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THE RELATION BETWEEN TICK-BORNE ENCEPHALITIS VIRUS AND THE WILD DUCK *(ANAS PLATYRHYNCHOS)*. I. ACUTE INFECTION

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Summary. — After subcutaneous infection with tick-borne encephalitis (TE) virus, wild ducks *(Anas platyrhynchos* L.*)* developed viraemia lasting from 3 to 9 days. The highest virus titre in adult ducks amounted to $10^{1.2}$ intracerebral (ic) mouse LD_{50}/0.03 ml on the 3rd day after infection (p.i.) and in subadult ducks to $10^{2.2}$ ic mouse LD_{50} on the 3rd and 5th day p.i. Virus neutralizing antibodies on day 21 reached titres from 1 : 64 to 1 : 128. From the blood of ducks infected by bite of *Ixodes ricinus* nymphs, the virus was irregularly isolated from 2 to 13 days p.i. The highest virus titre in the blood amounted to 10^2 ic mouse LD_{50}/0.03 ml on days 4 and 5. TE virus was recovered from the cerebellum of a duck dead on day 19 and from the spleen and liver of a duck dead on day 20. Virus neutralizing antibodies were found on days 7—21 in titres from 1 : 4 to 1 : 64. In some ducks no antibody was demonstrated in spite of previous viraemia and from the blood of one duck no virus was isolated, although it developed antibody.

Taking into account the limited contact of *A. platyrhynchos* with the vector *I. ricinus*, wild ducks can be considered to represent only occasional hosts of TE virus.

Introduction

Isolation of TE virus from the brain of *Anas querquedula* (Ernek, 1959) and from a mixture of liver and spleen of *Fulica atra* (Soběslavský *et al.*, 1960) led us to investigate the problem as to whether aquatic birds represent a reservoir of TE virus. This suggestion is also supported by the isolation of TE virus from the liver and spleen of *Clangula hyemalis* in Siberia (Bolotovsky, 1960) and from the organs of *Casmerodius albus* near the Caspian Sea in Azerbeidjan (Bashmakova *et al.*, 1967).

The results of van Tongeren and Timmers' (1960) experiments indicated that after subcutaneous injection of *Fulica atra* with TE virus, there developed viraemia lasting 7 to 9 days and amounting to 3—4 log LD_{50}/0.03 ml, with a maximum on the 4th—6th day. After experimental infection of domestic geese, Morozov (1965) succeeded in isolating TE virus from their

blood with the highest titre on the 7th day, and from the brain on the 16th day p.i. In experiments on domestic ducks, inoculated subcutaneously with large amounts of TE virus, we isolated the virus from the blood from the 1st to the 4th day in titres from $10^{2.2}$—10^3 intraperitoneal (ip) mouse LD_{50}/0.05 ml (Ernek, 1961). Subsequently we observed viraemia in subcutaneously infected wild ducks for up to 6 days, the titre reaching its maximum of 10^3—10^4 ip mouse LD_{50}/0.05 ml 4 days p.i. TE virus was simultaneously isolated from the brain, spleen and pancreas 8 to 14 days p.i. (Grešíková and Ernek, 1965).

The occurrence of virus neutralizing antibodies in sera from wild ducks shot or captured in west Slovakia in 1960 (Ernek and Mačička, 1961) as well as further findings in Czechoslovakia and south Hungary (Ernek *et al.*, 1967) seemed to confirm the assumption that aquatic birds could be involved in the circulation of TE virus. To confirm the results of isolation experiments, serological findings as well as previous studies, we attempted to infect wild ducks 1) subcutaneously with TE virus in doses of which it could be assumed that they approximately correspond to the amounts of virus transmitted by tick bite, and 2) directly by bite of experimentally infected nymphs of *Ixodes ricinus*.

Materials and Methods

Fourteen wild ducks *(Anas platyrhynchos)* were used; 4 were captured on a pond near Pohořelice in south Moravia and 10 were bred in the Pozďatín farm. They were kept individually in cages. Their food consisted of a moist mixture of corn groat and albumin mixture for poultry, maize, lucerne (fresh in summer, hay in winter), pressed scrap, grated carrot and always plenty of fresh water.

Four (2 adult and 2 subadult) ducks were injected subcutaneously with 0.1 ml of a 10^{-4} diluted 10% brain suspension from mice infected with TE virus (strain "Hypr" in the 54th mouse passage). The dose of virus inoculated to each duck corresponded to $10^{4.7}$ ic mouse LD_{50}.

Ten subadult ducks were infected by infectious nymphs of *Ixodes ricinus*. Transparent feeding capsules (Nosek, 1965) with polyurethane foam pads (Kaiser, 1966) (Fig. 1) were fixed to the necks of ducks by adhesive plaster; before fixing the capsules, the feather was cut and the skin defattened with ethanol. Six *I. ricinus* nymphs were placed into each capsule. They had molted from laboratory bred larvae, engorged on viraemic white mice infected with the 7th mouse passage of the strain J 13 of TE virus, isolated from a hedgehog by Kožuch *et al.* (1967). The engorged larvae were kept at 22—24° C. The premolting period larva-nymph lasted for 38 days, the prefeeding period of nymphs 70 days, and the whole virophoric period 111 days.

To demonstrate the virus in engorged nymphs, each was individually homogenized in 1 ml of Earle's solution supplemented with 5% heated calf serum and antibiotics (200 units of penicillin and 200 μg of streptomycin per ml). After centrifugation, the supernatant fluid was inoculated into white mice.

Blood for virus isolation was taken from vena ulnaris of the ducks into 1% heparin at approximately 2-day intervals beginning with the 2nd or 3rd day till the 14th day p.i. It was diluted 1 : 3 in the medium indicated above.

From organs of dead birds (brain, cerebellum, medulla oblongata, liver, spleen and pancreas) 10% suspensions were prepared in Earle's solution supplemented with 5% heated calf serum and assayed for virus.

Virus isolations from blood and organ and tick suspensions were attempted by ic inoculation of suckling white mice with 0.01 ml volumes. For blind passages, the mice were killed 9 and 10 days p.i. Blood and organ and tick suspensions from which virus had been isolated, were then assayed by titration in white mice weighing 6—8 g, inoculated ic with 0.03 ml volumes.

The viruses isolated were identified in neutralization tests in white mice weighing 6 g. The mice were inoculated with mixtures of virus and hyperimmune goat serum against TE virus with a neutralization index of 10000.

Blood for antibody assay was taken from subcutaneously infected ducks 21 days p.i., from ducks infected by *I. ricinus* nymphs 7, 14 and 21 days p.i. and from ducks which died on the day of their death. The virus neutralization tests were carried out in monkey heart cell cultures (Libíková, 1963). No virus neutralizing antibody was demonstrated in the ducks' blood before the experiments.

Table 1. Viraemia and virus neutralizing antibody in wild ducks subcutaneously infected with TE virus

Duck No.	Virus in blood (log ic mouse LD_{50}/0.03) on day					Antibody titre on day 21
	3	5	7	9	14	
36-subadult	2.2	2.2	< 1	0	0	128
37-subadult	2.2	0	0	0	0	64
38-adult	1.2	< 1	< 1	< 1	0	128
39-adult	< 1	< 1	< 1	0	0	64

0 means no virus isolated.

Results

TE virus was found in the blood of subcutaneously infected adult ducks from 3 to 9 days p.i. in a maxinal titre of $10^{1.2}$ ic mouse LD_{50}/0.03 ml on the 3rd day p.i. From the blood of subadult ducks, the virus was isolated from 3 to 7 days p.i.; the titre reached $10^{2.2}$ ic mouse LD_{50}/0.03 ml on days 3—5 (Table 1).

Relatively high levels of virus neutralizing antibodies were found in both adult and subadult ducks 21 days p.i. Within this period, the animals showed no pathological symptoms.

Nymphs of *I. ricinus*, placed in capsules on the ducks, did not engorge equally till the 3rd day. Some of them sucked for a short time, withering afterwards. All six nymphs were fully engorged only on one duck (No. 529), while only 1 engorged nymph was recorvered from ducks Nos 528 and 530, respectively. The levels of TE virus in engorged nymphs ranged from threshold values to $10^{3.2}$ ic mouse LD_{50}/0.03 ml (Table 2).

Viraemia in tick-infected tucks was demonstrated from 2 to 13 days p.i., with individual differences. It lasted 2—4 days in most cases. The maximal titre amounted to 10^2 ic mouse LD_{50}/0.03 ml 4 or 5 days after tick bite (Table 2).

Until day 17, no clinical symptoms of disease were observed in the ducks; four ducks died in the period from day 17 to day 20. Two of them showed clinical symptoms of meningoencephalitis, while the other two displayed no evident disease symptoms 2 hours before they were found dead. From the cerebellum of duck No. 523 dead 19 days p.i., showing clinical symptoms of meningoencephalitis, and from the spleen and liver of the duck No. 526 dead 20 days p.i., virus was isolated in a titre of $< 10^1$ ic mouse LD_{50}. It was identified in neutralization tests against hyperimmune goat serum as TE virus.

As early as 7 days after tick bite, TE virus neutralizing antibodies were found in 5 ducks in titres from 1 : 16—1 : 32; the antibody titre subsequently rose till day 21. In one of these ducks (No. 523), the antibody titre decreased on the 14th day to 1 : 4, with a subsequent rise to 1 : 16 on day 19, when the

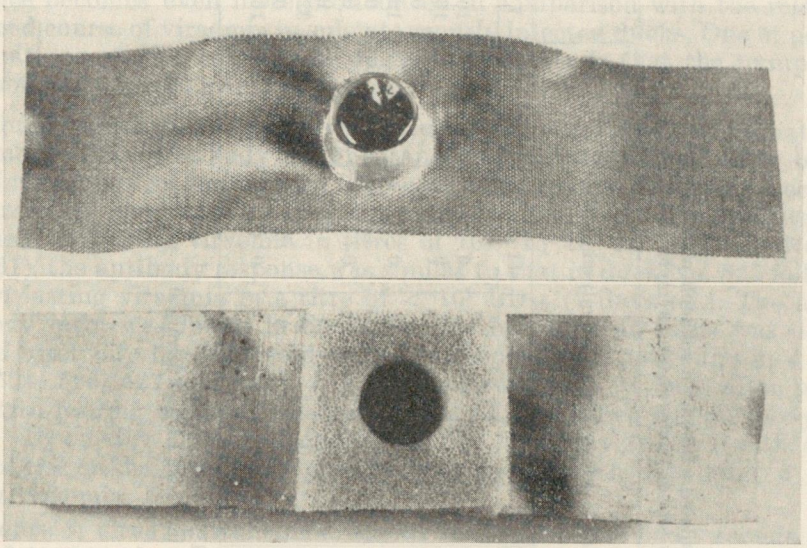

Fig. 1.
Feeding capsule for ticks

duck died. In 5 ducks we failed to demonstrate virus neutralizing antibody even in sera diluted 1 : 2 (Table 2); in 3 of them, no antibody was found on the day of their death.

Discussion

The viraemia in subcutaneously infected adult wild ducks reached a lower level and was more protracted than in subadult ones.

All subadult ducks bitten by infected nymphs of *I. ricinus* developed viraemia of different intensity and duration. These differences were probably due to inequal numbers of infected ticks which fed on the ducks, inequal amounts of transmitted virus, different sucking periods or individual susceptibility of the host. On the first day the majority of nymphs started to feed, then a part of them ceased feeding, withered and fell off within the first three days. The withered nymphs were not investigated for the presence of virus. The presence of virus in the blood of ducks (Nos 524, 530) from which we recovered 1 or 2 ticks without demonstrating virus in the latter could be explained so that viraemia was possibly caused by those nymphs which had

Table 2. Viraemia and virus neutralizing antibody in subadult wild ducks infected with TE virus by bite of Ixodes ricinus nymphs

Duck No.	Engorged *I. ricinus* nymphs recovered[1])	Virus in blood (log ic mouse LD_{50}/0.03 ml) on day[2])												Antibody titre on day[3])		
		2	3	4	5	6	7	8	9	10	11	12	13	14	7	14	21
522	3 (*1*)		0		0		<1		0		0		0		16	32	64
523*	4 (*3; 2.7; 0; 0*)		1.7		0		0		0		0		0		16	4	
524	2 (*0; 0*)	0	<1	2		0		0		0		0		0	0	0	0
525	5 (*3.2; 0; 0; 0; 0*)		1.1		1.7		0		0		0		0		16	32	64
526*	2 (*2.7; 2.2*)	0		0		0		<1		<1		0		0	0	0	
527*	2 (*<1; 0*)	0	0		<1		1		0		0		<1		0	0	
528	1(*0*)	0		0		0		0		0		0		0	0	0	0
529	6 (*3; 2.2; 2; 2; 1.2; <1*)	0		0		0		0		0		0		0	32	64	64
530*	1(*0*)	<1	<1	1.7		0		0		0		0		0	0	0	
531	1 (*2.7*)	<1	<1		2		0		0		0		0		16	16	16

* Ducks Nos 523, 526, 527 and 530 died on days 19, 20, 18 and 17, respectively.

[1]) In parentheses (*italics*): virus titres in individual nymphs (log ic mouse LD_{50}/0.03 ml); 0 — no virus detected.

[2]) 0 — no virus detected.

[3]) 0 — no antibody detected in serum diluted 1 : 2.

fed only for a short time and then withered. Evidence in favour of individual reaction of the ducks was offered by the finding that in duck No. 529, bitten by 6 infected nymphs, no virus was demonstrated in the blood; the transmission of virus by these nymphs was proved by the presence of virus neutralizing antibodies as early as 7 days after nymphs' bite. The individual responce becomes even more pronounced in comparison with the relatively balanced course of viraemia in subcutaneously infected ducks. One of possible explanations of such reaction of ducks to tick bite is that the nymphs fed on a host they rarely infest in nature.

A single injection of virus was sufficient for a satisfactory formation of virus neutralizing antibody in subcutaneously infected ducks. After bite by *I. ricinus* nymphs, individual differences in antibody formation occurred. The antibody level was up to 4 times lower than that after subcutaneous infection. Following viraemia in titres of $10^{1.7}$ to 10^{2} LD_{50} (ducks Nos 525 and 531), the antibody response was similar to that in duck No. 522 following a short-lasting viraemia in a titre of $< 10^{1}$ LD_{50} (7 days p.i.). The highest antibody level was found in duck No. 529 in which no virus was demonstrated after bite by 6 infected ticks. Antibodies were not found in 4 ducks (Nos 524, 526, 527 and 530), 3 of which died within 21 days after placing ticks into feeding capsules. Also duck No. 523, in which the antibody titre was 1 : 16 on day 7, then decreased on the 14th day to 1 : 4 with a subsequent rise on the 19th day, suddenly died on the 19th day, after a short-lasting viraemia.

Absence of virus neutralizing antibody in ducks which had viraemia might be explained so that the virus was localized in certain target organs (spleen, cerebellum and liver, from which TE virus was occasionally recovered) without serologically detectable immunity. The mechanism of this phenomenon remains obscure, however.

The question as to how the wild duck can take part in the circulation of TE virus in a natural focus is important from the ecological point of view. The majority of wild duck biotopes are not foci of TE. During the nesting period the duck's radius of action can spread into a TE focus, bur even this occurs only rarely.

Only a few reports on tick infestation of ducks are available. In Czechoslovakia this problem was investigated by Daniel and Černý (1963) and Balát (1964). The latter found larvae and nymphs of *I. ricinus* only on 5 ducks aged maximally 7 days out of 23 ducks aged up to 3 weeks. If a duck is nesting in an area where ticks occur, juvenile ducks can come into contact with ticks in the nest and on their way to the water.

On their flights, ducks are able to link various biotopes; they fly seeking for food, during and after their nesting period as well as during migration. Flights of both subadult and adult ducks after nesting were observed in various directions, also to the northeast; migrations are pronouncedly southward and westward. The ringed ducks found in Czechoslovakia were mostly of inland orgin. While migrating, some birds cross Czechoslovakia on their way from northeast, but they only rarely stay in our country in summer

months (Hudec, 1967). In the spring, ducks arrive to Czechoslovakia during a period when the possibility of their contact with ticks is minimal.

The results of our serological tests carried out in 1961 and 1967 indicated only a slight contact of ducks with TE virus. Viraemia in a titre of 10^2 LD_{50} demonstrated in some ducks after bite by infected nymphs of *I. ricinus* might eventually be sufficient to infect a further vector. In a convenient biotope the wild duck could occasionally be involved into the circulation of TE virus.

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