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THE ALTERNATION BETWEEN SPIROCHETE AND CYSTIC FORMS OF THE TICK-BORNE BORRELIOSIS AGENT: ITS RELATIONSHIP TO LYME DISEASE MORBIDITY DYNAMICS

ЧЕРЕДОВАНИЕ СПИРОХЕТ И ЦИСТ КАК ОСНОВА ЖИЗНЕННОГО ЦИКЛА ВОЗБУДИТЕЛЯ КЛЕЩЕВОГО БОРРЕЛИОЗА

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Ключевые слова: клещи *Ixodes*, клещи *Argas*, *Borrelia*, спирохеты, цисты, болезнь Лайма, заболеваемость

This work is dedicated to Joanne Rubel

ABSTRACT

The long established life cycle pattern in Spirochaetacea implies the alternation of a motile spirochete and a cyst depending on the situation. Adverse conditions like starvation in vitro, improper pH, low temperature, suppression by increased immunity or drug action in vivo (e.g. salvarsan, antibiotics), etc. are known to result in spirochete cystogeny, whereas such favorable conditions as, e.g., a rich medium in vivo, absence of vertebrate host immunity, blood replenishment by a soft or hard tick vector, etc. provoke the development of motile spirochete. The agent of Lyme disease, or tick-borne borreliosis, being a spirochete, is suggested to be no exception in the *Borrelia* — ixodid tick/vector — human patient system. Based on observations in 1996–2000 at a focus of tick-borne encephalitis and borreliosis near St. Petersburg, Russia, an analysis was performed of the occurrence frequency of motile *Borrelia burgdorferi* sensu lato in the taiga tick, *Ixodes persulcatus* Schulze, detected using darkfield microscopy, PCR and IFA techniques. With a total of 3610 ticks analyzed, a positive correlation of adult tick activity (April–July) with soil surface temperature over the season was demonstrated but a negative one with morbidity during May–July. At the same time, PCR positive but live spirochete-negative ticks were 3 times more abundant at the very beginning of the season (end of April beginning of May), when the highest morbidity rate was observed. This allows for the hypothesis to be advanced that, in the hibernating adult ticks, it is the spirochete cystic form that prevails, serving the main source of infection at least during that period. An increased frequency of occurrence of motile spirochetes toward the end of the season appears to suppress tick locomotor activity, shortens up to 2 times the lifespan of infected ticks, and decreases the number of *Borrelia*-infected tick females. This suggests that *Borrelia* is a true tick parasite, being its competitor in food consumption, i.e. the blood accumulated by the host at the previous stage. As cyst prevalence until early in the season followed by a steady rise in live spirochete population (especially non-pathogenic ones) toward fall correlates most spectacularly (Pearson's correlation index 1.000) with a drop in tick morbidity, the main infectious agent in PCR positive but live motile spirochete-negative ticks can be supposed to be the cystic form of *Borrelia*, especially so after overwintering, at the beginning of the season. A relapse of the disease can thus depend on cyst and motile spirochete alternation. A similar alternation process is apparently typical of *Ixodes* as well as of *Argas* ticks.

РЕЗЮМЕ

Накопленные за последнее столетие данные о трансформации подвижных спирохет (боррелий) в цистные формы под влиянием неблагоприятных для них условий среды (резкого изменения pH, понижения температуры, голодания in vitro и воздействия иммунной системы или антибиотиков in vivo), а также данные о реконверсии цистных форм снова в подвижных спирохет, позволили выдвинуть гипотезу, что аналогичные процессы происходят в системе

боррелия—иксодовый клещ—переносчик—больной человек. На основании наблюдений в течение 1996–2000 гг. в очаге клещевого энцефалита и боррелиоза в окрестностях Санкт-Петербурга (Россия) произведено сравнение частоты обнаружения живых спирохет в клещах, их принадлежность к 3 видам боррелий, патогенных для человека, (методом ПЦР и РНИФ) и заболеваемости клещевым боррелиозом с учетом влияния абиотических факторов среды (температуры подстилки). Установлено, что в течение сезона встречаемость клещей со спирохетами растет и положительно коррелирует с ростом температуры. Число клещей без спирохет с боррелиями патогенными для человека меняется существенно меньше и в начале сезона (в апреле) в 3 раза превышает таковые у особей со спирохетами. Все перечисленные параметры: встречаемость клещей с боррелиями и температура отрицательно коррелируют с заболеваемостью. Установлено, что выживаемость клещей с боррелиями в 2 раза ниже, чем незараженных. Этим вызвано уменьшение числа самок с патогенными боррелиями в сезоне. Отмечено уменьшение локомоторной активности клещей по мере роста экстенсивности заражения переносчиков спирохетами. Эти факторы положительно коррелируют с уменьшением заболеваемости.

Допущение, что экстенсивность заражения клещей цистными формами в течение сезона обратно таковому встречаемости подвижных спирохет, дало величину корреляции с заболеваемостью равную единице. Взрослые клещи, не успевшие напитаться кровью в течение сезона активности, погибают от истощения, ускоряемого паразитированием накапливающихся боррелий. У всех напитавшихся зараженных клещей по данным Бургдорфера [Burgdorfer, 1999] происходит бурное размножение спирохет. Напротив, в зимний период, по всей вероятности, происходит уменьшение количества спирохетных форм и накопление цистных, выявление которых возможно методом ПЦР. Так, в начале сезона число клещей положительных по ПЦР, но без спирохет, определяемых методом темнопольной микроскопии, в 3 раза превышало число клещей, у которых обнаруживались подвижные спирохеты в темном поле.

Таким образом, представляется вероятным, что чередование цистной и спирохетной форм является основой жизненного цикла боррелий не только в аргасовых, но и в иксодовых клещах.

INTRODUCTION

Most probably it is Ewing [1907] who was the first to describe a chain of granules which outlined a complete spirochete, *Treponema pallida*. Dutton & Todd [1907] supposed that sporocyst-like bodies found in the blood without detectable *Spirochaeta* (*Borrelia*) *duttoni* might actually represent a different form of spirochete, from which true spirochetes could develop. Balfour [1911] found spherical granules in the liver of spirochete-infected Sudanese fowl after bird treatment with salvarsan. This author was apparently the first to suggest that spirochete transformation into granules as being the microorganism's defense mechanism, which also provided the capacity of a disease relapse.

A direct confirmation of the alternation pattern of an agent's two life forms seems first to have been demonstrated by Skavinski who, in 1940, revealed the development of the lice typhus agent, *Spirochaeta obermeieri*, in the patient's blood [cf. Shcheulov, 1956]. Skavinski described these stages of development as follows: (1) a non-fever period — granular forms 0.2–0.25 μ, not motile; (2) their growth to 1μ, cocciform and motile in appearance; (3) 3–4 μ, motile amoeba-like bodies; 4) last pre-fever day — cells with tails and short spirochete-like cells with 2–3 spirals; (5) appearance of 5–6 short spirochetes from one amoeba-like cell; (6) normal-sized spirochetes and fever appear together, no granules but only spirochetes; (7) on the second day of fever, new granules (together with spirochetes) appeared; (8) after the fever's paroxysm — only granular forms again. Then the cycle might be repeated. Taking all this into account, Skavinski recommended salvarsan injections administered repeatedly every 2–3 months following a "false" recovery from the disease.

He did not analyze the agent's life cycle inside the louse, whereas Balfour suggested that the granular form of the spirochete might be infective to the soft tick, the vector of bird spirochetosis. Hindle [1912] confirmed this by tracing "coccoid bodies" transformed from *Spirochaeta* (now *Borrelia*) *gallinarum* in the Malpighian tubes and ovary cells of the soft tick genus *Argas*. This author described a very probable pattern of spirochete life cycle inside the soft tick body. Spirochetes ingested with host (= bird) blood either formed coccoid bodies, or got destroyed. The coccoid bodies remaining from spirochetes inside the cells were able to survive, to multiply and thereafter to be retransformed into typical spirochetes. Surviving in the "invisible"

form inside the tick cells was the main reason why, during an active epidemiological survey of tick-borne relapsing fever in Central Asia and the Caucasus, tick infection was only revealed in soft ticks (*Ornithodoros*) feeding on susceptible guinea-pigs. Working at the foci of borreliosis over the territory of the former Soviet Union, Pavlovsky [1949, 1952] emphasized that, to offer the right response to the infection, it was insufficient to detect spirochetemia after 5–7 days of incubation. He recommended, 20 d after tick attachment, to inject some blood of the attacked animal without detectable spirochetemia into the nasal cavity or under the eyelid of a fresh, "naive" animal, and to check for spirochetemia during the next 20 days. This presupposed that the blood of a healthy animal formerly attacked by ticks was able to retain "invisible" forms of *Borrelia*. Most probably, dormant intracellular "coccoid forms" of spirochetes kept the soft ticks *Ornithodoros papillipes* (Birula) infective during several years of hunger. For example, one of the *O. papillipes* females collected in 1931 was able to transmit spirochetes after 2 years of starvation (1941–1942, World War 2), with neither food offered nor warming, nor humification of its container [Pavlovsky & Skrynnik, 1945]. The female originating from the Dushanbe Region, Tajikistan was first fed periodically, once a year, and the first time it transmitted spirochetes to a guinea-pig was in 1933. Soft tick recurrence typhus was very well-known those days, with periods of fever with abundant spirochetemia followed by periods of remittance. Toward the end of the disease, the patient developed relatively strong immunity.

The hard tick borreliosis agent enjoys a different arthropod host, the tick genus *Ixodes*, whose clinic and life cycle are quite dissimilar. *Ixodes* ticks are capable of neither standing a too long starvation period nor repeating blood consumption during one stage of development. A complete disappearance of the spirochete form inside the active developmental stages is also atypical. Nevertheless, the data accumulated during the past two decades allow to conclude that *Borrelia burgdorferi* basically displays the same or a very similar life cycle in both invertebrate and vertebrate hosts as soft tick borreliosis agents do. Burgdorfer [1999], the discoverer of *B. burgdorferi* in its spirochete form, has demonstrated their "granule shedding" in ticks and that "the engorgement of infected ticks results in up to 100% infected ticks", with visible borreliae. Exactly the same picture was observed by Hindle [1912] in *Argas* ticks some 90 years earlier. The use of electron microscopy has recently allowed to locate both cysts and spirochetes of *B. burgdorferi* as associated with tick gut epithelium [Barbour & Hayes, 1986]. All these paramount similarities invite an analysis of the factors which might cause the process of alternation.

Unfavorable factors. Low temperatures and starvation are long known as such factors for tick survival [Hindle, 1912; Pavlovsky & Skrynnik, 1945]. Similar factors prove valid for spirochete cultures as well. In 1949, Geltzer described the appearance of "corn-like" bodies in an old, exhausted culture of a Caucasian *Borrelia* strain. These bodies were called by this author "dead spirochetes". Nonetheless, in a fresh culture they "revived", again becoming live motile spirochetes. A rough change in pH initiates a very quick (within minutes) transformation of *Borrelia* in distilled water into the cystic form [Brorson & Brorson, 1997]. Alban et al. [2000] have confirmed the old data of Geltzer [1949] that serum starvation induces *B. burgdorferi* morphological changes. Geltzer's [1949] observations have also been confirmed by Brorson & Brorson [1997]. In their experiments, a torpor state of the cysts was overcome after the medium was enriched by serum.

Another unfavorable impact is the use of benzylpenicillin. When affected by this antibiotic, *B. burgdorferi* spirochetes form spherical cyst-like structures [Schaller & Neubert, 1994]. Alban & Nelson [1999] note that cystic forms are much more resistant to antibiotics than spirochetes. According to these authors, the amount of tetracycline needed to inhibit the cysts is far greater than that achievable in humans.

Unfavorable impacts on *Borrelia* in vivo have been described many times. Relapsing soft tick-borne borreliosis has placed on the stage an immunity action and confirmed that the alternating spirochete and cystic forms might also be due to immunity pressure. Alerer et al. [1996] have shown that a hyperimmune serum action results in granule formation at *Borrelia* centers or their ends. Cystic forms have been revealed during remittance periods of Lyme disease not only in the skin [Alerer et al., 1996] but in brain tissue [MacDonald, 1988] and human spinal fluid as well [Brorson & Brorson, 1998a]. Such cysts appear at lower protein concentrations in the fluid and might be undetectable using PCR technique but, nonetheless, they are capable of reconversion when the protein concentrations are increased. Hulinska et al. [1994] suggest that cyst-like *Borrelia* forms may be spores because

their surface envelope shows a lectin WGA-positive reaction. Not only the cover quality of the cysts but their surface shrinking by up to 75% [Wolf & Wecke, 1994] reduces the reaction surface for antibodies.

All above permits to agree with Alban et al. [2000] in supposing that cyst formation might help *B. burgdorferi* in evading the detection and action of the immune system.

Cyst formation allows the spirochete to also evade a drug action, e.g. salvarsan [Balfour, 1911] and various antibiotics [Schaller & Neubert, 1994; Kersten et al., 1995; Kazragis et al., 1996]. Most of the cysts retain the capacity to get (re)transformed into spirochetes. In favorable conditions in vitro, i.e. in the presence of serum and proteins, *Borrelia* appeared from cysts even after a long period of survival in distilled water [Brorson & Brorson, 1998b, 1999], where they were previously converted into the cystic form within 1 min at a temperature of 4° C. Such cysts obtained in distilled water have recently been shown to become reconverted into normal motile spirochetes in intraperitoneally inoculated mice [Gruntar et al., 2001]. This capability is retained even after freezing and thawing the cysts. This latter observation seems of special importance, as the main *B. burgdorferi* vector in Russia, the taiga tick *Ixodes persulcatus*, is well-known to hibernating at all active stages of development at very low temperatures, sometimes below –20° C. The northern range limit of *I. persulcatus* has long been established as being determined by winter temperatures below –25° C [Shashina, 1981]. Quite possibly the numerous cocci long detected among dying/starving *I. persulcatus* ticks at the end of the season [Balashov et al., 1997] are *Borrelia* cysts, or "coccoid bodies" as described by Hindle [1912].

Favorable conditions. The main favorable conditions for reconverting the cysts into spirochetes are temperature and blood nutrition. According to Hindle [1912], spirochetes appear from the "coccoid bodies" after 5 days of keeping the soft tick at a temperature of 37° C. Pavlovsky & Skrynnik [1945] have demonstrated that one *Ornithodoros papillipes* female survived for 13 years (1931–1944) and, within this time period, it transmitted *Borrelia* 5 times when fed on nonimmune guinea-pigs. A retarded appearance of borreliae in guinea-pig blood (on 10th to 13th day) seems sufficient as a demonstration that mainly cystic ("cocoid") forms of borreliae were transmitted. In *Ixodes* ticks, the prevalence of darkfield-positive and PCR-positive specimens does not coincide, being rather disparate [Alekseev et al., 2001]. The cystic form infectivity [Gruntar et al., 2001] actually conforms to the highly important conclusion [Nordstrand et al., 2000] that the severe inflammatory reactions of the patients suffering from Lyme disease are out of all proportions compared to the low numbers of spirochetes at the lesion sites. At this early state of infection, no serious immune suppression of spirochetes can be taken into account.

The above suggests that the *Borrelia* cystic form facilitates pathogen survival in *Ixodes* ticks during the winter, this likely playing important roles in human infection.

In an attempt to discover possible correlations between abiotic factors, infected *I. persulcatus* tick abundance rates, and Lyme disease morbidity, the main objective of this study lies in testing the hypothesis that the cystic form of *B. burgdorferi* may be an important factor in the epidemiology of Lyme borreliosis. For this purpose, data accumulated during the 1996–2000 seasons at a focus of tick-borne borreliosis have been analyzed.

MATERIAL AND METHODS

Ixodes persulcatus ticks were collected by flagging at a tick-borne encephalitis and borreliosis focus in the vicinity of St. Petersburg, Russia from April to July (the seasons of 1996 to 2000). Such abiotic factors as air, soil surface and litter temperatures were constantly monitored [Alekseev & Dubinina, 2000]. All ticks collected (total N = 3610) were sorted, placed into tubes with differentiated humidity [Alekseev et al., 1998] and maintained at 21±1.0° C. Their locomotor activity was estimated on inclined "tickdrome", and an activity index was calculated according the author's equation [Alekseev, 1996; Alekseev et al., 2000]. The locomotor activity (LA) was tested the first time 24–48 h following the collection. During 1996–1998, the activity was tested twice and all ticks were dissected after 30 days of maintenance. This method permitted to calculate and compare the mean survival times of noninfected and infected ticks that died earlier. All specimens collected were dissected and their gut content was investigated using darkfield microscopy (DF) to detect live spirochetes (the seasons of 1996 to 2000). All spirochete-positive specimens were tested using PCR techniques [Alekseev et al., 2001] to obtain data concerning their infection by *Borrelia burgdorferi* sensu lato, *Borrelia afzelii*, *Borrelia garinii* and *Borrelia burgdorferi* sensu stricto. In 2000, all

ticks (1257) were studied using PCR regardless of their spirochete infection (DF-positive) as well as negative. This allowed to detect *Borrelia* infection in ticks without visible live spirochetes. Part of the ticks got randomly were investigated using the standard IFA method (the season of 2000) with polyclonal *Borrelia* antibodies.

The morbidity of tick-borne borreliosis was estimated based both on the general number of patients in St. Petersburg's hospitals (163, the season of 2000) and the epidemiological data concerning the people who suffered from Lyme disease deriving from the study focus of borreliosis (24, the seasons of 1996 to 2000).

To estimate the statistical significance of the data obtained, Student's *t*-test and the Pearson correlation matrix were analyzed using SYSTAT [Wilkinson, 1990].

RESULTS

The data obtained are summarized in Table 1. The values were compared with one another to detect their possible intercorrelations (Table 2). The dynamics of morbidity as the main subject of correlation analysis demonstrated two types of correlation with the other phenomena, i.e. negative or positive. An increasing temperature which positively correlated with a rise in the relative number of spirochetes detected during the season (0.996) showed a highly negative correlation with morbidity, i.e. –0.994. Spirochete prevalence data provided the highest possible negative correlation with the morbidity dynamics, i.e. –1.000. Evidence obtained using IFA and PCR techniques also revealed negative correlations with the morbidity dynamics, but lesser than DF-positive. This was not surprising, as the morbidity by itself depends on the pathogenic *Borrelia* abundance in the vector population. Nevertheless, an increase in IFA- and PCR-positive ratios during the season also correlated negatively with a decrease in Lyme disease-affected patients. A decrease in LA of the main vectors, i.e. infected females, during the season (tick ageing), and a decrease in the number of such females correlated positively with the morbidity decrease. This was not strange either, because calculating the longevity of naive and spirochete-infected ticks gave a very interesting and important result: the

Table 1. Lyme disease morbidity dynamics and the phenomena it may be accounted for.
Таблица 1. Динамика заболеваемости болезнью Лайма и феномены, которые могут являться ее причиной.

Phenomenon	Conventional symbols	Periods of the season						Correlation values with morbidity
		May		June		July		
		n	data	n	data	n	data	
Morbidity in the focus (1996–2000) and the St. Petersburg Region (2000)	MORB	78	41.7%	62	33.2%	47	25.1%	–
Frequency of occurrence of spirochetes according to darkfield microscopy (1997–2000)	DF	1017	26.7%	727	32.7%	195	38.0%	–1.000
PCR-positive results in the entire population (2000)	PCR1	468	29.9%	349	34.7%	72	34.7%	–0.873
PCR-positive results among DF-positive ticks (1997–2000)	PCR2	334	60.9%	260	74.3%	68	67.0%	–0.467
Mean monthly to of the soil surface, °C (1997–2000)	T	1465	11.7°C	840	15.7°C	235	18.3°C	–0.994
Mean number of females with pathogenic Borrelia calculated per 10 days (1998–2000)	BIF	177	13.0	102	11.3	17	5.7	0.951
Mean values of females locomotor activity calculated per 10 days (1998–2000)	LA	114	19.8	98	19.1	17	18.5	1.000
Hypothetical frequency of occurrence of cysts (100.0 — DF, %) (1997–2000)	CYST	–	73.3	–	67.3	–	62.0	1.000

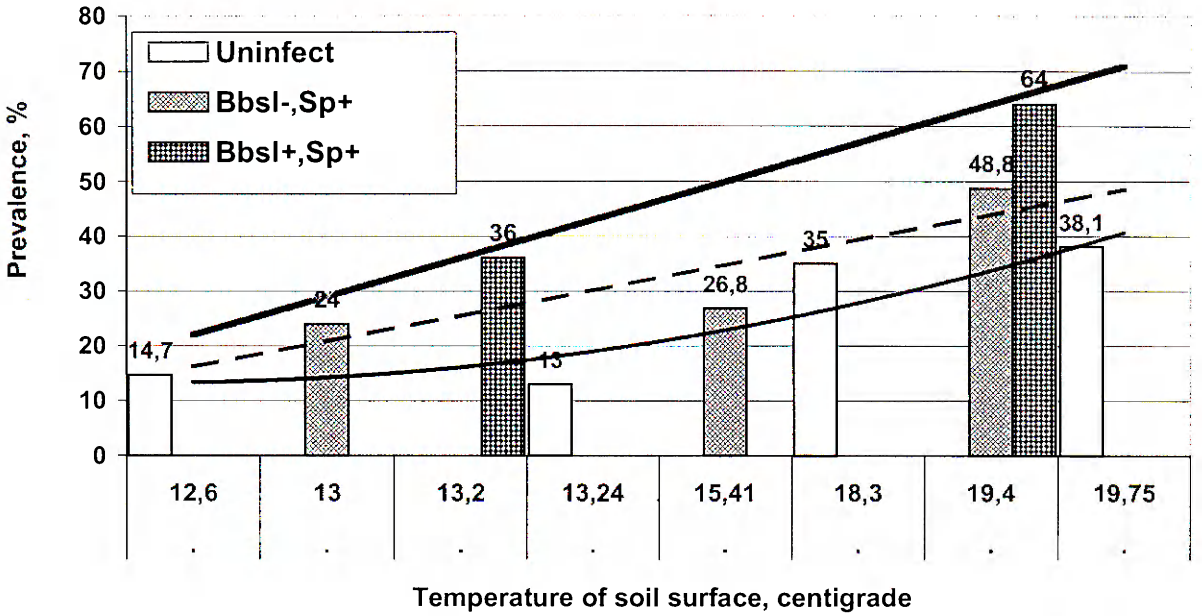

Fig. 1. Prevalence (%, and trend lines) of uninfected and infected ticks in dependence of temperature (1997). Bbsl-, Sp+ — prevalence of live spirochaetes detected by darkfield microscopy, PCR confirmation negative.
Рис. 1. Частота встречаемости (% и линия тренда) зараженных и незараженных клещей в зависимости от температуры. Bbsl-, Sp+ — встречаемость живых спирохет (по данным темнопольной микроскопии) при отрицательном ответе ПЦР.

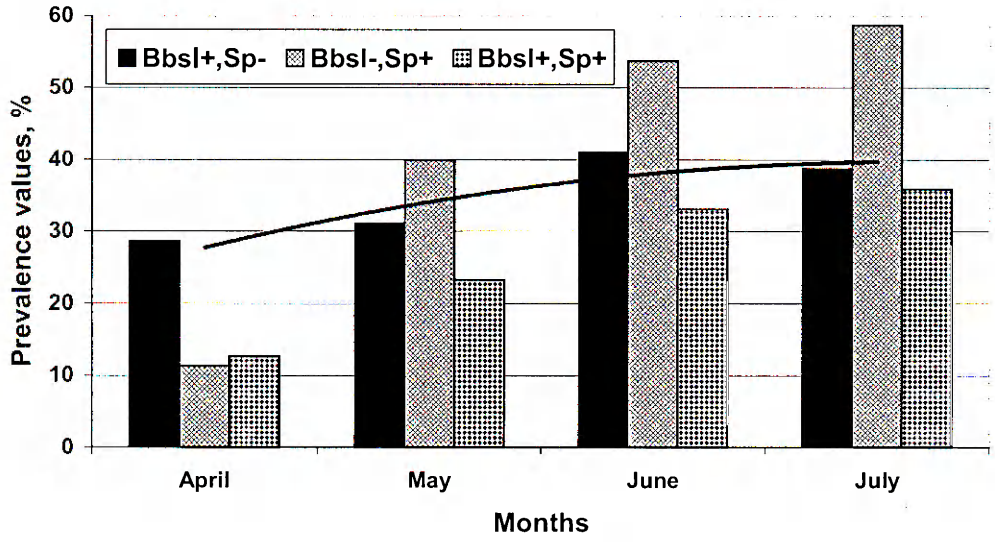

Fig. 2. *Borrelia* prevalence in *Ixodes persulcatus* population (season 2000) and trend line of PCR *Borrelia burgdorferi* s.l. positive tick (Bbsl+, Sp-) ratio changes. Bbsl+, Sp- — prevalence of PCR positive ticks, in which live spirochaetes were not detected by darkfield microscopy; Bbsl-, Sp+ — same as on Fig. 1; Bbsl+, Sp+ — DF and PCR positive specimens.
Рис. 2. Встречаемость боррелий в популяции *Ixodes persulcatus* (сезон 2000 г.) и линии тренда встречаемости *Borrelia burgdorferi* s.l. среди только ПЦР положительных клещей (Bbsl+, Sp-). Bbsl+, Sp- — встречаемость зараженных клещей (по данным ПЦР), среди особей, в которых не были обнаружены спирохеты методом темнопольной микроскопии; Bbsl-, Sp+ — то же, что на рис. 1; Bbsl-, Sp+ — встречаемость живых спирохет (по данным темнопольной микроскопии) при отрицательном ответе ПЦР.

mean lifespan of ticks before dissecting on the 31st day was quite different among infected and noninfected specimens, i.e. 13.7±2.7 (n=6) among the former and 22.9±1.5 (n=20) days among the latter category. The difference using Student's *t*-test was statistically significant ($p<0.01$). An increased frequency of occurrence of differently infected ticks with a temperature increase (Fig. 1) and during the season (Fig. 2) correlated with a decrease in the number of active infected vectors (Fig. 3).

Table 2. Pearson correlation matrix based on the Table 1 data (legend same as in the Table 1).
Таблица 2. Корреляционная матрица Пирсона, полученная на основе данных табл. 1 (условные обозначения те же, что и на табл. 1).

	BIF	CYST	DF	IFA	LA	MORB	PCR1	PCR2	T
BIF	1.000								
CYST	0.944	1.000							
DF	-0.944	-1.000	1.000						
IFA	-0.741	-0.479	0.479	1.000					
LA	0.942	1.000	-1.000	-0.471	1.000				
MORB	0.951	1.000	-1.000	-0.498	1.000	1.000			
PCR1	-0.680	-0.883	0.883	0.011	-0.887	-0.873	1.000		
PCR2	-0.172	-0.486	0.486	-0.535	-0.494	-0.467	0.839	1.000	
T	-0.913	-0.996	0.996	0.401	-0.997	-0.994	0.920	0.560	1.000

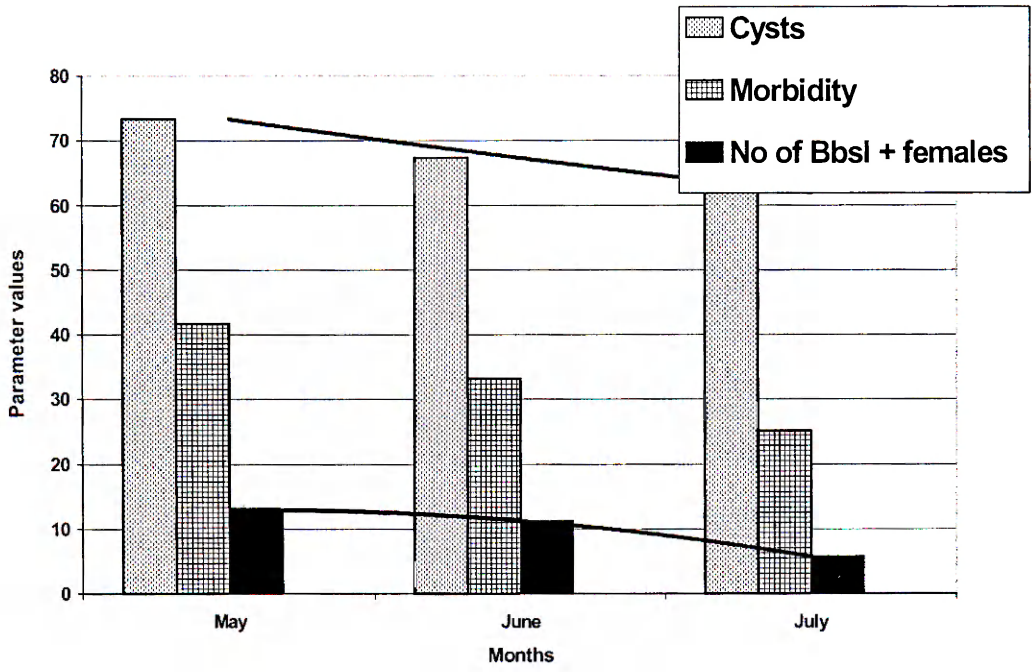

Fig. 3. Ratios of Lyme disease morbidity (seasons 1996–2000) hypothetical prevalence of cysts (seasons 1997–2000) and mean number of *Borrelia burgdorferi* s.l. positive *Ixodes persulcatus* females (seasons 1998–2000) with trend lines.
Рис. 3. Относительное число больных болезнью Лайма в сезоне (1996–2000 гг.), гипотетическая встречаемость клещей с цистами возбудителей (1997–2000 гг.) и среднее число зараженных патогенными боррелиями самок клещей *Ixodes persulcatus* (1998–2000 гг.).

DISCUSSION

The occurrence frequency of infected ticks increasing with a rising temperature (Fig. 1) corresponds very well with Hindle's [1912] data, i.e. at high temperatures the "coccoid bodies", or cysts, are transformed into motile spirochetes. Their low abundance at the beginning of the season, after hibernation, supports Brorson & Brorson [1997, 1998a, b] in that cystic *Borrelia* forms appear quicker at low temperatures. The revealed "granule shedding" of *B. burgdorferi* inside the tick gut cavity is noteworthy in this context [Burgdorfer, 1999]. It is no surprise that both longevity and LA drop over the second half of the season. The physiological ageing of *I. persulcatus* and their abundance in nature correlate with each other [Repkina, 1980]. Nevertheless, a 2 times shorter lifespan of *Borrelia*-infected ticks must be taken in considera-

tion. This might mean that motile spirochetes, whose frequency of occurrence and abundance increase during the season (Fig. 2), being blood-consumers just like their host, accelerate the processes of and *Borrelia*-infected host ageing and female elimination. The results analyzed and the comparisons with published evidence seem sufficient to set forth the hypothesis that the high morbidity at the beginning of the season is associated with tick infection by *Borrelia* cysts, which may be abundant in hibernating *I. persulcatus*.

Data in Fig. 2 seem to confirm this hypothesis. The number of ticks with DF-detected spirochetes was nearly 3 times less than that of PCR-positive (Bbsl+, Sp-, Fig. 2). At the same time, the quantity of ticks with the motile form of spirochetes was nearly the same at the beginning of the season (April) regardless of the presence or absence of PCR-positive specimens. This is no surprise as, in the adverse conditions like low temperature, immune or antibiotic action, *Borrelia* cysts are only detectable using PCR.

Numerous patients have been revealed that are negative serologically, with undetectable *Borrelia* in blood or cerebrospinal fluid but still PCR-positive [Mouritsen et al., 1996; Branigan et al., 1997; Grignolo et al., 2001]. Some infected mice free from *Borrelia* after an antibiotic treatment also appear PCR-positive [Malawista et al., 1994]. All these data as well as the highest correlation value between the hypothetical prevalence of cysts and the morbidity (1.000, Table 1 & 2, Fig. 3), confirm the viability of this hypothesis. "Unseen", microscopically omitted or neglected cysts may infect man. After the tick's engorgement on the vertebrate host the food supply is increased multifold. As a result, the cysts are transformed into motile borreliae [Burgdorfer, 1999].

The above results of five seasons of observation permit to interpret the data obtained in the framework of a new hypothesis. The cyst as the dormant form of a parasite is much safer to their host. The cyst's transformation to a motile form turns them into true, active parasites quite unsafe to both hosts involved, i.e. the tick and man.

The tick solves this problem either by a replenishment with blood or by the death from starvation. Tick mortality (Fig. 3) may be enhanced by an increased spirochete frequency of occurrence toward the end of the season (Fig. 2). The appearance of cysts [Balashov et al., 1997] is not able to improve the fate of the parasite in a dying invertebrate host (*Ixodes* tick). Nearly all vertebrate hosts of adult ticks (e.g. deer, hedgehog, hair) are tolerant to infection while rodents are too short-living. Man, the "dead-end" for the infection, being less tolerant and having less capacities to eliminate this parasite, pays off by chronic Lyme disease, in which both motile and cystic *Borrelia* forms alternate due to a periodically increased immunity or an insufficient antibiotic action.

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