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Tick-borne Meningoencephalomyelitis in Sweden*

By

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With 2 Figures

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During recent years the presence of meningoencephalomyelitis caused by agents related to the Russian spring-summer/louping ill group of tick-borne viruses have been reported not only from Russia (1) but also from Czechoslovakia (2, 3), Hungary (4), Slovenia (5), Austria (6, 7, 8), Poland (9), Sweden (10, 11), Finland (12), and Northern Ireland (13).

The first verified Swedish cases were in the summer of 1954. It was then noticed that some of the patients with aseptic meningitis or meningoencephalitis from whom no enteroviruses were isolated had been bitten by ticks some time before the onset of the disease, an observation that prompted search for evidence of infection with a virus related to that of Russian spring-summer encephalitis (RSSE). Through the courtesy of Dr. *Joseph E. Smadel*, Dr. *Jack Schmidt* of the neurotropic virus section of the Department of Virus and Rickettsial Diseases, Army Medical School, Washington, kindly carried out tests, in mice, for neutralizing antibodies against RSSE virus on the sera of three such patients. They all appeared to have significant levels of neutralization (neutralization indices of 2.2 logs or more) and in one case a significant rise in titer during convalescence was demonstrated (10). Later, we have found neutralizing antibodies against the closely related louping ill (LI) virus in the convalescent sera of 14 out of 34 other cases tested from the same year (unpublished results).

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When, in 1956, again a large proportion of the cases of aseptic meningoencephalitis in Stockholm were found to have been tick-bitten, a more comprehensive study was undertaken. Tissue culture methods were adopted for the assay of neutralizing antibodies as well as for the production of complement-fixing antigens, as elaborated on elsewhere (14). The results indicate that infections with viruses of this group not infrequently occur in the Stockholm region.

Materials and Methods

Specimens from cases with acute infectious disease involving the central nervous system (CNS). During the period of May 1 through December 31, 1956 240 patients with the clinical diagnoses of aseptic meningitis, meningoencephalitis, or poliomyelitis were treated at the Hospital for Infectious Diseases in Stockholm. The present study is based on the 176 of these patients from whom serum specimens collected on more than one occasion were available. This material included 38 paralytic cases. A single stool specimen was procured during the acute disease from 156 of the patients.

Specimens for epidemiological survey. In order to obtain preliminary epidemiological data single sera, intended for screening of neutralizing antibodies against LI virus, were collected from 44 healthy blood donors in Stockholm and 31 adult male patients living in the archipelago north of Stockholm who were treated at the Norrtälje Hospital for various diseases other than those involving the CNS. Similarly, sera were drawn from two groups of cows, over 5 years of age, one of which (comprising 22 animals) was raised in an area of the same archipelago region at the Baltic, the other (35 animals) in the typical agricultural plain country around Skara in the western part of southern Sweden.*

Finally, 113 ticks (*Ixodes ricinus*) were collected from islands in the Stockholm archipelago for a preliminary attempt at virus isolation.

All samples were stored at $-30°$ C until tested, sera following inactivation at 56° C for 30 minutes.

Tissue cultures. The tissue culture methods employed for enteroviruses and for tick-borne viruses, respectively, have been described in detail in previous papers. Thus primary cultures of human embryonic lung were used for isolations of enteroviruses from stool specimens (10), cultures of Detroit-6 cells for the growth of LI virus and of the HYPR strain of Czech tick-borne virus (14).

Virus isolations. The techniques used for isolation and identification of enteroviruses have been described elsewhere (10, 15).

The 113 ticks were ground with sand in about 3 ml of phosphate-buffered saline with antibiotics. Following clarification, 5 mice three weeks of age as well as 6 mice twelve hours of age were inoculated intracerebrally, adult mice with 0.02 ml, suckling mice with 0.01 ml. The mice were observed for 10 days whereupon two subsequent blind passages on the brain extracts were performed in mice of corresponding ages.

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Serological tests. Neutralization tests. Tests for neutralizing antibodies against LI virus were performed in stationary cultures of Detroit-6 cells (14).

A single convalescent serum from each of the 176 patients was first screened at an initial dilution of 1/4 against 1000 TCD_{50} of virus. The serum-virus mixture was incubated at 37° C for 1 hour before inoculation of volumes of 0.1 ml into three cultures; the final reading of the test was taken after 6 days at 35° C. Sera showing no evidence of neutralization were also tested undiluted against the same dose of virus.

Convalescent sera showing neutralization in the screening test were subsequently tested diluted 1/5, 1/25, and 1/125 simultaneously with the first serum drawn, the latter diluted 1/5 and 1/25. Again 3 cultures were used per dilution. The serum titer is recorded as the inverse of the highest initial dilution of serum that protected at least one of the three tubes. However, two serum dilutions were considered to differ in titer only when the difference in neutralizing capacity amounted to at least one full step of dilution.

Sera from blood donors in Stockholm and patients at the Norrtälje Hospital as well as all bovine sera were screened undiluted for neutralizing antibodies. Positive bovine sera were then titrated (1/5—1/125).

It should be mentioned that all sera of human or animal origin tested undiluted for neutralizing antibodies were simultaneously checked for cytotoxic properties in a control culture. However, no such effect was observed.

Complement-fixation tests. All patients were also tested for CF antibodies against tick-borne viruses. CF antigens were prepared from cultures infected with LI or HYPR strains. The LI antigen was used live, whereas the HYPR antigen was tested live as well as killed with 0.2 per cent β-propiolactone. This inactivation caused a twofold loss of antigen as measured in box titrations. There was a suggestion that the optimal serum titer simultaneously dropped about half a step. The methods of preparation were outlined in a previous paper (14).

The CF technic was that of *Fulton* and *Dumbell* (16) as modified by *Svedmyr, Enders* and *Holloway* (17). Thus two optimal units of antigen were used in all serum titrations. Titers are given as the reciprocal of the highest initial serum dilution giving at least 50 per cent inhibition of hemolysis.

CF tests against heat-inactivated poliovirus antigens were also performed (17, 15).

Results

Virological data on cases with CNS disease. Not less than 68 out of the 176 patients with signs of acute infectious disease involving the CNS were found to have neutralizing antibodies against LI virus in their convalescent sera. The outcome of the CF tests against tick-borne viruses coincided with this result, the same 68 patients — but none of the remaining 108 cases — giving positive reactions against at least one of the viruses used as antigen. No enteroviruses were isolated from the stool specimens (61 specimens available) procured from the patients with antibodies against tick-borne viruses and the CF test against heated poliovirus antigens [which appears to respond quite broadly to infections with various enteroviruses, such as ECHO 6 (15) and ECHO 9] gave

consistently negative or low ($\leq$ 8) titers without significant rise among the 64 patients tested.

The group lacking antibodies against LI virus, on the other hand, showed evidence of a varied etiology. Thus 12 polioviruses (one type 1, one type 2, ten type 3), 9 ECHO viruses (three type 6 and six type 9) and 9 unidentified cytopathogenic agents were recovered from a total of 95 stool specimens available. Furthermore, 47 out of 103 LI-negative patients tested had a CF titer against poliovirus antigens of at least 16, and 16 showed a rising titer ($\geq$ fourfold). The LI-negative group also included 8 cases with the clinical diagnosis of mumps and 1 of infectious mononucleosis.

The serological results, against tick-borne viruses, of the 68 patients having LI antibodies are recorded in Tables 1 and 2, the main tendencies are summarized in Table 3.

A rise in neutralizing titer against LI virus was recorded in 9 cases only. It should be noted, however, that some rises were probably missed since the titrations were not carried beyond 1/25 for acute phase sera or 1/125 for convalescent sera. Three of the convalescent sera had a titer of 5, 38 of 25 and 26 $\geq$ 125.

The CF titers against live LI antigen increased at least fourfold during the convalescence in 22 of the cases. Maximum titers were usually low, however (geometric mean 9, range < 2—32). With killed HYPR antigen convalescent titers were about 3 times higher (geometric mean 23, range 4—128) and the only patient with negative tests (< 2) against LI antigen (patient nr. 10 in Table 1) showed a rising titer with a maximum of 16. At least fourfold rises were found in 18 cases. The apparent difference in the frequency of rises obtained with the two antigens may be a matter of chance only. Thus significant rises were found with the LI antigen alone in 9 cases, with the HYPR antigen alone in 5 cases. Most of these cases showed a twofold rise against the other antigen.

It might be mentioned in this connection that CF tests with live and killed HYPR antigens resulted in almost identical results when performed simultaneously in a separate experiment on 28 sera from 7 patients. There was only a suggestive reduction in titer by about half a step with the killed antigen, in acute phase and convalescent sera alike. A similar minimal reduction of the optimal serum titer has, as mentioned above, been noticed several times in box titrations of inactivated antigens when compared with the active starting materials.

Apparently altogether 30 patients developed a significant rise in titer, whether in neutralization or against one or both of the CF antigens. It seems highly probable that infection with an immunologically related virus was the cause of the disease in these cases.

Table 1. Serologic data on 30 patients with significant rise in neutralizing and/or CF antibodies against tick-borne viruses

Case No.	CF titers at various intervals after onset of disease*					
	Before 2. onset	0–10 days	11–20 days	21–40 days	41–118 days	>1 year
1§			**16:** 4–2	**35:** 16–4		
2		**7:** 16–8		**24:** 64–32	**118:** 32–16	**556:** 16–8
3		**2:** 16–2			**50:** 32–8	**484:** 8–4
4§		**4:** 2–<2			**95:** 8–4	**479:** 2–4
		8: 2–<2				
5		**5:** 16–4		**31:** 32–16	**41:** 32–16	**480:** 4–<2
					89: 16–8	
					108: 16–4	
6		**5:** 8–<2	**15:** 8–<2	**25:** 8–2	**67:** 8–4	**463:** 4–2
7		**4:** 8–<2	**13:** 16–4		**67:** 8–4	**472:** 4–2
8		**5:** 8–4	**14:** 16–4		**55:** 32–8	**466:** 4–2
9		**2:** 16–2		**21:** 32–16	**55:** 32–16	**463:** 8–4
		7: 32–16				
10		**6:** 2–<2	**16:** 4–<2		**51:** 16–<2	**466:** 4–<2
11		**4:** 2–<2	**13:** 8–2	**24:** 8–4	**58:** 16–8	
12†	**–4:**<2–<2	**5:** 16–4	**15:** 16–8		**54:** 32–16	**449:** 16–16
	–3: 2–<2					
13§†			**19:** 8–8	**28:** 16–8	**65:** 16–8	**462:** 4–2
14		**2:** 8–<2	**12:** 16–4		**43:** 16–4	**453:** 16–8
15		**9:** 8–<2	**16:** 16–2	**26:** 32–16	**58:** 32–16	**455:** 8–2
16†		**4:** 8–<2	**14:** 16–4	**22:** 16–8	**63:** 64–16	**452:** 16–4
		6: 8–<2				
17†		**4:** 16–16	**15:** 32–16	**25:** 32–16	**53:** 32–8	**506:** 8–2
18			**12:** 16–2		**50:** 16–8	**448:** 8–4
19†		**5:**<2–<2	**16:** 16–4		**51:** 16–8	**450:** 2–2
20		**9:** 2–8	**19:** 32–16		**58:** 16–8	**450:** 4–2
21§		**5:** 8–8			**66:** 128–32	**442:** 16–16
22†		**4:** 8–4	**13:** 16–16		**54:** 32–16	**432:** 8–4
23§		**7:** 8–2	**17:** 32–8		**65:** 16–8	**435:**<2–<2
24		**6:** 8–2	**16:** 32–4			
25§†		**11:** 4–2	**19:** 32–16	**27:** 32–16	**61:** 32–16	**430:** 32–8
26§			**15:** 8–2	**21:** 32–8	**69:** 16–8	
				32: 16–8		
27§†		**4:** 4–2	**13:** 8–4		**48:** 8–4	**418:** 4–<2
28†		**4:** 16–2	**12:** 16–4		**50:** 64–16	
29		**6:** 16–2	**16:** 32–8		**56:** 32–8	**413:** 16–4
30§		**6:** 16–<2	**17:** 32–8		**53:** 32–4	**407:** 16–4

* The fig. before the colon refers to the interval between the second onset of disease (or the single onset in cases with monophasic course) and the day of procurement of the serum specimen. A minus sign before this figure indicates that the serum was taken before the second onset of disease. The fig. before the hyphen represents the CF titer against inactivated HYPR antigen, the fig. after the hyphen represents the CF titer against live LI antigen. ND = not done (no serum left). § Patients with this sign had a monophasic course of their illness, all others had a biphasic one. † These patients had a significant rise in neutralizing antibodies.

Table 2. Serologic data on 38 patients with antibodies against tick-borne viruses but without significant rise in titer

Case No.	CF titers at various intervals after onset of disease*				
	0–10 days	11–20 days	21–40 days	41–109 days	> 1 year
31§	**5:** 4–2	**18:** 4–2			**629:** 4–2
32	**6:** 32–16	**14:** 32–16		**88:** 32–16	**493:** 16–8
33	**9:** 32–4	**17:** 16–4		**63:** ND–8	**498:** 4–2
34	**6:** 8–2			**109:** 4–2	
35	**5:** 32–8			**76:** 64–16	**479:** 16–4
36	**5:** 32–8			**94:** 16–8	**477:** 8–4
37§		**16:** 8–2		**82:** 8–4	**487:** 4–2
38§	**6:** 32–4		**23:** 32–8 **35:** 16–8		
39		**19:** 8–4	**28:** 8–4	**99:** 8–4	**488:** 4–2
40	**4:** 16–4	**13:** 16–8		**68:** 8–4	**471:** 4–2
41	**6:** 16–8			**69:** 16–16	
42	**5:** 16–4	**15:** 32–8 **18:** 32–8			
43		**19:** 32–8	**29:** 32–8	**69:** 16–4	**476:** 4–4
44	**8:** 32–16	**17:** 32–16	**27:** 32–16	**58:** 16–8	**460:** 4–4
45	**6:** 8–2	**15:** 16–2		**52:** 16–2	**456:** 4–<2
46	**2:** 8–8	**13:** 16–8		**68:** 16–8	**452:** 8–4
47	**5:** 16–8	**11:** 16–16 **20:** 16–16		**64:** 16–8	**453:** 4–4
48	**4:** 32–8	**12:** 16–8 **19:** 32–16	**28:** 32–16	**72:** 32–16	**447:** 16–8
49				**59:** 32–16	**453:** 4–2
50§	**9:** 32–8	**19:** 32–8		**60:** 32–4	**450:** 4–<2
51		**14:** 32–8	**24:** 32–16	**67:** 16–8	**455:** 4–2
52§			**23:** 16–16 **34:** 32–16	**76:** 32–16	
53	**3:** ND–8	**19:** 32–16	**25:** 32–16	**68:** 32–16	**439:** 16–4
54	**5:** 16–8	**15:** 32–16		**60:** 16–8	
55	**7:** 32–16	**16:** 64–16		**59:** 32–32	**434:** 16–8
56	**2:** 16–8 **10:** 32–8		**19:** 32–16	**54:** 16–8	**434:** 8–4
57	**8:** 16–16	**16:** 32–16		**59:** 16–16	**430:** 4–8
58		**16:** 32–16		**62:** 32–8	**430:** 8–4
59§			**33:** 32–16		**436:** 8–4
60§			**30:** 16–8	**50:** 16–8	**442:** 16–16
61§			**28:** 8–4 **37:** 16–8		**436:** 4–4
62	**10:** 4–4	**19:** 4–2		**56:** 4–4	**422:**<2–<2
63§	**6:** 8–4	**15:** 16–8	**24:** 16–8		
64	**5:** 32–8	**14:** 16–16	**23:** 16–8	**53:** 16–16	**421:** 16–16
65	**4:** ND–2	**13:** 4–2	**22:** 4–4	**54:** 8–4	**418:**<2–<2
66	**8:** 16–4	**18:** 16–4		**58:** 16–4	**420:** 4–2
67§		**16:** 32–16	**25:** 32–8	**64:** 32–16	**422:** 4–4
68	**8:** 8–<2	**17:** 8–<2		**57:** 8–2	**409:**<2–<2

* For explanations see under Table 1.

Table 3. Summary of the serological patterns of 68 cases with antibodies against tick-borne viruses (only "significant" changes in titer are recorded)

Comparison between acute and convalescent phase sera			Number of patients showing patterns at left	CF titer of one year serum compared to maximum titer			
Neutralization	CF with killed HYPR antigen	CF with live LI antigen		Fall against both antigens	Fall against HYPR alone	Fall against LI alone	No change
Rise	Rise	Rise	6	3	—	—	2
	No change	No change	3	2	—	1	—
No change	Rise	Rise	7	3	2	—	—
		No change	4	2	—	—	—
		Negative	1	—	1	—	—
	No change	Rise	9	2	2	—	5
		No change	36	13	7	1	8
"Acute phase" serum lacking			2	2	—	—	—

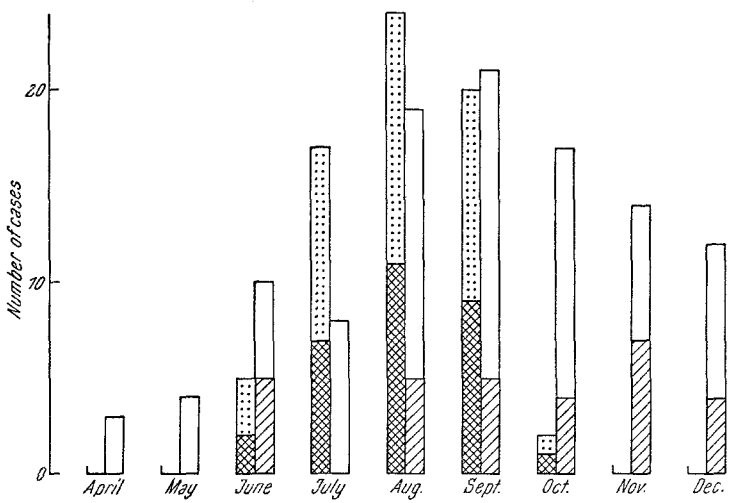
However, the same etiology seems probable also for part of the other material. This is suggested by the fact that the CF titers, also in most of those cases without significant titer rises, dropped from high peak values to lower levels within about a year after the disease. Whereas the geometric means of the peak values during the convalescence of these cases were 9 against the LI antigen and 21 against the HYPR antigen (to be compared with 9 and 26 for the cases with significant rises in titer), the corresponding figures were only 3 and 5, respectively, for the one year specimens (3 and 7 for cases with rise). Altogether 41 out of 56 patients investigated showed a fourfold or greater drop in titer against one or both of the antigens. It may be recalled, furthermore, that no evidence of another etiology was found in any of the cases with antibodies against tick-borne viruses.

It is noted that many cases retained a rather high CF titer even in late sera, however. The possibility therefore remains that many of the patients without significant titer rise, particularly those with low convalescent titers, had actually experienced an infection with a tick-borne virus at some time prior to the current disease.

Clinical data. A detailed account of the clinical observations on the cases with antibodies against tick-borne viruses will be given elsewhere (18). Here only the main data will be summarized concerning the 30 cases with significant rise in antibody titer against a tick-borne virus. Actually,

the clinical picture corresponded well to that described in Central European tick-borne encephalitis.

Thus, about 70 per cent had a biphasic course. The incubation period, i. e. the interval between a single known tick bite (16 cases) and the first onset of disease varied between 2 and 21 days (mean 7 days). The first phase lasted for 2—10 days (mean 6 days). It was characterized by moderate fever, headache, and muscle pains. After a free interval of 2—19 days (mean 8 days) the major disease followed, with fever lasting for 5—20 days (mean 10 days). Dominant symptoms in the second phase were high fever, splitting headache, neck rigidity, and vomiting. About 70 per cent of the patients had a definitely pathological electroencephalogram during the first week. 8 patients had transient paralysis (1 pharynx, 4 shoulder and/or upper arm, 1 shoulder and upper leg, 1 upper leg, 1 intestine). Lumbar puncture on admission revealed a moderate pleocytosis with mostly lymphocytes and often a relatively high protein content.



Cases with significant rise of antibodies against tick-borne virus.
Cases with antibodies against tick-borne virus, titer rise not found.
Cases without antibodies against tick-borne virus, excreting enteroviruses.
Cases without antibodies against tick-borne virus, not excreting enteroviruses.

Fig. 1. Distribution by month of the first onset of disease of 176 cases with signs of acute CNS infection in the summer and fall of 1956.

Epidemiological considerations. Fig. 1 records the seasonal distribution of the CNS disease material as indicated by the month of the first onset of disease. The maximum of patients with antibodies against tick-borne viruses, whether with titer rise or not, occurred in August; other cases, including those excreting enteroviruses, were frequent also during following months.

About 70 per cent of the patients with antibodies against tick-borne viruses (23 of those 30 with a significant titer rise) reported that they had, within a couple of weeks before their disease, been bitten by ticks when on vacation somewhere in the eastern part of southern Sweden, usually in the Stockholm region. The geographical locations where these tick-bites occurred are recorded on the map of Fig. 2. It should be noted

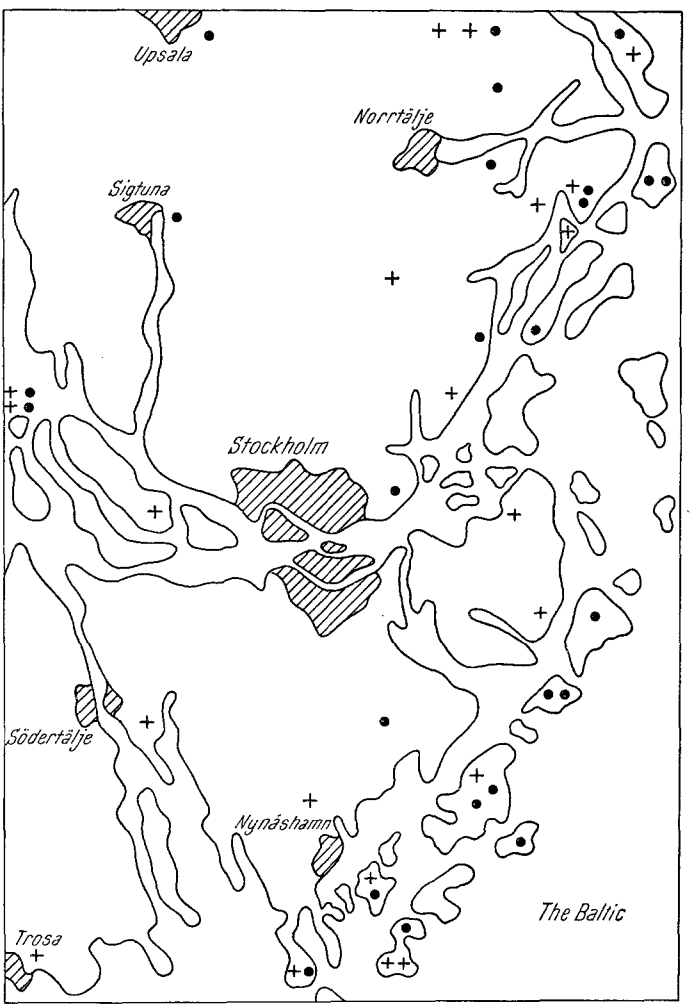


Fig. 2. Geographical location of places where 46 patients with antibodies against LI admitted to have been bitten by ticks.

+ indicates a patient with significant rise of antibody titer against tick-borne virus. Two further + patients not marked on the map had been bitten in other parts of Sweden.

● indicates a patient without rise of antibody titer.

that the frequency of reported tick bites for patients without antibodies was only 5 per cent.

The tick *Ixodes ricinus*, a known vector of this virus, is common in Sweden, at least in the south-eastern parts. A preliminary attempt was therefore made to isolate a virus from 113 *Ixodes ricinus* collected from the archipelago north-east of Stockholm; the result was negative, however.

A very preliminary attempt to trace the distribution of the virus by testing for neutralizing antibodies in residents as well as in cows was also made. Only one out of the 31 male adults from the archipelago north-east of Stockholm and none of the 44 blood donors from Stockholm neutralized the virus. A more distinct result was, however, obtained from the investigation of the bovine sera. Thus all 22 sera procured at 10 farms in the coastal region north-east of the town of Norrtälje contained neutralizing antibodies whereas only one of the 35 sera from south-western Sweden was positive. It should be mentioned that the titers of most bovine sera (nine had a titer of 25 and ten ≥ 125) were comparable to those found in human convalescent sera. It is a common experience that the cows in infested areas get extensively bitten by ticks; the frequency of immune cows may, therefore, possibly be regarded as an index of the infestation of the area with virus-infected ticks.

Discussion

The data presented above indicate that tick-borne meningoencephalomyelitis of the Central European type not infrequently occurs in the Stockholm region. A study of the distribution of the virus in the rest of Sweden is in progress.

It should be stressed that nothing is known so far about when this virus disease first appeared in Sweden; it seems quite probable that these cases previously were hidden in the group of acute infections of the CNS with undetermined etiology.

In endemic areas this etiology should certainly be considered in cases with the clinical diagnoses of aseptic meningitis, meningoencephalitis, or even paralytic poliomyelitis. In the individual case no etiological conclusion can be reached without suitable virological tests, however. For routine diagnosis of infections with tick-borne viruses the complement fixation reaction, with a satisfactory antigen, would appear to be the method of choice. Apparently the correlation of changes in CF titer to the interval after the first as well as the second onset of the disease may show a good deal of variation in individual cases, however. Therefore, although the titer may sometimes continue to rise over an extended period of time, it is important that the first serum specimen should be collected as early as possible in order to increase the possibility of conclusive

results of the diagnostic tests. As a suitable time for collection of the second specimen 3—4 weeks after the onset of the major disease may be recommended, as the peak titer should have been reached at this time in most cases.

For routine use a noninfective CF antigen is definitely preferable. With the aid of β-propiolactone a satisfactory antigen is easily prepared from tissue culture fluid infected with Central European tick-borne virus. The results obtained with this antigen were not significantly different from those reached with untreated tissue culture fluid containing live virus.

The CF titer does, however, drop rather rapidly and a negative CF test does obviously not exclude immunity. The immune status should rather be investigated by tests for neutralizing antibodies. Again tissue culture methods appear advantageous.

Summary

Out of 176 patients, hospitalized in Stockholm during the summer and fall of 1956 for acute infections of the central nervous system, not less than 68 had antibodies against viruses of the Russian spring-summer/louping ill group. During convalescence 30 of these cases showed a definite rise in antibody titer. The clinical picture was that described in Central European tick-borne encephalitis.

Neutralizing antibodies were determined in cultures of Detroit-6 cells. Infected tissue culture fluid, inactivated with β-propiolactone, was found suitable as complement-fixing antigen.

Addendum

Through the courtesy of Dr. *Pravin Bhatt*, Dr. *Khorshed M. Pavri* at the Virus Research Centre, Poona, India, has kindly tested paired sera from 12 of our cases with rising antibody titers against HYPR or LI virus, as well as the convalescent sera of 13 other cases lacking such antibodies. The sera were tested by him for CF and hemagglutination-inhibiting antibodies against four antigens of group B (Kyasanur Forest Disease [KFD] virus, which belongs to the RSSE-LI group, Japanese B, Egypt 101 strain of West Nile and Trinidad 1751 strain of Dengue II) as well as against Sindbis from group A. Cases negative in our hands were also negative, with both techniques, for all these antigens. The positive ones all had CF antibodies against the KFD virus (titers 4—16 in convalescent sera, 9 of which showed at least a fourfold rise) but were negative (<4) in CF tests against the other antigens. The corresponding HI titers against KFD antigen ranged between 20 and >160. On the other hand, negative results or low titers (<10—20) were obtained against other B group antigens, and all sera were negative (<10) against the A group strain.

These results of Dr. *Pavri* further corroborate our conclusion that a virus of the RSSE-LI group is prevalent in Sweden and demonstrate in addition the relative specificity of the CF reaction.

References

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