

THE RELATION BETWEEN TICK-BORNE
ENCEPHALITIS VIRUS AND THE WILD DUCK
(*ANAS PLATYRHYNCHOS*).
II. CHRONIC LATENT INFECTION

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Received March 4, 1969

Summary. — Chronic latent infection of wild ducks (*Anas platyrhynchos* L.) with tick-borne encephalitis (TE) virus was experimentally demonstrated. TE virus was irregularly, though repeatedly, isolated from the ducks' blood from the 4th till the 36th week infection (p.i.) in trace amounts up to maximal titres of $10^{1.2}$ intracerebral (ic) mouse $LD_{50}/0.03$ ml in the 11th or 14th week p.i. Virus neutralizing (VN) antibody persisted in subcutaneously (sc) infected ducks till the 52nd week p.i. in titres from 1 : 16—1 : 128. Ducks infected by bite of *Ixodes ricinus* ticks, when killed 20 weeks p.i., had VN antibody titres from 1 : 16—1 : 64. From a duck, which had formed no VN antibody and died with clinical signs of TE 37 days after bite by infectious *I. ricinus* nymphs, TE virus was isolated from the spleen and mesenteric lymph node. TE virus was isolated 24 hours after reinfection with $10^{4.5}$ ic mouse LD_{50} of TE virus from all ducks that had been sc infected 62 weeks previously. In one duck, TE virus persisted in the blood till the 9th day after reinfection. The levels of virus in the blood reached only trace values. All ducks reacted reinfection by a several fold increase in VN antibody.

Introduction

The possibility of the existence of a long-term reservoir of arboviruses among vertebrates has been studied by Reeves *et al.* (1958). After experimental infection of 9 bird species they found that Western equine encephalomyelitis (WEE) virus persisted in the organs of the bird hosts for up to 10 months; they isolated WEE virus from the blood of a sparrow 234 days p.i. Downs *et al.* (1955) isolated yellow fever virus from a patient on the 12th day of illness in the presence of antibody, although it has been reported that this virus disappears from the blood after 6, maximally 7 days. St. Louis encephalitis (SLE) virus persisted in *Molothrus ater* for 38 days after experimental mosquito-borne infection (Chamberlain *et al.*, 1957). Older work on the persistence of SLE virus for 4 weeks in the brains from resistant mice after intranasal infection (Webster and Clow, 1936) and for 162 days in the brains from passively immunized mice (Slavin, 1943) offered evidence of arbovirus persistence in vertebrates.

Comparatively little attention has been paid to the persistence of TE virus in animal hosts after the period of acute viraemia. This eventuality has been pointed out by van Tongeren and Timmers (1960) who recovered TE virus from the brains of *Fulica atra* one month after experimental infection. Webb (1961) isolated Kyasanur forest disease virus from the brain and skeletal muscles of a monkey dead 27 days p.i. One of us (Ernek, 1961) found TE virus in the brain of a domestic duck as late as 28 days after ic infection, in which case histological findings on the 21st day suggested only terminating encephalitis.

A peculiar form of TE virus persistence was found in the blood of hibernating animals (van Tongeren, 1959; Nosek *et al.*, 1961; Kožuch *et al.*, 1963).

Based on these findings we attempted to follow for a prolonged period of time the persistence of VN antibody and to recover in their presence the virus from the blood and organs of wild ducks infected sc or by bite of infectious *I. ricinus* nymphs.

Materials and Methods

Four and six wild ducks infected with TE virus sc and by bite of *I. ricinus* nymphs, respectively, from experiments described previously (Ernek *et al.*, 1969), were placed into a large cage in the courtyard of the Institute. Blood for VN antibody and virus assay was taken from the sc infected ducks every 7 days until the 30th week and then every 14 days until the 52nd week, and from ducks infected by tick bite at weekly intervals up to the 20th week.

In the 62nd week after primary inoculation, the sc infected ducks were reinfected sc with $10^{4.5}$ ic mouse LD₅₀ of TE virus (strain Hypr, 55th mouse passage). Blood for virus assay was taken at 2-day intervals, from day 1 till day 9, and on day 14. Blood for antibody assay was taken on days 7, 9 and 14 (day of reinfection = day 0).

Virus isolations were attempted from a duck which died in the course of the experiment, from ducks killed 10, 15 and 20 weeks after tick bite, and from ducks killed 64 weeks after sc infection (2 weeks after reinfection). The following organs were examined: brain, cerebellum, medulla oblongata, pancreas, liver and spleen, and in ducks infected by tick bite also the mesenteric lymph nodes.

The methods of virus isolation, identification and titration and of the VN test were the same as in previous experiments (Ernek *et al.*, 1969).

Results

Ten wild ducks, after the acute phase of TE infection, developed VN antibody in titres from 1 : 64—1 : 128 after sc infection and from 1 : < 2 to 1 : 64 after tick bite.

The antibody levels in the ducks were followed till the 52nd week after sc infection and till the 20nd week after tick bite; they showed considerable variations. After sc infection, the VN antibody titres reached values of 1 : 128—1 : 256 during the first 10—17 weeks and from 1 : 16 to 1 : 128 in the 52nd week; after tick bite they reaches values from 1 : < 2 to 1 : 64 (Figs 1 and 2).

TE virus was repeatedly isolated from the blood of the majority of ducks infected sc or by tick bite, at irregular intervals from the 4th till the 36th week, when VN antibody was simultaneously present in the blood. The titres of virus in the blood of ducks infected by tick bite reached only trace values, while in sc infected ducks they reached $10^{1.2}$ ic mouse LD₅₀/0.03 ml on the

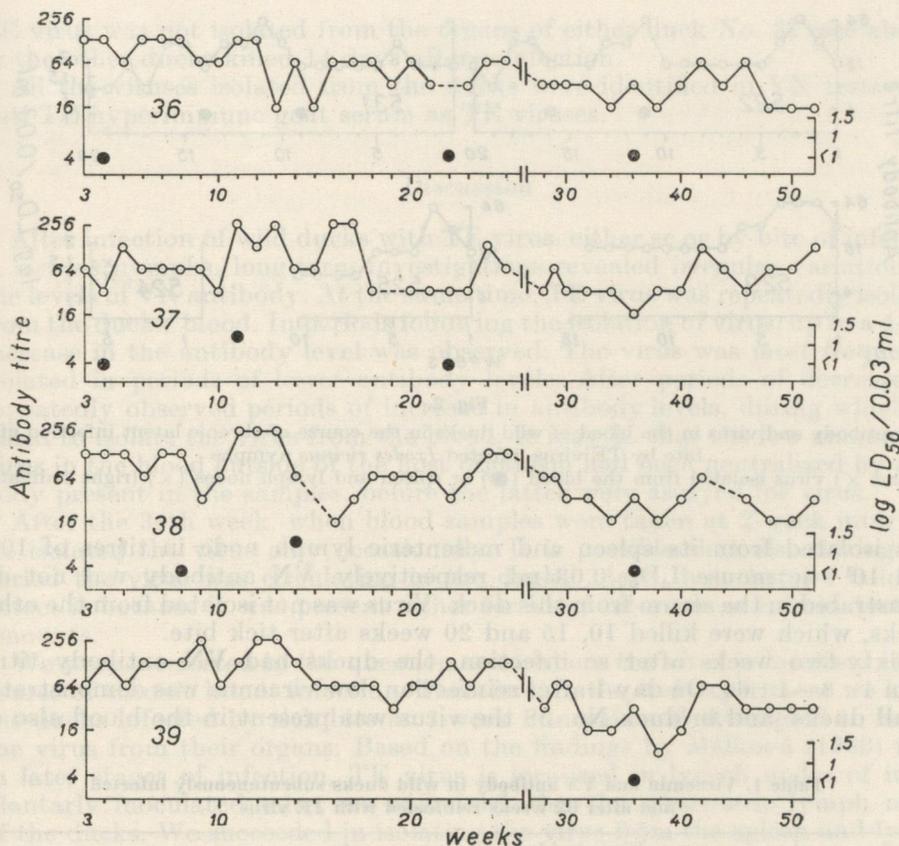


Fig. 1.

VN antibody and virus in the blood of wild ducks in the course of chronic latent infection after inoculation with TE virus

● Virus isolated from the blood (right ordinates)

11th and 14th week (Nos 37 and 38). Virus was not isolated from the blood of 3 ducks infected by tick bite (Nos 529, 525, 524) till the 10th or 15th week, when they were killed, or until death (5th week).

In two ducks, after reaching a maximum in the 3rd or 4th week, the antibody levels gradually decreased till the 7th week, after which they became stabilized at 1 : 16 or 1 : 8 till the 15th (duck No. 529) or 10th (duck No. 525) week, respectively, when the animals were killed (Fig. 2).

One duck (No. 528) was discarded after 7 weeks, when virus isolation attempts and antibody assays were repeatedly negative.

Duck No. 524 died 37 days after tick bite. The day before its wings were hanging down and the bird dragged them on the ground while it was moving; on the day of death the duck was unable to move and died in agony. TE virus

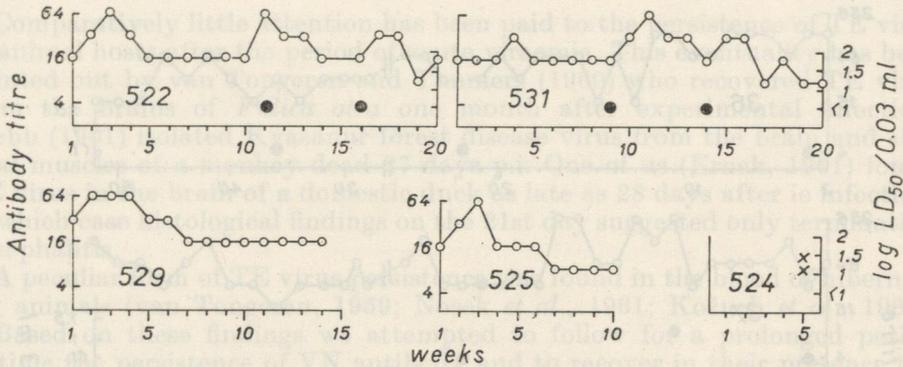


Fig. 2.

VN antibody and virus in the blood of wild ducks in the course of chronic latent infection after bite by TE virus-infected *Ixodes ricinus* nymphs

● and ×: virus isolated from the blood (●) or spleen and lymph nodes (×) (right ordinates)

was isolated from its spleen and mesenteric lymph node in titres of $10^{1.5}$ and $10^{1.2}$ ic mouse $LD_{50}/0.03$ ml, respectively. VN antibody was not demonstrated in the serum from this duck. Virus was not isolated from the other ducks, which were killed 10, 15 and 20 weeks after tick bite.

Sixty-two weeks after sc infection, the ducks had VN antibody titres from 1:8—1:64. On day 1 after reinfection, low viraemia was demonstrated in all ducks, and in duck No. 38 the virus was present in the blood also on

Table 1. Viraemia and VN antibody in wild ducks subcutaneously infected and after 62 weeks reinfected with TE virus

Duck No.	Virus in blood (log ic mouse $LD_{50}/0.03$ ml) on days after reinfection						VN antibody titres			
	1	3	5	7	9	14	before reinflection	on days after reinflection		
								7	9	14
36	< 1	0	0	0	0	0	8	32	32	16
37*	< 1	0	0				64			
38	< 1	0	0	< 1	< 1	0	16	64	128	64
39	< 1	0	0	0	0	0	16	64	128	128

* Duck died 5 days after reinfection.

0 = No virus isolated.

days 7 and 9. The virus titres reached values of $< 10^1$ ic mouse $LD_{50}/0.03$ ml.

After reinfection, all ducks appeared clinically healthy; on day 5, duck No. 37 died of intercutaneous disease.

The levels of VN antibody increased in all ducks that survived (Table 1).

TE virus was not isolated from the organs of either duck No. 37 (see above) or the other ducks killed 14 days after reinfection.

All the viruses isolated from the ducks were identified in VN tests with anti-TE hyperimmune goat serum as TE viruses.

Discussion

After infection of wild ducks with TE virus, either sc or by bite of infective *I. ricinus* nymphs, long-term investigations revealed irregular variations in the levels of VN antibody. At the same time, TE virus was repeatedly isolated from the ducks' blood. In periods following the isolation of virus, up to a 4-fold increase in the antibody level was observed. The virus was most frequently isolated in periods of lower antibody levels. After periods of decrease we repeatedly observed periods of increase in antibody levels, during which we failed to isolate the virus from the blood. It may be that the low amounts of virus in the blood outside of the host organism had been neutralized by antibody present in the samples, before the latter were assayed for virus.

After the 30th week, when blood samples were taken at 2-week intervals, we isolated the virus only occasionally. It is possible that also during this period the virus was circulating in the ducks' blood, but that we failed to detect it because it was present either for short intervals or in undetectable amounts.

We were interested in the question as to where the virus is localized in the course of chronic latent infection. Therefore, in the 2nd experiment we killed the ducks infected by tick bite at 5-week intervals and attempted to isolate the virus from their organs. Based on the findings by Málková (1960) that, in later stages of infection, TE virus is localized in lymph nodes of intraplantarly inoculated mice, we examined also the mesenteric lymph nodes of the ducks. We succeeded in isolating the virus from the spleen and lymph node of only one duck on the 37th day, when it died. This duck, in spite of that virus was demonstrated in its blood 4 days after tick bite in a titre of 10^2 ic mouse $LD_{50}/0.03$ ml, did not form VN antibody until death. The problem of virus localization in the presence of VN antibody in the course of chronic latent infection remains open. We incline to the opinion of Rivers (1952) that, in the latent phase, virus persists in some target organs intracellularly, wherefrom it is released into the blood circulation probably at low antibody levels. This provides a new antigenic stimulus, resulting in repeated antibody increase and elimination of virus from the blood. By such repeated "autosterilization" the host organism protects itself against spread of the infection. This assumption seems to be supported by the death of some ducks which, after the acute phase of infection, failed to develop antibody (Ernek *et al.*, 1969).

Low levels of TE virus were found 24 hours after reinfection of immune sc infected wild ducks. Only in one duck was the virus present also on the 7th and 9th day after reinfection. We failed to isolate the virus at later intervals, but as early as on day 7 there occurred a 4- to 8-fold increase in VN antibody.

The level of viraemia found in the course of chronic latent infection following transmission of TE virus by tick bite ($< 10^1$ ic mouse LD₅₀/0.03 ml) is insufficient for infecting ticks. Therefore, as well as because of the minimal probability of contact of wild ducks with infective ticks in a focus of infection, *Anas platyrhynchos* cannot be taken into account as a long-term reservoir of TE virus. It is even less probable that it could transport the virus for long distances during migration.

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