myocardium in all three cases, as well as prominence of the red pulp and of the littoral cells of the spleen.

Sections of the brain revealed a sparse meningeal exudate in one case (V.R.C. No. W 1889), and in the other two cases there was neuron degeneration, with moderate to marked glial cell satellitosis but without the overt features of an encephalitis. There were no inclusions in the brain or viscera of any of the three animals.

Group II.—The one animal in this group was estimated to have been dead about 12 hours at the time of autopsy. As the lungs and kidney showed numerous lesions of presumably parasitic origin, the animal was excluded from further consideration.

Group III.—There were eight animals in this group, four *M. radiata* and four *P. entellus*. The period between death and autopsy was about 24 hours in each case. There were varying grades of post-mortem autolysis recognizable grossly in the viscera of all eight animals.

Microscopic examination of viscera stained with hematoxylin and eosin revealed the following:

Liver.—There was a variable degree of autolysis in all of the eight livers. The parenchymal cells revealed the presence of pigment similar to that found in animals of group I. There were scattered focal necroses and a moderate to marked prominence of the Kupffer cells. Diverse numbers of inflammatory cells were encountered in the portal radicles. Three of the eight livers revealed the presence of rounded, acidophilic inclusions which were seen either in the liver cell cytoplasm or lying free in the sinusoids. Sections of one liver showed the presence of a number of infarcts, both old and recent, although in another numerous radicles of the central hepatic veins were thrombosed, with resulting stagnation of blood in the pericentral sinusoids.

Kidney.—As in the liver, there were different grades of autolysis in this organ, so that while the glomeruli retained their outlines with more or less distinctness, the convoluted tubules in the majority were rendered into indistinct eosinophilic smudges. In one case (V.R.C. No. W 650; N.P. A-61) it was difficult to be certain whether the histology of the kidney was that of severe degeneration
or post-mortem autolysis (Plate V, fig. 7). In the same kidney there were a number of focal inflammatory infiltrates occupying either a part or the entire extent of Bowman’s capsules and also scattered alongside the convoluted tubules. In another (V.R.C. No. W 536), peculiar crystalline structures were encountered in the lumina of indistinct tubules. In all these kidneys the cortex was in general much more severely affected than the medulla, and in the cortex, the tubules more than the glomeruli.

In six of the eight monkeys there were small or moderate-sized focal necroses in the ventricular myocardium. The spleens (in those animals without pronounced autolysis) revealed moderate to marked prominence of sinusoids and littoral cells, with occasional erythrocytophagocytosis in this organ.

In six of the seven animals in which the lungs could be studied, recently exuded plasma and erythrocytes in the pulmonary alveoli were present.

Sufficiently representative portions of the brain were available for study in all the eight animals. In all of these there were degenerative alterations in the larger neurons in the cortex and basal ganglia, with moderate prominence of satellite glia. Rare petechial hemorrhages (probably agonal) were found in two brains. In one (V.R.C. No. W 541-42) there was fairly pronounced evidence of eosinophilic degeneration of nerve cells of the basal ganglia with scattered diffuse polymorphonuclear leucocytes, an indication that these changes were premortal. None of the brains revealed distinct histological changes of an encephalitis and no inclusions were found in either nerve cells or glia.

**Group IV.—**The seven animals in this group consisted of three *M. radiata* and four *P. entellus*. All these animals had been dead for at least 48 hours, and some more than 72 hours, before being subjected to post-mortem examination. It was, therefore, not surprising that considerable post-mortem autolysis, as indicated by gas-filled vacuoles and blackening, had occurred to interfere with the subsequent interpretation of pathology.

Certain interesting features in one case (V.R.C. Nos. W 415-421; N.P. A-148 and A-157), a *M. radiata* monkey from Kyasanur Forest, merit separate mention. There was a fairly pronounced coagulative necrosis of the liver, with numerous Councilman bodies lying either within the effete liver cells or, more often, scattered free in the sinusoids (Plate V, fig. 8). There were hemorrhages in the pulmonary alveoli and sections of the brain disclosed distinctive histological features of a non-suppurative encephalitis (Plate V, fig. 9).

The tissues of the other six monkeys revealed fairly pronounced autolysis, but even in these autolytic tissues it was possible to recognise certain features that appeared to constitute pathology. Thus, two of these livers revealed focal necroses and in four there were cytoplasmic inclusions similar to those seen in the other groups. Focal necroses were seen in the ventricular myocardium in two cases. In each of the two brains available, there was a sparse to moderate meningeal exudate, with very prominent glial satellitosis around effete neurons,
RESULTS OF VIROLOGICAL STUDIES.

A detailed report dealing with the isolation and further study of viral agents from the animals in this series will be published separately (Bhatt et al., in preparation). It is necessary here only to mention that in seven (three M. radiata and four P. entellus) of the 22 animals, virus was isolated from one or more tissues or organs of the body, including the blood. Three of these animals belonged to Group I, two to Group III and two to Group IV. The isolations represent five from the brain, four from the lungs, three each from blood, liver and spleen, and one each from kidney, heart and skeletal muscle. In three monkeys (all dead for 24 hours or more at the time of autopsy) virus was present in only one organ, viz., the brain in one and the lungs in the other two. In a fourth monkey collected soon after death, virus was present in two organs—spleen and brain. In the remaining three animals virus was recovered from multiple organs and tissues. It is significant that virus was also present in the blood of these three animals. The results of the virological studies are summarized in Table II.

DISCUSSION.

In dealing with material of the type discussed in this paper, one encounters certain limitations. Principal among these in the present instance was the admixture of changes due to post-mortem autolysis in the tissues of a number of the animals. From Table I it is obvious that only six of the 22 animals could be considered to be in a state of preservation compatible with accurate interpretation of histopathology. In the rest there were varying grades of autolytic changes, including in some frank gas-vacuole formation. Another limitation was the presence in some of the monkeys of lesions in the viscera that appeared to be entirely unrelated to the disease process under study—for example, lesions due to helminthic parasites. Still another limitation was the incomplete state of the collections of viscera specimens, the reasons for which have already been stated. It was considered desirable, nevertheless, to examine as thoroughly as possible whatever material was available. The occurrence of an epizootic among forest monkeys, coupled with a concurrent epidemic among human beings living in or near the same areas (Work et al., loc. cit.), was a situation serious enough to warrant the maximum mobilization of effort to arrive at a possible etiology.

The criteria adopted for estimating the probable intervals between death and autopsy were based on findings both in and around these monkeys. That these criteria were generally reliable was evidenced when the tissue changes were subsequently correlated with the estimated intervals in individual animals. It might be pointed out that in some of the animals the pathological changes were characteristic enough to be recognizable as significant despite the presence of autolytic phenomena. For example, distinct encephalitic changes were present in an animal estimated to have been dead for between 48 and 72 hours.

In contrast to the apparent severity and virulence of the clinical epizootic, the histopathological changes in the tissues of the animals were generally mild. An analysis of the pathological lesions encountered, especially those in animals that were well preserved, makes it apparent that nothing in either the quality or the
### Table II
Correlation between virus isolations and pathological findings.

<table>
<thead>
<tr>
<th>Monkey.</th>
<th>Presence of virus in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. P.e. W 371-390 (30 mins.)</td>
<td>N.D.</td>
</tr>
<tr>
<td>2. M.r. W 1012-1019 (30 mins.)</td>
<td>N.D.</td>
</tr>
<tr>
<td>7. M.r. W 414-421 (48 hrs.)</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

**Pathological alterations:**

- N = Normal structure.
- N.D. = Examination not done.
- Questionable changes.
- Pathological alterations.
- +, ++ = Being more definite than +.
- ? = Changes due to autolysis observed.

**Virus studies:**

- + = Virus isolated.
- - = Virus not isolated.
- N.A. = Virus isolation not attempted.

*P.e. = Presbytis entellus.*
*M.r. = Macaca radiata.*
distribution of such changes was characteristic for any particular disease process. Excluding the inconstant brain lesions, the pathological process consisted of degenerative alterations in the larger parenchymatous organs, such as the liver and kidney, focal myocardial necrosis and variable degrees of erythrocytic and plasma exudate in the pulmonary alveoli. These alterations are not by themselves in any way specific, and could be part of any general toxemic or infective fever (Bell, 1956).

The alterations in the kidneys were more difficult to interpret than those in the liver. The difficulty stemmed from the known propensity of this organ to undergo rapid post-mortem autolysis (Maximov and Bloom, 1952). Even in those animals on which post-mortem examinations had been performed sufficiently quickly after death, the possibility of imperfect fixation and its consequences could not be ruled out entirely. While renal tubular 'damage' is characteristically seen in general parenchymatous degeneration, it is difficult to determine whether these tubular lesions constitute a similar condition until more is known of the pathogenesis of KFD. Significant in this respect is the absence of more frequent viral isolations from this organ.

Hemorrhagic phenomena were generally mild, though observed in more than half the number of animals studied. Fourteen of the 22 animals revealed the presence of recent hemorrhages in one or more organs. In ten there was either hemorrhage or a hemorrhagic exudate in the pulmonary alveoli. In three there was evidence of recent hemorrhage in the renal medulla. Petechial hemorrhages were observed in the brain in three, and in one case there was a sizeable hemorrhage in the adrenal medulla. Histological examination of the various tissues in which these hemorrhages had occurred did not reveal any structural alterations in the smaller vascular channels to explain them, and while caution must be exercised in interpreting observations on material of this type, it was considered reasonable to postulate that the defect in hemostasis lay probably in the blood itself.

In this connection it may be noted that similar hemorrhagic tendencies were observed both clinically and post-mortem in three fatal cases of KFD in human beings (Iyer et al., 1950). As in the monkeys, no structural alterations were observed in the blood vessels in the human cases to explain these hemorrhages. Reference to the available descriptions of the pathology of some of the closely related hemorrhagic fevers encountered in the Soviet Union (Gajdusek, 1953) indicates that an explanation for such hemorrhagic phenomena might be found in certain alterations in the bone marrow indicative of a suppression of maturation of some of the formed elements of the blood*.

As already mentioned, there appear to be certain similarities between the changes encountered in the animals in this series and those reported in some of the hemorrhagic fevers—Crimean and Omsk hemorrhagic fevers (Gajdusek, loc. cit., Chumakov, 1948, to be published), and Far Eastern hemorrhagic fever or, as it is

---

*Investigations of the hematological and biochemical features of KFD have been undertaken by Chatterjee et al., and results will be reported in due course.
described by the Russian workers, epidemic nephroseonephritis (Symposium, 1954; Hullinghorst and Steer, 1953; Steer, 1955). The main pattern of the pathological changes in both is, in the ultimate analysis, one of widespread toxemia, with the brunt of the attack falling on the larger parenchymatous organs. There is also the similarity of the degenerative character of alterations without more pronounced inflammatory response. The differences between KFD and the hemorrhagic fevers relate to the degree of these alterations and to certain details, such as an apparently less pronounced hemorrhagic tendency in the monkeys. While these differences are sufficient to support the contention that Kudasanur Forest Disease is an illness distinct from the previously known hemorrhagic fevers, the similarity in type and pattern of pathological changes suggests that the disease-producing agents have comparable pathogenic actions on the host. It may be noted here that a tick-borne agent closely related to RSSE virus has been shown to be responsible in Omsk hemorrhagic fever (Chumakov, 1948, to be published; MacLeod et al., 1956).

Involvement of the nervous system was infrequent in the monkeys. Of the 18 cases where sufficient brain tissue was available to warrant an opinion, only two animals showed distinctive histologic changes consistent with those of a viral encephalitis. Four other animals revealed either a sparse meningeal exudate or a degree of neuron degeneration and glial encephalitis higher than that in the remaining 12 but insufficient to be styled histologically as encephalitis. Such infrequent involvement of the nervous system appeared to correlate with the clinical illness, which in its dominant features, as already stated, resembled the hemorrhagic fevers rather than an encephalitis. In the two cases where distinctive encephalitis was observed histologically, virus was isolated from the brain alone and from the brain and one other organ, a fact which points to a more than coincidental association between the presence of virus in the tissue and the pathological changes.

In the five other animals from which virus was isolated, no consistent correlation was achieved between the presence of virus and histological damage. As indicated in Table II, the longer the interval between death and autopsy, the less chance there has been of virus isolation from multiple organs.

It may be mentioned that early in the course of these investigations it was strongly suspected that the cause of the epizootic in the monkeys was probably sylvan yellow fever, that being the only known disease in which forest monkeys are affected fatally in large numbers. In yellow fever, however, the histological changes in the liver are characteristic (Bugher, 1951), and as none of the animals in the series showed such changes, it was possible on the basis of histological examination to be certain that we were not dealing with yellow fever. This point is emphasized because of the serious implication for this country of detecting any positive evidence of the presence of active yellow fever.

Mention has already been made of the limitations of the materials forming the basis of this report. We feel safe in assuming that all the monkeys examined had suffered from the same illness because they were all found dead within a limited period of time and within a circumscribed area. Although evidence of autolytic changes was encountered in some of the animals, nevertheless the finding of similar
changes in animals with well-fixed tissues and those with poorly fixed tissues leaves open the possibility that these changes were present before death and had relation to the causative virus. Proof of these relationships can come, however, only from study of the disease in appropriate experimental hosts. Such experiments are in progress.

**Summary.**

1. A report is given of the pathological changes encountered in 22 monkeys of the species Presbytis entellus and Macaca radiata found dead in an epizootic in forested areas of Shimoga District, Mysore, during the early part of 1957.

2. Virological studies have revealed the presence of viral agents in the blood, liver, spleen, kidney, lung, heart and skeletal muscle, and the brain of some of these animals.

3. The main pathological alterations were in the nature of non-specific degenerative changes in the larger parenchymatous viscera. Two of the animals revealed distinctive histological features of a non-suppurative encephalitis, and in four others there were changes suggestive of encephalitis.

4. Hemorrhagic phenomena were observed in 14 of the 22 animals and consisted of focal hemorrhages or hemorrhagic exudations in the lungs, kidneys, brain, and adrenal medulla (one case).

5. Consistent correlation between the presence of virus in tissues and the type of pathological changes encountered was not possible except in the case of the brain. In two animals histological evidence of encephalitis coincided with the presence of virus in the brain alone and in the brain and one other organ, respectively.

6. The limitations of the materials under study have been borne in mind in the assessing of the significance of the changes observed. The general conclusion is that, in comparison with changes reported in other hemorrhagic illnesses of a similar type, the pathological lesions in these monkeys represent the effects of a widespread toxemia rather than those of any particular organ or tissue specificity of the causative virus.

**References.**


Work, T.H. (1958) ... ... Russian spring-summer virus in India. Kyasanur Forest Disease. Progress in Medical Virology, 1, 248-279.


KYASANUR FOREST DISEASE.
Part VIII.
ISOLATION OF KYASANUR FOREST DISEASE VIRUS
FROM NATURALLY INFECTED TICKS OF THE
GENUS HÆMAPHYSALIS.

By
HAROLD TRAPIDO, P. K. RAJAGOPALAN, T. H. WORK,
AND M. G. R. VARMA.

From
THE INDIAN JOURNAL OF MEDICAL RESEARCH.

CAMBRIDGE PRINTING WORKS.
DELHI.
1959.
KYASANUR FOREST DISEASE.

Part VIII.

ISOLATION OF KYASANUR FOREST DISEASE VIRUS FROM NATURALLY INFECTED TICKS OF THE GENUS HAEMAPHYSALIS.

HAROLD TRAPIDO*, P. K. RAJAGOPALAN†, T. H. WORK‡, AND M. G. R. VARMA**.

It has previously been reported (Work and Trapido, 1957) that the etiological agent of Kyasanur Forest Disease, which has been observed to affect men and monkeys in Shimoga District of Mysore State, India, has been identified as an arthropod-borne (arbor) virus of the ‘Group B’ of Casals and Brown (1954). The disease in man has been described by Work et al. (1957).

Our attention was first drawn to the disease on March 23, 1957, when a report was received of monkeys dying in a forested area of Shimoga District. From subsequent interviews with the local people, as well as from the records of the Mysore Department of Public Health, it was learnt that both villagers and monkeys in the adjoining forests were affected during the early months of 1956, and that the disease had become active again in January 1957. The aggregated reports of numerous villagers indicated a massive mortality of monkeys. The authenticity of these reports was confirmed by the number of monkeys either freshly dead or in various stages of decomposition that we observed during the first several days of field work. Viruses isolated from acutely ill human beings and from the tissues of recently dead monkeys were shown to be the same (Work and Trapido, loc. cit.).

To our knowledge the only precedent for the occurrence of massive monkey mortality in association with human disease was that provided by sylvan or jungle yellow fever in the New World. The setting in which the epizootic-epidemic was going on proved to be very reminiscent of that characteristic of sylvan yellow fever. The area involved, the southern portion of Sora Taluk and the northern edge of Sagar Taluk, is one of relatively high rainfall (60 to 90 inches annually) which supports in part broad-leaved evergreen tropical forest. The dead monkeys found were in all cases either within or at the edge of this forest. The persons affected with the disease were villagers living in close association with the forest. They were primarily agriculturists cultivating crops in clearings in the forest or housewives. However, certain of their activities, such as tending cattle in semi-cleared forest used for pasturage and gathering firewood, gave ample evidence of their exposure in the forest.

* Deputy Director, Indian Council of Medical Research Virus Research Centre, Poona, India and Staff Member, The Rockefeller Foundation. The Virus Research Centre was established by the Indian Council of Medical Research with the co-operation of the Rockefeller Foundation and the Government of Bombay.
† Research Assistant, Virus Research Centre, Poona.
‡ Director, Virus Research Centre and Staff Member, The Rockefeller Foundation.
** Research Officer, Virus Research Centre, Poona.
Kyasanur Forest Disease.

There were other elements, however, that were inconsistent with the epidemiological picture of sylvan yellow fever. As mentioned previously, the periods of overt human illness and monkey mortality during both 1956 and 1957 were the early months of the year, the dry season. While there was one report of monkeys dying in November 1956, just after the end of the monsoon, we had no records, at this time, of associated human cases. The dry season is the period when the lowest density of forest mosquitoes would be expected; experience gained in the study of the annual abundance cycles of forest mosquitoes in tropical America had shown that although population peaks might fall at different times in different species, all such peaks fell sometime within the rainy season. Since no data were available on the population dynamics of Indian forest mosquitoes, it remained to be determined whether the generalization arrived at in another part of the world would be valid here. Other factors that raised doubt as to whether the primary vector or vectors could be mosquitoes, were the restricted size of the area affected and the relative slowness of the disease’s spread. The disease was occurring in 1957 in the same villages as in 1956, and the only additions were villages no more than a few miles away from those involved in 1956. While moving waves of sylvan yellow fever have been known to linger in an area for a year or longer (Trapido and Galindo, 1956), they ordinarily move a hundred or more miles in this time.

It was clearly necessary to determine the characteristics of the sylvan mosquito fauna in a forest in which virus was known to be active. As there can be large fluctuations in mosquito populations in a short time, it was also necessary to accomplish the work rapidly. Fortunately, the investigations of the first several days had pin-pointed a locality in which it seemed certain that virus must currently be active. This was a portion of the Kyasanur State Forest about a half-mile square, adjacent to the village of Barage. In this forest two recently dead monkeys had been found and autopsied (virus was subsequently recovered from both); the remains of two other monkeys, dead for longer than two weeks, had also been seen here.

It was recognized that the combination of incidents occurring in so restricted an area presented a rare opportunity to resolve quickly the problem of the possible arthropod vector. The work of the insect collecting team of the Virus Research Centre based at Akividu in the Krishna-Godavari region of Andhra State was suspended on March 30, and this group was ordered to Shimoga District. The team arrived on April 2 and the arthropod collections began on April 3. At this time the laboratory identification of the etiological agent as a Group B arbor virus was not yet complete. When it was established several days later that such a virus was involved, the insect collecting crews of the Virus Research Centre at Vellore and at Poona were also transferred to the work in Kyasanur Forest.

In the first mosquito collections made in the forest during daylight fair numbers of Culex tritaeniorynchus and Culex whitmorei were taken resting on vegetation (40 of the former and ten of the latter in three man hours) but only a single mosquito, Aedes albopictus, was collected attacking a quiescent human subject during three hours. The first overnight mosquito collection, made during the night of April
4-5, produced 49 mosquitoes attacking two human subjects exposed from 18:00 to 06:00 hours. The species represented were *Culex vishnui*, 25; *Mansonella uniformis*, 16; *Culex whitmorei*, 4; *Anopheles leucophyrrus*, 2; and *Culex bitauviorhynchus* and *Anopheles hycanus*, 1 each. No *Phlebotomus*, *Simulium*, *Culicoides* or other biting Diptera were taken. As the exposure of local residents in the forest took place primarily during the daylight hours, the virtual absence of diurnal biting mosquitoes argued against their involvement as vectors at this time of year. Collections made on the forest floor during the daylight hours of the succeeding several days confirmed the general picture that while modest numbers of such species as *Culex vishnui* and *whitmorei* and *Mansonella uniformis* might be found resting on vegetation, mosquitoes attacking man were rare. To check on the situation in the tree tops, tree platforms were constructed and 24-hour collections made simultaneously on the ground and in the forest canopy. These revealed that there was no distinctive canopy mosquito fauna. The results of these and subsequently continued long-term studies of the mosquito fauna of this forest, which will be the subject of a future communication, indicated the desirability of pursuing collections of other biting arthropods as possible vectors.

We began the collecting of ticks and mites on April 15, using cotton flannel 'flags', one meter square, which had been made for the purpose. It was immediately found that larval and nymphal stages of ticks of the genus *Haemaphysalis* were abundant. On the third day of these collections two of the insect collectors reported sick with high fevers. Because of the suspicion that they might have contracted the disease under study (a suspicion which was subsequently confirmed by the isolation of virus from their blood), all work in Kyasanur Forest was immediately suspended. Several days later a third insect collector also fell sick with what proved to be Kyasanur Forest Disease. The course of the illness in these three men has been described (Work et al., loc. cit.). The infection of three men during less than two weeks of exposure proved only too clearly that virus was indeed active in this patch of the forest.

A short time later it was shown that the virus of Kyasanur Forest Disease belonged immunologically to the Russian Spring-Summer complex (Work et al., loc. cit.; Kulkarni et al.; to be published), and following protection of field personnel with RSS vaccine, the collections of arthropods in Kyasanur Forest were resumed on May 15. The present report will deal in detail only with results obtained with the collections made in Kyasanur Forest during the first three days of the tick-collecting operation.

The tick collections.—During the three days that flag-dragging tick collections were made (April 15 to 17) the following ixodid ticks were taken: of *Haemaphysalis*, one adult male each of the species *formosensis*, *spinigera* and *papuana* and 201 larvae and 589 nymphs which could not be determined to species. Also present in the collections were 93 larvae of *Ixodes* and 16 nymphs of *Amblyomma*; as in the case of the *Haemaphysalis*, these could not be determined to species. Among the *Haemaphysalis* nymphs two types appeared to be present, both of which were included in the pools from which virus was subsequently isolated. In later
collections, nymphs have been sorted into kinds and given arbitrary code numbers pending their association with the more readily identifiable adults. The taxonomy of forest-inhabiting ticks in India has not been systematically studied and it may be some time until all stages of ticks from this environment can be specifically determined with certainty. All ticks taken were unengorged, and presumably seeking a host when they attached to the flags. No mites were found on the flags.

Ticks were removed from the flags with a forceps and put into screw-capped vials or rubber-stoppered tubes, which were stored and transported on wet ice from the field laboratory to the Virus Research Centre at Poona.

The isolation of virus.—At Poona, the ticks were sorted and divided into pools. The individual pools were weighed in an ‘Ohaus’ balance. Each pool was triturated in a chilled mortar with a small amount of sterile alundum to ensure thorough crushing. The diluent used was 0.75 per cent bovine albumin in phosphate saline at pH 7.2, to which penicillin and streptomycin had been added in the proportion of 1,000 units of penicillin and 1 mg. of streptomycin per c.c. to suppress bacterial contaminants. The quantity of diluent used was minimal, between 1.5 c.c. and 3.0 c.c., depending on the weight of the pool. The aim was to get an amount of concentrated suspension (between $10^{-1}$ and $10^{-2}$ dilution) which would be sufficient for mouse inoculation and subsequent reinoculation. The suspensions were centrifuged at 2,000 r.p.m. for ten minutes. The supernate was removed into tubes in a wet-ice bath (2°C. to 4°C.) and each suspension inoculated into a litter of seven 2-day old mice. Each mouse received 0.02 c.c. of the inoculum intracerebrally and 0.03 c.c. subcutaneously. After inoculation, the balance of the suspensions was stored in a Revco deep freezer which maintains a temperature of about −55°C. The inoculated mice were observed every day.

Pool G 11333 was composed of 239 nymphs and 101 larvae of *Haemaphysalis* spp. taken on April 17. The infant mice inoculated with tick suspension from this pool became sick four to eight days after inoculation. One of the sick mice was sacrificed and a suspension made from its brain was passed into a fresh litter. All the infant mice of this second passage became sick three days after inoculation. Brain suspension from these was passed into two litters—the third mouse brain passage. All the brains from sick mice of these litters were harvested and a suspension made in 25 per cent rabbit serum in normal saline which was ampouled and lyophilized after one day's storage in a Revco deep freezer. The lyophilized virus that was stored had an adult mouse intracerebral titer of 7.0. This strain is designated as G 11333.

Pool G 11338 was composed of 169 nymphs and 63 larvae of *Haemaphysalis* spp. taken on April 15. All the infant mice inoculated with the suspension from this pool became sick five days after inoculation. The brain suspension from two of the sick mice was passed into a fresh litter of infant mice, which became sick three days later. Brain suspension from these was passed into three litters—the third mouse passage. Brains from sick mice of the third passage were harvested and a suspension made in 25 per cent rabbit serum in normal saline which was
ampouled and immediately stored in a Revco deep freezer. The lyophilized virus had an adult mouse intracerebral titer of 8.46. This strain is designated as G 11338.

Both virus strains are filterable and pathogenic to adult and infant mice.

In adult mouse intracerebral neutralization tests, serum from rhesus monkeys immunized against KFD virus neutralized 3.5 logs of strain G 11338 and more than 3.83 logs of strain G 11333. Third passage lyophilized mouse brain suspension which was the source of virus used in the tests; had titers of 10^8.5 (G 11338) and 10^6.83 (G 11333) in the presence of pre-inoculation serum from the immunized monkeys.

Strain G 11338 was reisolated by inoculation of the remainder of the tick suspension into a litter of infant mice after ten days' storage in the Revco deep freezer. The tick suspension that yielded strain G 11338 was also reinoculated into infant mice after ten days' frozen storage in the Revco deep freezer but none of the mice became sick.

**DISCUSSION.**

The isolation of two strains of the virus of Kyasanur Forest Disease from *Haemaphysalis* ticks by trituration of wild-caught unengorged specimens, as reported here, does not by itself conclusively establish these arthropods as vectors of the disease. But from July to October of 1957, 14 additional isolations of Kyasanur Forest Disease virus were made from adult *Haemaphysalis* ticks which could be identified to species (Bhatt et al., in preparation). There were twelve isolations from *H. spinigera* and one each from *H. tortuus* and *H. papuana*. All these isolations were from unengorged ticks taken in drags from Kyasanur Forest or the forests adjacent to the villages of Holekatte Hosur and Kannur, where both monkey deaths and human cases of Kyasanur Forest Disease occurred. There have been no isolations of Kyasanur Forest Disease virus from the substantial numbers of ticks processed from adjacent localities outside the epidemic area. There have also been no isolations from other genera of ticks taken either within or adjacent to the epidemic area although the genera *Rhipicephalus*, *Dermacentor*, *Amblyomma*, *Boophilus* and *Ixodes* are present.

While there remains to be accomplished the isolation of the virus by bite from naturally infected ticks, as well as the continued exploration of the possible rôle of other ticks and biting arthropods, the present evidence suggests the involvement of *Haemaphysalis* ticks, particularly *H. spinigera*, in the maintenance of the virus transmission cycle.

The established vector of the related Russian Spring-summer encephalitis virus is *Ixodes persulcatus*, but there is also evidence that *Haemaphysalis concinna* is involved (Silber and Soloviev, 1946). The vector of Omsk Haemorrhagic fever, another virus disease immunologically related to Russian Spring-summer encephalitis, is thought to be *Dermacentor pictus* (Chumakov, 1948). Thus, it appears that the viruses of the Russian Spring-summer encephalitis complex may be transmitted by a variety of ixodid tick genera in different places.
There has recently been isolated a virus similar or identical to that of Russian Spring-summer encephalitis from *Ixodes granulatus* taken off two species of Malayan forest rats, with which no human or animal disease has as yet been associated (Smith, 1956). This finding, together with the demonstration of antibodies in humans which are immunologically indistinguishable from those of Russian Spring-summer encephalitis in Saurashtra, and possibly other parts of India (Kerr and Gatne, 1954; Smithburn, Kerr and Gatne, 1954), suggest that there may be widespread in the Asian tropics a complex of Russian Spring-summer-like viruses and diseases which has gone unnoticed.

**SUMMARY.**

The details of two isolations of the virus of Kyasanur Forest Disease from pools of larval and nymphal ticks collected in Kyasanur Forest near Barage, Shimoga District, Mysore, have been reported. The ticks were unengorged specimens which attached to cloth flags dragged over the ground and low shrubbery. One pool was composed of 239 nymphs and 101 larvae of *Hemaphysalis* spp., the other of 169 nymphs and 63 larvae of *Hemaphysalis* spp. During the period July to October 1957, there were 14 additional isolations of virus from adult ticks, the species of which could be determined. Twelve isolations were from *H. spinigera*, one from *H. turturis*, and one from *H. papuana*. Preliminary negative evidence for the involvement of other hematophagous arthropods is briefly discussed.

**REFERENCES.**

Bhatt, P.N., Rajagopalan, P.K., and Isolation of Strains of Kyasanur Forest Disease Virus from *Hemaphysalis spinigera*, *H. turturis* and *H. papuana* ticks collected in the 1957 epidemic area.

_Bhalla, J., and Brown, L.V._ (1954) ...  
(See in translation).

Chumakov, M.P. (1948) ...  

Kerr, J.A., and Gatne, P.B. (1954) ...  

Kulkarni, K.G., Work, T.H., Clarke, D.H., and Casals, J.  
_Kyasanur Forest Disease V. Serological Studies with Kyasanur Forest Disease Virus. To be published._  
_Amer. Rev. Soviet Medicine, Special Supplement._

Silber, L.A., and Soloviev, V.D. (1946) ...  

Smith, Gordon C.E. (1956) ...  

_Church._ 72, p. 248.

Traphido, H., and Galindo, P. (1956) ...  

Work, T.H., Traphido, H., Narasimha Murthy, D.P., Laxmana Rao, R., Bhatt, P.N., and Kulkarni, K.G. (1957) ...  
Reprinted from
PROCEEDINGS OF THE NINTH PACIFIC
SCIENCE CONGRESS, 1957
Volume 17
PUBLIC HEALTH and MEDICAL SCIENCES

STUDIES ON TICKS AS POSSIBLE VECTORS OF
KYASANUR FOREST DISEASE

M.G.R. VARMA, HAROLD TRÁPIDO, and P.K. RAJAGOPALAN

Virus Research Centre, Poona, India.
Arthropod collections made in Kyasanur Forest during April 1957, when human cases of Kyasanur Forest Disease and monkey deaths were frequently being reported, demonstrated the virtual absence of any diurnal biting mosquitoes at ground level and the absence of a distinctive canopy mosquito fauna. No other biting Diptera such as *Phlebotomus*, *Simulium* or *Culicoides* were obtained attacking human subjects in the forest. These observations and the relatively slow linear spread of the disease suggested some other arthropod of limited range as the possible vectors of the disease.

Immature *Ixodid* ticks are most active in searching for hosts during the dry part of the year. Three categories of tick collections were initiated in April: (1) ticks collected in forests either from ground drags or by examination of vegetation when the ground was too wet for flag-dragging; (2) ticks collected from tree-trunks and a forest canopy collecting platform; and (3) ectoparasitic ticks on animals in the area. Between April and October 1957 these collections have produced more than 20,000 ticks of all stages.

*Haemaphysalis* larvae and nymphs comprised nearly 90% of the total number of ticks collected from drags in the forest during the dry month of April when human cases of the disease were occurring in numbers. They could not be identified as to species. But five types of *Haemaphysalis* nymphs, two of which were more common than the others, were represented in the collections. In May, about half the ticks collected from drags in the same area were immature *Haemaphysalis* (Table 1). No larvae and only limited numbers of *Haemaphysalis* nymphs were obtained in ground collections from early June through the middle of August, a period of heavy rainfall from the southwest monsoon. Correspondingly, no confirmed human cases of the disease were reported in July and August. With the cessation of the rains there was an increase in the population of immature stages of *Haemaphysalis*. Larvae and nymphs of these ticks collected in the latter half of August and in September comprised nearly 50% and 90% respectively of the total ground collections for this six-week period.

### Table 1.
Numbers of *Ixodes* and *Haemaphysalis* ticks taken in ground collections in Kyasanur Forest.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Number per 10 man-hour period</th>
<th>Total collection period in epidemic area</th>
<th>Total Number of ticks collected in epidemic area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>May</td>
<td>June</td>
<td>July</td>
</tr>
<tr>
<td><em>Ixodes</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae and nymphs</td>
<td>104.0</td>
<td>30.8</td>
<td>14.2</td>
</tr>
<tr>
<td><em>Ixodes</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>0</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td><em>Haemaphysalis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvae and nymphs</td>
<td>112.9</td>
<td>10.4</td>
<td>0.3</td>
</tr>
<tr>
<td><em>Haemaphysalis</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>1.6</td>
<td>17.7</td>
<td>52.1</td>
</tr>
</tbody>
</table>

88
Larvae and nymphs of *Haemaphysalis* constituted the majority of ticks ectoparasitic on langur and bonnet monkeys, both of which have been shown to be susceptible to the disease. Small numbers of the nymphs were collected from langurs in the margin of the epizootic area in April. An engorged *Haemaphysalis* larva was obtained from a moribund bonnet monkey caught in Kyasanur Forest in August. Kyasanur Forest Disease virus was subsequently isolated from this monkey. Numbers of *Haemaphysalis* larvae—in one case as many as 50—were found attached to the ears of three bonnet monkeys collected in the epidemic area in October. Out of a total of about 1,400 ticks collected from rats, squirrels and shrews trapped in the forest, mostly during the monsoon, only nine were *Haemaphysalis* larvae and eight *Haemaphysalis* nymphs. All except one nymph were engorged and all the 17 ticks were taken from *Rattus rattus wrightoni*.

*Haemaphysalis* adults started to appear in relatively increased numbers in ground collections by June. Of ticks collected in July and August from the floor of Kyasanur Forest, 75% in July and 50% in August were *Haemaphysalis* adults. In contrast only 6% of a total of more than 5,600 ticks collected there in September were *Haemaphysalis* adults (Table 1). *Haemaphysalis spinigera* was by far the most abundant species in these collections and made up about 70% of over 3,000 adults of this genus collected in the epidemic area. Less common were species provisionally identified as *Haemaphysalis papuana* and *Haemaphysalis turturis*. Apart from these ticks, adults of *Haemaphysalis formosensis*, *cuspidata*, *aculeata*, *cornigera* *typica*, *wellingtoni*, larvae of *Boophilus microplus*, nymphs of *Amblyomma* and adults of *Rhipicephalus haemaphysaloides*, *Dermacentor auratus*, *Amblyomma testudinarium* and *Aponomma gervaisi var. lucasi* were obtained from ground collections. *H. papuana* is recorded for the first time from India (Hoogstraal, 1957, personal communication).

*Haemaphysalis spinigera* adults were found on cattle and buffaloes in the epidemic area. It appears obvious that the cattle are infested with these *Haemaphysalis* ticks while grazing in the forest. Most of the species of adult ticks recovered from ground collections were also collected from cattle, although the predominant ectoparasites on these animals were nymphs and adults of *Boophilus microplus*. In May, about 13% of a total of 250 ticks collected on cattle in Baragi village at the edge of Kyasanur Forest were *H. spinigera* adults; by July the percentage had increased to 35. The majority of ticks on buffaloes in July were adults of this species.

*Ixodes* ticks, most of them larvae comprised about half the total number of ticks in ground collections in May and June (Table 1). By July their number had decreased. In August and September, *Ixodes* larvae and nymphs accounted for only 3-4% of the total number of ticks in grounds collections, although in September there was an increase in the number of *Ixodes* ticks collected per 10 man-hour period. Immature stages of *Ixodes* were collected in small numbers from a forest canopy collecting platform and from tree-trunks. They were also obtained from the skin and clothing of insect collectors exposed in the forest while performing routine arthropod collections. Whether this could be regarded as an instance of ticks attacking man is debatable because none were attached. The bulk of over 1,400 ticks collected from rodents and shrews were immature stages of *Ixodes*. Small numbers of *Ixodes* larvae and nymphs were taken from bonnet monkeys exposed as sentinels in Kyasanur Forest; in one instance, a bonnet monkey from which the virus was recovered, had 11 unfed *Ixodes* larvae on it. It has not been possible to identify, according to species, any of the larvae or nymphs of the *Ixodes* collected. It appears, however, that all of the nymphs examined to date are the same. Six adults of *Ixodes*—all provisionally identified as female *I. ceylonensis*—have been obtained so far, one from ground collections and five from rodents. The virtual absence of *Ixodes* adults in the collections made during the first six months of these investigations, cannot be explained at present. Perhaps they will appear in larger numbers before a year is completed. It should be noted that ticks of this genus had never been recorded from South India before *Ixodes kerri* was collected and described from a flying squirrel shot in North Kanara in 1954 (Rao, 1955).

Kyasanur Forest Disease virus has been isolated twice from unfed *Haemaphysalis* larvae and nymphs collected in the forest adjoining Baragi village in April (Trapido et al., in preparation). It has been recovered eight times from unfed mature *Haemaphysalis spinigera* collected in the forests adjacent to three villages in the epidemic area. Although this does not conclusively prove ticks to be the vectors, it nevertheless demonstrates that the virus is passed trans-stadially in ticks and is able to survive in them. During July and August when no human cases of the disease were seen, the virus was repeatedly isolated from unfed *Haemaphysalis spinigera* adults. This
suggests that the virus persists in the ticks during the inter-epidemic period, but is not transmitted to man either because there is minimal exposure of man to ticks at this time or because the ticks do not attack man. It is significant that so far all virus isolations from ticks have been from localities where human cases have been exposed, monkeys have died or there is serological evidence of virus activities. Collections of ticks from forests outside the epidemic area have not yielded any virus, although their tick fauna does not appear to be different from that of the epidemic area.

Information as to the host preferences of ticks which may play a role in the epidemiology of the disease is still not very extensive. From the present investigations it is known that adults of *Haemaphysalis spinigera* infest cattle and buffaloes in the epidemic area. What animals the immature stages of this species commonly feed upon, has not yet been established. Forest monkeys may be the important hosts. The small numbers of *Haemaphysalis* larvae and nymphs collected on rodents and insectivores trapped in the forest indicate the possibility that these animals are not commonly parasitized by these ticks. No ticks have yet been observed biting man in the epidemic area although the villagers admit to being bitten occasionally by ticks whilst working in the forest. Patients suffering from the disease, however, denied having noticed any tick bites prior to the onset of illness (Work *et al.*, 1957). This is in marked contrast to Russian Spring-Summer Encephalitis where up to 75% of the cases actually have a history of tick bite within the incubation period (quoted by van Rooyen and Rhodes, 1948).

In summary, the evidence which has incriminated ticks as possibly the vectors of Kyasanur Forest Disease is: (1) the numerical predominance of ticks over other blood-sucking arthropods in the affected areas during the epidemic season; (2) the repeated isolation of the virus from ticks in the epidemic area; and (3) the failure to isolate the virus from any ticks outside this area. Information as to what species of tick is the vector or whether more than one species is involved, must await further investigations. However, the recovery of the virus exclusively from *Haemaphysalis spinigera* adults, the infestation of monkeys and rodents which are known to be susceptible to the disease by immature stages of *Haemaphysalis* and the failure to isolate the virus from ticks other than those belonging to this genus, are suggestive. Final confirmation as to the role of ticks as vectors of the disease would be provided by the transmission of the virus to susceptible animals by the bite of unfed ticks collected in the epidemic area as well as by ticks experimentally infected in the laboratory.

**REFERENCES**

Hoogstraal, H., 1957, Personal communication.


Haemaphysalis megalaimae sp. n., a New Tick from the Small Green Barbet (Megalaima viridis) in India

P. K. Rajagopalan

Reprinted from The Journal of Parasitology
Made in United States of America
Haemaphysalis megalaimae sp. n., A New Tick from the
Small Green Barbet (Megalaima viridis) in India

P. K. Rajagopalan
Virus Research Centre,* Poona, India

ABSTRACT

Haemaphysalis megalaimae sp. n. is described from males, females, nymphs, and a larva collected from small green barbets (Megalaima viridis Boddaert) taken in Shimoga District, Mysore State, India. The species appears to be host-specific on the small green barbet. It is closely related to the Haemaphysalis hoodi–doenitzi–centropi group of bird parasitizing haemaphysalids but differs in several distinct morphological features.

While studying ticks of birds of Shimoga District, Mysore State, South India, several adults, nymphs, and one larva of a distinctive Haemaphysalis tick were collected from the small green barbet Megalaima viridis.

In consultation with Dr. Harold Trapido and Dr. Harry Hoogstraal these were recognized as close to, but differing from, Haemaphysalis centropi Kohls, 1949. The species has thus far been found only on one host species, Megalaima viridis, and is named for this genus of birds.

In the following description measurements given are the averages from three specimens.

Haemaphysalis megalaimae sp. n.

Holotype: Male, A 27097, collected on small green barbet Megalaima viridis from Sagar (elevation approx. 1900 feet), 1 February 1960.


Paratypes: Specimens listed in Table I other than the holotype and allotype.

The holotype and allotype are deposited in the collection of the Rocky Mountain Laboratory, Hamilton, Montana. Paratypes will be deposited in the collections of the Zoological Survey of India, Calcutta, the Virus Research Centre, Poona, the British Museum (Natural History), and the collection of Harry Hoogstraal.

Male (Figs. 1, 2, 5 to 8)

Body: Length from tips of palpi to posterior margin, 1.62 mm. Width, 1.11 mm. Brownish yellow in color, ovoid, widest at about the level of the spiracle.

Capitulum (Figs. 5, 6): Length from tips of palpi to tips of cornua, 0.37 mm. Length of basis, 0.17 mm. Width, 0.31 mm. Dorsal basis finely punctate, rectangular, lateral margins slightly divergent anteriorly, width about twice the length. Cornua moderate, sharp. Ventral basis broadly rectangular, with external corners rounded. Dorsal internal margins of palps straight. Palpal article 2 very salient laterally, flared, basal margin semi-circular in outline. Basal margin dorsally broken midway between inner and outer margins by commencement of more heavily chitinized distal portion of margin. Lateral margins acutely recurved from basolateral salience approximately to the level of junction between articles 2 and 3, thence converging to moderately rounded apex. Palpal articles 2 and 3 subequal in length, dorsally and ventrally. A posteriorly directed short, broad, triangular, bledelike spur situated at the distal margin of palpal article 3 on the ventral side. Three to four infrainternal bristles on article 2 and two infrainternal bristles on article 3, situated near ventral spur. Hypostomal teeth 5/5 apically, diminishing to 3/3 proximally, about ten per file. Length of hypostome 0.17 mm.

Scutum: Length, 1.41 mm. Punctuation fine, numerous, and evenly distributed. Prominent lateral grooves, beginning at a point between legs II and III and continuing posteriorly across two festoons on either side. Scapulae acute. Cervical grooves moderate, deep anteriorly, and slightly diverging posteriorly. Festoons long, well separated, eleven in number.

Spiracular plate (Fig. 7): Ovoid in shape, without dorsal process.

Genital aperture: At level of coxa II.

Legs: Coxae I to III with short ridgelike projections, that of coxa I larger and more prominent than II and III. Coxae IV with a short triangular spur. Distinct dorsal spur on trochanter I; trochanters I to III with weak ventral spurs, decreasing from I to III. Tarsi as illustrated in Figure 8.

Received for publication 18 December 1962.

*The Virus Research Centre is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation.
Figures 1 to 4.

*Haemaphysalis megalaimae* sp. n. Holotype (VRC-A27957) and allotype (VRC-A36352). Figures 1, 2. Male, dorsal and ventral views. Figures 3, 4. Female, dorsal and ventral views.
*Haemaphysalis megalaimae* sp. n. Figures 5, 6. Male, capitulum, dorsal and ventral views. Figure 7. Male, spiracular plate (A = anterior, D = dorsal). Figure 8. Male, tarsi I to IV, lateral view. Figures 9, 10. Female, capitulum, dorsal and ventral views. Figure 11. Female, genital area. Figure 12. Female, spiracular plate (A = anterior, D = dorsal). Figure 13. Female, tarsi I to IV lateral view.
Female (Figs. 3, 4, 9 to 13)

Body: Length (partially engorged) from tips of palpi to tips of posterior margin, 2.54 mm. Width 1.43 mm. Ovoid, broadest at the level of the spiracle. Marginal grooves distinct, beginning at about the level of leg II. Festoons distinct, about as wide as long, intervals between them dark.

Capitulum (Figs. 9, 10): Length from tips of palpi to tips of cornua, 0.40 mm. Length of basis, 0.16 mm. Width, 0.43 mm. Dorsal basis with posterior margin concave, width about three times the length (excluding cornua). Cornua moderate and blunt. Porose areas prominent, oval, slightly depressed, well separated, and with their longitudinal axes converging anteriorly. Palps similar to those of male, but palpal article 3 is shorter than 2 ventrally. Three to four slender infranarial bristles on article 2. Hypostome, length 0.24 mm. Teeth 5/5 per file distally diminishing to 4/4 proximally, about ten per file.

Scutum (Fig. 3): Length, 0.96 mm. Width, 0.87 mm. Lateral margins subparallel from level of anterior indentation to level of basal third of length, thence abruptly converging to moderately broad, bluntly rounded, posterior margin. Cervical grooves deep anteriorly, diverging posteriorly, extending to basal third of length of scutum.

Spiracular plate: As illustrated in Figure 12.

Genital aperture: At the level of coxa III (Fig. 11).

Legs: Coxae I and IV with broad ridgelike spurs, that of coxa I more prominent. Coxae II and III with broad ridgelike projections. Tarsi as illustrated in Figure 13.

Nymph (Figs. 14 to 19)

Body: Ovoid. Length of partially fed specimen approximately 1.6 mm.

Capitulum: Dorsal basis rectangular, posterior and lateral margins straight. Cornua present but weak. Posterior margin of ventral basis semicircular in outline. Palps about as broad as long. Palpal article 2 with lateral salience very much flared, its basal margin semicircular in outline. Palpal article 3 slightly shorter than 2 dorsally and ventrally. Palpal article 3 with a very small, barely perceptible, ventral projection. Two slender infranarial bristles on article 2, and one beside ventral projection of palpal article 3.

Hypostome: Teeth 3/3, about eight per file.

Scutum: Shape as illustrated in Figure 14, cervical grooves concave, deep anteriorly.

Spiracular plate: As in Figure 18.

Legs: Coxae with short, broad, ridgelike projections; that of coxa I most prominent. Trochanter I with slight ventral projection, II and IV unarmcd. Tarsi as in Figure 19.

Larva

Body: Ovoid.

Capitulum: Dorsal basis without cornua, about twice as wide as long, posterior margin straight. Palps short with slightly rounded lateral contour. Palpal article 3 with a small, barely perceptible, ridgelike ventral projection.

Scutum: As in nymph.
The host bird, *Megalaima viridis*, is exclusively arboreal and nests in tree holes. It seems likely that the life cycle of the tick must be completed on the bird and in its nest hole.

This species is closest to *Haem. centropi* from which it differs as follows: Males: The lateral salience of palpal article 2 is semicircular in outline in *megalaimae* whereas it is broadly angular in *centropi*; there are three or four infranentral bristles on palpal article 2 in *megalaimae*, as compared to six or seven in *centropi*; the width of the dorsal basis is twice its length in *megalaimae*, whereas the width is only about one and a half times the length in *centropi*. Females: The difference in the lateral salience as in males; *megalaimae* with three infranentral bristles on palpal article 2 as compared to eight to ten bristles in *centropi*.

The species also resembles the African *Haemaphysalis hodi* Warburton and Nuttall, 1909, and *Haemaphysalis doeniz* Warburton and Nuttall, 1909, described from Singapore but since recorded in Malaya (Andy et al., 1960). The males of *megalaimae* differ from those of *hodi* in the fact that two festoons are crossed by the lateral groove in *megalaimae* but only one in *hodi*; the female *megalaimae* has ovoid porose areas as compared to reniform porose areas in *hodi*. In both sexes the dorsointernal basal margin of palpal article 3 is recurved to form a point in *hodi* as illustrated (Figs. 7 and 8) by Warburton and Nuttall, a feature absent in *megalaimae*.

*Haemaphysalis doenitz* differs from *Haem. megalaimae* in the more elongate configuration of its palps in both sexes, as illustrated by Warburton and Nuttall (Figs. 9 and 10); in

---

**Table 1. Number and stages of Haemaphysalis megalaimae sp. n., collected from 13 small green barbets (Megalaima viridis) in Shimoga District, Mysore State, India.**

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Date collected</th>
<th>VRC arthropod number</th>
<th>Locality</th>
<th>No. of ticks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Larva</td>
<td>Males</td>
</tr>
<tr>
<td>1</td>
<td>1 Feb. 1960</td>
<td>A27957</td>
<td>Sagar</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>14 Apr. 1960</td>
<td>A37558</td>
<td>Malur</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>20 Apr. 1960</td>
<td>A35004</td>
<td>Kuppe</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>28 July 1960</td>
<td>A36332</td>
<td>Gidalagudi</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1 Nov. 1960</td>
<td>A39334</td>
<td>Hille-Marur</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2 Nov. 1960</td>
<td>A39339</td>
<td>Hille-Marur</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>20 Mar. 1961</td>
<td>A45138</td>
<td>Lingadahalli</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>7 April 1961</td>
<td>A45393</td>
<td>Kuppe</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>26 April 1961</td>
<td>A45561</td>
<td>Ulav</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>14 June 1961</td>
<td>A46173</td>
<td>Hille-Marur</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>14 July 1961</td>
<td>A44242</td>
<td>Sherrangbe</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>25 Oct. 1961</td>
<td>A31800</td>
<td>Bayur</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>20 June 1962</td>
<td>A54881</td>
<td>Hille-Marur</td>
<td>0</td>
</tr>
</tbody>
</table>

Total: 1 16 22 10 49

---

1 A total of 297 small green barbets were examined.
doenitzii the greatest width of palpal article 2 is subequal the length of article 2 while in megalaimae the greatest width of article 2 equals or exceeds the length of articles 2 and 3 combined.

Acknowledgments

The author is indebted to Dr. Harry Hoogstraal for providing the illustrations and to Dr. Harold Trapido and Dr. Hoogstraal for their critical help.

Literature Cited


Haemaphysalis kyasanurensis sp. n., a Member of the formosensis Group in Southern India and Ceylon (Ixodoidea, Ixodidae)

Harold Trapido, Harry Hoogstraal, and P. K. Rajagopalan

Reprinted from THE JOURNAL OF PARASITOLOGY
Vol. 50, No. 2, April 1964, p. 295–302
Made in United States of America
Haemaphysalis kyasanurensis sp. n., a Member of the formosensis Group in Southern India and Ceylon (Ixodoidea, Ixodidae)*

Harold Trapido,* Harry Hoogstraal,‡ and P. K. Rajagopalan§

ABSTRACT: *Haemaphysalis kyasanurensis* sp. n. is described from adult and immature stages from areas of southwestern India where Kyasanur Forest disease occurs and from Ceylon. This species is compared with syntype adults of *H. formosensis* Neumann of Formosa, a closely related species for which a diagnosis is provided, and with other samples from Assam and Burma. Adult palpi of the new species have a small, abrupt basolateral salience that is lacking in *H. formosensis*. Immature stages of the new species have more salient palpi than adults.

*Haemaphysalis kyasanurensis* sp. n. is described from males, females, nymphs, and larvae from areas of Shimoga District, Mysore State, India, in which Kyasanur Forest disease was first discovered in 1957 (Work, 1958) and from Ceylon. A diagnosis of syntype adults of *H. formosensis* Neumann is provided as a baseline for establishing differences between these two species, which are part of a clade in which *H. kyasanurensis* sp. n. of high rainfall areas of southwestern India and Ceylon is distinctly separated from *H. formosensis* of high rainfall areas of Formosa and Southeast Asia.

*Haemaphysalis kyasanurensis* sp. n. The Kyasanur Forest *Haemaphysalis* (Figs. 1 to 28)

Holotype

Male (VRC-A19890), from flag dray in Kyasanur Forest, near Barage, station 3, Shimoga

Received for publication 23 September 1963.

* From Research Project MR005.09-1402.3, Bureau of Medicine and Surgery, Navy Department, Washington, D. C. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

‡ Staff Member, the Rockefeller Foundation, Virus Research Centre, Poona, India. The Virus Research Centre is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation.

§ Department of Medical Zoology, United States Naval Medical Research Unit Number Three, Cairo, Egypt, UAR.

District, Mysore State, India, 28 January 1959, collected by V.R.C. personnel, Deposited in Rocky Mountain Laboratory (RML 39304).

Allotype

Female [VRC-A18125(4)], data as for holotype, except for station 2, 17 November 1958. Deposited in Rocky Mountain Laboratory (RML 39305).

Paratypes

Total 13♂♂, 14♀♀, 8 nymphs, 15 larvae. All but one collection are from Shimoga District, Mysore State, India, and from vegetation except for 10 reared larvae. Kyasanur Forest near Barage: 1♂ (taken with holotype), A19890, 28 January 1959; 1♂, A24393(4), 22 June 1959; 1♂, A24394(3), 22 June 1959; 1♂, A26241, 17 August 1959; 1♂, A30250(20), 23 February 1959; 1♂, A20182(13), 16 February 1959; 1♂, A15222(11), 29 July 1958; 1♂, A30297(2), 4 January 1960; 1♀ (bred from nymph), A17971(7)#1; 1♀, A35403(2), 30 May 1960; 3 nymphs, A36345(4), 11 January 1960. Sugar-Jog Road: 1♀, A21481(6), 18 March 1959; 1♀, A7358(c), 6 May 1957; 1♀, A7359, 6 May 1957; 1♀, A20006(3), 30 January 1959; 1♀, A7372(c), 10 May 1957. Near Koppalagadi: 1♂, A21426(8), 14 March 1959; 1♀, A21277(3), 28 February 1958. Near Bilisiri: 1♀, A20125(16), 10 February 1959. Near Kamur: 1♀, A5553(3), 27 April 1962. Near Hosur: 1♀, A32313(3), 11 March 1960. Sugar: 10 larvae (reared from ♀ from Indian wild dog, *Cuon alpinus dukanensis* Sykes), A54935, hatched 21 March 1960, P. K. Rajagopalan legiti. Ceylon: 1♂♂, 2♀♀, 2 nymphs, from Indian Crested Porcupine, *Hystrix i. indica* Kerr, Gammaduwa, Dansakande, Central Province, RML26874, 6 January 1939.

The above paratypes are deposited in collections of the Virus Research Centre (Poona), Zoological Survey of India (Calcutta), Rocky Mountain Laboratory, British Museum (Natural History), and Academy of Sciences (Leningrad).
Paratypes in Hongstraal collection (presented by Virus Research Centre): 1 δ, Barage, 27 December 1957 [VRC-A10989(8), HH5239]; 1 δ, Sagar-Jog road, 6 February 1959 [VRC-A20085(3), HH5237]; 1 δ, 1 η, Koppalagadde, 7 February 1959 [VRC-A20095(24), HH5240]; 1 η, Bilisiri, 28 February 1959 [VRC-A21291(16), HH5235]; 2 nymphs, Koppalagadde, 7 February 1959 [VRC-A20095(17), HH5238]; 1 nymph, Sagar-Jog road, 6 May 1958 [VRC-A12730(18), HH5236]; 5 larvae, Barage Station, 27 October 1958 [VRC-A17805(13), HH6081].

DESCRIPTION

Male (Figs. 1, 2, 5 to 9)
Mean length of seven specimens from apex of palpi to posterior margin of scutum measures 2.8 mm, mean width 1.7 mm. Color (unfed) yellowish.

Capitulum (Figs. 5 to 7): Basis capituli rectangular, approximately 1.7 times as wide as long (including cornua). Lateral margins and posterior margin between cornua straight; lateral margins posteriorly abruptly converging to form narrowly pointed cornua, which are at least as long as their views.

basal width and 3/5 as long as base of basis capituli. Surface with a few faint punctations. Ventrally with two or three very short setae on each side and a pair of short posthypostomal bristles. Palpi compact, subquadrate, with a slight, abrupt basolateral salience; each palp is 1.5 to 1.7 times as long as wide. Segment 1 visible as a short pedicle dorsally and ventrally, bearing one seta dorsally and one seta ventrally. Segment 2 1.2 times as wide as long; approximately 1.5 times as long as segment 3; basal margin convex and forming a slight abrupt salience at basolateral juncture; lateral margins anterior of salience short, straight, slightly converging to apex of segment; inner margin longer, almost straight from base to apex of segment; ventral outline similar except that basal margin is obtusely angled and inner margin is convex; setae numbering five dorsally, three ventrally; suprainternal setae numbering three, widely spaced, small, undifferentiated; infrainternal setae usually three, occasionally two, long, slender, widely spaced, undifferentiated. Segment 3 dorsally rather quadrate with straight sides and wide, truncate apex; basal margin dorsally almost straight (not elevated, lacking spur); ventral outline more triangular and with basal margin very slightly wider than apical margin of segment 2; ventral spur flat, very short, hardly reaching apical margin of segment 2; widely triangular, tapering to a more or less narrowly pointed apex; setae numbering four or five dorsally and three or four ventrally, two minute setae from inner margin dorsally and two long setae from inner margin ventrally. Hypostome short, stout, lateral margins convex; apex broadly rounded with extensive corona of approximately nine transverse rows of minute hooklets; dental formula 4/4, denticles in files of approximately 8 (inner) to 12 (outer); one or two extra denticles may be present medially.

Scutum (Fig. 1) flat or very slightly convex between deeply depressed lateral grooves and elevated lateral ridges; 1.5 times as long as wide (somewhat variable); lateral margins almost straight and parallel, or slightly convex, from level between coxae III and IV slightly broadened posterior margin, lateral margins anterior of coxae III abruptly converging to scapulae; scapulae bluntly rounded; anterior emargination very shallow. Lateral grooves long, distinct, extending anteriorly to level of coxae III, posteriorly enclosing two festoons (in some specimens appearing to enclose several festoons). Cervical grooves very narrow, converging from anterior emargination of scutum to short, deep, rounded cervical pits. Punctations practically obsolete, a few shallow punctations may be present. Surface glossy, otherwise with shallow depressions (as illustrated) or lacking such depressions. Festoons numbering 11, exceptionally long, distinctly separated.

Ventral surface (Figs. 2, 8): Spirocardia plates (Fig. 8) with rounded dorsal projection which forms a very small break in lateral scutal margin from dorsal view (Fig. 1). Genital grooves and anal groove as illustrated. Integument with very few, widely scattered, small setae. Other features as illustrated (Fig. 2).

Legs (Figs. 2, 9) robust: Coxae each with an elongate spur; spur on I longest, parallel sided, bluntly rounded apically; spurs on II, III, and IV shorter, subequal, elongately triangular. Trochanter I with a normal, pointed dorsal shield (Fig. 1); ventrally each with a short, triangular spur usually extending beyond margin of trochanter; on I or on IV this spur may be reduced to an elevated area of the trochanter surface. Tarsi (Fig. 9) II to IV narrowly elongate, gradually tapering distally; each with an apicoventral hook, which is small on I and long on II to IV; II and III with a small subbasal ventral ridge. Claws moderately large, variable. Palpters reaching to or almost to apical curvature of claws.

Female (Figs. 3, 4, 10 to 15)

This sex is quite similar to the male, except for usual sexual dimorphism and the marked elongation of the palpi. Length from apex of palpi to posterior body margin (unengorged) measuring approximately 3.2 mm, width 1.8 mm.

Capitulum (Figs. 10 to 12): Basis capituli with outline similar to that of male; corona shorter than those of male; porose areas shallow, subcircular, widely spaced, moderate size. Palpi quite elongate; each palpus twice as long as wide; basolateral salience similar to that of male. Segment 1 forming a pedicle that ventrally is exceptionally long; lateral margin ventrally half as long as segment 2; setae as in male. Segment 2 similar to that of male except that it is more elongate; dorsal setae numbering four, ventral setae two, suprainternal setae two, infrainternal setae usually three, occasionally two. The ventrobasal margin of segment 2 may be slightly convex (Fig. 11) or obtusely angular, as illustrated for the male (Fig. 6). Segment 3 dorsally more elongate than in male, almost as long as segment 2, with lateral and inner margins gradually converging distally to form a less truncate apex than in male; ventral spur similar to that of male but usually blunter apically. Hypostome elongate, slightly longer than palpi, lateral margins slightly convex, apex bluntly rounded; corona moderate; dental formula as in male but with one to four more denticles in each file.

Scutum (Fig. 5) glossy, subcircular, length and width subequal, margins rounded. Cervical grooves as in male except that shallow, diverging grooves extend from cervical pits to beyond midlength of scutum. Punctations shallow, indistinct, small and medium size, moderately numerous, widely spaced.

Dorsal and ventral integumental areas (Figs. 3, 4), genital area (Fig. 13), and ovoid spicularia plates (Fig. 14) as illustrated. Legs (Figs. 4, 15) similar to those of male.

Nymph (Figs. 16, 17, 20 to 24)

Differs greatly from adults in that the palpi are very widely salient basolaterally. Length from apex of palpi to posterior body margin (unengorged) approximately 1.4 mm.
Capitulum (Figs. 20 to 22); Basis capituli dorsally approximately twice as wide as long; margins straight; dorsal cornua as in males; ventrally with a short, bluntly rounded cornu in each basolateral corner (discernible in mounted material, appears as an angle in unmounted material); a short seta just anterior of each basolateral juncture, and a pair of comparatively large posthypostomal bristles. Palpi with a wide basolateral salience. Segment 1 small, largely concealed, with no visible setae. Basal margin of segment 2 forming a wide salience over coxae I; straight dorsally and slightly convex ventrally. Lateral margins gradually converging from basal salience to narrowly pointed palpal apex. Inner margin dorsally and ventrally essentially straight from base to apex. Segment 2 approximately 1.5 times as wide (basally) as long and 1.5 times as long as segment 3; juncture between these segments straight; ventral spur of segment 3 approximately as in female. Setae numbering six (7 to eight) dorsally and six ventrally; infrainternal and suprainternal setae each single. Hypostome (Fig. 22) slightly longer than palpi elongate, lateral margins slightly convex, apex bluntly rounded; a small corona present; dental formula 2/2 (may have two or three extra median denticles anteriorly); denticles broad, in files of 9 to 11. Scutum (Fig. 16) slightly shorter than wide, margins rounded to midlength, thence converging to bluntly rounded posterior margin. Cervical grooves deep and parallel or slightly converging, shallow and diverging on posterior half of scutum. Punctations rare, obscure. Spiracular plates (Fig. 23) as illustrated.

Legs (Figs. 17, 24) moderately stout. Coxae I with a large, elongately triangular basal spur; II, III, and IV each with a deltoid spur successively decreasing in length. Trochanters lacking ventral spurs. Tarsi (Fig. 24) moderately stout and somewhat more abruptly tapering than in adults; apicoventral hooks absent. Claws and pulvilli as in adults.

Larva (Figs. 18, 19, 25 to 28)

Palpal salience intermediate between those of adults and nymphs. Length (unengorged) from apex of palpi to posterior body margin approximately 0.70 mm, width 0.52 mm.

Capitulum (Figs. 25 to 27). Basis capituli quite similar to that of nymph, no ventral setae noted. Palpi 1.6 times as long as wide. Segment 1 small, concealed. Segment 2 with basal margin slightly convex dorsally and ventrally; basolateral juncture acute and lateral margins converging in a straight line from this juncture to narrowly pointed palpal apex; palpal outlines and ventral spur of segment 3 otherwise similar to those of nymph. Setae dorsally numbering one on segment 1 and five on segments 2 and 3; suprainternal and infrainternal setae each single. Hypostome (Fig. 27) similar to that of nymph.

Scutum (Fig. 18) 1.4 times as wide as long; lateral margins anteriorly gradually rounded, posteriorly more abruptly converging and concave to short, bluntly rounded posterior margin. Cervical grooves straight, parallel, extending to scutal mid-length. Punctations numbering four, each with a small seta. Other features of dorsal (Fig. 16) and ventral (Fig. 19) body integument as illustrated.

Legs (Figs. 18, 19, 28) stout. Coxae I with a large basal spur similar to that of nymph; II with a very short, wide spur; III with a minute, barely discernible inner spur. Tarsi (Fig. 28) narrowly elongate, more or less abruptly tapering. Claws and pulvilli as in nymph.

Related species

H. kyanurenensis sp. n. of southwestern India and Ceylon is closely related to H. formosensis Neumann (1913) of Formosa and southeastern Asia, a seldom collected species whose immature stages remain unknown. Both species are moderately large and share structural characters that easily separate the formosensis group from others in this genus. H. kyanurenensis sp. n. differs from H. formosensis in having palpal segment 2 longer with a distinct, abrupt basolateral salience; typically three rather than four infrainternal setae; a shorter ventral spur of segment 3; 4/4 rather than 6/6 dental formula; longer cornua; fewer scutal punctations; coxal spurs longer, more slender and pointed; longer and narrower tarsi, etc.

Adults of H. formosensis will be more completely described in a subsequent paper. Immature stages of H. formosensis remain unknown and it will be of interest to determine whether they possess the widely salient palpi that are so distinctive of larvae and nymphs of H. kyanurenensis sp. n.

DIAGNOSIS

Haemaphysalis kyanurenensis sp. n. (adults)

A moderately large haemaphysalid (♀ 2.8 mm; ♂ 3.2 mm in length). Basis capituli widely rectangular; cornua (♂) longer than their basal width. Palpi with a slight but distinct, abrupt, basolateral salience; compact in ♂, elongate and with a fairly long pedicel in ♀; segment 3 with a very short, flat, broad, pointed ventral spur, palpal apex truncate in ♂ but more tapered in ♀, dorsoabaxial spur or elevation lacking; suprainternal setae numbering three; infrainternal setae numbering two or (typically) three, long, undifferentiated, widely spaced. Hypostome short, stout, with extensive corona, dental formula 4/4 (a few extra median denticles may be present). Scutum (♂) flat, glossy, broad; punctations rare, practically obsolete;
lateral grooves long, usually enclosing two festoons; festoons long; (♀) subcircular; punctations shallow, moderately numerous. Coxae each with a prominent elongate spur; spur of I longest, parallel-sided, apex bluntly rounded; II to IV narrowly triangular, subequal. Trochanters each with a ventral spur (occasionally reduced to elevated ridge on I or IV). Tarsi elongate, gradually tapering, each with an apicoventral hook.

*Haemaphysalis formosensis* (adult syntypes only)

A moderately large, wide haemaphysalid (♂, 2.8 mm; ♀, 3.3 mm in length). *Basis capituli* widely rectangular; cornua shorter than their basal

---

width. Palpi lacking basolateral salience; compact in ♂, elongate and with a fairly long pedicle in ♀; segment 3 with a short, flat, broad, tapering ventral spur, dorsobasal margin may be slightly elevated laterally but lacks spur or projection; suprainternal setae numbering three; infrainternal setae numbering three or (typically) four, undifferentiated, moderately closely spaced. Hypostome short, stout, with moderate corona; dental formula 6/6. Scutum (♂) flat, moderate number of shallow punctations in certain areas (sometimes obscure); lateral grooves long, enclosing one or no festoons; (♀) outline distinctly wider than long. Coxae each with a heavy, peglike, slightly tapering dorsal and ventral views (drawn from mounted specimen). 27. Larva hypostome, ventral view. 28. Larva tarsi I to III, lateral view.
spur with bluntly rounded apex. *Trochanter* (♂) each with a ventral spur, those on I and IV may vary; (♀) lacking ventral spurs. *Tarsi* short, stout, gradually tapering, each with an apicoventral hook.

**ACKNOWLEDGMENTS**

We are grateful to Dr. G. Owen-Evans of the British Museum (Natural History) and to Professor A. Brizard of the École Vétérinaire, Toulouse, for allowing us to study the syntype series of *H. formosensis*. Mr. G. M. Kohls of the Rocky Mountain Laboratory kindly provided specimens of *H. kyasanurensis* sp. n. from Ceylon for description. We are also indebted to personnel of the Virus Research Centre, Poona, who over a period of years participated in the collection and rearing of the new species.

**LITERATURE CITED**


REARING OF *IXODES PETAURISTAE* WARBURTON, 1933, IN LABORATORY

P. K. RAJAGOPALAN

*Reproduced from*
THE INDIAN JOURNAL OF MALARIOLOGY
Vol. 17, No. 4, December 1963

CAMBRIDGE PRINTING WORKS
DELHI
1965
REARING OF IXODES PETARISTAE WARBURTON, 1933, IN LABORATORY.

BY

P. K. RAJAGOPALAN

(Virus Research Centre, Poona, India.)*

[December 11, 1963.]

Though several species of ticks of the genus Haemaphysalis have been reared in the laboratory successfully, difficulties had been experienced in regard to Ixodes petaristae, a species of tick occurring commonly in the evergreen forests of Sagar and Sorab talukas of Shimoga District, Mysore State. In view of the infestation of small mammals in the forests by this species of tick, and the recent experimental evidence suggesting its potentiality as a vector of Kyasanur Forest Disease, it was necessary to study the biology of this species in great detail. Further taxonomic studies of the developmental stages, most essential for the investigations on the epidemiology of the disease, were not possible without successful laboratory rearing of this species.

The successful rearing of Ixodes petaristae was finally achieved by careful manipulation of the environment in which they were kept, and the selection of appropriate hosts for the purpose of feeding.

Two species of rats, Rattus rattus wroughtoni and Rattus blanfordi, were trapped alive in the forests and were kept in wire-cages placed over trays containing water. Over a hundred fully engorged nymphs of Ixodes dropped into these trays during the next few days. These nymphs were picked, dried on a filter-paper and were transferred individually to shell vials (2 inch × 1 inch), the open ends of which were covered with bolting silk and placed in desiccators provided with a 20 per cent solution of KOH. The engorged nymphs were observed to be crawling for about six to nine days after which they became quiescent. A waxy secretion, which appeared to facilitate the nymphs to adhere to the glass surface of the tube was found on their ventral surfaces. In this quiescent stage, they were found for periods ranging from 31 to 62 days, after which they started moulting into adults which were identified as Ixodes petaristae.

The moulting adults were held in shell vials, covered with bolting silk, in the same desiccators provided with a freshly prepared solution of 20 per cent KOH. The moulting adults, though very inactive in the beginning, were found crawling...
inside the tubes and appeared to thrive very well at room temperature (the rainfall and temperature data are summarized in Table I).

Table I.

Monthly rainfall, mean monthly maximum and minimum temperatures at Sagar (Shimoga District).

<table>
<thead>
<tr>
<th></th>
<th>Rainfall, in inches.</th>
<th>Maximum temperature (degrees Fahrenheit)</th>
<th>Minimum temperature (degrees Fahrenheit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jul. 1963</td>
<td>30-19</td>
<td>78°</td>
<td>70°</td>
</tr>
<tr>
<td>Aug. 1963</td>
<td>28-47</td>
<td>78°</td>
<td>69°</td>
</tr>
<tr>
<td>Sep. 1963</td>
<td>7-52</td>
<td>82°</td>
<td>69°</td>
</tr>
<tr>
<td>Oct. 1962</td>
<td>9-03</td>
<td>85°</td>
<td>70°</td>
</tr>
<tr>
<td>Nov. 1962</td>
<td>2-27</td>
<td>83°</td>
<td>69°</td>
</tr>
<tr>
<td>Dec. 1962</td>
<td>1-70</td>
<td>85°</td>
<td>61°</td>
</tr>
<tr>
<td>Jan. 1963</td>
<td>0-00</td>
<td>85°</td>
<td>64°</td>
</tr>
<tr>
<td>Feb. 1963</td>
<td>0-58</td>
<td>85°</td>
<td>57°</td>
</tr>
<tr>
<td>Mar. 1963</td>
<td>0-50</td>
<td>90°</td>
<td>57°</td>
</tr>
<tr>
<td>Apr. 1963</td>
<td>0-06</td>
<td>93°</td>
<td>66°</td>
</tr>
<tr>
<td>May. 1963</td>
<td>5-82</td>
<td>93°</td>
<td>70°</td>
</tr>
<tr>
<td>Jun. 1963</td>
<td>7-59</td>
<td>82°</td>
<td>72°</td>
</tr>
</tbody>
</table>

The actively moving adults were transferred, one male and one female, to a petri-dish (60 mm. wide). The male tick was observed, moving actively and within a few minutes it was seen attaching itself to the female, with their ventral surfaces against each other. The male was seen slowly piercing the female genital aperture with its hypostome. The male palps were slowly pushed on either side of the hypostome, which was now deeply embedded in the female genital aperture. In this position, the pairs were seen coupled for five to seven hours. (It appears that fertilization does take place during the ‘coupling process’ inside the petri-dish, because these females laid fertile eggs after feeding and without coming into contact with any other males later).

These females were fed on a young Malabar giant squirrel (*Ratufa indica*), which in nature is a common host for the adults of this tick. (Baby monkeys of the species *Macaca radiata* and the common three striped palm squirrel, *Funambulus tristriatus tristriatus*, were also successfully used to feed adults). The female ticks were released in a metallic pill box cemented on to the back of the host and were seen to attach to the body of the host after about 24 to 36 hours. Only in two instances were males also released in the pill box along with the females. The females appeared to feed slowly for the first two or three days, after which they engorged very rapidly, swelling to about ten times the size of an unfed adult. The fed females detached nine to 13 days after they were released into the pill box, and were picked up, transferred individually to shell vials (2 inch × 1 inch) which were provided with a piece of filter paper and were placed in desiccators with 20 per cent KOH. The oviposition took place after about ten days and lasted several days.

Each female laid about 2,500 eggs (one egg mass was counted and numbered 2,686 eggs). The eggs were transferred with a brush to sterile baked clay pots,
about 2 inch in diameter and 3 inch high, filled to about half height with sterilized forest soil. The clay-pots were then placed in a tray (12 × 12 × 2 inch) containing water. By absorbing water through the porous clay walls, the soil in the pots attained almost the same consistency as the forest soil during the monsoon season. The object was to simulate as much as possible the natural conditions since *Ixodes* larvae were obtained from the forest floor in large numbers during the monsoon months.

The larvae started hatching after about 80 days, some after as long as 93 days, and started crawling out of the clay-pots after another week. The active larvae were transferred to 16 × 100 mm. tubes, closed at one end with plaster of paris and the other with bolting silk. The plaster of paris was kept wet always, and the larvae appeared to thrive well in such tubes.

Forest shrews and rats were employed as hosts to feed the larvae. The hosts were kept in wire-cages (9 × 9 × 9 inch) over trays containing water and the ‘questing’ larvae were transferred, with a brush, to the back of the host. The larvae, which dropped from the animals into the trays with water before attaching, were collected with a brush, dried on a filter-paper for a few seconds and replaced on to the back of the host. Most of the larvae were found crawling into the fur of the host, and many could be seen after a few hours attached to the ears and head of the host animal.

The engorged larvae, which detached after 4 to 5 days, were collected with a brush from the water and transferred to a sterile 50 ml. beaker, containing a circular piece of filter-paper and covered with bolting silk. The beakers, containing the engorged larvae, were placed in the centre of a white enamel tray. A wire-frame was provided in the tray to support lint pieces, which were kept constantly moist, so that the beakers containing the ticks were in a moist environment. The mortality among the engorged larvae was negligible.

Nymphs were found emerging after 25 to 30 days, and were seen actively crawling inside the beaker after two to three days. After a further 11 to 12 days, the nymphs were transferred with a brush on to rats and shrews. The animals were restrained inside a tight-fitting wire-gauze cover for a few hours to restrict their movement and to allow the nymphs to attach, after which the host animals were held in individual cages, over trays containing water. The engorged nymphs dropped after 4 to 5 days, and were picked up with a brush, dried on a filter paper, and transferred individually to shell vials (2 × 1 inch) covered with bolting silk. The shell vials, containing the engorged nymphs, were placed in a desiccator with 20 per cent KOH for moulting.

Following are the details of the time taken during the different phases of rearing:
Rearing of *Ixodes petauristae* Warburton, 1933, in Laboratory.

**LIFE CYCLE OF *IXODES PETAURISTAE REARED IN THE LABORATORY* (FROM NYMPH TO NYMPH).

<table>
<thead>
<tr>
<th>Event</th>
<th>Duration in Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Engorged nymphs dropped</td>
<td>0</td>
</tr>
<tr>
<td>Engorged nymphs quiescent after</td>
<td>6 — 9</td>
</tr>
<tr>
<td>Adults emerged after</td>
<td>31 — 62</td>
</tr>
<tr>
<td>Adults started feeding after</td>
<td>30</td>
</tr>
<tr>
<td>Adult fed for</td>
<td>9 — 13 (on <em>Ratus indica</em>)</td>
</tr>
<tr>
<td>Females oviposited after</td>
<td>9 — 15</td>
</tr>
<tr>
<td>Females continued laying eggs for</td>
<td>8 — 37</td>
</tr>
<tr>
<td>Larvae hatched after</td>
<td>80 — 93</td>
</tr>
<tr>
<td>Larvae started feeding after</td>
<td>15 —             (on rats and shrews)</td>
</tr>
<tr>
<td>Larvae fed for</td>
<td>4 — 5</td>
</tr>
<tr>
<td>Engorged larvae quiescent after</td>
<td>6 — 7</td>
</tr>
<tr>
<td>Nymphs emerged after</td>
<td>25 — 30</td>
</tr>
<tr>
<td>Nymphs started feeding after</td>
<td>11 — 12</td>
</tr>
<tr>
<td>Nymphs fed for</td>
<td>4 — 5 (on rats and shrews)</td>
</tr>
</tbody>
</table>

**Duration of life cycle**

238 — 288 days

The taxonomical studies on the larval and nymphal stages and a redescription of the adult, *Ixodes petauristae*, are being presented separately.

**SUMMARY**

Details of a successful method of rearing all the stages of *Ixodes petauristae* in the laboratory are given. Essentially the technique consisted of keeping the ticks in a very moist environment and the use of appropriate natural hosts for feeding. Eggs and larvae were held on wet mud, and the nymphs in the beaker surrounded by wet lint. The larvae and nymphs were fed on rats and shrews, and the adults on the giant squirrel. The complete life cycle took 238 to 288 days.

**ACKNOWLEDGEMENT**

Grateful thanks are due to Dr. Jorge Boswell, M.D., for his encouragement and advice.
A GUIDE TO THE IDENTIFICATION OF ALL STAGES OF THE HAEMAPHYSALIS TICKS OF SOUTH INDIA

By H. Trapido,1 M. G. R. Varma,2 P. K. Rajagopalan,3 K. R. P. Singh 2 and M. J. Rebello2

Virus Research Centre,4 Poona, India

(Received 2nd December 1963)

CONTENTS

Introduction ........................................ 250

Comments on characters used in the keys
 Key to larvae ...................................... 253
 Key to nymphs ...................................... 254
 Keys to males and females ......................... 257
 Key to larvae ...................................... 257
 Key to nymphs ...................................... 258
 Key to males ....................................... 259
 Key to females ..................................... 260

Comments on species included in the keys
 Haemaphysalis aculeata Lavara, 1905 ............... 261
 Haemaphysalis bispinosa Neumann, 1897 ........... 261
 Haemaphysalis centropi Kohls, 1949 ............... 263
 Haemaphysalis cornigera shimoga Trapido & Hoogstraal, 1964 .... 263
 Haemaphysalis cuspitata Warburton, 1910 .......... 263
 Haemaphysalis intermedia Warburton & Nuttall, 1909 .... 263
 Haemaphysalis kyasanurensis Trapido, Hoogstraal & Rajagopalan, 1964 .... 265
 Haemaphysalis leachii (Audouin), 1827 ............ 265
 Haemaphysalis megalaimae Rajagopalan, 1963 ........ 265
 Haemaphysalis minuta Kohls, 1950 ................. 265
 Haemaphysalis papuana kineari Warburton, 1913 .... 266
 Haemaphysalis spinigera Neumann, 1897 ............ 266
 Haemaphysalis turturis Nuttall & Warburton, 1915 .... 266
 Haemaphysalis wellingtoni Nuttall & Warburton, 1907 .... 266

Comments on species not included in the keys
 Haemaphysalis campanulata Warburton, 1908 ........ 266
 Haemaphysalis flav Neumann, 1897 ................. 266
 Haemaphysalis howletti Warburton, 1913 ............ 267
 Haemaphysalis kuchensis Hoogstraal & Trapido, 1963 .... 267
 Haemaphysalis paraturturis Hoogstraal, Trapido & Rebello, 1963 ... 267
 Haemaphysalis silicefelis Hoogstraal & Trapido, 1963 .... 268

Acknowledgements .................................... 268

References .......................................... 268

1 Staff member, the Rockefeller Foundation.
2 Department of Entomology, London School of Hygiene and Tropical Medicine, formerly at the Virus Research Centre.
3 Indian Council of Medical Research.
4 The Virus Research Centre is jointly maintained by the Indian Council of Medical Research and the Rockefeller Foundation.
INTRODUCTION

Investigations of the natural history of the virus of Kyasanur Forest disease (KFD) since its discovery during 1957 in Shimoga District, Mysore State, India (Work & Trapido, 1957; Work, 1958) have concentrated much attention on ticks of the genus *Haemaphysalis* in the region, as virus has repeatedly been isolated from them.

The basic work on the *Haemaphysalis* ticks has been the monographic treatment for the world by Nuttall & Warburton (1915) and for the region here considered the revision of the Indian Ixodidae by Sharif (1928). Recently there has been opportunity for one of us (H. T.) to re-examine the type specimens of almost all the *Haemaphysalis* species attributed to the Indian fauna and many of the related south Asian taxa. This has necessitated certain alterations in the nomenclature and the description of new species and subspecies. The publication of this guide has been held in abeyance until names which are likely to be stable have become available in a series of recent papers (see References). The review of south Asian *Haemaphysalis* material in various European collections, on which many published host and locality records are based, has revealed numerous errors of identification which are being dealt with in separate publications.

The original descriptions of most species of ticks have usually been based on adults and not infrequently on only one sex or even one specimen. Very little work has been done previously in rearing and obtaining associated larvae, nymphs and adults of ticks of the Oriental region. Such descriptions and/or illustrations of larvae and nymphs of *Haemaphysalis* of the Oriental region as have appeared in the literature have been based with few exceptions on the circumstantial evidence that the larvae and/or nymphs were collected on a host in company with the adults with which they were presumptively associated. The *Haemaphysalis* species of the Palearctic region to the north have been very much more fully studied, illustrations and keys to adults being available in Pomerantzev (1950) and for larvae and nymphs in Pospelova-Shtrom (1940). The relative richness of the Oriental region in *Haemaphysalis* species may be appreciated when one notes that, in the vast expanse of the U.S.S.R., Pomerantzev (1950) lists only 11 species, while there are 14 species presently known from an area of about ten miles square in Shimoga District.

The realisation of the involvement of these ticks in a virus-transmission cycle has necessitated the development of means of identifying larvae and nymphs as well as adults and has also brought out the need for a guide to their recognition. During the past several years, work in this direction has gradually progressed by laboratory rearing of immature stages taken in nature and by rearing the progeny of field-caught adults.

The material studied and illustrated, except as noted in the comments on the individual species, has originated from the KFD area in the western part of Shimoga District of Mysore State in south India. The keys include all species taken in the KFD area but, to broaden the usefulness of the guide, supplementary comments are provided to permit the recognition of other species (in each case known from only one to several specimens) recorded from elsewhere in south India.

The adults are mostly readily identified and at least partial illustrations of them are available in the literature. As larvae and nymphs for the most part have not been described and illustrated, and much of the published information is in error or inadequate for distinguishing between species, these stages are here illustrated as well as included in keys.

The illustrations for the keys to nymphs have been prepared with the aid of the camera lucida. The camera lucida could not be used effectively for the larvae as they are too small (of the order of 0·6 mm. in total length) to project
a useable image with the highest magnification feasible for the binocular dissecting microscope (7.5× objective and 15× ocular). Slide mounts of larvae for viewing through the compound microscope at higher magnifications would not be helpful, as, after mounting, the outlines of the palps and other characters

Fig. 1.—Hypothetical nymphs, illustrating principal characters and terminology used in the keys.

related to the form of structures are altered. The present keys and illustrations are based on unmounted material, this being the condition in which there is need to identify specimens, either alive, immobilised by cold or anaesthetised, or freshly dead.

The principal characters used and parts referred to in the keys are named on drawings of hypothetical nymphs in fig. 1.

**COMMENTS ON THE CHARACTERS USED IN THE KEYS**

Within restricted geographical regions *Haemaphysalis* ticks are, with only few exceptions (see comments on *H. leachii*), stable in appearance, although
clinal variation may be apparent in species of wide distribution, as for example in the case of *H. papuana* Thorell, which occurs from New Guinea to India.

---

Fig. 2.—Dorsal view (above) and ventral view (below) of the capitulum, coxa I and trochanter I of larvae of *Haemaphysalis* spp.
Identification of the HaemaphysalisTicks of South India

(Trapidio, Hoogstraal & Varma, 1964). Characters used in the keys have been found to be consistent for specimens from south India.

Key to larvae

All characters used in the key to larvae relate to the parts of the capitulum, either dorsally or ventrally, or the coxae and trochanters of the first pair of legs. It has not been possible to find useful characters in the other appendages, although in the nymphs and adults these have distinctive features.

For ready comparison of the larvae of the various species the illustrations of this stage are all displayed together (fig. 2), with species most alike arranged side by side.

The most useful character for division of larvae into two almost equal sized major groups is the presence or absence of the dorsal cornua. Specimens may be arbitrarily classed as being with or without cornua, although the various species show a graded series from those with very strong cornua, as much as half the length of the dorsal basis capituli as in H. cuspidata, to others with weak cornua. Some species may have a thickening and darkening of the cuticle at the posteroexternal angle of the basis, but unless this angle is developed into a distinct projecting point they are classed as without cornua. Reference to the illustrations (fig. 2) will clarify the condition for each species.

Other characters used in the key are all based on viewing the specimens in ventral aspect.

The relative proportions of the palps and the appearance of their external profile are characters of importance in species recognition. Reference to the illustrations in fig. 2 will clarify the conditions described in the text of the key.

In all species the ventral surface of palpal segment 3 is provided with a projection or spur. The length and form of this provides important distinguishing characters. Extreme forms are seen in H. aculeata with a very long stout spur on the one hand, and H. leachii and H. megalaimae on the other, with an almost imperceptible projection. The length of this spur is expressed in the keys in relation to how far it extends posteriorly toward the basal margin of the palpal segment 2 which it overlays. The measure of the length of this spur in relation to the second palpal segment is made by viewing the specimen at right angles to the spur and palp. Care must be exercised that the specimen is viewed in this way, as if the specimen is curled and the spur viewed at less than a right angle it will appear shortened and thickened. In practice, when carrying out routine identification of larval collections, the relative length, stockiness and sharpness of this spur in the various species become fixed in mind. Inexperienced persons, or those who have not recently examined larval material, would do well to examine and familiarise themselves with a series of known specimens before attempting to make judgments on the relative proportions of this spur in unknown larvae.

The first coxa in all species has some development of a posteriorly directed spur or ridge-like projection. The best developed of these may be seen in H. cuspidata. Species such as H. bispinosa or papuana kinneari have the spur much reduced, and in H. megalaimae it is hardly perceptible. It is particularly difficult to express the relative length of this spur as it curves gradually out from the margin of the coxa and its base cannot be distinctly defined. It may, however, be compared with the ventral spur of palpal segment 3 and this is done in couplet 12 for the separation of H. bispinosa and wellingtoni. Both these species have relatively narrow palps and they appear in general facies much alike. However, the greater development of the spur of coxa 1 and the smaller ventral spur of palpal segment 3 in wellingtoni are, in combination, distinctive from the small spur of coxa 1 and the somewhat larger spur of palpal segment 3 in bispinosa.
Key to nymphs

Nymphs provide a greater diversity of characters for the differentiation of species than do larvae. Their larger size also permits identification more readily.

Fig. 3.—Dorsal view (above) and ventral view (below) of nymph of *Haemaphysalis spinigera*, *H. leachi* sp. 2, *H. megalaimae* and *H. centropti*. The horizontal bar beside coxa 1 is 0.1 mm. in length.
at convenient working magnifications. Nevertheless, the distinction between certain of the species, for example *H. intermedia* and *wellingtoni*, requires very close observation and preferably direct comparison with reared specimens of known parentage.

The presence or absence of dorsal cornua does not provide as useful a character for a major separation of nymphs as in the case of the larvae, only two species, *H. aculeata* and *cuspidata*, having distinctively large cornua. The cornua of the latter have a narrow and elongate form which alone distinguishes this from all other species in the area. Only *H. leachii* has the cornua of the dorsal basis capituli essentially absent. The term “ventral cornua” with reference to a projecting point at the posteroexternal angle of the ventral basis capituli was introduced by Nuttall & Warburton (1915) and used by Sharif (1928); as it is a unique character for nymphs of *H. leachii* in this series it is used in our key. It provides a convenient means of avoiding confusion with the nymph of *H. spinigera* which *leachii* superficially resembles.

The character of greatest utility in dividing nymphs into two major categories is the outline of the palps, viewed either dorsally or ventrally. One group of eight species (*spinigera*, *leachii*, *megalaimae*, *centropi*, *papuana kimneari*, *cornigera shimoga*, *kyaanarensis* and *minuta*) have the palps broad and flaring; that is, the lateral salience of palpal segment 2 is well developed but without a distinct recurvature. The other six species (*aculeata*, *cuspidata*, *turturis*, *bispinosa*, *intermedia* and *wellingtoni*) have narrower palps giving the capitulum a compact appearance.

The ventrobasal margin of palpal segment 2 undergoes a number of distinctive variations characteristic of individual species. Of the species with a broad salience, both *H. spinigera* and *leachii* have the ventrobasal-external margin prolonged into a spur; *papuana kimneari*, *cornigera shimoga*, *kyaanarensis* and *minuta* have the juncture of this margin with the external margin formed into an approximate right angle, either relatively sharp or blunt; while in *megalaimae* and *centropi* the margin at this locus is rounded. The spur of the ventrobasal-external margin of palpal segment 2 in *spinigera* varies in sharpness, some specimens having it distinctly narrower and sharper than that illustrated (fig. 8). The relative sharpness of this spur appears related to the sex of the adult which will emerge, the spur of a “pre-male” being narrower and sharper than that of a “pre-female”. Other distinctive features of the ventrobasal margin of palpal segment 2 are seen in *H. leachii* and *megalaimae*, where there is an “overlapping” of the margin; in *leachii* the external margin extends internally over the internal margin while in *megalaimae* the reverse is true. Careful examination in good oblique light is necessary to see this character, and it is not used in the key, but may be seen in the illustrations (fig. 8).

Among the species with a compact capitulum there are distinctive characters in the external profile of the palps. Of particular usefulness is the presence or absence of an “abrupt recurvature”. The drawings of the nymphs of *H. turturis* and *intermedia* illustrate this condition, while that of *H. bispinosa* demonstrates the situation in which the external profile of the palps is essentially straight, without recurvature (see figs. 5 & 6).

As among the larvae, the nature of the ventral spur of palpal segment 3 is a valuable character. Its length in relation to palpal segment 2 is judged when the specimen is viewed as described for the larvae.

The internal margin of palpal segment 2, dorsally and ventrally, is provided with setae. Dorsally, all species in this series have one such seta, but ventrally the number, thickness and spacing of the setae differ and are of use in recognising species. We follow Sharif (1928) in referring to these setae of the ventral internal margin of the palps as “infrinternals” setae. All species have a single seta at or near the juncture of palpal segment 3 and 2 (it appears
Fig. 4.—Dorsal view (above) and ventral view (below) of nymph of *Haemophysalis papuana kinneari*, *H. cornigera shimoga*, *H. kyasanurensis* and *H. minuta*. The horizontal bar beside coxa I is 0.1 mm. in length.
to arise from segment 3) and as this does not vary, the number of infrainternal setae on segment 2 only are referred to in the key. The two slender, widely-spaced infrainternal setae of *H. turturis* and *biopinosa* are particularly useful in separating these species from *H. intermedia* and *wellingtoni*, which have four broader, closely-set setae (figs. 5 & 6). *H. kyasanurensis* is unique in the series with only one infrainternal seta (fig. 4).

The form of the spurs of all four coxae are of use. Thus in the case of *H. kyasanurensis* and *minuta*, whose capituli are similar in form, the well-developed sharp spurs of coxae II, III and IV of *kyasanurensis* readily distinguish it from *minuta* (fig. 4).

**Keys to males and females**

As the characters used in the keys to adults are ones generally applied in the tick literature, or relate to features already discussed above for larvae and nymphs, no further comments need be made here.

**Key to Larvae (see fig. 2)**

1—With cornua ... ... ... ... ... ... ... ... 2
   Without cornua ... ... ... ... ... ... ... ... 8

2—Cornua strong, about $\frac{1}{2}$ length of dorsal basis; spur of coxa I and
   ventral spur of palpal segment 3 elongate ... ... cuspida
cornua moderate or weak, $\frac{1}{2}$ or less length of dorsal basis ... ... 3

3—Palps conical without distinct recurvature of external profile of seg-
   ment 2; and spur of coxa I strong ... ... ... ... 4
   Palps with recurvature of external profile; and spur of coxa I either
   present but weak, or virtually absent ... ... ... ... 5

4—Palpal segment 3 with ventral spur long and stout, almost reaching
   basal margin of palpal segment 2; palps narrowly conical aculeata
   Palpal segment 3 with ventral spur minute, extending about $\frac{1}{3}$ distance
   to basal margin of palpal segment 2; palps broadly conical kyasanurensis

5—Dorsal basis with breadth not more than twice length, the posterior
   margin curving abruptly to form pronounced cornua ... ... 6
   Dorsal basis with breadth about $2\frac{1}{2}$ times length, the posterior margin
   curving gradually to form weak cornua ... ... ... ... 7

6—Ventral spur of palpal segment 3 extending $\frac{1}{2}$ distance to basal margin
   of palpal segment 2; salience greater; external profile of palps less
   strongly recurved ... ... ... ... ... ... ... ... spinigera
   Ventral spur of palpal segment 3 extending $\frac{1}{3}$ distance to basal margin
   of palpal segment 2; salience lesser; external profile of palps more
   strongly recurved ... ... ... ... ... ... ... ... turturis

7—Ventral spur of palpal segment 3 small, reaching less than $\frac{1}{2}$ distance
   to basal margin of palpal segment 2; coxa I with spur virtually
   absent ... ... ... ... ... ... ... ... ... ... minuta
   Ventral spur of palpal segment 3 strong, reaching about $\frac{1}{3}$ distance to
   basal margin of palpal segment 2; coxa I with distinct spur
   cernigera shimoga

8—Salience broad, palpal segment 2 twice or more breadth of 3 ... ... 9
   Salience reduced, palpal segment 2 only slightly broader than 3 ... 10

9—Palp segment 3 with prominent ventral spur reaching $\frac{1}{3}$ distance to
   basal margin of segment 2 ... ... ... ... papauna kimneari
   Palp segment 3 with ventral spur minute or obsolete leachii sp. 2

(L 1671)
10—Palpal segment 3 with ventral spur reduced to a slight ridge-like projection arising anterior to basal margin ... ... ... *megalaimae*

Palpal segment 3 with distinct ventral spur ... ... ... 11

11—Ventral spur of palpal segment 3 prominent, reaching $\frac{1}{2}$ or more distance to basal margin of palpal segment 2 ... ... *intermedia*

Ventral spur of palpal segment 3 short and blunt, reaching about $\frac{1}{2}$ distance to basal margin of palpal segment 2 ... ... 12

12—Coxa I with spur strong and sharp; ventral spur of palpal segment 3 shorter and more slender than the following; (spur of coxa I longer than ventral spur of palpal segment 3) ... ... *wellingtoni*

Coxa I with spur reduced and blunt; ventral spur of palpal segment 3 thick and blunt; (spur of coxa I subequal to or shorter than ventral spur of palpal segment 3) ... ... ... *bispinosa*

**KEY TO NYMPHS (see figs. 3-6)**

1—Salience broad, flared ... ... ... ... ... ... ... 2

Salience less well developed, with recurvature in some species ... ... ... ... 9

2—Ventralbasal-external margin of palpal segment 2 with retroverted spur ... ... ... ... ... ... ... 3

Ventralbasal-external margin of palpal segment 2 without retroverted spur ... ... ... ... ... ... ... ... 4

3—Ventral basis without cornua; ventral spur of palpal segment 3 larger, reaching about $\frac{1}{2}$ distance to basal margin of palpal segment 2 ... ... *spinigera*

Ventral basis with small but distinct sharp cornua; ventral spur of palpal segment 3 minute, reaching about $\frac{1}{2}$ distance to basal margin of palpal segment 2 ... ... ... ... *leachii* group

4—Basal margin of palpal segment 2 rounded both dorsally and ventrally ... ... ... ... ... ... ... 5

Juncture of ventrobasal margin of palpal segment 2 and externobasal margin an angle, blunt or sharp ... ... ... ... ... ... ... ... 6

5—Palpal segment 3 with slight ventral projection only; coxae III and IV with slight ridge-like projections ... ... ... ... *megalaimae*

Palpal segment 3 with ventral distinct sharp spur; coxae III and IV with small sharp spurs ... ... ... ... ... ... *centropi*

6—Coxa I with reduced, broad, rounded spur; coxae II to IV with broad ridge-like projections only ... ... ... ... ... ... ... ... 7

Coxa I with well-developed conical spur; coxae II to IV with distinct, sharp spurs ... ... ... ... ... ... ... ... ... ... 8

7—Ventral spur of palpal segment 3 almost twice as long as its basal breadth, peg-like, blunt; spur of coxa I less prominent, length equal to or less than ventral spur of palpal segment 3 ... ... *papuanus kinneari*

Ventral spur of palpal segment 3 only slightly longer than its basal breadth, tapering, pointed; spur of coxa I more prominent, longer than ventral spur of palpal segment 3 ... ... *cornigera shimogai*

8—Spur of coxa I elongate, narrow, length almost twice basal breadth; spurs of coxae II to IV reduced but retaining relatively narrow form of coxal spur I; palpal segment 2 with a single infratemporal seta ... ... ... ... ... ... *kyasanurenensis*

Spur of coxa I subtriangular, length only slightly greater than basal breadth; spurs of coxae II to IV reduced but retaining subtriangular form of coxal spur I; palpal segment 2 with two infratemporal setae ... ... ... ... ... ... *minuta*
9—Ventral retroverted spur of palpal segment 3 extending more than \( \frac{3}{4} \) distance to basal margin of palpal segment 2; strong dorsal cornua, \( \frac{3}{4} \) or more length of basis ... ... ... ... 10
Ventral retroverted spur of palpal segment 3 extending less than \( \frac{3}{4} \) distance to basal margin of palpal segment 2; dorsal cornua present but less than \( \frac{1}{2} \) length of basis ... ... ... 11

10—Coxal spurs strong, progressively diminishing in size from I to IV; cornua only slightly longer than their basal breadth ... aculeata
Coxal spur I strong, coxae II to IV with ridge-like projections; cornua greatly elongate, approximately twice as long as their basal breadth ... cuspidata

11—Coxae III and IV with ridge-like projections only; ventral trochantal spurs present; two slender, well-spaced infrainternal setae ... 12
Coxae III and IV with distinct sharp spurs; ventral trochantal spurs absent or obscure; four feathery, closely-set infrainternal setae ... 13

12—Palps broader, external profile with pronounced recurvature; palpal segment 3 with ventral spur strong, extending \( \frac{1}{2} \) distance to basal margin of palpal segment 2 ... ... ... ... turritis
Palps narrower, external profile without recurvature; palpal segment 3 with ventral spur short, blunt, extending about \( \frac{1}{2} \) distance to basal margin of palpal segment 2 ... ... ... ... bispinosa

13—In ventral aspect, external profile of palps with abrupt recurvature; dorsobasal margin of palpal segment 3 extending beyond internal margin of palpal segment 2 ... ... ... ... intermedia
In ventral aspect, external profile of palps without distinct recurvature; dorsobasal margin of palpal segment 3 reaching internal margin of palpal segment 2 only ... ... ... ... wellingtoni

**Key to Males**

1—Salience broad and with ventrobasal-external margin of palpal segment 2 developed into a prominent spur or projection ... ... ... 2
Salience not broad, or if broad then ventrobasal-external margin of palpal segment 2 rounded ... ... ... ... ... ... ... ... 5

2—Coxa IV with greatly elongated sharp spur(s), much exceeding length of other coxal spurs ... ... ... ... ... ... ... ... 3
Coxa IV without greatly elongated sharp spur(s) ... ... ... ... ... ... ... ... 4

3—Coxa IV with single spur ... ... ... ... ... ... ... spinigera
Coxa IV with double spur ... ... ... ... ... ... cornigera shimogae

4—Dorsal external margin of palpal segment 2 developed into a prominent spur ... ... ... ... ... ... ... ... ... ... teachii group
Dorsal external margin of palpal segment 2 rounded ... ... ... ... ... ... ... ... ... ... minuta

5—Cornua very well developed, as long as dorsal basis ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 6
Cornua less well developed, not exceeding \( \frac{1}{2} \) length of dorsal basis ... ... ... ... ... ... ... ... ... ... 7

6—Spurs of coxa I and trochanter I spatulate; ventrally, palpal segment 2 one and a half times as long as segment 3 ... ... ... ... ... ... aculeata
Spurs of coxa I and trochanter I pointed; ventrally, palpal segment 2 subequal to length of segment 3 ... ... ... ... ... ... ... cuspidata

7—Salience broad, rounded, breadth of palpal segment 2 greater than length of ventral basis ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 8
Salience less well developed, breadth of palpal segment 2 less than length of ventral basis ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 10
8—Breadth of palpal segment 2 subequal to its length; palpal segment 3 with dorsointernal retroverted spur extending about ¼ distance to basal margin of palpal segment 2 ... ... ... ... Wellingtoni
Breadth of palpal segment 2 distinctly greater than its length; palpal segment 3 without dorsointernal retroverted spur ... ... ... ... 9

9—Basal margin of palpal segment 2 with blunt rounded corners; six or seven feathery, closely-set infrainternal setae ... ... Centropi
Basal margin of palpal segment 2 semicircular in outline; two or three slender, well-spaced infrainternal setae ... ... Megalaimeae

10—Palpal segment 2 with salience reduced, extending little beyond lateral margin of basis ... ... ... ... ... ... ... ... ... ... 11
Palpal segment 2 with about half its breadth extending beyond lateral margin of basis ... ... ... ... ... ... ... ... ... ... 12

11—Palpal segment 3 with ventral retroverted spur slightly longer than its basal breadth; ventrobasal margin of palpal segment 2 meeting internal margin at an acute angle; coxa I with spur subtriangular, length about equals breadth at base; dorsobasal margin of palpal segment 3 with a small median spur ... ... ... Pupana kinneari
Palpal segment 3 with ventral retroverted spur rudimentary; ventrobasal margin of palpal segment 2 truncate; coxa I with spur elongate, peg-like, length about twice breadth at base; dorsobasal margin of palpal segment 3 without spur ... ... ... Kyasornurensis

12—Palpal segment 3 with a dorsal, broad, median, ridge-like projection slightly overlapping apical margin of palpal segment 2 ... ... Turturis
Palpal segment 3 with a distinct dorsal retroverted spur ... ... ... 13

13—Scutum elongate, punctations few and shallow; palpal segment 3 with dorsal, median, elevated spur; infrainternal setae slender, well-spaced ... ... ... ... ... ... ... ... ... ... Bispinosa
Scutum ovate, punctations numerous and deep over entire surface; palpal segment 3 with dorsal, sharp, triangular, subinternal, retroverted spur; infrainternal setae feathery, close-set ... ... ... Intermedia

KEY TO FEMALES

1—Salience broad, palpal segment 2 broader than long ... ... ... ... ... 2
Salience not broad, or if broad, palpal segment 2 not broader than long 7

2—Palpal segment 3 with ventral retroverted spur prominent, length about twice its basal breadth ... ... ... ... ... ... ... ... ... ... 3
Palpal segment 3 with ventral retroverted spur reduced, length subequal to or less than its basal breadth ... ... ... ... ... ... ... ... ... ... 5

3—Dorsobasal margin of palpal segment 3 without spur ... ... Leachii group
Dorsobasal margin of palpal segment 3 with median retroverted spur 4

4—Palpal segment 2 with ventrobasal external margin forming a sharp spur ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... Spinigera
Palpal segment 2 with juncture of ventrobasal and external margin a blunt angle ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... Cornigera Shimoga

5—Dorsobasal margin of palpal segment 3 with median retroverted spur ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... Minuta
Dorsobasal margin of palpal segment 3 without spur ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 6

6—Basal margin of palpal segment 2 with blunt rounded corners; eight to ten broad, closely-set, infrainternal setae ... ... ... ... ... Centropi
Basal margin of palpal segment 2 semicircular in outline; three slender, well-spaced, infrainternal setae ... ... ... ... ... Megalaimeae
IDENTIFICATION OF THE *HAEMAPHYSALIS* TICKS OF SOUTH INDIA

7—Ventrally, palp segment 2 about twice length of segment 3; spurs of coxa I and trochanter I elongate, spatulate ... *aculeata*

Ventrally, palp segment 2 less than twice length of segment 3; spurs of coxa I and trochanter I not spatulate ... ... ... 8

8—Palp segment 3 with dorsal and ventral retroverted spurs elongate, the ventral spur reaching to or beyond basal margin of palp segment 2 ... ... ... ... ... 9

Palp segment 3 with ventral retroverted spur not reaching basal margin of palp segment 2 ... ... ... ... ... 9

9—Profile of capitulum broadly deltoid; palp segment 2 with juncture of ventrobasal and external margin a rounded angle; palp segment 3 with sharp, dorsobasal-internal retroverted spur extending about 1/2 length of palp segment 2 ... ... ... ... 9

Profile of capitulum not broadly deltoid; palp segment 2 with basal margin not angled; palp segment 3 with dorsobasal-internal margin either without spur, or if spur present not extending 1/4 length of segment 2 ... ... ... ... ... 10

10—Salience reduced, palp segment 2 extending little beyond lateral margin of basis ... ... ... ... ... ... ... ... ... 10

Palp segment 2 with 1/2 or more of its breadth extending beyond lateral marginal of basis ... ... ... ... ... ... ... ... ... 11

11—Palp segment 3 with ventral retroverted spur prominent, sharp, extending approximately 1/4 distance to basal margin of palp segment 2; dorsobasal margin of palp segment 3 with small median spur; coxae II to IV with small blunt spurs *papuan* *kinneari*

Palp segment 3 with ventral spur short, only slightly overlapping apical margin of palp segment 2; dorsobasal margin of palp segment 3 without spur; coxae II to IV with distinct sharp spurs ... ... ... ... ... ... ... 12

kyasanurensis

12—Palp segment 3 with a dorsal broad projection slightly anterior to basal margin ... ... ... ... ... ... ... ... ... 12

Palp segment 3 with a well-developed dorsal retroverted spur ... 13

13—Punctations of scutum few and shallow; palp segment 3 with dorsal median elevated spur; four or five slender, well-spaced infradental setae ... ... ... ... ... 13

Punctations of scutum numerous and deep, distributed over entire surface; dorsobasal margin of palp segment 3 with sharp triangular subinternal retroverted spur; six or seven feathery, close-set infradental setae ... ... ... ... 14

*intermedia*

Comments on Individual Species Included in the Keys

*Haemaphysalis aculeata* Lavarría, 1905

The nymphs and larvae have not previously been figured or described in literature.

*Haemaphysalis bispinosa* Neumann, 1897

... is a common species widely distributed in the Oriental region. The illustrated by Nuttall & Warner (1915, fig. 362a), the progeny of an female, matches our reared material in essential features. The nymphs of this species (fig. 392), based on a specimen taken from a company with a male and female of *bispinosa* at Madras, has the general
Fig. 5.—Dorsal view (above) and ventral view (below) of nymph of *Haemaphysalis aculeata*, *H. cuspidata*, *H. turturis* and *H. bispinosa*. The horizontal bar beside coxa I is 0.1 mm. in length.
appearance of our reared material but differs in one important regard. The
dorsointernal margin of palpal segment 3 is represented as projecting posteriorly
to form a point. This feature is also remarked in their text. Our reared
nymphs lack this character (see fig. 5). The larva and nymph from Australia
illustrated by Roberts (1963, fig. 21), are similar to our specimens.

_Haemaphysalis centropi_ Kohls, 1940

This species was described from Burma, Malaya and the Philippines based
on adults from birds, principally crow-pheasants of the genus _Centropus_.
Although one of us (P. K. R.) has examined a series of more than 8,000 birds
in the study area, including more than 150 crow-pheasants, the species was taken
only once—a male on a common myna (_Acridotheres tristis_). This specimen
and another male taken on a small whitethroat (_Sylvia minula_) at Hingolghad,
Gujarat State, have been confirmed as _H. centropi_ by Dr. H. Hoogstraal on
comparison with type material. This is the one species known from the study
area of which larval material has not been obtained. The nymph which is
included in the key and illustrated was taken on a crow-pheasant (_Centropus
sinensis_) near Poona.

_Haemaphysalis cornigera shimoga_ Trapido & Hoogstraal, 1964

The nymphs and larvae of the nominate subspecies have not been figured
or described in the literature. In recently describing the Indian race, _shimoga_,
Trapido & Hoogstraal have illustrated slide-mounted larvae and nymphs.

_Haemaphysalis cuspidata_ Warburton, 1910

Nutall & Warburton (1915) figure and briefly describe the larva and nymph
of _H. cuspidata_ based on specimens taken in association with two females on
a palm civet (_Paradoxurus niger_) at Colombo, Ceylon (from the type series
of Warburton, 1910). Allowing for differences in angles of view and method of
drawing, their illustration of the nymph (fig. 369) is reasonably approximated
by our experience with and drawings of reared material. Slight differences do
appear in the representation of the length of the ventral spur of palpal segment 3
and the extent of the development of the spurs or ridge-like projections of coxae II,
III and IV. There is, however, a strong element of doubt about the identity
of the larva they illustrate (fig. 370). The larva of _H. cuspidata_ is one of the
most distinctive in our series with strongly developed cornua, ventral spur of
palpal segment 3 reaching the basal margin of palpal segment 2, and a strong
spur on coxa 1 (see fig. 2). These features are not apparent in the Nutall &
Warburton drawings.

_Haemaphysalis intermedia_ Warburton & Nutall, 1909

Until now this taxon has been referred to in the literature as _H. parva_
Neumann, 1908. Morel (1963) has shown the name _parva_ to be unavailable,
and Trapido & Hoogstraal (1963) have concluded that _H. intermedia_ is the next
available name. Hoogstraal & Trapido (1963c) have re-examined and re-identified
various lots which had been confused in the literature, and also redescribed and
illustrated all stages.

Nutall & Warburton (1915) describe the nymph and larva of _intermedia_ (cited
as _parva_) in a sentence. "Nymph and Larva: These only differ from the
corresponding stages of _H. bispinosa_—in having stronger cornua and more hairy
dorsum and legs." In fact, the cornua are absent in the larvae of both _H.
bispinosa_ and _intermedia_ (see fig. 2), and while cornua are present in the nymphs
they are essentially the same in both species (see figs. 5 & 6). We do not
find the relative 'hairiness' of the two species of use in their separation.
(1928) briefly describes the principal features of the nymph of *intermedia* (cited as *parva*) without indicating the source of his material. He remarks that the ventral spur of palpal segment 3 is longer than that of *bispinosa* and that there

![Diagram](image)

*intermedia*  
*wellingtoni*  
*kutchensis*  
*leachii sp. 1*

Fig. 6.—Dorsal view (above) and ventral view (below) of nymph of *Haemaphysalis intermedia*, *H. wellingtoni*, *H. kutchensis* and *H. leachii sp. 1*. The horizontal bar beside coxa I is 0.1 mm. in length.

are four feathery infranternal bristles on palpal segment 2, characters confirmed in our reared material. We do not, however, find Sharif’s statement that the
cornua of *intermedia* (cited as *parva*) are longer than in *bispinosa* to be true of our material (see figs. 5 & 6).

**Haemaphysalis kyasanurensis** Trapido, Hoogstraal & Rajagopalan, 1964

This recently described species is closely related to *Haemaphysalis formosensis* Neumann (1913), the immature stages of which are unknown. In the description of *H. kyasanurensis* the larva and nympha are illustrated from slide-mounted reared material.

**Haemaphysalis leachi** (Audouin), 1827

Sharif (1928) recognised two varieties of this species in India, the typical form and the variety *indica* Warburton (1910). The name *leachi* has been used for ticks from Africa, Transcaucasia, southern Asia, Australia and New Zealand. Nuttall & Warburton (1915) remarked the wide range of variation to which the species is subject, and Hoogstraal (1938) has recently described and illustrated extreme forms found in Egypt.

The species is rare in our Mysore collections, having been taken once on a mongoose and once on a jackal. Adults and nymphs have also been gotten from a fox collected in Kutch, Gujarat State, in a dry semidesert situation some 800 miles north-west of the Mysore study area.

Larvae reared from the Kutch females (designated as species 1) and those reared from females off a mongoose from Mysore State (designated as species 2) have striking differences in the form of the salience and the basal margin of palpal segment 2. The ventrobasal-external angle of palpal segment 2 is developed as a prominent spur in species 2 while there is only a minute spur at this locus in species 1 (see fig. 2). The reared nymphs show less pronounced differences, but they may be separated by the more prominent carpal spurs of species 1 (see figs. 3 & 6). The nymph of species 1 also resembles that of *spinigera* but is readily distinguished from it by the smaller ventral spur of palpal segment 3 and the obsolete dorsal cornua. Only the larva and nymph of the form taken in Mysore (sp. 2) are included in the key, but illustrations of the larva and nymph of species 1, not yet known from the area, are included for comparison. A study of variations of all stages from a diversity of hosts and geographical localities will be necessary before the taxa can be assigned specific or subspecific names with any assurance.

**Haemaphysalis megalaissa** Rajagopalan, 1963

This species, closely related to *H. centropi*, was found by one of us (P. K. R.) sparingly on the small green barbet (*Megalaima viridis*) in the course of a study of ticks ectoparasitic on birds in the study area. It is known from no other host. The association of sexes and immature stages is based on the finding of distinctive entities on a single host species. As the *Haemaphysalis* fauna of this restricted area has been studied in detail, and other recognised species can be eliminated from consideration, it seems likely that these associations of the stages and sexes will be confirmed when rearing has been accomplished.

**Haemaphysalis minuta** Kohls, 1950

Kohls (1950) described this species from males only, off jungle fowl in Ceylon. Santos Dias (1956) described what he supposed to be the female based on material at the Musée d'Histoire Naturelle, Paris, also from Ceylon. He does not, however, note the character of the presence in the female of a dorsal median retroverted spur of palpal segment 3 found in our reared associated material and here used in the key to females. This spur is not present in the males. The immature stages of this species have not previously been described or figured.
Haemaphysalis pupuana kinneari Warburton, 1913

Ticks attributed to *H. pupuana* Thorell, 1882, are described and figured by Nuttall & Warburton (1915) in their monograph of the genus from New Guinea, Borneo, the East Indies and Malaya. Anastos (1950) gives other records from south-east Asia. In the course of the present work it was realised that *H. kinneari* described by Warburton, from a single female taken on a tiger from an Indian locality near the area where this study was carried on, represented a western subspecific population of *H. pupuana*. The situation is fully dealt with by Trapido, Hoogstraal & Varma (1964). They conclude that the larva and nymph circumstantially attributed to *H. pupuana* by Nuttall & Warburton (1915, figs. 340, 341), are not of that species, and provide illustrations of slide-mounted reared larvae and nymphs of *H. pupuana kinneari*.

**Haemaphysalis spinigrã** Neumann, 1897

While this is the most abundant species of the KFD area, the nymphs and larvae have not previously been figured or described in the literature.

**Haemaphysalis turris** Nuttall & Warburton, 1915

This name is based on the description of a single male specimen found on a dove in Ceylon. During the present work detailed studies have been made of the holotype specimen (deposited in the Berlin Museum) and other material from India and Ceylon. Specimens of *turris* were found to have been misidentifie ted under other names in various collections. The results of these studies are reported by Trapido, Hoogstraal & Varma (1963) who also provide illustrations of all stages, those of the larvae and nymphs based on reared slide-mounted specimens.

**Haemaphysalis wellingtoni** Nuttall & Warburton, 1907

The nymph and larva of *H. wellingtoni* are figured and briefly described by Nuttall & Warburton (1915, figs. 419, 420), based on material taken in company with males on a domestic fowl in Malaya. Our reared material corresponds in essential features with their illustrations.

**Comments on Species not included in the Keys**

**Haemaphysalis campanulata** Warburton, 1908

This species was described from China, and all records cited by Nuttall & Warburton (1915) are from China and Japan with the exception of one lot of the Nuttall collection recorded from Satharangapara, Travancore (now Kerala State), south India. A search of the Nuttall collection now lodged at the British Museum (Natural History) by one of us (H. T.) has failed to reveal this lot. In the light of the confusion which has surrounded the group to which this species belongs, we question this record. Sharif (1928) reports the species from north Indian localities but it has not been encountered by us.

**Haemaphysalis flava** Neumann, 1897

The description of this species is based on material from Japan although Neumann attributed it to, with doubt, specimens from Ceylon in the Paris Museum. One of us (H. T.) has examined this Ceylon collection and found the specimens to be *Haemaphysalis intermedia* Warburton & Nuttall, 1909, *sensu* Trapido & Hoogstraal (1963). Indian records of *H. flava* have all been from the north of the subcontinent with the exception of a single half-century-old record from Madras (Nuttall & Warburton, 1915). The species has not been
encountered by us. The male and female have been described and illustrated by Nuttall & Warburton (1915), and Sharif (1928) describes and figures the nymph, although he does not indicate if it is a reared specimen or one circumstantially associated with adults.

**Haemaphysalis howletti** Warburton, 1913

Mr. Vijai Dhanda of the Virus Research Centre has recently obtained, in the vicinity of Poona, material identified by Dr. Harry Hoogstraal as *H. howletti* (V. Dhanda. Description of immature stages of *Haemaphysalis howletti* Warburton, 1913 (Ixodoidea: Ixodidae), with redescription of adults. *In preparation*). This species was described from Rawalpindi, north-western India (now Pakistan). In the adult keys it will run out to couplet 12. It may be distinguished from the species following that couplet (*turturis, bispinosa* and *intermedia*) by the absence of a spur or projection on the dorsobasal margin of palpal segment 3. Other distinctive features are the subequal spurs of coxae I and IV and the progressive increase in size of ventral trochanter spurs from I to IV. Descriptions, illustrations and distinctive features of the larvae and nymphs will be given by Dhanda.

**Haemaphysalis kutchensis** Hoogstraal & Trapido, 1963

This recently described species (Hoogstraal & Trapido, 1963b) has not been found in the study area and thus is not included in the keys. However, as the immature stages are frequent ectoparasites of birds in the region to the north and west through which migrants pass to south India, illustrations of the larva (fig. 2) and nymph (fig. 6) based on reared material are included for comparison. The larva will key out with that of *H. intermedia* and they are difficult to separate; characters distinguishing *kutchensis* are the slightly more slender and longer ventral spur of palpal segment 3, and the broader angle at which the ventrobasal margin meets the internal margin of palpal segment 2. The nymph also keys out with *H. intermedia* but may be distinguished from it by the internally directed spur of coxa IV which is directed posteriorly in *intermedia* and the slightly more slender and sharper ventral spur of palpal segment 3. The males and females key out to couplet 12, but may be conveniently separated from the species grouped after this couplet (*turturis, bispinosa* and *intermedia*) by the appearance of the dorsobasal margin of palpal segment 3. This margin lacks the median spur of *bispinosa*, the subterminal spur of *intermedia* or the elevated broad projection of *turturis*. It is distinguished by a rounded projection at the basointernal angle of segment 3 extending well beyond the internal margin of palpal segment 2.

**Haemaphysalis paraturturi** Hoogstraal, Trapido & Rebello, 1963

Apart from the specimens recorded as *Haemaphysalis bispinosa var. intermedia* by Sharif (1928), we have this species from a jungle cat (*Felis chaus*) from 25 miles north-west of Hyderabad (taken in company with *H. silvafelis* and *intermedia*) and also from Poona, a fed nymph which emerged as a female, taken on a crow-phaasant (*Centropus sinensis*) and two males from dogs. We also attribute to this species a nymph from a goat, taken near Poona.

The nymph keys out with that of *H. intermedia* from which it differs by the presence of three moderately slender infrainternal setae instead of the four broad, closely-set setae of that species. Of the species in the keys, *H. turturis* is the one of which the adults are closest to those of *paraturturi*; they may be most readily separated by the number and form of the infrainternal setae, which are slender, well-spaced apart, and four or five in *turturis* but broad, closely-set, and seven to nine in *paraturturi*. 
Haemaphysalis silvafelis Hoogstraal & Trapido, 1963

This newly described species (Hoogstraal & Trapido, 1963a) is known only from six males and one female taken on a jungle cat (Felis chaus) 25 miles north-west of Hyderabad, Andhra State. The male and female will key out with H. turturis but may be separated from that species by the absence of ventral trochanteral spurs, the presence of seven broad, rather than four or five slender, infranternal setae, and the less well developed dorsal ridge-like projection of palpal segment 3. Characteristic of both sexes and serving to separate this species from H. paraturturis (as well as from turturis) are the large, coarse punctations densely scattered over the scutum.

Summary

Investigations of the natural history of the virus of Kyasanur Forest disease since its discovery during 1957 in Shimoga District, Mysore State, south India have concentrated much attention on ticks of the genus Haemaphysalis in the region, as virus has repeatedly been isolated from them.

Keys are provided for larvae, nymphs and adults of both sexes of the 14 species of Haemaphysalis that have been taken in the area, with supplementary comments on six other species of the genus recorded, or likely to occur, elsewhere in south India. Illustrations are given showing the characters of the larvae and nymphs that are used in the keys.

Acknowledgements

We have been in constant consultation with Dr. Harry Hoogstraal of the Naval Medical Research Unit Number Three, Cairo, and Dr. Glen Kohls of the Rocky Mountain Laboratory, Hamilton, Montana, on the many taxonomic problems which arose during the work leading to the preparation of this guide, and we are indebted to them for much help and advice. The illustrations accompanying the keys have been prepared by Mr. R. V. Mittapelly under the technical supervision of two of the authors (H. T. and M. J. R.).

The final draft of the guide was prepared by the senior author while a guest at the Bureau of Animal Population, Oxford, and this author is grateful to the Director of the Bureau, Mr. Charles Elton, for the facilities afforded him.

References

Anastos, G. (1950). The scutate ticks, or Ixodidae, of Indonesia.—Ent. amer. 30 pp. 1–144.


© Commonwealth Agricultural Bureaux, 1964

TICKS ECTOPARASITIC ON MONKEYS IN THE KYASANUR FOREST
DISEASE AREA OF SHIMOJA DISTRICT, MYSORE STATE, INDIA

HAROLD TRAPIDO, M. K. GOVERDHAN, P. K. RAJAGOPALAN, AND M. J. REBELLO
The Virus Research Centre, Poona, India

Reprinted from American Journal of Tropical Medicine and Hygiene
Vol. 13, No. 5, pp. 723-725
September, 1964
Copyright © 1964 by The Williams & Wilkins Co.
Printed in U.S.A.
The attention of the Virus Research Centre was first drawn to the activity of the agent subsequently named Kyasanur Forest disease (KFD) virus in 1957 by reports of monkey deaths in a forested area of Shimoga District, Mysore State.\(^1\) \(^2\)

In the course of studies of the epidemiology of this tick-borne virus, monkeys obtained at irregular intervals under a variety of circumstances were examined to establish the identity of tick species ectoparasitic on them. As there is a marked seasonal cycle of various stages of the tick species in the region, it was thought desirable to examine a sample of monkeys on a regular basis throughout the year. Thus, during the 1-year period from November 1959 through October 1960 an attempt was made to collect and examine host samples of uniform size each month. The present paper reports the findings of ticks on these two series of monkeys, the one collected on a monthly basis over a 1-year period, the other consisting of dead, sick, and shot monkeys examined at irregular intervals from 1957 to 1961.

Hoogstraal and Theiler\(^3\) have recently summarized records of ticks of several genera and a number of species parasitizing primates other than man in Africa, but there are relatively few literature records of ticks on subhuman primates in the Oriental region. Audy et al.\(^4\) did not find any ticks on a series of macaques, leaf-monkeys and lorises examined in their survey of the host distribution of ticks in Malaya. Kohls,\(^4\) in his summary of ticks of Malaya and Borneo, gives circumstantial evidence of *Haemaphysalis cornigera* and *Amblyomma testudinarium* on a red monkey (*Presbytis rubicunda*) based on the finding of the ticks on human beings who had handled the monkey. In Indonesia *Haemaphysalis koningsbergi* has been recorded on the slow loris (*Nycticebus coucang*) and *H. hylobatis* on the Siamang (*Hylobates syndactylus*).\(^5\) So far as we are aware, at the time these studies were begun the literature contained only a single report of an identified tick on a monkey in India, an adult *Haemaphysalis bispinosa* on "*Macaca sinica*" at Maddathory, Travancore, as well as one Ceylon record of *H. bispinosa* on a "black monkey."\(^7\)

The senior author has been able to find in the Nuttall collection now at the British Museum (Natural History) the specimen on which the Ceylon record is based and reidentified it as *H. intermedia*. At the time of the Nuttall and Warburton work on the genus *Haemaphysalis*, several species were confused under the name *bispinosa*, and the identity of the tick recorded from Maddathory is therefore open to question. Incidental to a study of monkey malaria, Donovan\(^8\) remarked finding ticks on monkeys at the foot of the Nilgiri Hills in South India, but they were not identified. A preliminary list of ticks found on monkeys in the KFD area has been given by Trapido.\(^9\)

Recent work on the systematics of the *Haemaphysalis* ticks of South Asia has necessitated changes in the nomenclature of some taxa. The names used in this paper follow a recently prepared guide to the *Haemaphysalis* of South India\(^10\) in which these changes are incorporated.

The area in which KFD virus is known to be active, as evidenced by human cases and dead monkeys, is at an elevation of 580 to 650 meters in rolling hill country east of the crest of the Western Ghats which is locally termed the "malaad." Over a distance of 50 km eastward from the western margin of the area close to the crest of the ghats, annual rainfall drops sharply from 300 cm or more to 75 cm. The annual rainfall distribution is typical of the tropical monsoon pattern with about 80% of the annual total falling in the period from mid-June to mid-September. Monthly mean temperatures vary from a minimum of 22°C in January to a maximum of 28°C in April at the height of the dry season.

The vegetation cover reflects the rainfall gradient with tropical broadleaved evergreen
forest to the west and a transition eastward to semi-deciduous or deciduous forest mixed with thorn bamboo.

Two species of monkeys are common in the area, the langur (Presbytis entellus) and the bonnet macaque (Macaca radiata). While no precise measure of the total populations and relative numbers of these animals is available, it may be said that before the area was affected by KFD the population was large, and the number of Presbytis was substantially greater than that of Macaca. During the period from 1957 to the middle of 1961 included in this study, records of approximately 600 dead Presbytis and 100 dead Macaca were accumulated.

Ticks were most often found on the ears, about the face, in the soft skin at the base of the fingers and toes, in the axillae and groin and about the buttocks.

At the beginning of the work the immature stages of the ticks of the genus Haemaphysalis, which are the commonest ticks on monkeys, could not be identified to species but later these were associated, with adult forms described in the literature. As the material from the 1-year study was all preserved to permit later authentication based on more complete association of immature stages with known adults, all ticks of the genus Haemaphysalis in this series are identified to species.

**MONKEYS EXAMINED IN THE 1-YEAR SAMPLE**

In this series of shot monkeys collected on a routine monthly basis from November 1959 to October 1960, the aim was to examine approximately 30 monkeys each month. This number was chosen as one that could reasonably be attained, would not reduce the local monkey population too severely, and would give a useful picture of the tick species attacking monkeys and their seasonal fluctuations. The number of monkeys actually examined in this series approximated the goal, with monthly examinations of from 26 to 32 hosts and a total of 357 animals examined during the year (Table 1).

The ticks enumerated in Table 1 were in all instances found attached except for one collection from Macaca examined in January 1960. In this instance, while note was made in the field that some ticks were attached and others crawling, these were not preserved separately. As the species present, Haemaphysalis spinigera, cuspidata and aculeata and Dermacentor auratus, had all been found attached at one time or another on other monkeys in the series, the ticks in this collection have been included in the tabulation.

As shown in Table 1, of 232 Presbytis examined, 85 were positive for ticks while of 125 Macaca, 42 were positive. On the 127 monkeys positive of both species, 891 ticks were found. The relative number of monkeys positive for ticks varied widely in different months of the year, with a high proportion positive from October to February and a peak in November when 25 of 26 monkeys examined were positive. Ticks were found in all months except the monsoon period from June to September (120 monkeys examined).

Ticks of the genus Haemaphysalis made up 78%, and Dermacentor 20%, of all ticks taken. The large proportion of H. spinigera on monkeys (70% of all ticks and 90% of all Haemaphysalis) is of special interest since this is a species from which KFD virus has been frequently recovered. Only 29 specimens of H. turturis, from which virus has also been frequently obtained, were found on the monkeys. Small numbers of six other species of Haemaphysalis were taken. Ticks of the genus Ixodes, which are common ectoparasites of rodents in the area, were represented by only a single collection of 15 specimens taken in May.

Larval ticks were found on monkeys in all months except April and the four monsoon months June to September, but with a distinct peak in the post-monsoon months, October and November. Nymphs were present in the collections in all but the monsoon months. Adult ticks were rare on monkeys, only two specimens having been taken, one Haemaphysalis spinigera in January and one H. turturis in May.

A closer analysis of the occurrence of separate and mixed infestations of larvae and nymphs of H. spinigera is of interest for what it may reveal about opportunity for KFD virus to move from one tick stage to the next through simultaneous feeding on a common host which is known to circulate high titers of virus. The frequency with which spinigera larvae and nymphs were found as separate and mixed infestations on monkeys in each month of the study year is shown in Table 2. In October, when the post-monsoon crop of larvae appears, 291 larvae were
<table>
<thead>
<tr>
<th>Month</th>
<th>Haemaphysalis sp.</th>
<th>Amblyomma sp.</th>
<th>Dermacentor auratus</th>
<th>Rhipicephalus haemaphysoides</th>
<th>Total all species</th>
<th>No. hosts pro/no. hosts exam.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov. 1960</td>
<td>164</td>
<td>22</td>
<td>0</td>
<td>164</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Dec. 1960</td>
<td>21</td>
<td>7</td>
<td>0</td>
<td>28</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Jan. 1960</td>
<td>38</td>
<td>11</td>
<td>0</td>
<td>38</td>
<td>48</td>
<td>1</td>
</tr>
<tr>
<td>Feb. 1960</td>
<td>36</td>
<td>10</td>
<td>2</td>
<td>22</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Mar. 1960</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>16</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Apr. 1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>May 1960</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>June 1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July 1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aug. 1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept. 1960</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oct. 1960</td>
<td>291</td>
<td>0</td>
<td>1</td>
<td>292</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>525</td>
<td>97</td>
<td>1</td>
<td>575</td>
<td>118</td>
<td>2</td>
</tr>
<tr>
<td>Subtotal</td>
<td>349</td>
<td>8</td>
<td>0</td>
<td>381</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Macaca</td>
<td>176</td>
<td>89</td>
<td>1</td>
<td>194</td>
<td>107</td>
<td>2</td>
</tr>
</tbody>
</table>

* L = larvae, N = nymph, A = adult.
### TABLE 2

Incidence of separate and mixed infestations of larvae and nymphs of *Haemaphysalis spinigera* on *Presbytis entellus* and *Macaca radiata* obtained in 1-year sample

<table>
<thead>
<tr>
<th>Hosts with</th>
<th>Total hosts infested</th>
<th>Total hosts examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae only</td>
<td>Larvae and nymphs</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>No. hosts pos.</td>
</tr>
<tr>
<td>Nov. 1959</td>
<td>92</td>
<td>12</td>
</tr>
<tr>
<td>Dec. 1959</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Jan. 1960</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Feb. 1960</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Mar. 1960</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Apr. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May 1960</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>June 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aug. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oct. 1960</td>
<td>291</td>
<td>16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>418</td>
<td>40</td>
</tr>
</tbody>
</table>

### TABLE 3

Incidence of separate and mixed infestations of larvae and nymphs of *Dermacentor auratus* on *Presbytis entellus* and *Macaca radiata* obtained in 1-year sample

<table>
<thead>
<tr>
<th>Hosts with</th>
<th>Total hosts infested</th>
<th>Total hosts examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larvae only</td>
<td>Larvae and nymphs</td>
</tr>
<tr>
<td></td>
<td>No. larvae</td>
<td>No. hosts pos.</td>
</tr>
<tr>
<td>Nov. 1959</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Dec. 1959</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Jan. 1960</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Feb. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mar. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apr. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>June 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>July 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aug. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept. 1960</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oct. 1960</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

taken on 16 monkeys but no nymphs were found. In the months November, December and January, 20 monkeys were infested with larvae only (116 specimens) and 10 monkeys with nymphs only (19 specimens), but 22 monkeys were found with mixed infestations aggregating 107 larvae and 51 nymphs. In the subsequent dry season months, February through May,
TABLE 4
Frequency of distribution of ticks on Presbytis entellus and Macaca radiata obtained in 1-year sample

<table>
<thead>
<tr>
<th>No. ticks</th>
<th>Number of monkeys in each category</th>
<th>Haemaphysalis spinigera</th>
<th>All Haemaphysalis</th>
<th>All species</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>N</td>
<td>L</td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>11</td>
<td>25</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>11</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>4</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6-10</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>11-20</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>21-30</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>31-40</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>49</td>
<td>81</td>
<td>54</td>
</tr>
</tbody>
</table>

Maximum no. ticks on one host........ 77 6 77 6 77 32

*L* = larva, N = nymph.

there were no mixed infestations of larval and nymphal *spinigera* found in the sample totaling 125 monkeys examined, although 21 monkeys were found during this period with either larvae or nymphs separately. A parallel analysis for *Dermacentor auratus*, the next most abundant species (Table 3), reveals a similar pattern, with November, December and January being the months in which mixed infestations of larvae and nymphs of this species were found.

The frequency distributions of three categories of ticks on monkeys—*Haemaphysalis spinigera*, all species of *Haemaphysalis* combined, and all species of ticks combined—are given in Table 4. About half (25/49) the monkeys with *Haemaphysalis* nymphs had infestations of only one nymph and all but one (98%) had five nymphs or less. The maximum number of nymphs of *spinigera* on a monkey was six. The greater tendency for larvae to be grouped on individual hosts is illustrated by the fact that only 65% (40/62) of the larval infestations were of one to five larvae. Two monkeys had more than 51 larvae attached; these were both *Macaca* taken at the time of larval abundance in October, one with 58 and the other with 77 larvae.

The data are further examined for what they may reveal about possible host preference of *H. spinigera*. While the number of *Presbytis* examined (222) was somewhat less than twice the number of *Macaca* (125), there were about 11 times as many *H. spinigera* nymphs on the former (89) as on the latter (8). But as the size of the samples of the two species of monkeys varied widely from month to month and the prevalence of nymphs also varies seasonally, this figure alone is not evidence of a significantly higher frequency of attachment of *H. spinigera* nymphs on *Presbytis*. Closer examination of the original data suggests, however, that such a relationship may exist. Data from November are excluded since in that month 24 of 26 monkeys examined were *Presbytis*. During the months December through March, a period of prevalence of *H. spinigera* nymphs, the tick-host relationships were as follows:

<table>
<thead>
<tr>
<th>Monkey</th>
<th>No. hosts examined</th>
<th>Hosts positive</th>
<th>Avg. no. ticks per positive host</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td><em>Macaca</em></td>
<td>43</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td><em>Presbytis</em></td>
<td>76</td>
<td>33</td>
<td>43</td>
</tr>
</tbody>
</table>

It will be seen from this summary that both the proportion of hosts positive and the number of ticks per positive host were substantially higher for *Presbytis*. Whether these differences are due to chance, a preference for one monkey species as a host, or differential exposure due to habit differences, cannot be definitely said.

Because of the grouping of larvae on individual animals this stage is less suited to a similar analysis and such is therefore not attempted here.

MONKEYS EXAMINED AT IRREGULAR INTERVALS, 1957–1961

The identity of ticks on monkeys shot at irregular intervals and those found sick or dead is summarized in Tables 5, 6 and 7. The tick findings on sick and dead monkeys are not strictly comparable with those on healthy, freshly collected and examined individuals. Sick animals and those found dead, which may be presumed to have been sick for some time prior to death,
TABLE 5
Monthly incidence of ticks on shot Presbytis entellus and Macaca radiata obtained at irregular intervals, 1957-1961

<table>
<thead>
<tr>
<th>Month</th>
<th><em>spinetosquamata</em></th>
<th><em>larvata</em></th>
<th><em>bipinnata</em></th>
<th><em>heteroloma</em></th>
<th><em>minnesota</em></th>
<th><em>scaleata</em></th>
<th><em>cystidiospora</em></th>
<th><em>species undetermined</em></th>
<th><em>Total Haemaphysalis</em></th>
<th><em>Amblyomma spinigerum</em></th>
<th><em>Dermacentor auratus</em></th>
<th><em>Rhipicephalus</em></th>
<th><em>Ixodes sp.</em></th>
<th><em>Total all species</em></th>
<th><em>No. hosts pos.</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan.</td>
<td>10</td>
<td>4</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Feb.</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mar.</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Apr.</td>
<td>5</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>8</td>
<td>10</td>
<td>27</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>May</td>
<td>3</td>
<td>20</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>June</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>July</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>0</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Aug.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oct.</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>98</td>
<td>0</td>
<td>98</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Nov.</td>
<td>390</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>328</td>
<td>331</td>
<td>8</td>
<td>858</td>
<td>74</td>
<td>5</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Dec.</td>
<td>390</td>
<td>15</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
<td>328</td>
<td>727</td>
<td>17</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>515</td>
<td>39</td>
<td>1</td>
<td>15</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Subtotal</td>
<td>159</td>
<td>9</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
<td>231</td>
<td>72</td>
<td>0</td>
<td>90</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* L = larva, N = nymph, A = adult.
† One *Ixodes petauristae* female.
‡ One *Ixodes ceylonensis* female.
TABLE 6

Monthly incidence of ticks on dead Presbytis entellus and Macaca radiata obtained at irregular intervals, 1957-1961*

| Month | spiničera | tortuus | lutescens | bispinosus | granulatus | aculeatus | cryphocephalus | kyasanurinus | Total | Haemaphysalis | Amblyomma sp. | Dermacentor sp. | Ixodes sp. | Total all species | No. hosts pos./no. hosts exam. |
|-------|-----------|---------|-----------|------------|------------|-----------|----------------|-------------|-------|--------------|-------------|----------------|-------------|----------------|------------------|------------------|
|       | L | N | A | L | N | A | L | N | L | N | L | N | L | N | L | N | L | N | L | N | A | L | N | A |
| Jan.  | 4 | 10 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 | 0 | 15 | 2 | 3 | 5 | 0 | 1 | 1 | 0 | 5 | 11 | 0 | 5 | 11 | 0 | 6/6 |
| Feb.  | 100 | 169 | 0 | 12 | 26 | 3 | 3 | 4 | 0 | 1 | 2 | 0 | 15 | 2 | 3 | 5 | 0 | 1 | 135 | 208 | 3 | 30 | 10 | 0 | 2 | 2 | 165 | 222 | 3 | 25/27 |
| Mar.  | 0 | 15 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 19 | 0 | 0 | 2 | 0 | 1 | 1 | 0 | 20 | 0 | 8/8 |
| Apr.  | 2 | 15 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 15 | 2 | 0 | 3 | 0 | 2 | 18 | 2 | 4/5 |
| May.  | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 9 | 0 | 1 | 0 | 1 | 0 | 1 | 9 | 0 | 1/3 |
| June. | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 2 | 1 | 1/1 |
| July. | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1/1 |
| Aug.  | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 6 | 0 | 1 | 0 | 16 | 0 | 1/1 |
| Sept. | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/0 |
| Oct.  | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/0 |
| Nov.  | 1 | 10 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 4 | 0 | 16 | 1 | 0 | 0 | 1 | 16 | 20 | 0 | 1/3 |
| Dec.  | 107 | 219 | 1 | 12 | 27 | 5 | 4 | 6 | 0 | 4 | 2 | 0 | 15 | 2 | 3 | 5 | 14 | 1 | 2 | 0 | 159 | 264 | 6 | 30 | 13 | 0 | 19 | 3 | 6 | 3/3 |
| Total | 16 | 93 | 0 | 11 | 3 | 0 | 0 | 1 | 0 | 3 | 2 | 0 | 15 | 0 | 1 | 5 | 1 | 0 | 46 | 105 | 0 | 11 | 57 | 0 | 10/12 |
| Subtotal | 91 | 126 | 1 | 12 | 24 | 5 | 4 | 5 | 0 | 1 | 0 | 2 | 0 | 14 | 1 | 1 | 0 | 113 | 159 | 6 | 30 | 13 | 0 | 19 | 3 | 143 | 194 | 6 | 41/44 |

* Bodies examined at varying periods after death.
† L = larva, N = nymph, A = adult.
TABLE 7

Monthly incidence of ticks on sick Macaca radiata obtained at irregular intervals, 1957–1959

<table>
<thead>
<tr>
<th>Month</th>
<th>Haemaphysalis</th>
<th>Rhipicephalus sp.</th>
<th>Total all species</th>
<th>No. hosts pos./no. hosts examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spinigera</td>
<td>torturi</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>popusa</td>
<td>knemeri</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>wondering</td>
<td>wellingtoni</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>aculeata</td>
<td>cupida</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L* N L N L N L N L N L N L N</td>
<td>L N L N L N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feb</td>
<td>0 27</td>
<td></td>
<td>0 27</td>
<td>0 0 27</td>
</tr>
<tr>
<td>Mar</td>
<td>57 31</td>
<td>0 5 3 2 1 0 2 0 4</td>
<td>61 44</td>
<td>61 44</td>
</tr>
<tr>
<td>Dec</td>
<td>14 6</td>
<td>14 6</td>
<td>14 6</td>
<td>14 6</td>
</tr>
<tr>
<td>Total</td>
<td>71 64</td>
<td>0 5 3 2 1 0 2 0 4</td>
<td>75 77</td>
<td>75 78</td>
</tr>
</tbody>
</table>

*L = larva, N = nymph.

could be expected to have shown abnormal behavior which would affect their tick exposure. It is also possible that the sick monkeys were less effective in their grooming activities. Another source of error in judging what might be thought of as the usual tick fauna on monkeys is that in the case of the dead animals there would have been time for ticks to detach. Furthermore, negative data cards were not invariably filled out when dead monkeys were found or irregularly shot monkeys casually examined.

Despite these qualifications, the general composition of the tick fauna on monkeys derived from this body of data is similar to that from the 1-year host sample.

Thus about 90% of all ticks found on these animals were of the genus *Haemaphysalis* and, of the *Haemaphysalis* identified to species, 86% were *H. spinigera*. In addition to the eight species of *Haemaphysalis* found on monkeys of the 1-year sample, *H. kyasanurensis* was recorded in the dead monkey series.

A large difference appears in the relative numbers of larval and nymphal *Haemaphysalis* on the shot and dead monkeys when the total figures are examined: on the shot monkeys there were 885 larvae and 74 nymphs, on dead monkeys 159 larvae and 264 nymphs (see Tables 5 and 6). The numbers of hosts examined varied widely, however, from month to month, and Tables 5 and 6 show that 41 dead monkeys were examined during the first 3 months of the year, when nymphs are prevalent, as compared with only 8 shot monkeys.

Adult ticks were sparse in number, only six having been taken on the dead, seven on the shot, and none on the sick monkeys. These included two *Haemaphysalis spinigera*, nine *H. torturi* and one each of *Ixodes ceylonensis* and *I. petauristae*.

It will be recalled that in the 1-year sample of monkeys no ticks were found during the monsoon months of June to September. In the present series, the 3 dead and 29 shot monkeys examined during these months yielded 33 ticks. On the three positive dead monkeys examined in June, July and August one nymph of *H. wellingtoni*, an adult *H. torturi*, one larval *Haemaphysalis* sp. and 11 larval *Ixodes* were found (Table 6). Three positive shot monkeys collected in June and July yielded one nymph of *H. wellingtoni* and 18 larval *Ixodes* (Table 5). It is of interest that while on an over-all basis *H. spinigera* was the commonest tick on monkeys, this species was not among those found during the monsoon months. It is also noteworthy that 29 of the 33 ticks on monkeys during this period were larval *Ixodes*. The peak period of *Ixodes* larval abundance falls before the monsoon in May, and on small mammals, on which *Ixodes* immature stages are most regularly taken during the year, larvae persist in small numbers through the ensuing monsoon months. The larval *Ixodes* found on monkeys fit this pattern. The one lot of *Ixodes* larvae found in the 1-year sample of monkeys consisted of 15 specimens on one monkey in May (Table 1). In the irregularly obtained shot monkey series a single larva was taken in April, 45 larvae on 6 monkeys in May and 18

*Ixodes kerri*, which was described from nearby the area of this study, is a synonym of *I. petauristae*. 
on one monkey in July (Table 5); while in the
dead monkey series 11 larvae were present on
one monkey in August (Table 6).

DISCUSSION

All ticks identified on monkeys have proved to
be species known from other hosts, and it thus
appears that monkeys of the KFD region lack a
specialized tick ectoparasite fauna. Ticks on
monkeys were primarily larvae and nymphs, only
a small number of adults having been found.
There are two possible explanations for the low
incidence of adult ticks on monkeys: (1) mon-
keys are not favored hosts for the adult stage, or
(2) adults are large enough to be readily seen
and picked off in the course of grooming, while
the smaller larvae and nymphs are not. In a com-
parable study of ticks on human beings, to be
reported separately, immature stage ticks were
relatively common while adults were very rare,
although the hosts denied having picked ticks
off themselves. This would favor the view that
the adult stages of ticks of the KFD area do not
commonly attack primates.

The relatively large proportion of H. spinigera
on monkeys is probably less an indication of
any marked preference for these hosts than a
reflection of their general abundance in the
forests of the area together with a lack of defi-
nite host specificity; this species has been taken
in one circumstance or another on almost all
tick-positive warm-blooded animals examined in
the area. There is, however, some indication of
a higher attachment rate of spinigera nymphs
on Presbytis entellus than on Macaca radiata.

SUMMARY

The species composition and seasonal incidence
of ticks on two species of monkeys (Presbytis
entellus and Macaca radiata) occurring in the
Kyasanur Forest disease (KFD) epizootic area of
Shimoga District, Mysore State, India, are
presented.

The bulk of the ticks were larvae or nymphs
even though a substantial number of monkeys
were examined during the monsoon when adults
are prevalent on vegetation in the forest. The
dominant genus of ticks on monkeys was Haema-
physalitis and the commonest species, H. spinigera,
from which KFD virus has repeatedly been
isolated. Altogether, 9 of the 14 species of
Haemaphysalitis known from the area were found
on monkeys. Other genera taken were Dermacentor,
Amblyomma, Ixodes and Rhipicephalus.

There was a wide seasonal variation in the in-
festation rate of monkeys, with a peak in Novem-
ber when ticks (predominantly larvae) were
found on almost all monkeys, and a period dur-
ing the monsoon from June to September when
ticks were rare or absent on monkeys.

In a series of monkeys collected in approxi-
ately equal numbers in each month of the
year, mixed infestations of Haemaphysalis spinigi-
era larvae and nymphs were found on indi-
vidual hosts during November, December and
January.

ACKNOWLEDGMENTS

The series of monkeys shot on a regular
schedule during a 1-year period were obtained
by one of us (M. K. G.), while many persons
certified to the accumulation of the host
series shot at irregular intervals or found dead
and sick. Our thanks are due to villagers who
reported dead and sick animals seen by them and
to field personnel of the Virus Research Centre
and the Mysore Department of Public Health
who followed up these reports.

REFERENCES

1. WORK, T. H., and TRAPIDO, H., 1957. Sum-
mary of preliminary report of investigation
of the Virus Research Centre on an epidemic
disease affecting forest villagers and wild
monkeys of Shimonga District, Mysore.
2. WORK, T. H., 1958. Russian spring-summer
virus in India. Kyasanur Forest Disease.
Ticks (Ixodidae, Ixodidae) parasitizing lower
primates in Africa, Zanzibar, and
4. AUSB, J. R., NADEH, M., and LEH
Boog-Liat, 1960. Malaysian parasites, XLIX.
Host distribution of Malaysian ticks (Ixo-
didae). Studies Inst. M. Res. Malaya, 29:
225-246.
XVIII. Ticks (Ixodidae) of Borneo and
Malaya. Studies Inst. M. Res. Malaya, 28:
65-94.
6. AXARKER, G., 1950. The scutate ticks or Ixodi-
dae of Indonesia. Entomologia Americana,
30 (new ser.): 1-144.
7. NUTTALL, G. H. F., and WARRINGTON, C.,
1915. The genus Haemaphysalis. In Ticks, A
8. DONOVAN, C., 1919. Malaria of monkeys at
the foot of the Nilgiris during the months of
May and June, 1919. Indian J. M. Res.,
7: 717-721.


11. Virus Research Centre, Poona, India. Annual Reports.