

Interactions of some intracellular *Hyalomma asiaticum* pathogens in organism

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ABSTRACT

Aim: The aim of this study was to study the interaction of intracellular pathogens of tick-borne encephalitis (TBE) and salmonella in *Hyalomma asiaticum* tick body during the bloodsucking of imago and nymphs on laboratory animals with viremia and bacteremia. With a simultaneous infection from *H. asiaticum* ticks by two intracellular pathogens (CE and salmonella virus), the virus titer decreases after 30–40 days, and then, the ticks die. **Materials and Methods:** The study was conducted on the ticks of a laboratory line, free from pathogenic flora. Tick encephalitis virus was used to infect ticks with the “Sofin” strain and the bacterial culture *Salmonella typhimurium* LT-2. **Results:** During an infection of nymphs, *H. asiaticum* virus persisted in their body for the entire observed period, both at the infection of nymphs with a mixed infection and at the infection with TBE virus. Salmonella persisted in the body of sexually mature *H. asiaticum* ticks with a mixed infection up to 12 days. **Conclusions:** With a mixed infection of ticks by two infection pathogens at the same time (TBE and salmonella virus), the inhibition of both TBE and salmonella virus growth and development takes place.

KEY WORDS: Borrelia, *Hyalomma asiaticum*, Salmonella, Tick-borne encephalitis

INTRODUCTION

It is known that different pathogens affect the behavior of ticks and their pathogens that are pathogenic to humans. For example, the presence of tick-borne encephalitis (TBE) virus stimulates their activity, and borrelia, on the contrary, decreases it. Moreover, with ticks, inhibited by Borrelia, the situation is a reversed one - they are less common on an animal feeder and on a man.^[1] Thus, there is a marked antagonism between two types of tick-borne pathogens. It is also suggested that the ligands of borrelia can block the penetration of virions into tick cells through membranes. Moreover, apparently, this blockade can be carried out not only in the intestines but also in other tissues, including hemocytes.^[2]

The interaction of pathogens in a tick organism has been studied poorly, especially in the case, when both pathogens are intracellular (CE and salmonella

virus). There are only few experimental works on mixed infections of TBE and salmonellosis.^[2-6] The aim of the study was to study the interaction of intracellular pathogens of TBE and salmonella in *Hyalomma asiaticum* tick body during the bloodsucking of imago and nymphs on laboratory animals with viremia and bacteremia.

The Collection of Studied Ticks, Tick Encephalitis Virus, and Bacterial Culture *Salmonella typhimurium*

The experimental model was represented by *H. asiaticum* Sch. et Schl., 1929 (Acarina, Parasitiformes, and Ixodidae). The study was conducted on the ticks of a laboratory line, free from pathogenic flora. Tick encephalitis virus was used to infect ticks with the “Sofin” strain and the bacterial culture *S. typhimurium* LT-2.

The Study of Intracellular Pathogen of Tissue Encephalitis and Salmonella Interaction in Tick Organism on the Animals with Viremia and Bacteremia

The mixed infection of white mice was made intraperitoneally. The titrated 10 brain suspension of

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diseased mice and the bacterial culture of Salmonella were used to infect ticks. The mites were fed on infected mice. The infection with the virus and bacteria was checked only among completely soaked ticks. The virus was identified in ticks by the titration on white mice. Two sexually mature mites and three nymphs were taken for titration. The ticks treated with saline and 96 alcohol were ground in a porcelain mortar, and 1 ml of physiological saline was added, which contained 1000 units of kanamycin to release the suspension from Salmonella. Then, the suspension was centrifuged at 3000 rpm for 10 min, and the dilutions were made from 10⁻¹ to 10⁻¹⁰. Then, mice were infected by each dilution (5 mice per each) with an intracerebral introduction.^[4,6] The infestation of ticks with salmonella was tested according to the same scheme, and 10-fold dilutions of the suspensions were sown on agar plates by 0.1 ml. The crops were incubated in a thermostat at 37°C for 2–5 days, and then, the number of grown colonies was taken into account.^[4,6]

Infected ticks were kept in tubes with the humidification at room temperature.

The experiments were repeated 2–3 times.

DISCUSSION OF OBTAINED RESULTS

The results of the studies are presented in Tables 1 and 2. We have established that sexually mature *H. asiaticum* ticks can be a reservoir for CE virus, preserving it for the entire follow-up period (60 days). The number of viruses and salmonella decreased in ticks as the length of stay in their body increased. In control group, *H. asiaticum* was infected only with TBE virus, the virus in their organisms persisted for a longer time, and after 2 months, its titer was 1 g LD 50/0.03 ml–4.8 ml [Table 1].

The amount of viruses in sexually mature *H. asiaticum* ticks infected with two infections (TBE and salmonella) decreased 12–24 h after infection, making 1 g LD 50/0.03 ml–4.4 ml after 12 h and 4.1 ml after 24 h and 1 g LD 50/0.03 ml–4.9 ml after 48 h. Later, an increase in titer was noted on the 3rd–6th days. In the following terms (after 12, 18, 24, 30, and 40 days of infection), the virus titer fell and did not reach the initial level. 60 days after infection, the TBE virus could not be isolated. Just as in the experiments with imago, the nymph of *H. asiaticum* noted a slight increase in the virus titer 3–6 days after the infection. After 60 days of observation, the titer at mixed infection (TBE and salmonella) was lower than in the control group and did not reach the initial infectious dose. The studies of bacteria survival dynamics at their joint residence in the organism of ixodids showed that, within 12 days, the ratio of their amount in the invertebrate microcell was reduced sharply.

Salmonella with mixed infection in sexually mature mites persisted up to 18 days [Table 2]. The number of bacteria decreased significantly after 12, 24, 48, and 72 h, amounting to 1.5 × 10³, 60, 27, and 6 CFU/ml, and 6 days later, 6 colonies, after 12, 18, 24, 30, 40, and 60 days, respectively, Salmonella was not sown. They were stored up to 18 days in the body of *H. asiaticum* salmonella nymphs when they were coinhabited with CE virus.

When ixodid ticks were applied to the body, only Salmonella was different. Thus, *H. asiaticum* in imago had the decreased number of Salmonella during the period from 12 h to 72 h, from 1.0 × 10⁸ CFU/ml to 8.9 × 10³. In the following 18 days after infection, the amount of salmonella among ticks decreased to 1.1 × 10⁴–5.3 × 10³ cfu/ml. By the 24th, 30th, and 60th days, salmonella was sown from the ticks in the amount of 4.3 × 10², 140, 87, and 68, respectively. The release of ticks from salmonella was not observed

Table 1: The change of TBE virus titer (in lg LD 50/0.03 ml) in *H. asiaticum* ticks

The period between tick infection and study (titration method)	Tick development stage			
	Imago		Nymphs	
	With simultaneous infection by the virus and salmonella	When infected only with TE virus	With simultaneous infection by the virus and salmonella	When infected only with TE virus
0 h	6.8	7.6	6.5	7.7
12 h	4.4	6.1	6.2	7.0
24 h	4.1	5.3	4.9	5.1
48 h	4.9	5.5	4.8	5.0
72 h	5.0	7.4	5.9	6.4
6 days	5.9	7.9	6.1	7.6
12 days	4.8	8.1	5.7	6.9
18 days	4.0	6.2	6.0	6.6
24 days	4.1	5.9	4.3	5.8
30 days	3.9	5.2	4.3	5.6
40 days	2.5	5.0	4.1	5.4
60 days	Absent	4.8	3.6	5.1

H. asiaticum: *Hyalomma asiaticum*, TBE: Tick-borne encephalitis

Table 2: The change of viable *S. typhimurium* bacteria number among *H. asiaticum* ticks with simultaneous infection by salmonella (1.0×10^8 of microbial cells per 1 specimen) and TBE virus and the infection by salmonella only

The period between tick infection and study (sowing method)	Tick development stage			
	Imago		Nymphs	
	With simultaneous infection by TE virus and salmonella	When infected only with salmonella	With simultaneous infection by TE virus and salmonella	When infected only with salmonella
0 h	1.0×10^8	1.0×10^8	1.0×10^8	1.0×10^8
12 h	1.5×10^3	1.0×10^6	1.5×10^2	2.1×10^6
24 h	60	6.4×10^5	1.2×10^2	1.5×10^5
48 h	27	2.9×10^5	1.1×10^2	4.0×10^4
72 h	8	8.9×10^3	86	1.2×10^4
6 days	6	1.1×10^4	24	8.0×10^3
12 days	No growth	9.0×10^2	No growth	7.6×10^3
18 days	No growth	5.3×10^2	No growth	4.8×10^3
24 days	No growth	4.3×10^2	No growth	1.2×10^3
30 days	No growth	140	No growth	4.7×10^3
40 days	No growth	87	No growth	215
60 days	No growth	68	No growth	150

H. asiaticum: *Hyalomma asiaticum*, *S. typhimurium*: *Salmonella typhimurium*, TBE: Tick-borne encephalitis

by the end of 60 days. Similar data were obtained from infected *H. asiaticum* nymphs.

Hence, with a mixed infection, the viability of the virus among ticks is lower than in control. Salmonella lost vitality after 12 days in the organism of adult ticks with mixed infection and after 18 days in the body of nymphs, but their number was higher. The results of the experiments confirm the conclusion reached earlier that the persistence of viruses and bacteria in ticks depends not only on the properties of pathogen strains but also on the protective mechanisms of ticks [4,6,7,8]

The works on mixed infections (TBE and *Borrelia afzelii*) concerning the interaction of extracellular and intracellular pathogens in the body of *Ixodes ricinus* ticks are of great interest. The authors conclude that the offspring of abnormal (changed under the influence of environmental changes) and normal female ticks is more likely to be more sensitive to TE infection and, in connection with this, more epidemically dangerous. When the tick of the TE virus and the bacterium enters the organism mutually, an antagonistic interaction between pathogens is possible.^[3,9]

Alekseev *et al.* and Mishaeva *et al.* found that the TE virus stimulates the activity of ticks, and *Borrelia*, on the contrary, reduces them. *Borrelia*, which causes a less severe bacterial infection, competing with the TE virus, partially suppresses and limits both the spread and the severity of the viral disease. The authors consider this phenomenon as the first factor in the history of science detecting the suppression of bacteria virus in the same organism - in a carrier tick.^[1,3]

CONCLUSIONS

With a simultaneous infection of *H. asiaticum* by two intracellular pathogens (TE and salmonella virus),

the virus titer decreases after 30–40 days, and then, ticks die. In control, where the ticks are infected with only one TBE virus, the virus was released during the entire observed period (60 days). A similar picture was observed in the change of virus amount among ticks, which, however, was slightly higher than during the infection contamination with a double infection and slightly lower than an original infectious dose.

When nymphs are infected by *H. asiaticum*, the virus in their body persisted for the entire observed period, both during the infestation of nymphs with a mixed infection and during the infestation with one virus of TBE. However, the amount of the virus with a mixed infection after 60 days of observation was two orders lower than during the infection with one virus and did not reach the infectious dose.

Salmonella with mixed infection persisted in the body of sexually mature *H. asiaticum* ticks up to 12 days. In control, during the infection by one bacterial culture, the salmonellae persisted for the whole observed period, and their number was 150 cfu/ml. Similar data were obtained during the infection by *H. asiaticum*. Salmonella died during the infection with a mixed culture after 18 days, while they remained viable in the control.

With a mixed infection of ticks by two pathogens of infection at the same time (TBE virus and salmonella), the inhibition of both TBE virus and salmonella growth and development takes place.

SUMMARY

Thus, as a result of the conducted studies, with a mixed infection, the viability of the virus among ticks is lower than in the control. Salmonella with mixed infection lost vitality after 12 days in the organism of adult ticks and after 18 days in the body of nymphs,

but their number was higher. The results of the experiments confirm the conclusion made earlier that the persistence of viruses and bacteria among ticks depends not only on the properties of pathogens strains but also on the protective mechanisms of ticks.

CONFLICTS OF INTEREST

The authors confirm that the presented data do not contain conflicts of interest.

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