VII International Potsdam Symposium on Tick-borne Diseases (IPS-VII) 2003

Dear colleagues,

It is our pleasure to welcome you all to the VII International Potsdam Symposium on Tick-borne Diseases (IPS-VII) in Berlin. The meeting takes place at the Federal Institute for Risk Assessment which has been reorganised and now harbours the German National Reference Laboratory for Tick-borne Diseases.

With its main topics – Tick-borne Diseases incidence on the increase, epidemiology and ecology, prophylaxis and vaccination, immunology, diagnostics and strain differentiation – IPS-VII is going to discuss again urgent problems of this field of research.
The question as to whether tick-borne human and animal diseases are actually on the increase concerns us all. The answer to this question is essential for deciding whether research programmes with far-reaching consequences should be approved and new strategies in the control of these diseases developed.

Also, the topic of vaccination against TBE should be of particular interest since two manufacturers of TBE-vaccines as well as independent scientists are going to present their data on current and future commercially available TBE vaccines and on the current situation in Lyme borreliosis vaccination.

I am very pleased to welcome colleagues and many friends from more than 25 European countries, overseas and the Far East. It has been again our aim to encourage young scientists from eastern Europe, and it is our intention to continue and intensify this kind of support.

The fact that IPS has, for the first time in its existence, been open to submissions of oral presentations has fortunately resulted in new dimensions as to number and content of the scientific contributions submitted. This IPS has reached a new level with approximately 80 scientific contributions. This means, however, that for reasons of time we were unable to include by far all the presentations submitted, although many contributions would have been suitable for oral presentation. We had to be very strict to decide which contributions should orally be presented. The schedule leaves enough time to discuss poster presentations. The idea to hold parallel sessions was rejected since this would have affected the character of IPS as a scientific meeting with a distinct personal touch.

The Proceedings of IPS-VII – like those of IPS-VI – will be published in the International Journal of Medical Microbiology (IJMM). Therefore, all authors of oral papers are asked to send their manuscripts in electronic form as soon as possible to (j.suess@bfr.bund.de). The manuscripts will be presented as described under http://www.urbanfischer.de/journals.

Also, on behalf of my colleagues Olaf Kahl and Peter Kimmig, I wish to express our hope that IPS-VII will be successful, that there will be good contributions and a vivid exchange of ideas and a warm and pleasant atmosphere.

Berlin, February 2003

Jochen Süss

Abstracts

Evidence that climate change has caused 'emergence' of tick-borne diseases in Europe?

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Tick-borne diseases are not new in Europe, but there have been significant increases in their recorded incidence over recent decades as increasingly robust enzootic cycles spill over into human populations. This may be due partly to public health activities (better surveillance and diagnosis), but also to real increases in rates of contact between humans and infected ticks. Causes for the latter may be non-biological (sociological) or biological, principally the impact of humans on tick habitats and wildlife hosts, and changing climate. Climate has a direct impact on tick populations and pathogen transmission dynamics, but there is very little direct evidence that climate changes to date are responsible for the up-surge of tick-borne encephalitis (TBE) or Lyme borreliosis (LB). In Sweden, the northward spread of ticks and gradual rise in TBE cases since the 1980s have been equivocally related to milder winters, springs and autumns. At the same time, significant host species such as deer have increased in abundance. Although non-competent to transmit the pathogens, deer crucially support tick populations. High densities of deer have been described both as having a zooprophylactic effect and as being responsible for the emergence of LB - only one, probably the latter, can be correct. In many eastern European countries, however, increases in TBE during the early 1990s were extremely abrupt, and coincided with the end of communist regimes. Greater human exposure to infected ticks, through agricultural and sociological changes may have played an important part. It is clear that non-uniform patterns of "emerging" zoonoses are due to geographically variable interactions of climate, wildlife and politics. The same may also be said of emergence in an evolutionary sense - the origin of new species of tick-borne flaviviruses in a cline across the northern hemisphere can be related to satellite imagery revealing distinct environmental conditions. Even this depended on changing host availability driven by sociological forces.

Global climate change and the emergence/reemergence of infectious diseases

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The relationship between climate and human health has been studied for a long time. Weather conditions depend on annual seasonal variations and interannual variability driven by atmospheric phenomena such as the El Niño/Southern Oscillation (ENSO) which is the major source of global climate variability or the North Atlantic Oscillation (NAO). Global warming since the 1950s or long-term changes in climate are superimposed on periodic variability. Starting in the 1970s, the Southern Oscillation Index underwent a marked intensification and an increased frequency. There is still a debate about the relationship between climate change and ENSO intensification. Other issues in the debate on climate change are the impact that these changes will have on human health and whether climate change is caused by human activities. From the recent emergence and reemergence of infectious diseases worldwide, it was inferred that long-term global warming might affect changes in disease trends.

Outbreaks of vector and water-borne diseases (e.g., malaria, dengue fever, cholera) show seasonal patterns indicating the dependence of vectors and pathogens on climate and weather. The incidence of these diseases has increased during the past decades. While several studies support the role of interannual and short-term climate variability in the temporal dynamics of certain infectious diseases (e.g., the correlation of the activity of cholera, rift valley fever, Murray Valley encephalitis, bluetongue virus, and hantavirus pulmonary syndrome to the ENSO), it remains to be seen whether or not there has been any documented change in human disease trends in response to long-term climate change. Clinical malaria cases in Kenya and dengue hemorrhagic fever in Thailand, the most important infectious diseases worldwide, show significant variation in case numbers which do not correlate with ENSO activity: while both disease incidences have a periodicity of 36 months, ENSO has a 4-year periodicity. Moreover, malaria resurgence in Kenya is not linked to climate change. Rather than climate change, variations in environmental, social and epidemiological factors appear to be more plausible explanations for the resurgence of malaria in Kenya.

Human granulocytic ehrlichiosis (HGE) in Europe

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A MEDLINE literature search on HGE revealed that the European story about this tick–borne zoonosis, caused by *Anaplasma phagocytophila*, began in 1995 with an article on the presence of serum antibodies to *A. phagocytophila*. At present, there are seroepidemiological reports indicating the presence of infection with HGE agent(s) for several European countries; seroprevalence rates range from zero or very low to up to 25 %. The proportion of seropositive persons increases with age and is higher in forestry workers, patients with Lyme borreliosis and tick–borne encephalitis, and/or persons with a history of tick bite(s) than in subjects who are not (or who are less) exposed to ticks. Seroconversion of up to 11 % over a single tick season was established in highly exposed persons. Knowledge about the causative agent of HGE in Europe is limited and based on PCR findings; only recently a report on the successful isolation of *A. phagocytophila* from a European patient with HGE appeared. Deer and small mammals are likely natural reservoirs for granulocytic ehrlichiae. A study from Slovenia revealed that red and roe deer are often infected with *A. phagocytophila*. None of the *A. phagocytophila groESL* and 16S rRNA gene sequences from deer were identical to those obtained from human patients; however the sequences from red deer were more closely related to human sequences than those from roe deer. *Ixodes ricinus* is a recognized vector of *A. phagocytophila* in Europe but no transmission studies have been reported. Several articles on the presence of the agent in *I. ricinus* from different European countries have appeared during the last six years; some revealed high or even 100 % homology of genetic material in examined ticks with the causative agent of HE; in the others genetic diversity was established. The prevalence of *A. phagocytophila* in questing *I. ricinus* ticks is usually higher in adult ticks than in nymphs and ranges from zero or very low to >30 %. Pronounced differences between countries and marked variability by localities were established.

The first proven case of human disease originated from Europe (Slovenia) and was published in 1997. Until November 2002 about 50 patients (all but one were adults) with confirmed HGE and several patients fulfilling criteria for probable HGE had been reported. The majority of the cases originated from central Europe (Slovenia) and Scandinavia (Sweden and Norway) but individual reports from the Netherlands, Spain, Poland and Check Republic also appeared. All these patients presented with an acute febrile illness that as a rule occurred after a tick bite; the majority had leukopenia and/or thrombocythopenia, elevated concentration of C–reactive protein and mild abnormalities of liver function tests results. The small number of patients does not permit reliable conclusions about the clinical features of European HGE; however, there is an impression that at least in central Europe (but maybe not in Scandinavia) the disease is, in clinical terms, only mild to moderately severe and (most likely) self–limited. In a prospective study on febrile illnesses occurring after a tick bite in Slovenia, the etiology was delineated in 54/130 (49 %) patients. The most frequent diagnosis was tick–borne encephalitis, followed by Lyme borreliosis and HGE, while in the subgroup of patients with leukopenia and/or thrombocytopenia, HGE ranked second after the initial phase of tick–borne encephalitis.

Conclusions: A relatively high proportion of population with HGE serum antibodies and the presence of *A. phagocytophila* (like) agent(s) in ticks, small mammals and deer as found in several European countries do not concord with the rather low number of patients with proven HGE. The discordance may indicate inadequate awareness among European physicians, limiting registration and reporting of the disease, and/or the presence of and the infection of humans with nonpathogenic *A. phagocytophila* (like) strains present in ticks. Additional studies are needed to better define the biological and public health significance of HGE in Europe.

Natural foci of tick-borne diseases (TBD) and epidemiological situation in Latvia during the last decade

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The highest peak of tick-borne encephalitis incidence in Latvia was observed in 1994 and 1995, however the highest increase of Lyme borreliosis incidence has been noticed since 1998. The observations in the natural foci of the diseases during this period showed comparatively high levels and noteworthy annual changes in activity and spread of two *Ixodes* tick species common in Latvia - *Ixodes ricinus* L. in the whole territory and *Ixodes persulcatus* P.Sch. in the eastern part of the country. Since 1999 there has been a significant decrease in TBE morbidity and a slight decrease in Lyme borreliosis morbidity which correlates, to a certain degree, with the annual trend of tick activity in the monitoring sites. The tick observations also pointed to the landscape and vegetation type as well as rodent species as being the likely determining factors behind adult and nymphal tick density in these particular areas.

The mean annual tick infectivity rate with *Borrelia burgdorferi* s.l. stated in the Biomedical Research and Study Centre University of Latvia by means of a nested PCR method of amplification of a part of *OspA* gene was 20-45 %. The highest tick infectivity level was found in the eastern part of the country. Typing of *Borrelia*-positive DNA samples by PCR-RFLP analysis of 16S - 23S rDNA intergenic spacer pointed to *B.afzelii* as the dominant strain (76 %). It is followed by *B. garinii* (18 %), *B. burgdorferi* sensu stricto (2 %) and *B. valaisiana* (2 %). According to the epidemiological data, during the last several years most of the patients (about 80 %) contracted a local skin form of the disease. There could, however, be a problem with underreporting of the chronic course of LB.

The prevalence of the TBE virus was determined by specialists of the Laboratory of Virological Investigations of the Public Health Agency using ELISA assay of ticks collected in the fields and replete ticks removed from humans and domestic animals. It pointed to a 6 to 20 times higher replete tick infectivity level compared with fasting ones. While annual field-collected tick infectivity rate during the last decade varied from 1 to 10 % (with the exception of 1995 – 28.4 %), the mean replete tick infectivity level was about 30 %. Typing of TBEV isolated from ticks and patient serum samples in collaboration with German and Swedish scientists revealed the presence of three virus subtypes - *Neudoerfl, Vasilchenko* and *Far Eastern* subtype.

Initial investigations of *Ehrlichia phagocytophila* genogroup (by nested PCR targeted the 16S rRNA gene) revealed the presence of *Ehrlichia* sp. in *I. ricinus*. The quality of the results was controlled by HGE agent-positive samples kindly donated by Dr. K. Hartelt, State Health Office Baden-Württemberg, Germany.

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A TBE ceiling in Central Europe has moved upwards during the last 30 years: possible impact of global warming?

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Climate change is expected to have wide-ranging consequences for human health including alterations in the spread of vector-borne diseases. Climate change impact models suggest that the largest changes in the potential for disease transmission will occur on the fringes in terms of both latitude and altitude. The evidence that climate change has increased the risk of these diseases in Central Europe is weak, however, because of the relatively subtle changes in climate to date and the overriding impact of the other human-driven environmental changes.

To examine the surmise of the global warming effect, we analysed retrospectively the geographic/temporal pattern of cases of tick-borne encephalitis (TBE) registered in the Czech Republic since 1970. Using a geographic information system, over 8700 notified places of contraction were pinpointed on a map and overlaid with a digital elevation model to estimate the vertical distribution of the cases. Series of yearly disease ceilings (assessed alternatively as the respective maximum altitude or mean altitudes of the upper 5 or 10 cases) were tested against the null hypothesis of random elevation course, and analysed for correlation with concomitant factors (yearly TBE incidence rate, mean yearly temperature, population density of mice and roe deer).

The Spearman rank correlation test as well as the test of mean stepwise difference and sample variance ratio showed that the TBE ceiling has systematically moved upwards in the course of the last three decades. An average ascending rate within this period was 5.97 m yearly; this correlates well with a concurrent mean temperature increase of ca. 0.043 ºC per year, relying on the model of standard atmosphere. The TBE ceiling correlated significantly with TBE incidence data (the Pearson correlation coefficients were 0.48, 0.65 and 0.64 for the maximum altitude, and mean altitudes of the upper 5 and 10 cases, respectively). The correlation between the TBE ceiling and the mean yearly temperature was not synchronous but delayed with a lag of 1 – 2 years; the closest correlation was shown between the maximum altitude and the mean yearly temperature recorded a year before (Pearson corr. coeff. 0.54). Although TBE incidence correlated with the mouse population density that was observed 1 - 2 years before, neither the TBE ceiling seems to be influenced by mouse population dynamics nor do the population dynamics correlated with mean yearly temperatures. To some extent, TBE incidence as well as mean altitudes of the upper 10 cases also correlated also with the data on harvested roe deer (Spearman corr. coeff. 0.38).

Overall, the fluctuations in TBE incidence and TBE ceiling proved to be synchronous processes that correspond to temperature changes. Although the dependence of TBE on temperature is not straightforward and various factors could be involved, climate warming has a clear impact on vertical disease distribution in Central Europe.

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An attempt to elucidate increased incidence of tick-borne encephalitis and shift to higher altitude in the Czech Republic

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In 1993 the incidence of tick-borne encephalitis (TBE) showed a sharp rise in Central Europe and has remained high since – with some slight fluctuation. This increase is clearly evident in the Czech Republic where TBE has been a notifiable disease since 1951 and serologically confirmed TBE cases have been recorded since 1971. Roughly this increase could be characterized as twofold in 1993-2001 in comparison with 1984-1992 (5,240 : 2,441) human cases. As yet in the Czech Republic the TBE increase has been manifested by: a) a higher number of cases in areas well known for TBE occurrence in humans; b) re-emergence in areas where TBE human cases were not observed or only sporadically, for a long time; c) emergence of TBE in places unknown previously (including high elevated areas). This phenomenon has not been fully elucidated yet and we would like to contribute to a better understanding of the cause by comparing the present situation with historical data. Besides TBE epidemiological data (1965-2001) we use the twenty-year, all season dynamics data of *Ixodes ricinus* host-seeking activity (1953-1972) supplemented by the data in 1992. The fluctuation of annual averages of *I. ricinus* occurrence was irregular. When compared with meteorological data (Czech Hydrometeorological Institute) these irregularities can be explained by different meteorological conditions in particular years. In corresponding long-time series, the peaks of *I. ricinus* occurrence and TBE incidence were reached at the same time. Analyses of relevant meteorological data showed that joint *I. ricinus* and TBE maximal occurrence values had been preceded by mild (or warm) autumn seasons allowing a prolongation of *I. ricinus* activity (including development) until November, at least, thus resulting in higher tick levels the following year. Based on these data we conclude that the increased TBE incidence rates reported in 1993 and thereafter are attributable to a more abundant occurrence of *I. ricinus* ticks and that their higher abundance is due to modified climatic conditions in the last decade. A situation of this kind has appeared in the past as well; however, it was rare and in isolated years only. At that time, although the tick population had been more abundant, the following year it returned to its usual level again. In the 1990s the prolonged mild autumn for several years consecutive led to the permanent occurrence of more abundant populations of *I. ricinus.*

The same conclusion explains the shift in *I. ricinus* occurrence and TBE distribution to higher altitudes in the Sumava and Krkonose Mountains. The studies performed in cooperation with the staff of the Sumava and Krkonose National Parks during 2001-2002 confirmed the existence of local *I. ricinus* populations of up to 1100 m a.s.l. When comparing these present and historical data, it can be concluded that the vertical limit of the *I. ricinus* local population has shifted towards higher altitudes, accompanied by the risk of tick-borne diseases. This is documented by the first human cases newly registered in Sumava Mts. (at an altitude of 900 m a. s .l.).

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Socio-economic conditions and other anthropogenic factors influencing tick–borne encephalitis incidence in the Czech Republic

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Laboratory confirmed cases of tick-borne encephalitis (TBE) have been reported in the Czech Republic since 1971. Peaks of incidence occurred at two to five year intervals. Incidence in the eighties was generally lower than in seventies. In the nineties there was a sharp increase in incidence that reached the peak in 1995 (7,2/100000). TBE cases occurr from April to November with maximum incidence in July. During the last decade the second peak of incidence occurred in most of the years in September and/or October. In the last decade the extension of the TBE season towards the spring and autumn period has been observed. In addition to a general increase in TBE incidence in the last decade, some other changes in the epidemiology of TBE were observed in the Czech Republic. During the whole period, sex-specific incidence was higher in men than in women (man to woman ratio 1.5:1). In recent years age-specific incidence has increased steadily in children and adolescent age groups. In the ten-year age groups from 25 to 65 remain practically at the same level (6-8/100 000). In older persons it decreases to 2-3/ 100 000.

Ticks mainly bite the patients during their recreational activities. A very small proportion (less than 1 %) acquires the infection through the alimentary route. We have tested the hypothesis whether the increase of TBE incidence in the nineties occurred due to economical or social changes after the velvet revolution 1989.

Between 1991 and 195 unemployment largely remained on the same level (between 2 – 3 %). During the next years the percent of unemployed persons increased rapidly to 9.3 % in 1999 (7.8 % in 2001). This trend differs significantly from the trend of TBE incidence that peaked in 1995. No correlation was found between the district incidence of TBE and the district percentage of unemployment in the period 1997 – 2001 ($r = - 0.20$). The percentage of unemployed persons among the TBE cases was 1-3 % in contrast to the Czech Republic figures which were 5 - 9 % for the same period. Gross domestic product in US $ per capita increased from $ 2600 in 1991 to $ 5000 in 1995. Since that year it has varied between $ 4800 – $ 5600. This trend, therefore, differs from the trend of TBE incidence as well. The percentage of forest men and other persons working in the forests among the TBE cases in the period 1997 - 2001 was 0.5-1 %.

The behavioral and socio-economic aspects of TBE cases remained stable despite the political changes which have occurred in the Czech Republic since the beginning of the nineties . They are not, therefore, responsible for the increased TBE incidence.

In the industrial areas which are most heavily polluted with SO_2 in North Bohemia, a sharp increase in TBE incidence in the past seems to be connected with the measures aiming to eliminate the SO_2 discharge of brown coal stations.

Tick-borne encephalitis (TBE) in Germany – Epidemiological data, development of risk areas and virus prevalence in engorged and unengorged ticks

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In Germany, high-risk areas for TBE are found in Bavaria and Baden-Wuerttemberg and low-risk areas in Hesse, Thuringia, Saxony, and Rhineland-Palatinate. 100 – 300 autochthonous clinical cases were recorded annually and more than 95 % of these cases had been acquired in high-risk areas. The number of cases registered annually is stable on a high level with a slight upward tendency in recent years.
In the last 5 years, however, 12 further districts had to be defined as risk areas. These areas were found to expand slowly to the north, in particular in Bavaria and Thuringia. Long-term studies conducted between 1997 and 2002 in free-living unengorged *I. ricinus* from high-risk areas have revealed neither an increase of virus prevalence during this 6-year period nor any significant seasonal variations.

The epidemiological situation in the different risk areas for TBE was found to vary considerably:

1. Establishment of completely new low-risk areas.
2. Reactivation of formerly active areas with endemic latency.
3. High-risk areas with stable viral activity over long periods.
4. High-risk areas which slowly expand and merge with low-risk areas.
5. High-risk areas which have developed into endemic areas or become inactive.

Long-term application has shown that both epidemiological methods have to be used for the characterization of natural foci of TBE, evaluation of autochthonous TBE cases and virus detection in ticks by means of molecular-biological techniques. The advantages and disadvantages of both methods may be complementary and are discussed.

In a further study, virus prevalence in *I. ricinus* engorged with human blood was examined. Exposure of humans had taken place in some districts near Passau in Bavaria. In the autumn of 2001, virus prevalence of unengorged free-living nymphs in this area was 0.38 (0.08 – 1.1) % and of adults 1.17 (0.03 – 6.38) %. Surprisingly, virus prevalence in engorged ticks from the same area and collected during the same period was significantly higher (nymphs 6.9 % and adults 9.3 %). Virus-positive engorged ticks were found only in districts known as risk areas. Nucleotide and deduced amino acid sequence data of the PCR products have confirmed the only presence of virus prototype Neudoerfl.

Problems in the study and prophylaxis of mixed infections transmitted by Ixodid ticks

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Recent knowledge about the structure and functioning of natural foci allows the conclusion that **most ecosystems usually include several pathogenic or conditionally pathogenic microorganisms as their essential components.** In the overwhelming majority of cases, a natural focus in which only one pathogen circulates is an orthodox abstraction that has no analogues in nature. Of infections ecologically linked with ixodid ticks, the pathogens causing TBE, borrelioses, ehrlichioses, tick-borne and other rickettsioses, and tularemia may circulate in the same ecosystem under various landscape–geographic conditions existing in Eurasia. The same is also observed on other continents. The wide distribution of tick-borne infections with natural focality is a natural phenomenon accounted for by the principles of relationships between different pathogens within the vector and in the ecosystem as a whole. In an individual tick, the micropopulations of various viruses, rickettsias, bacteria, and other microorganisms form a kind of community, or parasitocenosis. Even the same tick species (or other vector) in areas with different landscape–ecological conditions may carry microcommunities differing in the composition of their constituent microorganisms. The greater the species range of a vector, the more variable the corresponding parasitocenoses. Even more complex parasitocenoses are formed in each individual reservoir host. **Different pathogens in the same tick do not usually interfere with each other because they usually occupy certain organs, tissues or even cell structures that serve as specific ecological niches. This allows the parasitic systems formed by the agents of tick-borne infections to be relatively autonomous and provides conditions for the existence of mixed natural foci.** The spread of a certain type of mixed natural foci depends primarily on the degree of sympatry of the corresponding pathogens and their specific requirements for abiotic and biotic environmental factors. Mixed tick-borne infections are by no means unusual, and the probability of acquiring them is very high. Hence, **any disease developing after a tick bite should be regarded as a potential mixed infection.** The spread of mixed diseases makes it necessary to fundamentally revise the entire system of views on infections transmitted by ticks. It is also necessary to develop a comprehensive approach to the study, diagnosis, and therapy of these etiologically different diseases and a rational strategy of their prophylaxis. As a person bitten by a tick runs the risk of being infected by several pathogens together or separately, rational prophylactic measures, both specific and unspecific, must be aimed at protecting people from the entire complex of infections transmitted by ticks.

Symbionts and pathogens in ticks: prevalence of W*olbachia* sp., *Ehrlichia* sp., *Rickettsia* sp. and *Babesia* sp. in Germany

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Tick-borne diseases are well known in Germany. With ongoing research a broad range of bacteria and parasites in ticks, which possibly can lead to various human diseases, are recognized. The prevalence of *Ehrlichia* sp., *Rickettsia* sp. and *Babesia* sp. in ticks is an important factor for the emergence of human Ehrlichiosis, Rickettsiosis and Babesiosis. Therefore, nymphs and adult ticks were collected and examined for the presence of *Ehrlichia* sp. (genogroup: *Ehrlichia phagocytophila*) (n = 5424 ticks), *Rickettsia* sp. (n = 1187) and *Babesia* sp. (n = 2486). For the detection of *Ehrlichia* sp., DNA from the 16S rDNA gene was amplified by nested PCR and hybridized with a DIG-labeled oligonucleotide probe. The examination of *Rickettsia* sp. was performed by single PCR. A partial sequence of the citrate synthase gene was amplified. As a target for the detection of *Babesia* sp., DNA from the 18S rDNA gene was amplified, also by single PCR. All positive PCR products were sequenced to control the specificity.

The prevalence of *Rickettsia* sp. in ticks (n = 1187) from 3 areas was 8.9 % (105) with a range from 13.3 % to 5.6 %. Sequencing showed exclusively *Rickettsia helvetica*. In about 0.3 % of *Rickettsia*-positive ticks, double infection with *Ehrlichia* sp. was found. *Babesia* sp. was detected in 1.0 % (26) of all ticks (n = 2486) which originated from 3 different areas. By sequencing 96 % were identified as *Babesia divergens;* only one PCR product was identified as *Babesia microti*. *Ehrlichia* sp. could be detected by PCR in 1.9 % (103) of all examined ticks (n = 5424) from 11 investigation areas. However, not all positive PCR products hybridized using DIG-labeled oligonucleotide probe. Thus, the result of sequencing indicated that only 1 % (54) belonged to *Ehrlichia phagocytophila* and nearly half of these PCR products (0.9 %) were identified as *Wolbachia* sp.

Wolbachia sp. is known as a symbiont of human pathogens (i.e. *Onchocerca volvulus*). It also influences the reproductive cycles of arthropodes. Therefore, the findings on ticks and their role should be further investigated.

The infection of ticks with *Ehrlichia phagocytophila, Rickettsia helvetica* and *Babesia* sp. demonstrates their possible role as a source of infection for humans in Germany. However, due to the lack of documented clinical cases their significance in human disease remains unknown but has to be considered in the differential diagnosis of tick-borne diseases.

Serologic evidence for *Babesia* infections in tick-infested humans in Midwestern Germany

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Objective. Babesiosis is considered to be an emerging tick-borne disease in humans world-wide. However, most studies on the epidemiology of human babesiosis to date have been carried out in North America, and there is little knowledge on the prevalence of infection and frequency of disease in other areas. The aim of this study was to investigate the prevalence of *Babesia* infections in a human population in Germany.

Methods. A total of 467 sera collected between May and October 1999 from individuals living in the Rhine-Main area were tested for the presence of IgG and IgM antibodies to antigens of *Babesia microti* and *Babesia divergens* by indirect fluorescent antibody (IFA) tests. These sera were derived from 84 Lyme borreliosis patients suffering from erythema migrans, 60 asymptomatic individuals with positive borreliosis-serology, and 81 individuals with a history of tick bite. Cut-off values for discrimination between seronegative and seropositive results in the IFA tests were determined using sera from 120 healthy blood donors and 122 patients suffering from conditions other than tick-borne diseases [i. e. malaria, $n = 40$; toxoplasmosis, $n = 22$; syphilis, $n = 20$; Epstein-Barr virus infection, $n = 20$; presence of anti-nuclear antibodies, $n = 20$]. The overall specificities of the IFA tests for *B. microti* and *B. divergens* were estimated to be $\geq$ 97.5 %.

Results. Positive IgG reactivity against *B. microti* antigen (titer, $\geq$ 1:64) or *B. divergens* antigen (titer, $\geq$ 1:128) was detected significantly more often ($p < 0.05$) in the group of tick-exposed patients ($n = 26$ out of 225 individuals: 11.5 %) than in the group of healthy blood donors ($n = 2$ out of 120 individuals 1.7 %). IgG antibody titers $\geq$ 1:256 against at least one of the babesial antigens were found significantly more often ($p < 0.05$) in tick-exposed patients ($n = 9$ out of 225 individuals: 4 %) than in the control groups ($n = 1$ out of 242 individuals: 0.4 %). In the human population investigated here, the overall seroprevalences for *B. microti* and *B. divergens* were 5.4 % (25/467) and 3.6 % (17/467), respectively.

Conclusions. The results obtained here provide evidence for concurrent infections with *Borrelia burgdorferi* and *Babesia* species in tick-exposed humans in mid-western Germany. They also suggest that infections with *Babesia* species in the German human population are more frequent than previously believed and should be considered in the differential diagnosis of febrile illness occurring after exposure to ticks or blood transfusions, in particular in immunocompromised patients.

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First report on the compatibility of seven tick-borne pathogens in *Ixodes persulcatus* (Acarina, Ixodidae)

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More then one thousand adult *Ixodes persulcatus* Schulze ticks were collected by flagging in the forest in the vicinity of St. Petersburg during the 2000 season. All ticks were investigated by PCR using species-specific primers to detect TBE virus, *Borrelia*, *Ehrlichia* and *Babesia* infection.

Seven pathogens: TBE virus, 3 species of *Borrelia* (*B. afzelii*, *B. garinii*, *B. burgdorferi* sensu stricto), 2 species of *Ehrlichia* [HME-agent (*E. muris*), HGE-agent] and *Babesia microti* were identified. The total number of infected ticks was 33.2 % (417 out of the 1257 analyzed). More then 1/3 of all specimens were multiinfected (37.6 % - 157 out of 417). Among them 135 ticks (86 %) were dually infected; 22 specimens (14 %) contained 3 species of pathogens simultaneously.

Borrelia burgdorferi s. s. (34), and *Babesia microti* (7) were only encountered in mixtures, whereas other multiinfected specimens were encountered either in mixtures or as monoinfected ones (Table).

Table. Prevalence of mixed infected *Ixodes persulcatus* ticks.

Species of pathogens	No. of multiinfected among infected ticks	
	abs.	%
Borrelia afzelii	110/290	37.9
Ehrlichia, HGE	8/12	66.7
Borrelia garinii	115/171	67.2
Ehrlichia, HME	46/62	74.2
TBE virus	16/20	80.0
Borrelia burgdorferi s.s.	34/34	100.0
Babesia microti	7/7	100.0

All mixed infected ticks contained either *B. afzelii* (110) or *B. garinii* (47). Thus all pathogens were compatible with the gut extracellular parasites represented by genus *Borrelia* species. By contrast, intracellular parasites such as *Ehrlichia* or *Babesia* were not encountered in any of the specimens. The total number of ticks infected by one of these microorganisms was 81. No data on *Ehrlichia* and *Babesia* coinfection were detected in the literature, which was analyzed.

Mathematical analysis of the hypothesis of the incompatibility of these pathogens demonstrated that all the above-mentioned intracellular parasites belong to different clusters and that their qualities are determined by different factors (cluster and multifactor analyses were made using the programme STATISTICA for Windows). The possible causes and physiological mechanisms of incompatibility of intracellular parasites are discussed.

Tick-borne encephalitis in Japan

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A tick-borne encephalitis (TBE) patient has not been reported for many years in Japan. In October 1993 in Oshima district in Hokkaido, a severe case of encephalitis case in a farmer's wife was diagnosed as tick-borne encephalitis. Serological examination of paired sera showed a rise in neutralization (NT) antibody titer to Russian spring summer encephalitis virus, a far-eastern subtype of tick-borne encephalitis (TBE) virus. TBE virus isolates were obtained from the blood of sentinel dogs, spleens of wild rodents and Ixodes ovatus ticks. Sequence analysis of envelope protein genes identified these isolates as the far-eastern subtype TBE virus. The results provide the evidence that TBE is endemic in a certain area of Japan.

To identify when these TBE viruses emerged in Hokkaido, we estimated the divergence time of TBE virus strains isolated in Oshima and Far East Russia. We isolated TBE virus in Khabarovsk in 1998 and determined the nucleotide sequence of viral envelope protein genes of virus isolates from Oshima and Khabarovsk. From the synonymous substitution rate of these virus strains, the lineage divergence time of these TBE virus strains was predicted to be about 260 – 430 years ago phylogenetically.

Furthermore, we compared the virulence of TBE virus isolates from Oshima and Khabarovsk by mouse model. The results showed that TBE virus isolates from Oshima and Khabarovsk possess similar virulences. We evaluated the immune response of European vaccine against Japanese TBE virus strain for man and mouse. European TBE vaccine was found to be effective against the TBE virus prevalent in Hokkaido.

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Lyme Borreliosis spirochetes in ticks from Mainland Portugal

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In Portugal, *Ixodes ricinus* ticks have shown DNA of several spirochetes belonging to the *Borrelia burgdorferi* sensu lato complex, with major differences in genotypic diversity between ecozones. Some isolates were also obtained, confirming the circulation of pathogenic strains in these ticks. *Ixodes ricinus* is considered to be a widespread species although it has a very limited range of suitable habitats across the country.

Here we present preliminary results from a nationwide survey of questing *I. ricinus* and other Ixodidae populations in Portugal. The sample points were selected using Geographic Information Systems, according to type of vegetation, accessibility and prevalence of human cases. Data are presented on the spatial and temporal (seasonal dynamics) trends in tick abundance and patterns of tick infection with *Borrelia*. All ticks collected were examined for the presence of *B. burgdorferi* sensu lato by isolation of the bacterium, and direct PCR amplification of the 5S-23S intergenic spacer region followed by reverse line blot assay and nucleotide sequencing.

So far, seven *B. burgdorferi* sensu lato strains have been isolated from a region near Lisbon (Mafra). Genotyping of these isolates showed five *B. garinii* strains, one *B. valaisiana* and one *B. lusitaniae*. Detection of borrelial DNA also evidenced the presence of *B. afzelli* in this area and *B. lusitaniae* in another region in the South (Grândola). Dynamics of *I. ricinus* ticks showed bimodal activity. Regarding other ixodidae species, only *Hyalloma* spp. (so far) was reported to have *B. burgdorferi* sensu lato DNA.

The findings of this study support the idea that *B. burgdorferi* sensu lato is maintained in nature in different geographic areas across Portugal. The question now is what contributes to that (reservoirs and other vectors).

Biodiversity in canine babesiae: molecular and epidemiological investigations

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Currently, canine babesiae are considered to belong to 2 species, *Babesia gibsoni* and *B. canis*. This view is mainly based on the morphological features of the parasites in stained blood smears. “Small” piroplasms are diagnosed as *B. gibsoni* while larger parasites are considered to be *B. canis*.

We have investigated canine blood samples containing small piroplasms from Japan, Malaysia, Sri Lanka, California and Spain by DNA sequencing and phylogenetic comparisons of the 18S rDNA. An unexpectedly high genetic heterogeneity was found suggesting separate species status for the pathogens from Asia, America and Europe. Phylogenetic analyses using distance matrix, maximum parsimony and maximum likelihood methods revealed that the parasites from Asia genotypically clustered with the classical *Babesia* spp., while the pathogens from California were most closely related to the genus *Theileria*. The piroplasms from Spain were attributed to a new species most closely related to *B. microti*. Based on this genotypic characterization, distinct phenotypic features of these 3 species have to be anticipated, especially biological and epidemiological features.

In large canine babesiae, 3 subspecies of *B. canis* are currently recognized based on differences in vector specificity and pathogenicity: *B. canis canis*, *B. canis vogeli* and *B. canis rossi*. To determine whether genotypic features exist to confirm this taxonomic separation, rDNA Internal Transcribed Spacers (ITS1, 5.8S, ITS2) were analyzed in isolates with known or suspected vector specificity. Three genotypic groups were detected correlating with vector specificities and supporting the separation of the large canine babesiae into 3 subspecies, possibly even 3 species. The distinction between the 3 subspecies is not only of clinical interest because of differences in pathogenicity between the 3 *B. canis* subspecies; it is also of epidemiological importance because of differences in the potential of the parasites to become established in Germany. To rapidly differentiate between the 3 subspecies, a diagnostic Real Time PCR was developed. This test is currently used in an epidemiological investigation and first results are presented.

Reservoir host identification by analysis of host-seeking ticks

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Ticks are among the most important vectors of human and animal disease in the northern hemisphere. Identification of reservoir hosts is a prerequisite for effective prevention and control of tick-borne zoonoses. However, it presents many difficulties such as the need to trap large numbers of wild animals and to perform laboratory transmission experiments. A more efficient method consists of the molecular analysis of the blood meal remnant of ticks infected by the particular pathogen of interest. Vertebrate DNA is identified by PCR amplification using universal primers targeting a part of the 18s rDNA, followed by DNA/DNA hybridisation (Reverse Line Blot). This method identifies the host at the subgroup level, e.g. Ruminantia, Leporidae, Canidae, Murinae, Arvicolinae (mammals), Galliformes, Passeriformes (birds). By targeting the cytochrome b gene it is possible to further identify the host to species level. Analyses of the remnants of the blood meal in unfed nymphs developed from larvae fed on gerbils that were infected with *Babesia microti* successfully identified the host at subgroup level (Muridae) and thus demonstrated the origin of the infected blood meal. It was also shown that host DNA can be identified up to 9 months after the emergence of the nymph. This means that wild host-seeking ticks are appropriate subjects for this method. Results of analyses performed on nymphs collected in well-defined Irish habitats (particularly concerning host diversity and density) will be presented in order to demonstrate the validity of this approach for reservoir host identification of certain tick-borne zoonoses.

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Geographical and seasonal variation in detecting *Borrelia burgdorferi* sensu lato in rodents of North East Austria

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Background In order to learn about the infection rate of rodents with agents of Lyme borreliosis in Austria - a country endemic for this disease complex - we started with field studies in the north eastern part of Austria.

Methods Life traps were set out in north eastern Lower Austria every six weeks during a year consecutively in one of three different areas. Rodents were collected, identified and the number and type of their ecto-parasites ascertained. Samples of heart, urine bladder and brain were removed under sterile conditions and transferred to BSK medium in order to culture borrelia. Heart muscle was also used for DNA extraction. Real time polymerase chain reaction was performed with borrelia universal primers and with species-specific primers using the TaqMan system.

Results 938 mice were caught, most frequently *Apodemus flavicollis* (44 %), followed by *Clethrionomys glareolus* (34 %), *Microtus arvalis* (9 %), *A. sylvaticus* (7 %) and *Mus musculus* (6 %). Significant differences were seen in the total number of catch per area (Hohenau, Ernstbrunn, Vienna equal 10 : 9 : 2) and in the distribution of the various rodent species in the different catchment areas. Borrelia were more frequently cultured from bladder wall than from heart muscle and only once from brain. Heart specimens of 226 animals were borrelia PCR positive (24 %) and most frequently of the rodent species *A. flavicollis* (43 %) and *C. glareolus* (38 %). *B. afzelii* was most frequently identified, followed by *B. burgdorferi* sensu stricto and by mixed infection of *B. afzelii* and *B. burgdorferi* sensu stricto. *B. garinii* was only detected once in a sample of *M. arvalis*. In about 20 % of borrelia PCR positive samples the identification of one of the three genomic species could not be ascertained with the test panel used.

Conclusion The rodent species *A. flavicollis*, *M. arvalis* and *C. glareolus* in particular proved to be reservoir animals for the Lyme borreliosis agents *B. afzelii* and *B. burgdorferi* sensu stricto. It is worth noting the absence of *B. garinii* which evidently has no relevant reservoir in rodents in the areas investigated.

Borrelia burgdorferi LuxS/AI-2-mediated quorum sensing

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The spirochete *Borrelia burgdorferi* is transmitted to humans and other warm blooded animals through the bites of infected *Ixodes* spp. ticks. The establishment of *B. burgdorferi* infection involves numerous interactions between the bacteria and a variety of vertebrate host and arthropod vector tissues. Different bacterial proteins are required at specific points of this infectious cycle, and precise regulation of the synthesis of such proteins is essential for successful infection to occur. Our studies indicate that these spirochetes utilize a quorum sensing mechanism to control protein expression patterns that involves the chemical signal autoinducer-2 (AI-2). Through this mechanism, a population of Lyme disease spirochetes may synchronize production of proteins needed for infection processes. AI-2 is produced by the *B. burgdorferi* LuxS protein, which we have demonstrated to be a functional enzyme. Addition of AI-2 to cultured *B. burgdorferi* dramatically alters the expression of more than 50 different proteins. Among the AI-2 regulated proteins are many previously identified surface proteins that serve functions during mammalian infection. These studies suggest that *B. burgdorferi* uses AI-2 as an important signaling molecule to control expression of proteins during the natural infectious cycle of the Lyme disease spirochetes.

Randomized, phase II, multicenter dose-finding studies of a modified tick-borne encephalitis vaccine in children: Evaluation of safety and immunogenicity of two vaccinations with FSME-IMMUN® "new"

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Two randomized, phase II, multicenter, double-blind studies were conducted to identify the optimal dose of a modified tick-borne encephalitis vaccine (FSME-IMMUN® "new") in children. The safety and immunogenicity of two vaccinations (21-35 days apart) with either 0.3 µg, 0.6 µg or 1.2 µg of antigen were investigated in children aged 6-16 years (n = 639) and in children aged 1-6 years (n = 639).

Children 6-16 years: Seroconversion rates after the second vaccination, as determined by enzyme-linked immunosorbent assay (ELISA), demonstrated that the 0.6 µg and 1.2 µg doses were highly immunogenic (96.3 % and 98.5 % respectively), whereas the 0.3 µg dose induced a substantially lower seroconversion rate (88.4 %). Local and systemic reactions after the first and second vaccination occurred at a low frequency. No vaccine related SAE was observed during the study. After the first vaccination, fever was reported in 4.5 % of children in the 0.3 µg dose group, in 3.3 % in the 0.6 µg dose group and in 3.4 % in the 1.2 µg dose group. Fever was predominantly mild with no severe cases reported.

Children 1-6 years: Both the 0.6 µg and the 1.2 µg doses were highly immunogenic, inducing a seroconversion rate after the second vaccination of 98.1 % and 100 % respectively. By contrast, the 0.3 µg dose induced a lower seroconversion rate (93.2 %). Local and systemic reactions after the first vaccination occurred at a low frequency, with no dose-dependent response observed between the three study groups. No vaccine related SAE was observed during the study. Fever (measured rectally) after the first vaccination occurred at a comparable rate between study groups, namely 19.8 % in the 0.3 µg dose group, 16.3 % in the 0.6 µg dose group and 15.9 % in the 1.2 µg dose group. These fever cases were predominantly mild, with no severe cases. As anticipated, fever occurred at a higher frequency among younger children (1 year olds: 33.3 %, 2 years: 19.7 %, 3 years: 12.8 %, 4 years: 13.6 %, 5 years: 6.3 %).

Conclusions: The 1.2 µg dose was determined to be the optimal dose for children aged 1-16 years because (1) the 1.2 µg and 0.6 µg doses induced a sufficiently high seroconversion rate - according to the predefined criteria - whereas the 0.3 µg dose did not; (2) the 1.2 µg dose was found to be non-inferior to the 0.6 µg dose with respect to fever rate after the first vaccination. The study results demonstrate that, at the optimal dose of 1.2 µg TBE antigen, FSME-IMMUN® "new" has been shown to be safe and highly immunogenic for the vaccination of children aged 1-16 years.

Safety and immunogenicity of FSME-IMMUN® "new" vs. Encepur® in adults

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A prospective, randomized, multicenter, single-blind phase III study was performed to assess the safety of two vaccinations (21-35 days apart) with FSME-IMMUN "new" (five consecutive lots) compared to that of ENCEPUR with polygeline (two lots) in healthy volunteers (n=3966) aged 16 to 65 years. The safety of the third vaccination with FSME-IMMUN "new" (six months after the first vaccination) was investigated in a follow-up study on the same population and TBE antibody titers were analyzed pre- and post-vaccination in a subgroup of volunteers.

Following the first vaccination, the probability of occurrence of fever (≥ 38.0°C) was 0.8 % in the FSME-IMMUN "new" study group and 5.6 % in the ENCEPUR study group. Fever was mainly mild. Local and systemic reactions (other than fever) after the first vaccination occurred with a lower frequency at all severity grades in the FSME-IMMUN "new" study group than in the ENCEPUR group. The most frequently reported symptoms were:

	FSME-IMMUN® "new" (n=2977)	ENCEPUR® (n=989)
Headache	5.7 %	15.7 %
Muscle pain	4.8 %	15.1 %
Joint pain	1.3 %	8.2 %
Malaise	4.5 %	14.7 %
Fatigue	6.2 %	15.1 %

When analyzing the tolerability of the third vaccination with FSME-IMMUN "new" (n=3705), similar results were shown in both study groups of volunteers previously vaccinated with FSME-IMMUN "new" and ENCEPUR with respect to fever rate (mild 0.5 % vs. 0.3 %), as well as local reactions (29.7 % vs. 31.4 %) and systemic reactions (10.4 % vs. 12.9 %). Furthermore, the immunogenicity results of a subgroup of volunteers (n=564) demonstrated similar seroconversion rates (as determined by ELISA and neutralization test) before and after the third vaccination in the FSME-IMMUN "new" group (45.2 % vs. 99.0 %) and in the ENCEPUR group (49.3 % vs. 98.6 %)

Conclusions: The results of both studies demonstrate that: (1) FSME-IMMUN "new" is safe and highly immunogenic, (2) all five production lots FSME-IMMUN "new" are consistent with respect to a low adverse events rate, (3) FSME-IMMUN "new" induces considerably lower adverse reaction rates than ENCEPUR and (4) two vaccinations with ENCEPUR can be successfully followed by a third vaccination with FSME-IMMUN "new".

Long-term immunity after vaccination against tick-borne encephalitis with *ENCEPUR*® using the rapid vaccination schedule

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Purpose of the study

Tick-borne encephalitis (TBE) virus transmitted by ticks is highly endemic in the Czech Republic. TBE vaccines have been licensed in the Czech Republic for more than 10 years. We present our own experience on TBE vaccination with Chiron's former and currently marketed TBE vaccines specialising on long-term immunity by using the rapid immunization schedule. Furthermore, we have evaluated the tolerability of booster vaccination with Chiron's new TBE vaccine.

Summarized description of the study

The study was conducted in Hradec Kralove/ Czech Republic in 2002. 157 adult subjects with complete primary TBE vaccination according to the rapid vaccination schedule (i.e. on days 0, 7, 21) and who had already received their first booster with Chiron's marketed TBE vaccine (*Encepur*®) entered the study. The second booster (*Encepur*® *adults*) was administered 36 months after the first booster vaccination. Study objectives were to evaluate the immunogenicity and safety of Chiron's new TBE vaccine. Blood samples were taken prior to booster and 1 month later. In 145 out of 157 subjects evaluable blood samples from both measurements were available. The immunogenicity was measured by the ELSIA assay (Enzygnost® Anti-TBE virus). All 157 subjects were included in the safety analysis.

Results and conclusion

Prior to second booster immunization with Chiron's new TBE vaccine, TBE antibodies (GMTs) had remained on a high level and were far above the detection limit of the used ELSIA test. All (100 %) of subjects still were seropositive prior to the second booster immunization. From the sharp/moderate increase of TBE antibodies following the second booster vaccination, long-lasting immunity can be concluded. The majority of vaccinees reported only mild and transient systemic reactions upon vaccination. The booster vaccination with Chiron's new TBE vaccine was well tolerated by the vaccinees. Neither febrile post-immunization reactions, nor unexpected adverse events or serious adverse events were reported.

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TBE booster immunisation in adults - first experience with Chiron's new Tick-borne encephalitis (TBE) vaccine.

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Objective:
To evaluate immunogenicity and safety of the first booster vaccination with Chiron's new TBE vaccine in adults primarily immunized according to the rapid immunization schedule.

Methods:
A total of 222 adult subjects, all of whom received primary immunization with either Chiron's new or the former TBE vaccine in a preceding clinical trial, were enrolled in this extension study. The subjects received their first booster with Chiron's new TBE vaccine (Encepur® adults) 15 ± 3 months after primary immunization according to the rapid immunization schedule. Neutralizing TBE antibody titers were determined prior to and 21 days after booster immunization. Selected postimmunization reactions were recorded for 7 days following the booster. All other adverse events were monitored throughout the study period.

Results:
Prior to first booster immunization, TBE antibodies (GMTs) had remained on a high level and were far above the detection limit of the used neutralization test. All subjects of the per protocol population, who were primarily immunized with the new TBE vaccine formulation, and all but one subject of the control group were still seropositive prior to the booster immunization. All subjects showed a sharp increase of TBE antibodies following the booster. Postimmunization reactions were not frequent except for pain at the injection site. Only very few febrile reactions (< 1 %) below or equal 38.5°C were reported. No serious or unexpected adverse events related to vaccination were reported.

Conclusion:
These successful results in terms of both immunogenicity and safety indicate that the TBE vaccination with this polygeline-free TBE vaccine can be used safely in adults. Long-lasting immunity can be concluded from the strong immune response following the booster.

Stimulation of the immune system by different TBE-virus vaccines

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Tick-borne encephalitis (TBE) is one of the most frequent viral diseases in Europe transmitted by arthropods. This virus belongs to the Flaviviridae and is endemic mainly in Central and Eastern Europe. It raises a significant health problem in at least 13 European countries. Different vaccines against TBE-virus have been developed. A thiomersal free and also an albumin-free vaccine [Ticovac® (Baxter Hyland Immuno, Vienna)] was approved by the Austrian health authorities in 2000. Contrary to previous experience, 779 cases of fever occurred following the first vaccination of children under 15 years of age and febrile convulsions even occurred in 62 children of up to two years old. After this experience, the vaccine was changed and albumin was added again. Fever appeared now only in a few cases and no febrile convulsions were observed. The new Encepur Kinder® (Chiron-Behring, Marburg) from 2002 is a TBE-vaccine for children without any protein as the stabilizer but with a relatively high concentration of sucrose whereas the former vaccine Encepur K® from 1991 contained polygeline as the stabilizer.

The induction of the immune system by the different TBE virus vaccines was compared in an in-vitro test in order to find an explanation for the unexpected fevers. The whole blood was stimulated with a suspension of complete vaccine. Cytokines TNF-α, IL-Iß, IL6, and IL-8 were determined from heparin/EDTA-plasma and culture supernatants using Immulite (DPC Biermann GmbH, Bad Nauheim, Germany) according to prescribed procedure.

It was shown that Ticovac® and the new Encepur Kinder®, which contains no albumin or other proteins as the stabilizer, can induce relatively high amounts of TNF-α and lower amounts of IL-1β. Increase of both cytokines is first observed after an incubation period of 4 hours. The maximum is reached after 15 hours. After 26 hours, it has reverted to the original value. The behaviour of both cytokines is parallel to the febrile phases in children up to two years old. Albumin or other proteins like polygeline and also immunoglobulins prevent a rise of cytokines.

After inhibition of the MHC II complex of macrophages by monoclonal antibodies, the release of cytokines clearly decreased. Is this phenomenon the consequence of the effect of a viral superantigen?

TBE-vaccine monitoring in Austria – results of a post-marketing sentinel project

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Modern vaccines have to pass a well-documented registration process and must fulfil strict scientific criteria, including clinical studies on different levels. Nevertheless, it is crucial to monitor adverse events and reactogenicity of vaccines in the post-marketing phase.
Prior to the TBE-vaccination season 2002, the Research Institute of Vaccine Adverse Events (RIVAE) invited 504 practitioners and paediatricians from all over Austria to join a TBE-vaccine sentinel program. They were informed about the new TBE-vaccines licensed, potential side effects of the TBE-vaccine and the process of documenting vaccine adverse events in forms or on the Internet.
By the end of the vaccination period, we had received information about the occurrence of vaccine side effects from 187 doctors (37.1 % in total: 30.5 % practitioners and 69.5 % paediatricians). 25,905 vaccinations were administered by these doctors during the period from 2 to 7 February and 107 reports were sent to the RIVAE.
The age distribution of the reports showed a clear tendency for adverse events to occur in younger age groups: 31 % of all reports concerned events in infants, 34 % in toddlers up to the age of 2 years and 7 % in children aged 3-11 years. Most of the reported events were related to the first vaccination (48.6 %), approximately 10 % to the second and third vaccinations and 23 % to booster vaccinations. Whereas local reactions were rather rare (19 reports) and predominant in older children and adolescents, systemic reactions occurred more frequently in infants and toddlers: 22 reports concerned agitation and persistent crying. 63 persons complained about fever: mild fever (38 - 39°C) was reported in 45 cases, moderate fever (39 - 40°C) in 15 and severe fever was seen in 3 patients. No fever convulsions were observed or reported. 15 persons consulted the practitioner or paediatrician because of side-effects of the vaccination. Two children were hospitalised, one because of a severe fever reaction and the other because of collapse with cyanosis and apathy.

TBE-vaccine monitoring in Austria was able to gain objective and representative data on vaccine adverse events during a well-defined period of time. The rate of side-effects was moderate and no serious vaccine adverse events were reported indicating the low reactogenicity of the new TBE-vaccines now available.

An alternative to standard vaccination: Immunization with tick salivary antigens prevents tick-borne encephalitis virus infection of Balb/c mice

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An attractive alternative to the standard vaccines against arboviruses would be a TRANSBLOK™ vaccine designed against vector antigens blocking the transmission of one or more arboviruses. A vaccine candidate used in our study is based on the sequence of the protein (designed 64p) from the tick *Rhipicephalus appendiculatus* salivary glands. For the immunization of Balb/c mice two forms of recombinant 64p protein were used: a small N-terminal fragment (Trp2) of 51 amino acids cloned as a glutathione-s-transferase (GST)/histidine tag fusion protein, and a full-length sequence consisting of 133 amino acids cloned as a GST fusion protein (Trp5). One month after immunization with only one dose, Balb/c mice were challenged with tick-borne encephalitis (TBE) virus infected female *Ixodes ricinus* ticks accompanied with uninfected males and nymphs. Fed nymphs were tested for acquired TBE virus infection and mice were observed for signs of disease and death.

About half of the mice survived in experimental (Trp2 and Trp5 immune) groups (38 resp. 53 %) compared with much lower survival (15 and 16 %) in control groups (untreated and GST immunized). The infection rate in *I. ricinus* nymphs was greatly reduced (15 resp. 9 %) on experimental mice compared with control animals (58 resp. 44 %). All or almost all animals supported transmission in control groups while only 56 resp. 32 % of immune animals supported TBE virus transmission. In contrast, even when one dose of standard TBE vaccine protected mice against lethal challenge (88 % survival), it did not block virus transmission (71 % of TBE vaccinated mice supported the transmission).

In conclusion, tick-derived recombinant protein immune Balb/c mice were largely protected against lethal tick delivered TBE virus infection and virus transmission was blocked suggesting 64p protein is a good candidate for a TRANSBLOK™ vaccine.

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Decreasing tick-borne disease risk in the US by intervening in the natural cycle: lessons from the woods thus far

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Recent withdrawal of a commercial ospA vaccine has prompted reevaluation of prevention strategies for Lyme borreliosis in the US. Promising progress towards reducing transmission risk lies in the development of methods that decrease entomological risk index (ERI), the major determinant of Lyme disease risk. ERI is the absolute density of infected ticks, which is a function of both tick population density per area and the proportion of ticks infected with *Borrelia burgdorferi*. Thus, both vector control and management of the host community to reduce the average reservoir competence represent possible approaches for decreasing ERI. Here we give an overview of proposed intervention strategies, present data from field studies still in progress, and highlight key questions about tick population dynamics that deserve further investigation in order to improve strategies for reducing Lyme borreliosis transmission risk.

Lyme disease vaccine in the US and Europe: public and medial disputes discredit a well-founded and most successful protection strategy

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The first generation Lyme disease vaccine LYMErix™, consisting of outer surface protein (Osp) A from the European *B. burgdorferi* (*B. b.*) s. s. strain ZS7, was approved by the FDA in 1998 and has since been applied in over 1.5 million doses to > 500,000 vaccinees. In 2002 LYMErix™ was withdrawn from the market for as yet unknown reasons. However, the fact that the published study on the successful phase III clinical trial with LYMErix™, headed by a leading scientist, was followed within only a week by another publication, co-authored by the same person, suggesting vaccine-induced autoimmune sequelae, may have contributed to this decision. Unverified reports in the media on putative side effects following vaccination have been contravened by a detailed examination by the FDA of more than 900 „cases of adverse side effects" and a number of recently published studies. Thus, to date all the evidence from clinical trails and post marketing medical surveys of vaccinees establishes LYMErix™ to be safe and effective. For Europe, a trivalent OspA vaccine has been developed and successfully tested in the phase II clinical trial.

The OspA-based vaccine formula is only suitable for prophylaxis but not therapy of Lyme disease. This is due to the fact that the target structure for the vaccine-induced bactericidal antibodies, OspA, is expressed by spirochetes exclusively when harboring ticks, but not mammalian hosts. Antibodies with specificities to other Osps (encoded by > 150 independent genes in the *B. b.* genome) that are generated in the course of a *B. b.* infection are not capable either to eliminate persisting spirochetes. Therefore, unorthodox approaches that do more than just mimicking natural immunity are in demand. In this respect, our unexpected finding that an artificially generated mouse immune serum was highly effective in curing chronic *B. b.* infection in mice subsequently led to the identification of a number of additional promising *osp* genes. These novel *B. b*. genes are expressed by spirochetes persisting in the mammalian host, but are poorly immunogenic. However, when applied as recombinant antigens, monospecific antibodies were generated with the capacity to prevent, upon passive transfer, subsequent *B. b.* infection in mice. This approach opens further avenues to disclose hitherto unrecognized Osps that may serve as suitable targets for immunotherapy. Some of these newly identified Osps are also immunogenic during *B. b.* infection in humans and thus raises expectations for developing immunotherapeutical regimens against Lyme disease.

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State-of-the-art serological techniques for the detection of antibodies against Tick-Borne Encephalitis Virus

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Introduction: The detection of antibodies against tick-borne encephalitis virus (TBEV) plays an important role in diagnosing infections with TBEV and monitoring vaccination. We have developed and evaluated three state-of-the-art anti-TBEV test systems.

Methods: (a) For the indirect immunofluorescence test (IIFT), TBEV-infected cells were fixed as BIOCHIPs into the reaction fields of a microscope slide. The slides were incubated under standardized conditions (TITERPLANE technique). (b) For the ELISA, microplate wells were coated with a TBEV extract. (c) In the EUROASSAY test system, immunoblot strips coated with a line of TBEV extract and a control band were fixed into the test fields of a microscope slide. The incubation was performed using the TITERPLANE technique, and results were evaluated visually.

Sera from 112 patients with clinically characterized TBEV infections were investigated for anti-TBEV antibodies using the three test methods. To analyse potential cross-reactions, sera from 27 patients with clinically characterized dengue or yellow fever were also investigated. Sera from 100 healthy blood donors were included as controls.

Results: With respect to clinical data, the IIFT, ELISA and EUROASSAY test systems yielded sensitivities of 91-95% for detection of anti-TBEV antibodies. The specificities (including dengue and yellow fever samples) amounted to 91 – 94 %.

Discussion: The new anti-TBEV IIFT, ELISA and EUROASSAY systems are efficient, standardized methods for the sensitive and specific detection of antibodies against TBEV. Furthermore, with IIFT the TBEV substrate can be supplemented with further BIOCHIPs, for example *Borrelia burgdorferi*, enabling a detailed antibody profile to be obtained with a single incubation.

Immune Evasion of *Borrelia burgdorferi*: Expression of Complement Regulator-Acquiring Surface Proteins contributes to Complement Resistance in the Human Host

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The innate immune system, particularly the human complement system plays an important role in the elimination of invading pathogens. *Borrelia (B.) burgdorferi* possesses the ability to inhibit this host defence system. As we have shown previously, complement resistance of *B. burgdorferi* is mediated by surface-expressed complement regulator-acquiring surface proteins (CRASPs). These borrelial proteins bind the two important human complement regulators factor H and factor H-like protein 1 (FHL-1). Acquisition of these host immune regulators allows inhibition of early steps of complement activation directly on the surface of *Borreliae*, thereby preventing formation of destructive activation products.

Further studies aimed at identifying and isolating the bacterial ligands interacting with factor H and FHL-1 led to the identification of five distinct CRASPs expressed by *B. afzelii* and *B. burgdorferi* s.s. isolates. CRASPs are differentiated according to their size and binding property for the human complement regulators. Using deletion mutants of factor H and FHL-1, the binding region of both proteins was localized mainly to the C-terminal end. Attachment to this region is of physiological relevance because the regulatory activity of factor H and FHL-1 resides in their N-terminus.

In order to characterize CRASPs in more detail, we have cloned and identified BbCRASP-3 as a new member of the polymorphic Erp (OspE/F-related) protein family. Using recombinant BbCRASP-3, several deletion mutants and three additional OspE-related proteins a consensus factor H-binding motif of nine amino acids was identified. This motif is localized at the C-terminus of all OspE-related proteins analyzed so far. The presence of negatively and positively charged amino acids suggests that the binding of factor H to BbCRASP-3 and Erp proteins is of ionic nature.

Studying the influence of environmental stimuli on the expression of CRASPs, a thermoregulation for some CRASPs could be demonstrated. Up-regulation of distinct CRASPs at 37 °C suggests that this regulatory mechanism is of relevance for bacterial survival during transmission and adaptation in the human host. Thus, the detailed characterisation of additional CRASPs on the molecular level is expected to identify new virulence factors and potential vaccine candidates (support by DFG Br 446/11-4 and Zi 342/5).

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Significant Improvement of the Recombinant *Borrelia* IgG Immunoblot for Serodiagnosis of Early Neuroborreliosis

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We previously described a recombinant IgG immunoblot for serodiagnosis of Lyme borreliosis containing recombinant p83/100 (derived from *B. afzelii* strain PKo), p58 (*B. garinii* PBi), p39 (*B. burgdorferi* sensu stricto B31, PKo, and PBi), OspC (B31, PKo, PBi), internal flagellin fragment (PKo, PBi) and DbpA = Osp17 (PKo) as antigens (1). In the present study we investigated the use of three additional recombinantly expressed proteins, VlsE (*B. burgdorferi* s.s. PKa2), DbpA and OspC (*B. garinii* strains PBr and 20047). The respective genes could be amplified by PCR. After cloning these PCR products, the proteins were overexpressed in *E. coli* and purified.

Sera from patients with acute neuroborreliosis are especially suitable for the evaluation of possible test improvements because it is an early manifestation and causative strains are heterogeneous. A clinically well defined panel of sera from 36 patients with acute Lyme neuroborreliosis and 67 controls were investigated and compared with the previously used recombinant immunoblot (1) and the whole cell lysate immunoblot (antigen strain PKo) (2).

Diagnostic sensitivity could be significantly increased without loss of specificity compared with the previously used recombinant immunoblot (86.1 % versus 52.7 %) and was even higher than the conventional whole cell lysate immunoblot (which was positive in only 63.8 % of the patients). The increase in sensitivity was mainly due to VlsE and DbpA. Only one additional positive result was detected in neuroborreliosis sera by OspC from strain 20047. New tests show that VlsE from the European species *B.afzelii* PKo and *B.garinii* PBi could even improve on the results shown here (3). The new recombinant IgG immunoblot considerably improves serodiagnosis with respect to sensitivity and standardization and can be recommended as a confirmatory immunoblot that should be given preference over the conventional blot.

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3. Göttner, G Abstract Potsdam meeting 15/16. March 2003

Disappearance of specific immune response after successful therapy of chronic Lyme borreliosis

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512 consecutive patients suffering from chronic Lyme borreliosis have been treated according to a standardized therapy regimen, which was developed on the basis of results from a controlled trial (1, 3). Follow-up was performed for at least 6 (maximum 16) years.

Inclusion Criteria:
Patients suffering from chronic Lyme borreliosis. They had to fulfil the following criteria (2):

1) Myalgia and arthralgia and / or arthritis, not explainable with a concurrent diagnosis, and / or Acrodermatitis chronica atrophicans.
2) Sweating and fatigue as signs of active infection.
3) A specific immune response against Borrelia burgdorferi s. l.

Treatment:
First treatment was Cefotaxime 40 mg/kg i.v. (twice a day) for 15 days. In the event of a clinical relapse Cefotaxime 40 mg/kg (twice a day) was administered for 20 days. Cases showing another relapse were treated with an increased dose of 60 mg/kg Cefotaxime (twice a day) for 20 days. 512 Patients received the first treatment, 131 needed a second treatment, 48 a third and 27 a fourth.

Results:
485 out of 512 patients could be followed up for at least 6 years after successful clinical therapy. 469 (96.7 %) could be cured according to clinical criteria. 16 (3.3 %) patients could not be cured. 12 of them had developed allergic reactions and did not respond to alternative therapy regimens. Four patients remained symptomatic despite appropriate and repetitive treatment.
Westernblot band patterns after 72 months remained positive for 77 patients (16 %). For the other patients, the specific immune response within 72 month (24-66 months) did disappear. In most patients non-specific/cross-reactive bands disappeared, too (see Table).

Discussion:
Nearly all patients could be clinically cured after one or more intravenous therapies with Cefotaxime. 16 patients could not be cured and had persisting clinically symptoms. Westernblot band patterns mostly showed a disappearance of specific immune response after 24-66 months. The successfully treated patients with disappearing clinical symptoms showed a concurrent disappearance of specific immune responses. Unsuccessfully treated patients remained positive. This provides further evidence that IgG antibodies disappear after successful therapy and that their occurrence is clear evidence of persistent active infection and not just previous disease.
The smaller group of patients (16 %) remaining seropositive without clinical signs or symptoms needs further observation. We have not observed clinical relapses more than three years after initial successful treatment.

Borrelia burgdorferi s.l. OspA-types are widely spread in Bavaria but show distinct local patterns

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Borrelia (B.) burgdorferi sensu lato is the agent of Lyme borreliosis, the most common tick-borne disease in the northern hemisphere. OspA, a major outer membrane protein and vaccine candidate for Europe, can be used to identify different OspA-types that may be associated with distinct clinical manifestations.

We developed a seminested PCR targeting the *ospA*-gene. Restriction fragment length polymorphism analysis of the amplicons (five different enzymes) allowed a reliable differentiation in *B. burgdorferi* sensu stricto (s.s.) (OspA-type 1), *B. afzelii* (type 2), all five *B. garinii* OspA-types (3-7), *B. valaisiana*, and *B. lusitaniae,* as well as multiple infections with different OspA-types.

A total of 529 *Ixodes ricinus* ticks were collected from 3 different sites in Southern Bavaria with different anti *B. burgdorferi* antibody prevalence rates in the general population. Total infection rate (nymphs and adults) was 14.4 % with regional variations between 6.7 % and 28.6 %, respectively. The local infection rates showed no correlation to the antibody prevalence rate in the general population. The most common species found were *B. garinii* (61.6 %), followed by *B. afzelii* (24.7 %), and *B. burgdorferi* s.s. (11 %). The most common OspA-types were types 6 (30 %), 2 (28 %), 4 (22 %), and 1 (10 %). Notably, even the recently described genotype A14S was present in one region. Only *B. lusitaniae* and OspA-type 7 were not detected. Significant differences were found in the local distribution of OspA-types 4, 6, and 2. Noticeably, *B. garinii* OspA-type 4 was frequently found at one location. This OspA-type has rarely been isolated from ticks in Europe so far although it has been frequently isolated from the cerebrospinal fluid of patients with neuroborreliosis. The rate of double infections with borreliae of different OspA-types increased from larvae to nymphs and adults without preferences for any combinations of OspA-types.

The most striking finding was that - except OspA-type 7 – all clinically relevant species and even OspA-types were present in the study area, suggesting a broad distribution of various OspA-types. Furthermore, we found evidence for a focal predominance of individual OspA-types with possibly different pathogenic potential. Those data are an essential epidemiological basis for the development of (OspA-) vaccines for Europe and for local risk assessment after tick bite.

Is leishmaniasis becoming endemic in Germany?

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In 1999 the first proof for the presence of phlebotomine sandflies (Diptera, Psychodidae) succeeded in Germany. Four sandflies of the species *Phlebotomus mascittii* were trapped by CDC light-traps at three different locations along the Rhine river-valley in Baden-Wurttemberg. In 2001 - 2002, a further 120 sandflies of the same species were found at various locations in the same land. The northern-most town positive for this sandfly species is Baden-Baden. At all locations, except one sylvatic place near the village Istein, the sandflies were trapped inside villages associated with people and animals.

Since the past 10 years some cases of suspected autochtonous leishmaniasis had been reported from Germany. Sandflies seem to be the most probable causative vector for *Leishmania*-infections in human, dog and horse. One of those cases was reported in 1998/99 to local veterinarians of the village of Gehrweiler (Rhineland-Palatinate). The whole summer-period 2001 a CDC light-trap was running in Gehrweiler. As a result four sandflies of the species *Phlebotomus perniciosus*, a proven vector of *Leishmania* were caught.

The geographical distribution of phlebotomine sandflies in Germany is uncompleted. Until now *P. mascittii* has never been experimentally demonstrated to be a vector for *Leishmania*. But at least two German cases of autochtonous leishmaniasis, a child spending a holiday stay in the area of Füssen (Bogdan et al., 2001) and a horse that never left the region of Augsburg (Koehler et al., 2002), strongly indicate the presence of a sandfly vector-species at least in Bavaria.

Test systems for tick repellents

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Although ticks can transmit a variety of pathogens to humans, an efficient vaccine is currently only available against TBE-virus. Prevention of tick-borne disease (TBD) thus has to rely on additional measures on an individual or community basis including tick habitat management, improving public knowledge, etc. Repellents offering protection against tick bites can be an important prophylactic component of a TBD management strategy.

Since available repellents are not always satisfactory, there is ongoing research on safe and effective products. Evaluation of new repellents needs testing of the candidates at different phases of product development. The present talk discusses the advantages and disadvantages of such repellent assays and focuses on two recently developed test systems performed with the European tick, *Ixodes ricinus*. The first one is an in vitro system suitable for the screening of extracts, pure substances or formulations. It consists of a heated rotating drum, serving as a moving object for questing ticks (Dautel et al., 1999). This system makes use of the natural behaviour of certain ticks, clinging to a passing by host. The second test involves volunteers, thus being only suitable for the investigation of repellent formulations safe for humans. This test is a modification of the US EPA guidelines for tick repellent tests and is, to a certain degree, more rigorous than the former. It proved able to distinguish the efficacy of several commercial tick repellents available in Germany. Test criteria were developed allowing the discrimination of stronger and weaker repellents.

Poster

1 TBE (Tick-borne encephalitis) in Italy

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The first Italian case of TBE was documented in 1975 in Tuscany and a TBE focus later detected near Florence in 1978. Since then, two more endemic areas and five more TBE foci have been recognized in the northeast, namely in the provinces of Trento (Trentino-Alto Adige) and Belluno (Veneto). The disease initially appeared to be very rare, only 18 cases having occurred in the period 1975-1991, but a different scenario has presented itself in recent years, with 84 new cases diagnosed from 1992 to 2001. Thus, a total of 102 indigenous TBE cases has been recorded in the spell 1975-2001. By comparing the median number of TBE cases/year recorded in the period 1975-1991 and in the period 1992-2001, respectively, an almost eight-fold increase in the incidence of the disease can be estimated. In line with the rest of Europe, even in Italy TBE predominates in males (M/F ratio=72.5 %), can occur in any age (age range=16-73 years) and tends to be acquired during outdoor leisure activities. Unlike other countries, however, the disease incidence is seasonally distributed throughout the year, with the exception of January and March, and displays a biphasic peak in July and October. Only 58 % of Italian TBE patients recalled a recent tick bite. Of the 62 patients studied in Veneto, 56 % presented with a flu-like syndrome, 24 % with clinical isolated meningitis, and another 20 % with meningoencephalitis, meningoencephalomyelitis, polyradiculoneuritis or meningomyelitis. The global incidence of encephalitis was 16 %. No fatalities occurred; however, five patients required respiratory support in ICU and persistent motorial deficits affected four subjects. A number of patients without meningeal irritation signs displayed pleocytosis on CSF analysis. Transient leuco-thrombocytopaenia was detected in 5/7 subjects (71.4 %) studied in the first febrile phase of the disease. The bio-molecular investigations performed in the provinces of Belluno and Trento did not reveal any significant differences between local strains and Western *TBEV* (Neudörfl strain) (Hudson PJ et al., 2001).

2 Atlas of tick-borne encephalitis in the Czech Republic: predictive maps of high risk areas and tick-borne encephalitis cases distribution

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The main prerequisite for the prevention of *Ixodes ricinus* tick attacks and tick-borne encephalitis (TBE) infection is the detailed, accurate and reliable prediction of habitats presenting a high epidemiological risk in large areas. For this purpose an Atlas of predictive maps of *I. ricinus* high tick occurrence sites and of TBE risk assessment, together with the maps of TBE dissemination in the Czech Republic (period 1971-2000), was prepared for the Public Health Service. The methods of GIS and remote sensing (RS) are used to solve this problem. A basic source of information is the importance of different plant communities as indicators of suitable ecological conditions for high tick occurrence and for TBE virus circulation in nature. This is a good indication of the influence of both biotic and abiotic environmental factors. Satellite data covering all the Czech Regions (52 000 km^2) were obtained by LANDSAT 5 TM scanner (with spatial resolution of 30 m) in six visible and near infrared spectral bands. Full satellite 180x180 km scenes recorded in July to August were used. A mosaic of such scenes covering the whole Czech territory was available for the project. Nine forest categories were recognized with a different structure and species composition corresponding to the different occurrence of *I. ricinus* ticks. Epidemiological TBE maps, based on human cases contracted in the territory under study, were further exploited for the evaluation of risk in particular forest categories. High resolution RS data also permitted the spatial structural analysis and characterization of the mosaic pattern of TBE natural foci under study. Predictive maps were produced both in digital form (CD-ROM) and in printed forms with a scale of 1:200 000 for general epidemiological information and a scale of 1:25,000 for detailed local orientation.

Both printed and digital forms of this Atlas are demonstrated.

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3 Tick-borne encephalitis in the South of the Russian Far East

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The Far East is one of the regions on the Eurasian continent, where for the first time was tick-borne encephalitis (TBE)occurred as a new disease for the first time. More than 2000 cases of tick-borne encephalitis were registered in the south of the Russian Far Eastern region (Primorsky Territory) from 1990 to 2002. During the 1990s, a steady growth in the reported TBE cases was observed in the Far Eastern region characterized by the most severe cases of this disease with a mortality rate of 8 - 27 %.

In the Primorsky Territory there is a typically long epidemic season beginning in April (from 1.8 %, before 4.8 %), maximum sick TBE accounts for June (before 40 %) and for July (before 33 %), single events are registered before October.

The incidence of infection with the TBE virus in persons after being bitten by a tick was studied by means of detecting antigen to blood in ELISA and virus to blood. The mean factor percentage of antigenic to blood beside for those persons changed during these years from 19 % to 34 %. High (60 - 68 %) or low (20 - 16 %) to factors at the end of the epidemic season corresponded to high (33 - 40 %) or low (6 - 8 %) degree infection of persons at the beginning initially following season. Each year to April the percent of the persons with antigenic to blood occurred the reduction (in 2 - 3 times), that conditioned the loss to viral population in ixodid ticks for period winter diapause.

109 strains of the virus TBE were isolated using the author's method from 645 tests of blood with antigen. These were strains, which possessed the pantropic property to various cells of white mice of 2-h daily age, including brain cells (neurotropic strains) as well as strains, which possessed the electoral tropic to cells immunocompetence organs (aneurotropic strains).

These strains were characterized at antigenic and molecular levels by sequencing the structural E-protein region. They can be attributed to virus TBE far eastern subtype.

It has been established that both neurotropic and aneurotropic strains of the TBE virus are more often capable of causing the inapparent forms of TBE among people. Studies by Russian scientists performed in recent years have indicated, however, that the vast majority of those exposed to TBE acquired benign or inapparent infections with an average inapparent-to-manifested ratio of 34:1. The clinical infection TBE among people manifests itself in the paralitic forms in 40 - 60 %, meningeal form - in 10 - 20 % and febrile form - in 20-40 %. Focal forms of the disease were often registered with the mortality rate reaching 60 %.

The high degree of infection of virus TBE persons bitten by the ixodid ticks and high disease TBE indicates the need for timely preventive action, in particular - specific vaccination. The experience of using the vaccine "Encepur" has shown its expressed immunity. The observation is continued not only with regard to immunity but also to the epidemiological efficiency of this and other vaccines under study.

4 Human Granulocytic Ehrlichiosis – a biological hazard in forestry and agricultural workers in Southwestern Germany?

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Introduction: Several studies showed an increased risk of infection for professionally exposed individuals with regard to tick-borne diseases (TBD). In south-western Germany, the occupationally increased risk of infection with *tick-borne encephalitis virus* (TBEV) or *Borrelia burgdorferi* s. l. (B. b.) was demonstrated for forestry and agricultural workers by means of several studies. Due to these epidemiological connections, professionally exposed individuals may be used as indicators for emerging or rare tick-borne pathogens. A respective sample, already investigated in studies on anti TBEV and anti LB seroprevalence, was examined with regard to prevalence of antibodies against *Anaplasma phagocytophila* (A. p.), the agent of human granulocytic ehrlichiosis (HGE).

Methods and Material: Sera of forestry and agricultural workers, collected in the years 1996-1997, were tested for IgG-antibodies against HGE. Additionally, data gathered by means of a standardized questionnaire on sociodemography, professional biography, leisure time activity, former TBD, and vaccinations were available. Serological testing (IgG) was performed by means of an immunofluorescence test (A.p. infected HL60 cells). Statistical analysis included descriptive, bivariate and multivariate analysis, using the software SPSS 10.

Results: The sample consisted of n= 518 subjects (197 forestry and 321 agricultural workers; 457 men, 61 women). The anti A. p. seroprevalence (IgG, positive 1: > = 80) in the total sample reached 2.1 % (11 / 518 positives).

Seroprevalence was not dependent on age, sex or any of the further sociodemographic factors, the leisure time parameters or the TBD history. Profession resulted as the only significant risk factor in a multivariate model built on the bases of bivariate findings (ANOVA: $F = 7.0$, $df = 1$, $p < 0.01$): All 11 seropositive individuals were agricultural workers – none of the forestry workers showed a positive result. Inside the agricultural group a (not significant) trend for a higher risk in subjects working on farms with more pasture land and a lower risk on farms with more arable land was detected.

Discussion: The anti A. p. seroprevalence is significantly higher in the agricultural sample than in a forestry sample. Two interpretations may be possible in order to explain the absence of positive results in forestry workers and the presence in the agricultural group: either agricultural workers apply less occupational safety measures and are, therefore, more exposed or the risk of infection is closely connected to pasture agriculture.

5 Tick-transmitted diseases in the Ukraine

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Tick-transmitted diseases are a problem for practically all countries worldwide. The Ukraine is a country with various climate and geographical landscape zones which are characterized by corresponding species of ticks and their warm-blooded hosts. It determines the possible formation of natural foci of different infections under the mentioned conditions (tick-borne encephalitis, viral haemorrhagic fevers, lyme borreliosis, coxiellosis etc.).

Tick encephalitis. In recent years about 40-50 cases in humans have been registered annually, usually in western regions and the Crimea. In eastern regions this disease has not been registered. All the same, the numerous patients with “neuroinfection” as well as the prevalence of *Ixodes ricinus* in the tick fauna permit consideration of this nosology as prospective for the Ukrainian forest and forest-steppe landscape zones.

Lyme Borreliosis. This pathology was first formally registered in 2000. In 2001, 99 cases (0.2 per 100,000 population) were registered on the basis of clinical and epidemiological data and according to the immune enzyme analysis. In the potential natural foci (Kharkiv region) 3.4 % *I. ricinus* were infected.

Coxiellosis. Annually about 10 cases in humans are registered. The formal registration data do not reflect the real spread of infection. It is confirmed by the significant incidence of *Coxiella burnetii* observed in domestic animals and the relatively high infectivity of certain occupational population groups (2.1-16.7 %). Intensive *C. burnetii* circulation was confirmed by our isolation of *C. burnetii* strain “Ukraine-1” which was added to the National Strain Collection.

Other endemic tick-borne diseases. Like Marseilles fever (infectious epidemic exantema), Crimean-Congo fever etc. The loimic potential of these infectious foci remains and thus requires adequate ecological epidemiological surveillance.

Key words: Tick-transmitted diseases – Lyme Borreliosis – Coxiellosis.

6 Titration of *Borrelia burgdorferi* sensu lato in selected *Ixodes persulcatus* on the territory of Western Siberia, Novosibirsk region

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The Borrelia Burgdorfery sensu lato complex is now considered to comprise at least ten genospecies and genomic groups and seven of them have been recorded as occurring in Eurasia.

The circulation of Borrelia genospecies in the Asian part of Russia is still poorly studied. The present work was aimed at the development of a finger printing method for B. B. s. l. that can be used for the timely diagnosis and therapy of borreliosis.

We developed the primer finger printing system on a conservative region of Flagellin gene.

To do this, DNA was isolated from several clinical samples (bioptates) taken from manifestation sites of symptoms resulting from Ixodes persulcatus bites. The spirochetes B. burgdorfery s. l. were detected by PCR targeting the FLA-gene region. The obtained fragment of 291 b.p. was sequenced. The analysis of the obtained sequences with the BLAST system allowed the detection of B. garinia and B. afzelia genotypes among them.

Primers for the detection of genotypes of the B. B.s. l.-B. garinia, B. afzelia and B. b. s. l. complex were developed on the basis of analysis of the obtained primary sequences of local isolates that were compared with EMBL data libraries. The genotyping system presents a “nested” PCR.

We analyzed 67 selected ticks I. persulcatus (Novosibirsk region) using the developed PCR-analysis. Spirochetes B. b. s. l. were detected in 43 ticks (63.8 %), twenty of which (29.7 %) had the B. garinia genotype, 16 ticks (28/8 %) had the B. afzelia genotype, B. Burgdorfery sensu stricto was not detected, and a mixed B. garinia and B. afzelia genotype was detected in 4 ticks.

7 Tick-borne encephalitis and Ixodes tick-borne borreliosis mixed infection in Tyumen region

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Tick-borne encephalitis and ixodes tick-borne borreliosis have common epidemiological characteristics such as area, season, vectors. Tick-borne mixed infection by virus and borrelia vary in different landscape zones of Tyumen region from 1.6±0.5 % to 3.0±0.8 % (Kolchanova, 1996). Data obtained in 1997-2001 were used to determine the mixed infection rate in patients. The diiagnosis of "tick-borne encephalitis" or "ixodes tick-borne borreliosis" was based on clinical observations and on duplicate serum investigation: in NDRIF for detecting antibodies to borrelia and in RSHA for detecting antibodies to the virus of tick-borne encephalitis. The cut-off titre in NDRIF was 1/40. The media-geometric titer of antibodies to borrelia vary in different landscape zones from 1/45 to 1/60, to the virus – from 1/28 to 1/34. The average data for many years showed 48-9 cases of ixodes tick-borne borreliosis for 100,000 population. The rates of mixed infection vary in different zones from 4.7 % to 12.1 %. Patients with tick-borne encephalitis mixed with ixodes tick-borne borreliosis demonstrate a more prolonged incubation period and two times as many complaints about weakness, headache, mialgy, arthralgy.

Thus, in the Tyumen region a high rate of mixed infection tick-borne encephalitis and ixodes tick-borne borreliosis with severe clinical forms was found. It is necessary to detect antibodies to borrelia in the serum of all encephalitis patients and to the virus – in the serum of all patients with borreliosis. The clinical-epidemiological peculiarities of the mixed infection must be investigated in detail.

8 Mixed infections issue: Ixodid tick-borne Borrelioses and Opisthorchiasis in Western Siberia

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Opisthorchiasis and tick-borne infections, Ixodid tick-borne Borrelioses (ITBB) in particular, represent a very topical problem of infectious pathology in some of the regions of Russia. Tyumen region is one of them in the territory of Western Siberia. Morbidity rates for these diseases are: 31.1 cases of ITBB and 490.5-667.3 cases of opisthorchiasis per 100 000 people. These rates are correspondingly 6 and 20 times higher than the average in Russia. Tyumen region has 46.6 % of the overall number of opisthorchiasis cases in Russia. Opisthorchiasis and ITBB sympatry often overlap which opens up the possibility for mixed Opistorchiosis - Borreliosis diseases to expand widely. In Tyumen region this is fostered by a high level of invasion of fish with opisthorches and ticks with borrelia: the invasion rate of *Cyprinidae* fish (dace, roach) varies from 14.8 to 100 % (Stepanova T. F., 1988) and the prevalence in *Ixodes persulcatus* ticks by borrelia in the southern part of Tyumen region - from 11.5 + 3.1 to 68 + 1.2 (Kolchanova, 1996, 1999). What's more, 3 % of these ticks are infected with tick-borne encephalitis virus (TBE) as well. The preliminary analysis revealed, that in general around 47.9 + 0.2 % of patients, verminated with opisthorches, have antibodies to borrelia (Stepanova T.F., 1988).

In order to detect the incidence of mixed diseases and various forms, we examined 222 people. The diagnoses were based on anamnesis, clinical and laboratory data. The borrelia antibodies, immune M- and G-globulins present were determined by indirect immunofluorescent reaction, immune M- and G-globulins using the immunoferment method have been used to detect immune M- and G-globulins to TBE. In order to diagnose opisthorchiasis, faeces and bile have been examined for eggs of opisthorches. Serum has also been studied using an immunoferment method to define the level of M and G antibodies to antigens of opisthorches. Mono- infections were identified in just 22.1 % of the overall number examined while mixed infections have been found in 56.3 %; 21.2 % of the cases did not have antibodies for these infections at all. Half of the patients diagnosed with a mixed pathology had a mixture of ITBB and opisthorchiasis; the combination of ITBB and TBE was detected less frequently (20.8 % of the overall number of diseases), and TBE along with opisthorchiasis (7.2 %). 24 cases were found to manifest a combination of three diseases in a row: ITBB, TBE and opisthorchiasis.

On the basis of clinical manifestations mixed infection cases involving Opisthorchiasis and Ixodid tick-borne Borrelioses, it was concluded that they have a longer incubation period, the patients have more complaints, a global immunity malfunction is described and the symptoms are far more serious than with mono-infections.

9 Distribution of soft ticks and their infection with Borrelia in Hamadan province, Iran

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Abstract:
Tick-borne diseases like tick-borne relapsing fever are a public health problem in Iran. They are more prevalent in the northern part of the country. In order to determine the distribution of soft ticks, Argasidae, and their infection with *Borrelia* species in Hamadan province, 53 villages were randomly selected. A total of 4805 ticks were collected directly from human dwellings, poultry and animal shelters. They belong to the genus *Argas* and *Ornithodoros*. Of these ticks, 52.3 % were *Argas persicus*, *A. reflexus* (2.6 %), *Ornithodoros canestrinni* (2 %), *O. lahorensis* (41.4 %), *O.tholozani* (1.77 %). The most prevalent species was *O. lahorensis* and the least prevalent *A.persicus*.

Examination of ticks with *Borrelia* revealed that *O.tholozani* are infected with *Borrelia persica*. The infection rate and disease prevalence coincide in the region. The results will be discussed in more detail in terms of preventive measures.

10 *Dermacentor marginatus* and Erythema migrans

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Lyme borreliosis is the most common and significant arthropod-borne zoonosis in the northern hemisphere, caused by a spirochete *Borrelia burgdorferi*. The predominant tick species that transmit *Borrelia burgdorferi* are the Eurasian species *Ixodes ricinus* and *Ixodes persulcatus* and the North American species *Ixodes scapularis* and *Ixodes pacificus.*

The aim of this study is to present two cases with serologically confirmed Erythema migrans, associated with the bite of *Dermacentor marginatus* tick.

Results. A man and a woman were treated for suspected early Lyme borreliosis at the Department of Infectious diseases, Medical University, Plovdiv, Bulgaria in March 2000. Both the patients had a similar clinical manifestation: Erythema migrans presented a median of 6 days after the tick bite with homogeneous configuration rather than peripheral erythema with partial central clearing and the associated symptoms were mild. The patients had positive IgG antibody response to *B. burgdorferi* and responded promptly to doxycycline treatment.

Conclusion. In some areas, apart from *Ixodes* ticks, some other tick genera such as *Dermacentor* might be implicated as secondary vectors in *Borrelia burgdorferi* transmission to humans. Further studies are needed to elucidate the probable impact of *Dermacentor marginatus* tick on the clinical presentation of Erythema migrans.

11 *Borrelia burgdorferi* sensu lato isolated from engorged *Ixodes ricinus* ticks infestating birds in Slovenia

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Introduction:
Birds are an important reservoir of *B. burgdorferi* sensu lato in nature. Larvae and nymphs infestate birds, which are often infected with borreliae. Birds are most frequently infected with *B. garinii*, either alone or in combination with *B. valaisiana*. *B. afzelii* was found in only one bird species. *B. burgdorferi* sensu stricto has not yet been found in birds.

Materials and Methods:
Forty engorged *Ixodes ricinus* ticks were collected from birds in central Slovenia in the autumn of 2001. Ticks were collected from four species of birds: black bird (*Turdus merula*), song thrush (*Turdus philomelos*), robin (*Eritacus rebecula*) and hedge accentor (*Prunella modularis*). Ticks were decontaminated in 70% ethanol and sterile saline and put in MKP medium. We cultivated borreliae at 33^{o}C for nine weeks. Growth in culture was monitored weekly by dark-field microscopy. Species of *B. burgdorferi* were identified by pulsed-field gel electrophoresis (PFGE) of DNA prepared from cultures using the gel insert method. Borrelial DNA was isolated, digested with *Mlu*I restriction endonuclease and put on gels. After PFGE gels were stained with ethidium bromide and visualised under UV light. Stained bends of restricted DNA were characteristic for different species of *B. burgdorferi*.

Results and conclusions:
B. burgdorferi sensu lato was isolated from 13 ticks. *B. valaisiana* was isolated from 7 ticks, all removed from three black birds (*Turdus merula*). *B. valaisiana* was also isolated from the skin of one of those black birds. *B. afzelii* was isolated from the skin and from a tick of one hedge accentor (*Prunella modularis*). In five ticks collected from various bird species we isolated *B. garinii*. Ticks from birds in Slovenia are infected with *B. burgdorferi* sensu lato in a similar manner to Switzerland.

12 The zoonotic reservoir for *Borrelia burgdorferi* sensu lato in the district of Mazury Lakes, North-East Poland

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The objectives of the present study were: (1) to determine the phenological patterns of rodents *Clethrionomys glareolus* and *Apodemus flavicollis* and seasonal patterns of their abundance as well as infestation rate of *I. ricinus* in these rodents; (2) to assess the prevalence of *Borrelia burgdorferi* s. l., *B. garinii* and *B. afzelii* infection in a population of ticks and its hosts in woodland habitats of northern Poland.

The studies were carried out on a monthly basis in heterogenous deciduous woodland at Urwitałt near Mikołajki on the Mazury Lakes, between May and October 2001. The prevalence of tick infection with *B. burgdorferi* s. l. was determined using IFA and PCR methods. Two genospecies *B. garinii* and *B. afzelii* were determined by nested PCR.

A total of 196 rodents (112 *C. glareolus* and 84 *A. flavicollis*) were trapped and 1349 *I. ricinus* ticks (1061 from *A. flavicollis*, 288 from *C. glareolus*) were collected. There was an increase of rodents in the autumn with maximum density in October for *C. glareolus* and in September for *A. flavicollis.* The infestation rate of inspected animals was higher than 98 % for both species. Only two stages of *I. ricinus* were found on trapped rodents: larvae (80 % for *C. glareolus,* 84.5 % for *A. flavicollis*) and nymphs (7.5 % for *C. glareolus*, 15.5 % for *A. flavicollis)*. The average level of infestation with ticks in rodents appears to decrease from spring to autumn. Both methods of *B. burgdorferi* s. l. detection, PCR and IFA, showed that larvae collected from both species of invested rodents were infected by these spirochetes. The increased infection of *B. burgdorferi* s. l. was observed from spring to autumn: from 12.5 % to 15.6 % for ticks collected from *C. glareolus*, and from 5.6 % to 8.8 % for ticks collected from *A. flavicollis*. The infection rates of larvae examined by PCR were 5 % in spring and 8 % in autumn for ticks collected from *C. glareolus*, and 10 % and 5 % for *A. flavicollis*. A total number of 329 ticks were analysed for the detection of genospecies (184 for *B. garinii* and 145 for *B. afzelii*). From larvae collected from *C. glareolis*, spirochetes of *B. garinii* was found in 3 % and *B. afzelii* in 1.1 % of inspected ticks. Correspondingly, in larvae collected from *A. flavicollis* the percentage of infected ticks was 1.5 % for *B. garinni* and 1.7 % for *B. afzelii*. The examined ear biopsies were *Borrelia* positive in 2.3 % and 3.9 % of the samples from *C. glareolus* and *A. flavicollis*, respectively.

This study confirms that in the northern part of Poland, *C. glareolus* and *A. flavicollis* significantly contribute to maintaining an extremely high abundance of larvae of *I. ricinus* infected with *B. burgdorferi* s. l. and both species of rodents may represent significant reservoir hosts for *B. burgdorferi* s. l. as well as for *B. garinii* and *B. afzelii*.

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13 Alternation between spirochete and cystic forms of the Borreliosis agent may exist in human and in the tick vector

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The long established life cycle pattern in Spirochaetacea implies the alternation of a motile spirochete and a cyst depending on the situation. Adverse conditions are known to result in spirochete cystogeny, whereas favorable conditions promote the development of motile spirochete. It is suggested that tick-borne borreliosis agent is no exception. Based on observations in 1996-2000 at a focus of tick-borne encephalitis and borreliosis near St. Petersburg, Russia, an analysis was made of the occurrence frequency of motile *Borrelia burgdorferi* s. l. in the 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. PCR positive but live spirochete-negative ticks were 3 times more abundant in April, May, at the very beginning of the season, 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 which then acts as the main source of infection at least during that period. The increased incidence of motile spirochetes toward the end of the season appears to suppress tick locomotor activity, shortens by 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. This is because 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 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 after over-wintering, at the beginning of the season. A relapse of the disease may, therefore, depend on cyst and motile spirochete alternation.

14 Studies on the transovarial transmission of Borrelia burgdorferi sensu lato in the Ixodes persulcatus tick

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The possibility of vertical transmission of *B. burgdorferi* sensu lato in *I. persulcatus* ticks was studied in the progeny of 20 spontaneously infected females collected from the vegetation in an active ITBB focus located in the Perm oblast, where *B. garinii* and *B. afzelii* were shown to circulate. After feeding and egg laying under laboratory conditions, individual females were studied by the PCR method with primers specifically recognizing the conservative sequences of the *B. burgdorferi* 16S rRNA gene. Eggs taken from 19 egg masses and unfed larvae hatched from 17 egg masses were studied by the methods of inoculation into the BSK II medium, microscopic analysis of fixed smear preparations, and PCR with the above primers. The presence of *Borrelia* DNA was detected in 16 out of 20 engorged females (80.0 %) as well as in 65 out of 178 samples containing 20 eggs each (36.5 ± 7.2 %) and in 81 out of 378 samples containing 10 eggs each (21.4 ± 4.2 %). The respective rates of individual egg infection in egg masses, calculated by V.N. Beklemishev's formula (1963), were 0.4 – 8.05 % and 0.5 – 23.0 %. PCR analysis of 370 eggs (one egg per sample) and 781 unfed larvae hatched from the same egg masses (1, 20, 40, and 50 larvae per sample) failed to reveal the presence of *Borrelia* DNA. Negative results were also obtained in experiments on inoculating the BSK II medium with the suspensions of eggs and larvae (12 samples by 50 eggs and 60 samples by 10 larvae). Five egg-derived cultures (41.7 ± 14.2 %) and 16 larva-derived cultures (26.7 ± 5.7 %) (from three and seven females, respectively) contained immobile spiral forms of *Bacillus* spp. Microscopic analysis of 1683 smear preparations of eggs and 1416 preparations of unfed daughter larvae revealed spirochete-like cells in 7 (0.4 ± 0,3 %) and 13 (0.9 ± 0.5 %) preparations, respectively; typical *Borrelia* cells were found in seven preparations of larvae (0.5 ± 0.4 %). The spirochete-like cells in larvae were morphologically identical to those in eggs. Only one out of 16 infected females transmitted *Borrelia* vertically, through the eggs to the larval progeny. The infection rate in this progeny was about 7 %, and the prevalence of *Borrelia* in individual larvae was 0.4 – 0.8 cells per 100 microscopic fields. These data show that the transovarial transmission of *Borrelia* in *I. persulcatus* ticks is possible but its probability is very low.

15 PCR-RFLP: A potential technique to identify blood meal donors in vector arthropods

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Knowledge about the feeding behaviour of vector arthropods responsible for the transmission of vector-borne diseases is important because it can serve to improve vector control measurements as well as disease eradication strategies. Recent models proposed for the description, for example, of the epidemiology of African trypanosomosis include as the key parameters for understanding transmission, the biting rate of tsetse (Diptera: Glossinidae) and the probability of tsetse feeding on different hosts.

Information on host-vector interactions can be obtained from studies on the natural feeding habits of different species of tsetse. For this purpose serological techniques have been developed using host-specific antisera to identify the source of vertebrate blood from the intestinal tract of flies caught in the wild (Weitz 1963; Staak *et al*., 1981; Clausen *et al*., 1998). Repeated absorption of antisera with the most cross-reacting antigens will yield highly host-specific antisera. Cross-reactivity between members of different groups of animal families can be eliminated whereas, even after repeated absorptions, a slight cross-reactivity will remain between phylogenetically closely related species e.g. cross reactivity between domestic pigs, bush pigs, and warthogs. This results in a high percentage identified as suidae of unknown species.

Recently, a combined polymerase chain reaction - restriction fragment length polymorphism analysis (PCR-RFLP) - was established for the differentiation of material from closely related animal species in food products (Meyer *et al*., 1995, Bellagamba *et al*., 2001). The aim of this study was to adapt this technique for the identification of blood meals from tsetse.

The primers described are complementary to the conserved region of the cytochrome b gene (*cyt b*) of the vertebrates mitochondrion DNA (mt DNA) resulting in a unique but variable 359 bp-PCR product. The selection of appropriate restriction endonucleases sites was based on comparison of mt DNA sequence data of vertebrates drawn from the search engine of the National Centre of Biotechnology Information (NCBI) available on the web (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide). Sites for all restriction enzymes that cut the 359 bp sequence were identified by means of the free programme *NEB cutter V1.0* (http://tools.neb.com/NEBcutter/index.php3) designed by New England Biolab (NEB).

Theoretical and practical examples for the identification of important mammalian hosts of tsetse by PCR-RFLP will be presented.

16 Genetic variants of *Anaplasma phagocytophilum* in sheep in Norway

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Tick-borne fever (TBF) caused by *Anaplasma phagocytophilum* (formerly *Ehrlichia phagocytophila*) has been recognised for decades as a disease in sheep in tick (*Ixodes ricinus*) infested areas in Norway. *A. phagocytophilum* is also known to cause disease in domestic and wild ruminants, horses and humans in this country. The main problem with *A. phagocytophilum* infection in sheep is the concomitant immunosuppression. Down-regulation of the immune response may be responsible for the susceptibility to secondary infections and fatal disease caused by pathogens such as *Pasteurella haemolytica / trehalosi.*

A. phagocytophilum containing blood samples from Norwegian sheep has been analysed by sequencing the *Anaplasma* 16S rRNA and *groESL* heat shock operon genes. At least four 16S rRNA gene variants of *A. phagocytophilum* were found. Experimental inoculation studies of three of these variants indicate that two of them induce only a mild clinical reaction, no neutropenia and moderate degree of bacteraemia whereas the third variant causes severe clinical reactions. Sequence analyses of the *groESL* operon revealed a higher degree of genetic diversity than those performed on the 16S rRNA gene.

17 Ecology, epidemiology and genetics of spotted fever group rickettsiae and new data of their study in Russia and Kazakhstan

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Rickettsiae are characterized by a wide range of pathogenicity - classic and new pathogens, endocytobiotes of eukariotic cells. The evolutional role of *Rickettsiaceae*, as a possible ancestor of mitochondria, draws interest to its genetic study. Progress in the study of rickettsiae has shown a widening mainly of representatives of spotted fever group (SFG) (about 50 are now known). SFG *rickettsiae* are tick-borne microorganisms with effective transovarial and transstadial transmission. The main hosts are ticks of genera *Dermacentor*, *Rhipicephalus, Haemophysalis, Ixodes, Amlyomma* and some insects. The strategy of the maintenance of tick microorgamisms is promoted by vector type of transfer and tropism to endothelial cells of vessels or blood cells of animals. Interesting clusters may be male-killing and other neither SFG nor typhus group rickettsiae. Not all of them are connected with blood feeding.

The evolution of SFG was closely connected with the evolution of vectors. The common root of Eurasiatic and American tick-borne rickettsiae is determined in common co-evolution. Three main SFG pathogens - *R.rickettsii, R.conorii* and *R. sibirica* - belong to the same subgroup of tick-borne rickettsiae. The main ecological and epidemiological characteristics of SFG rickettsioses are re-emerging (long-standing cycles of epizootical activity), anthropogenic influence, connection of morbidity with seasonal tick activity, quantitative and qualitative heterogeneity of its populations and coexistence of different tick's microbes (rickettsiae, borreliae, ehrlichiae etc.).

The study of non-pathogenic *Rickettsia* supposes the application of new tools of detection and isolation (tick experimental models, sensitive cell lines, monoclonals, genetic methods). The real role of new rickettsial genotypes in infectology is determined deficiently (for example - *R. slovaca*, which caused TIBOLA was revealed in Russia. The interference between rickettsiae with different virulence may determine alterations in its populations and levels of morbidity.

Differences in the SFG screening of ticks from active foci of Siberian tick typhus (STT) and STT - free territories in Russia and Kazakhstan were shown in antigenic, biological and genetic characteristics. Up to now, nine species of tick-borne rickettsiae have been detected in Russia and Kazakhstan and *E.muris* – in three regions of the Asian part of Russia. Cases of HGE were detected in Novosibirsk and Altai regions of Siberia. New rickettsia closely related with *R.canada* was detected in *Ixodes persulcatus* in different regions of Russia and was named *Rickettsia tarasevichiae s.nov*. (Shpynov et al., 2002). The most widespread SFG rickettsiae are *R.sibirica* (Siberia, Russian Far East), R.RpA4. The list is not exhaustive.

18 Randomized, phase II dose-finding studies of a modified tick-borne encephalitis vaccine in adults: Evaluation of the safety and immunogenicity of FSME-IMMUN® "new"

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Two clinical studies were conducted to identify the optimal dose of a modified tick-borne encephalitis vaccine (FSME-IMMUN® "new") in adults. A prospective, randomized, double-blind, phase II dose-finding study with the FSME-IMMUN® "new" vaccine was performed in volunteers aged 16 - 65 years (n = 405) to evaluate the immunogenicity and safety of two vaccinations (21 to 35 days apart) with three vaccine doses (0.6 µg, 1.2 µg and 2.4 µg antigen). The safety and immunogenicity of the third vaccination (6 months after the first vaccination) were investigated in a follow-up study on the same study population.

Antibody response to vaccination was assessed by enzyme-linked immunosorbent assay (ELISA). Seroconversion rates in the three dose groups (0.6 µg, 1.2 µg and 2.4 µg) were 85.1 %, 96.2 % and 97.0 %, respectively after the second vaccination, which the majority (73 %) of the volunteers received exactly 21 days after the first vaccination. The geometric mean concentration (GMC) was significantly higher after the 2.4 µg dose (GMC: 631.3 VIEU/ml, 95 % C.I.: 561.3; 710.0) than after the 1.2 µg dose (GMC: 465.8 VIEU/ml, 95 % C.I.: 414.7; 523.1). Seroconversion rates after the third vaccination were 96 %, 99.2 % and 100 % with the 0.6 µg, 1.2µg and 2.4 µg doses respectively. The GMC was 1503.0 VIEU/ml (95 % C.I.: 1253.5; 1802.2) in the 2.4 µg dose group and 1267.7 VIEU/ml (95 % C.I.: 1067.7; 1505.3) in the 1.2 µg dose group.

No unexpected AEs or vaccine-related serious adverse events were observed during either study. Local and systemic reactions were mainly mild and not dose-dependent, with an overall fever rate of <1 % (0 % in the 2.4 µg dose group) after the first vaccination.

Conclusions: The 2.4 µg dose is the optimal dose of the FSME-IMMUN® "new" preparation in adults, as it was found to: (1) induce a superior immune response, (2) be non-inferior to the 1.2 µg dose with respect to fever rate after the first vaccination and (3) be well-tolerated with respect to local and systemic reactions. The results of both studies demonstrate that the FSME-IMMUN® "new" vaccine is safe and highly immunogenic in adults.

19 Cloning and Sequencing of the V-regions of Protective Murine Monoclonal Antibodies against Tick-Borne Encephalitis Virus

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Tick-borne encephalitis is a viral disease of the central nervous system. The distribution of the TBE virus almost the entire southern part of the nontropical Eurasian forest belt, from Alsace-Lorraine in the west to Vladivostok in the east. TBE incidence may vary from year to year but has increased significantly in the Siberian and Russian Far East during the last twenty years. The main treatment for TBE is immunoglobulins from human sera but this involves a possible biological risk. That is why it is necessary to find another possible approach to the specific treatment of TBE. The development of humanized antibodies might help to solve this problem. Because such antibodies are mainly human and only complementary determing regions (CDRs) retain mice nucleotide sequences, they are less immunogenic and might be used as a specific medicine for TBE therapy.

The first stage of the creation of huminized antibodies is cloning and sequencing of variable (V) genes of murine monoclonal antibodies (Mabs) possessing neutralising and protective activity. The following computer analysis of the nucleotide sequences obtained led to the identification of their CDRs and a strategy for humanization.

Murine neutralising MAbs specific to glycoprotein E of TBE virus were assayed to select protective antibodies. Several MAbs have been shown to possess protection against TBE infection on a mouse model. Murine MAbs with protective activity were used for cloning and sequencing of their V-genes. Total mRNA was extracted from murine hybridoma cell lines. cDNA fragments encoding variable domains of the heavy (H) and light (L) chains of protective Mabs were obtained using the RT-PCR technique. The nucleotide sequences of the genes were determined and analysed by Kabat database to identify their CDRs.

20 Human Miniantibodies against Tick-Borne Encephalitis Virus

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Tick-borne encephalitis (TBE) flavivirus is a highly pathogenic cause of serious disease in humans. The specific medicine for TBE therapy might be very important for Russia where TBE virus infections have so far occurred in some regions. Since there is no specific treatment for TBE, gamma-globulin from the donor's blood is now used. By means of phage display technology, human antibody fragments can be obtained by affinity selection against target antigens. These antibodies have several potential advantages over immunoglobulins derived from immunized individuals and animals. They are less immunogenic, can easily be produced in very large quantities, have lower retention times in tissues and more rapid blood clearance.

A combinatorial phage library of human single-chain antibody fragments, scFv, (Medical Research Council Centre, Cambridge, England) was panned against recombinant E protein of the TBE virus. Approximately 10^{12} pfu of bacteriophages were used in each of three subsequent rounds of selection. Phage populations after each round of biopanning were tested in ELISA for their binding with recombinant E protein of the TBE virus. The increasing optical density in ELISA confirmed the enrichment of the library with antibodies specific to this antigen. Individual clones from the enriched library were assayed for their binding with recombinant E protein of the TBE virus in ELISA. Approximately 20% clones were positive in ELISA. The binding characteristics of the mini-antibodies obtained were studied with subsequent dilution of either phage antibodies or antigen. Clones selected in ELISA were then tested in immunoblotting to confirm their specificity. The capability to bind E protein of the TBE virus was shown for all selected mini-antibodies. It was also shown that all clones bind recombinant E protein of the TBE virus.

21 Cytokine activity upon experimental tick-borne encephalitis immunization.

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Experimental double (day 1, day 9) immunization of BALB/c mice with tick-borne encephalitis vaccine (Moscow, Russia, lot 547) resulted in adequate vaccine protection (100 %) against the subsequent challenge with the lethal dose of the tick-borne encephalitis virus (strain 205). Appearance of IgM was registered on day 3 and IgG on day 7 after the first immunization. The maximum level of IgG (1:640) was found on day 14 after the second immunization.

Experimental immunization was accompanied by the production of the following cytokines: IL-1β , IL-2, IL-4, IL-6, IL-10, IL-12, TNF-α, and IFN-γ, which were measured in the serum of mice. The profiles of changes of TNF-α, IL-1β and IL-6 were very similar: growth after the first immunization followed by dropping (below day 0 baseline) on day 3 after the second immunization. The concentration of IFN-γ gradually increased during the immunization. IL-12 showed two peaks on day 1-after the first and day 3 after the second immunization. Both IL-2 and IL-4 had maximum levels on day 7 after the first immunization. IL-10 showed two close maximums on day 1 after the first and the second immunization.

Thus, the first immunization with TBE vaccine stimulated production of all measured cytokines, but the second one affected only IL-10 and IL-12. All cytokines concentration returned to the pre-vaccination level (day 0) on day 14 after the second immunization with exception of IFN-γ, which reached its maximum.

22 Tick-borne encephalitis virus propagation into the brain after intraperitoneal challenge of mice

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Tick-borne encephalitis virus (TBE) is one the most important human infections of the central nervous system in several European countries and Russia. Studies of the pathogenesis of certain neurotropic viral infections suggest that cells of olfactory neuroepithelium play a special role in the propagation of these viruses in the central nervous system upon peripheral infection. The spread of the TBE virus into the central nervous system and propagation in organs and blood of mice-infected intraperitoneal routies was studied. In this study we used PCR and PFU assays to detect the virus in mouse organs and tissues. PCR assay allows the detection of replicative viral RNA in olfactory bulbs and blood as early as 10 h after ip challenge with TBE virus. Plaque-forming unit assay on the monolayer of embryonic swine kidney cell culture indicates virus circulation in blood 1 day after challenge. Primary virus detection was also observed in the spleen 1 day after challenge, and in mouse brain and in nasal cavity 3 days after infection. Ultrastructural and histological studies indicated destructive changes in cortical and olfactory nervous tissues (oedema, necrosis, hemorrhages).

The results obtained point to olfactory TBE viral RNA propagation in the brain after ip challenge. A hypothesis on the effectiveness of intranasal immunization against peripheral TBE virus challenge was proposed. Cationic solid lipid nanospheres containing killed TBE viral particles induced TBE specific humoral IgG in the blood serum of intranasally immunized mice. Animals were partially protected against intracerebral TBE virus challenge, and average time to death was increased by 3.2 days compared with non-immune control group.

23 Neurological sequelae in children and adolescents after Tick-borne Encephalitis.

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Tick-borne Encephalitis (TBE) in children is –apart from a few case-reports of severe TBE-disease in the literature - considered to be a benign disease with complete recovery without sequelae. To date, no systematic studies focusing on children with former mild to moderate TBE-infection regarding course and outcome as well as possible sequelae have been undertaken.

We studied 19 children who had suffered from TBE-Meningitis or Meningo-encephalitis as well as 19 healthy controls, and compared them with the previously described features of TBE-infection in our endemic area in south-west Baden-Wuerttemberg.

None of the children examined had severe neurological or neuropsychological sequelae. One child developed significant clinical depression shortly after the illness. EEGs from children with TBE were significantly slower on follow up than control EEGs. Children, who had suffered fromr TBE, had a higher likelihood of having impairment of attention and psychomotor speed. For four of the ten subsystems in the neurological examination, children after TBE had lower scores than control children. Due to the small number, it was difficult to identify risk factors and predictors for an adverse outcome. Boys and children with meningoencephalitis had a higher likelihood of being classified as differentially impaired in neuropsychological testing. An abnormal EEG and persistent attention deficits at follow up were associated with poorer performance on some subsystems in the neurological examination and on selected subtests of the neuropsychological battery.

In summary, this study suggests that TBE in children might cause sequelae along the lines of minor neurological dysfunctions or neuropsychological impairment even in mild to moderate disease. Further studies with a larger number of patients are required in order to characterize sequelae in more detail.

24 The colour of clothes and tick attachment

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To prevent tick bites personal precautions, such as wearing light-coloured clothes, are generally recommended. It is believed that ticks are more visible and easier to detect on light-coloured clothes than on dark-coloured clothes. Accordingly, the risk for tick-transmitted disease will decrease. However, studies confirming the advantages of light-coloured clothing have not been found. Also, we do not know if clothing influences the tick when choosing its hosts.
The present study was undertaken to evaluate the preventive recommendations to wear light-coloured clothing and, furthermore, to determine whether light- and dark-coloured clothing influenced the attachment of host seeking ticks, i. e. *Ixodes ricinus*.

In the autumn of 2001 the study was conducted in two geographic areas, both situated in the archipelago of the south-eastern part of Sweden. A total of ten participants (5 male, 5 female) exposed themselves to ticks in extreme tick infested habitats. Randomised into two groups the participants were exposed in a standardised manner by walking in squares measuring 25 x 25 meters. In total, they were all exposed 12 times, twice in each square; once with the light- and once with the dark-coloured clothes (vice versa when entering the next exposure area). Every exposure lasted for 3 ½ minutes. After the exposure all the nymphs and adult ticks on their clothes were collected and counted.

In total, 892 nymphs and adult ticks were collected and of these 552 (62 %) were found on the light-coloured clothes and 340 (38 %) on the dark-coloured clothes. The total mean in the number of ticks found between both groups differed significantly, with a mean difference of 21.2 more ticks on the light-coloured clothes ($p = 0.003$, 95 % CI 9.37 - 33.03).

Taking in account the total number of ticks/participant when wearing light versus dark coloured clothes, all the participants had more ticks on the light coloured clothes in all the exposure occasions. In view of these results, the recommendation to wear light-coloured clothes as a personal precaution in tick endemic areas must be questioned.

25 Analysis of the Heterogeneity of the Immunodominant Surface Protein VlsE among the Three European Genospecies of *Borrelia burgdorferi* s. l.

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Borrelia burgdorferi sensu stricto (*B. b*.), the causative agent of Lyme borreliosis in the USA exhibits the immunodominant surface protein VlsE, which undergoes antigenic variation based on 16 recombination cassettes. Each cassette has 6 conserved (CR1-CR6) and 6 variable regions. CR6 codes for a 26 aa long immunodominant peptide which is used in serodiagnostics. Here we investigated the diverse group of European *Borrelia* species *B. b*., *B. afzelii* (*B. a*.) and *B. garinii* (*B. g*.) with respect to genetic and antigenic heterogeneity of VlsE.

To determine whether *vlsE* sequences are present in the genome of different European *B. Burgdorferi* sensu lato strains, we localized the gene on the borrelial plasmids by PFGE and Southern Hybridization using a *B. b.* B31 *vlsE* probe. In 10 of the 13 strains tested (3 *B. b*., 2 *B. a*., 7 *B. g*. and 1 *B. valaisiana*) we found a plasmid varying in size from 19 to 30 kb that hybridized with the *vlsE* probe.

The *vlsE* genes of 3 human isolates PKo (*B. a*.), PBr (*B. g*., OspA-serotype 3), and PBi (*B. g*., OspA-serotype 4) were sequenced and compared to the *B. b.* B31 sequence. Alignment of protein sequences revealed that the main structure of the protein is identical with 6 conserved and 6 variable regions respectively. CR2-CR5 are conserved among all species; the CR1 region is conserved among *B. a.* and *B. g*., but differs from CR1 of *B. b*.. The immunodominant CR6 regions of PKo, PBr and PBi differ in 4 to 5 aa in comparison to CR6 of B31, a finding with possible diagnostic relevance.

VlsE is weakly expressed in borreliae cultured in modified Kelly medium under standard conditions (33°C; microaerophil). VlsE was not detectable using the conventional Western blot technique and only traces of VlsE were detected using the highly sensitive chemoluminescence detection method. However, VlsE became visible in the conventional Western blot after 18 hours of coincubation with HL60 cells. This indicates that VlsE is upregulated in the presence of eukaryotic cells in vitro too. This implies that conventionally cultured borreliae are not suitable as antigens to detect anti-VlsE antibodies with sufficient sensitivity in diagnostic Western blots.

Recombinant VlsEs from the *B. burgdorferi* sensu lato strains PKa2, PKo and PBi displayed different reactivities with 40 sera of early neuroborreliosis. Only one serum showed no reactivity with any VlsE. Three sera reacted only with VlsE of PBi, one serum only with VlsE of PKo and one only with VlsE of PKa2. This is an indication that the combination of different VlsE homologues as antigens might improve serodiagnostic tests.

26 Diagnostic Value of a new C6 and C10-Peptide ELISA for the Serodiagnosis of Early Lyme Borreliosis - Comparison to the European Two-Step Protocol

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C6 Peptide, an immunodominant 26 amino acid sequence of the surface protein VlsE and C10 Peptide from OspC were recently published as very sensitive and specific borrelial antigens for serological diagnosis in all stages of Lyme Borreliosis. Because of its accuracy it was proposed as substitute for the currently recommended two-step protocol with a sensitive sceening ELISA followed by a specific confirmatory immunoblot.

Methods:
Sera from 88 patients with early localized infection (well defined typical erythema migrans < 4 weeks duration) and 50 healthy blood donors as controls were evaluated with the following assays: C6 Peptide ELISA for the detection of IgG antibodies and a C10 Peptide ELISA for the detection of IgM antibodies (Immunetics, USA) in comparison to Flagellum IgG/IgM ELISA (Dako, Denmark) and µcapture IgM Flagellum ELISA (IDEIA Dako, Denmark), European B. b. s. s. Sonicate IgG/IgM ELISA (Genzyme Virotech, Germany) and Immunoblot with antigens from 3 genospecies of B. burgdorferi (Triple Blot, Viramed, Germany).

Results: Sensitivity

Method		EM-pts (N)	1st step ELISA positive or borderline	2nd step Immunoblot positiv	Confirmation of ELISA by Immunoblot
µcapture	IgM	83	58 %	42 %	72 %
C10- peptide	**IgM**	**88**	**27 %**	**25 %**	**93 %**
C6-peptide	**IgG**	**88**	**38 %**	**28 %**	**74 %**
C6 plus C10	**IgM/IgG**	**88**	**53 %**	**43 %**	**81 %**
Flagellum	IgM/IgG	87	44 %	35 %	80 %
Sonicate	**IgM/IgG**	**87**	**77 %**	**62 %**	**81 %**

Specificity: 50 blood donors from an endemic area were also examined by these assays. Specificity of C10 Peptid ELISA was 100 %, of C6 Peptid ELISA 88 %, of Virotech ELISA IgG 88 %, of IgM 86 %, of Viramed Immunoblot IgG 85 % and of IgM 94 %.
Further data of pts with early disseminated Lyme Borreliosis, follow up after therapy, cross-reactivity in syphilis and autoimmune diseases will be presented at the meeting.

Conclusion:
In this pilot study with sera from patients with early localized Lyme Borreliosis, the new C6 and C 10 Peptide ELISA did not show improved sensitivity in comparison to the presently recommended two step-protocoll with screening ELISA and confirmatory immunoblot with 3 European genospecies as the test antigens.

27 Diagnostic quality of Lyme disease serology: Lessons from the German proficiency testing program established in 1999

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Objective: External quality control surveys are an important tool in regulating the quality of infection serology in general and of borreliosis serology in particular. We report on the results of a Lyme disease proficiency testing program established in 1999 which is regularly organised two times a year by our institutions in close cooperation with the Institute of Standardisation in the Medical Laboratory (INSTAND).

Methods: From 1999 to 2002 between 229 and 350 microbiological laboratories participated in each of the seven surveys that were held so far. In addition, between 26 and 30 laboratories from 13 other European countries also participated in each trial. In each survey two serum samples (Lyme disease sera, syphilis sera, blood donor sera) which had been unambiguously characterised by six reference laboratories as containing or not containing antibodies against the Lyme disease spirochete were sent out to determine the accuracy of the diagnostic methods used in the participating laboratories. Moreover, the laboratories reported interpretative statements whether or not the test constellation suggested a possible borrelial infection and if an early or late phase of the specific antibody response was suspected.

Results: Test results were found to be in part highly variable and clearly correlated to the manufacturers and the applied test methodology. Obviously, IgM tests were more difficult to handle than were IgG-tests. ELISA testing was more reproducible and proved to be more sensitive and specific than IFA and IHA testing. Quantification of test results and reporting of specific immunoblot bands also showed high variability. Moreover, a high number of false positive and false negative test results were reported for some assays by the participants.

Qualitative results of Lyme serology proficiency testing program (correct results in %)

Test	08/1999 (n=226)	03/2000 (n=334)	11/2000 (n=247)	03/2001 (n=337)	09/2001 (n=265)	03/2002 (n=350)	09/2002 (n=301)
IHA	94.5	91.7	22.7	57.9	35.7	80.3	92.3
IFA (IgG)	86.4	93.6	81.8	65.1	34.2	53.8	97.1
IFA (IgM)	66	80.5	23.1	62	63.6	75	85.2
ELISA (IgG)	95	94.3	93.5	79	60.5	66.2	99.5
ELISA (IgM)	84	88.6	78	85	77.5	87.7	83.2
Immunoblot (IgG)	81	89	71.5	83.6	61.3	64.5	98
Immunoblot (IgM)	74	89	81	74	66.3	77.9	71.9
Diagnostic Interpretat.	96.2	85	74.4	81.4	32.8	74.7	88.6

Conclusion: In the view of our results further standardisation of Lyme disease serology is not just desirable but is urgently needed. Moreover, stronger criteria must be applied to approve the available test kits.

28 Species-specific diagnosis of Neuroborreliosis

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Lyme borreliosis is a bacterial infection caused by the tickborne spirochaete *Borrelia burgdorferi*. In Europe, at least 3 different genospecies of *Borrelia burgdorferi* are known to be involved in Lyme borreliosis : *B. b. sensu stricto, B. garinii or B. afzelii*.
B. garinii and *B. afzelii* are the prevalent species in Europe, *B. burgdorferi* is rare in Europe, but prevalent in the USA.

Depending on which species is the causative organism for the Lyme borreliosis, the clinical manifestations vary.
Infections with *B. burgdorferi s. s.* tend to lead to arthritis, those with *B. afzelli* to acrodematitis chronica atrophicans, whereas neuroborreliosis is caused by *B. garinii* in most of the cases.

In laboratory diagnosis the detection of intrathecally produced Borrelia-specific IgG and IgM antibodies is the most evident method in respect of proof for neuroborreliosis.
Borrelia-specific antibody titers are determined in serum-liquor pairs and are brought into correlation with the total immunoglobulin concentration in serum and liquor, respectively. The calculated Borrelia-specific antibody-index allows distinction between antibody migration from blood to liquor by normal diffusion (AI<1.3) and those specific antibodies that are intrathecally produced (AI>1.5).
The index calculation requires a test for quantitative determination of IgG and IgM as both immunoglobulin classes are involved in antibody response in neuroborreliosis.

In the case of a positive antibody index derived from serum-liquor pairs by Elisa, final confirmation by WB is recommended.

In the following study, serum-liquor pairs from clinically defined cases of neuroborreliosis have been examined according to this two-step protocol comprising Elisa screening for IgG and IgM, followed by Western Blot, whereby the respective immunoglobulin concentrations in serum and liquor have been equalized by appropriately calculated dilution.
Intrathecally produced antibodies could be demonstrated by stronger bands in liquor than in serum or by the additional presence of specific bands in liquor that were not detectable in serum.

This study proved that the applied two-step method –including the calculation scheme based on Prof. Reiber- is an adequate and useful means for determining neuroborreliosis.
At the same time, the use of the Microgen test system (Elisa and Western Blot) including antigens from all three Borrelia genospecies, supports the above-mentioned statement that the genospecies *B. garinii* plays a prominent role in neuroborreliosis.

29 Serological description of Estonian patients with Lyme disease, comparison with control sera from Estonians and Laplanders from North Sweden

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Introduction:
The rate of infections with borrelia or other pathogens having cross-reactive epitopes with *B. burgdorferi*, may influence the diagnostic value of serologic tests in different populations. The aim of the study was to compare the immunoblot (IB) pattern of Lyme disease patients and control sera from Estonians and Laplanders.

Material and methods:
Serum samples were taken from 27 Estonian patients with Lyme disease enrolled in the study. 15 presented with typical erythema migrans, three had a chronic form of the disease and one had neuroborreliosis. For comparison, samples were obtained from 10 donors and 4 patients with other neurological diseases (Estonian control sera). Sera from 50 Laplanders from North Sweden (supplied by K. Ornstein from Lund University), where people never usually come into contact with ticks, were tested by IB. Sonicated lysate of *Borrelia afzelii* (strain ACA1) was used in IB as the antigen source. According to experiments with positive control sera, the lysate contained all borrelia-specific antigens: p83/100, Oms66, p41, BmpA, OspB, OspA, OspC, p18, and also bands at 75, 57/59, 50, 47, 43, 17 and 14 kDa (tested with known positive sera). Serum samples were tested in a dilution of 1:100. Bound IgG and IgM were visualised using anti-human IgG or anti-human IgM (Dako) conjugated with horse radish peroxidase (HRP) at a dilution of 1:1000.

Results and discussion:
The sera were regarded to be IgM IB positive if two of the three bands: OspC, BmpA, p41 were detected. IgG IB positive were sera reactive with at least three borrelia-specific bands. ELISA was performed by clinical laboratory using commercial kit (IDEIA^{TM}IgM and IDEIA^{TM}IgG, Dako). Altogether 20 patients had positive borrelia serology with either test or Ig class. However, 6 patients with clinically defined erythema migrans remained serologically negative. On the whole, ELISA results correlated well with the IB test. Two ELISA IgG positive cases were not confirmed by IB, one ELISA IgG negative serum ws IB positive. IgM antibody results did not overlap in 4 cases. From the Estonian control sera one had an IgG IB positive result and two an IgM IB positive result. No-one had Lyme disease in their anamnesis. All sera from North Sweden remained negative (no patient had more than one band at borrelia specific locations). 12 from Laplander sera reacted at p41 and one at p22 (OspC). These reactions plausibly indicate cross-reactivity with other antigens. It was apparent that Laplander sera had far fewer bands at borrelia specific sites than Estonian control sera (the mean number of bands per patient 0.3 *vs* 1.7). In the endemic areas asymptomatic infections of borrelia seem to be rather common and this further complicates the serodiagnosis of Lyme disease. Also the rate of other infections giving rise to cross-reactive antibodies may be more common in Estonians.

30 Chosen proinflammatory cytokines (TNF-alfa, interleukins L-6 and IL-8) release assay in the supernatant of neutrophils taken from patients with Lyme arthritis stimulated by antigens of three *Borrelia burgdorferi* strains

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The purpose of this study was to evaluate the concentration of proinflamatory cytokines – interleukin-6 (IL-6), interleukin–8 (IL-8) and tumor necrosis factor (TNF)-alfa measured in supernatants of neutrophils taken from patients with diagnosed Lyme arthritis after stimulation by antigens of three genomic groups *of Borrelia burgdorferi (B.sensu stricto, B.afzelii, B.garinii).*

Materials and methods
Neutrophils were obtained from the systemic blood of 18 patients diagnosed with Lyme arthritis. The incubation of three genomic strain neutrophils stimulated by *Borrelia burgdorferi* cells lasted for 5 days and on the sixth day of the experiment, the concentration of proinflamatory cytokines was measured using an ELISA method. The medium with granulocytes was treated as the negative control. The medium with granulocytes and the addition of granulocytes-stimulating factor were treated as positive control. The obtained results were compared with positive and negative control groups.

Results
TNF-alfa concentrations were significantly higher in the cultures stimulated by three strains of *B. burgdorferi* spirochete than in a negative control, but also significantly lower than in a positive control. The comparison of TNF-alfa concentrations among these three groups indicates a significantly lower concentration of this cytokine at *B. afzelii* antigen stimulation when compared to the cultures stimulated by *B. garinii* and *B. sensu stricto* antigens.

Similarly, IL-6 concentrations obtained in the cultures stimulated by three *B. burgdorferi* spirochete strains were significantly higher than in a negative culture. A significantly lower concentration was found at *B. garinii* and *B. afzelii* stimulation in comparison with a positive control. However, B. *sensu stricto* antigen stimulation induced IL-6 synthesis comparable to a positive control. A significantly lower concentration of this cytokine was reported at *B. afzelii* and *B. garinii* stimulation when compared to the cultures stimulated by *B. sensu stricto* antigens.

Similarly, IL-8 concentrations were significantly higher in the cultures stimulated by three *B. burgdorferi* strains than in a negative control. The IL-8 concentrations compared with positive control as well as the comparison of results between three examined groups were found to be statistically insignificant.

Conclusions:
1/The antigens of three analyzed *B. burgdorferi* strains are potent stimulators of neutrophils to synthesize IL-8.
2/ *B. sensu stricto* antigens stimulate neutrophil IL-6 synthesis more potently than *B. afzelii* and *B. garinii*.
3/An increase in TNF-alfa concentration in the neutrophil cultures is more significantly induced by *B. garinii* and *B. sensu* stricto than by *B. afzelii* antigens.

31 Concentration of IFN-γ, IL-6, IL-12 and IL-15 in serum of patients with Neuroborreliosis

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Objectives

To evaluate the concentration of IFN-γ, Il-6, Il-12 and Il-15 in the serum of patients with neuroborreliosis presenting as meningitis.

Material and methods

The study group consisted of 25 patients, aged from 21 to 64 years. Neuroborreliosis was confirmed by detection of IgM and/or IgG antibodies in serum and/or cerebrospinal fluid by ELISA (Biomedica, Austria. Before and after 4 weeks of treatment with cefotaxime, the serum concentration of IFN-γ, Il-6, Il-12 and Il-15 was measured with ELISA kits (Quantikine, R & D Systems, USA and Human Interleukin-12, Endogen, USA). The control group consisted of 10 healthy volunteers.

Results

In control sera, moderate concentrations of IFN-γ ($\bar{x}$ = 0.9 pg/ml), Il-6 ($\bar{x}$ = 1.04 pg/ml), Il-12 ($\bar{x}$ = 5,19,15pg/ml) and Il-15 ($\bar{x}$ = 0.29 pg/ml) were observed. Inflammatory changes (cytosis of 38-80 cells per mm3, protein concentration 32,0-91,0 mg/dl) were initially observed in the csf of all patients but were resolved after treatment. All measured serum cytokine concentrations in neuroborreliosis patients were significantly, many times higher than in control sera (IFN-γ $\bar{x}$ = 6.77 pg/ml, IL-6 $\bar{x}$ = 5.67 pg/ml, IL-12 $\bar{x}$ = 319.39 pg/ml, IL-15 $\bar{x}$ = 0.96 pg/ml). After 4 weeks of cefotaxime treatment, cytokine concentrations decreased significantly to: IFN-γ $\bar{x}$ = 1.82 pg/ml, IL-6 $\bar{x}$ = 2.93 pg/ml, IL-12 $\bar{x}$ = 217.4 pg/ml and IL-15 $\bar{x}$ = 0.11 pg/ml. However, they remained significantly higher than in the control group despite normal csf parameters and a lack of clinical symptoms of neuroborreliosis.

Conclusions

Significant and substantial increase of serum concentrations of IFN-γ, Il-6, Il-12 and Il-15 was observed in neuroborreliosis patients when compared to control group and persisted even after the end of 4-week antibiotic therapy.

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32 Concentration of IFN-γ, IL-6, IL-12 and IL-15 in cerebrospinal fluid of patients with neuroborreliosis

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Objectives
To evaluate concentration of IFN-γ, Il-6, Il-12 and Il-15 in cerebrospinal fluid (csf) of patients with neuroborreliosis presenting as meningitis.

Material and methods
The study group consisted of 25 patients, aged from 21 to 64 years. Neuroborreliosis was confirmed by detection of IgM and/or IgG antibodies in serum and/or cerebrospinal fluid by ELISA (Biomedica, Austria. Before and after 4 weeks of treatment with cefotaxime, csf concentration of IFN-γ, Il-6, Il-12 and Il-15 was measured with ELISA kits (Quantikine, R&D Systems, USA and Human Interleukin-12, Endogen, USA). The control group consisted of csf fluids of 10 patients with discopathy.

Results
In control csf, moderate concentrations of IFN-γ ($\bar{x}$ = 0,93 pg/ml), Il-6 ($\bar{x}$ = 0,79 pg/ml), Il-12 ($\bar{x}$ = 1,11pg/ml) and Il-15 ($\bar{x}$ = 0,27 pg/ml) were observed. Inflammatory changes (cytosis of 38-80 cells per mm3, protein concentration 32,0-91,0 mg/dl) were initially observed in csf of all patients, but resolved after treatment. Concentrations of all measured cytokines were significantly increased in csf of all neuroborreliosis patients before treatment (IFN-γ $\bar{x}$ = 13,10 pg/ml, Il-6 $\bar{x}$ = 9,8 pg/ml, IL-12 $\bar{x}$= 167,19 pg/ml, Il-15 $\bar{x}$= 7,71 pg/ml), compared to controls. After 4 weeks of antibiotic treatment csf cytokine concentrations decreased significantly (IFN-γ to $\bar{x}$ = 4,32 pg/ml, Il-6 - $\bar{x}$ = 4,56 pg/ml, IL-12 – x = 43,67 pg/ml, Il-15 - $\bar{x}$ = 4,8 pg/ml), but still remained significantly higher than in control group.

Conclusions
Significant and substantial increase of csf concentrations of IFN-γ, Il-6, Il-12 and Il-15 was observed in neuroborreliosis patients when compared to control group. It persisted after the end of 4-week antibiotic therapy, in spite of clinical recovery and normal csf parameters.

33 Cross-reactivity of leptospiral antibodies in Borrelia Western blot in acute stage of Leptospirosis in dogs but not in leptospiral vaccinated dogs

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Western blot is a common diagnostic tool for the serological detection of Borrelia antibodies in dogs to determine possible infection and/or vaccination status. Beside the controversial and difficult interpretation of such Western blot results in canine borreliosis, cross-reactivity to other spirochetes even worsens the diagnostic reliability.

In order to evaluate the influence of leptospiral antibodies caused by acute Leptospirosis or leptospiral vaccination (Canimed SHL®; containing inactivated 10^8 *L. canicola* and 10^8 *L. icterohaemorrhagiae* / Merial / France) to Borrelia Western blot results, we tested four 8-week old puppies (littermates) that suffered from acute Leptospirosis for borrelia Western blot bands. We also tested two healthy adult, tick free Beagles prior and after leptospiral vaccination boostering for Borrelia Western blot bands (recomBlot Borrelia IgG® / Mikrogen / Germany).

Leptospirosis was confirmed by PCR of kidney and liver of two puppies and positive serological micro-agglutination test (*L. australis, L. copenhagii, L. Jez bratislava, L. sejrö*) of three puppies as well as by PCR of one rat that was the suspected transmitter.

In all four puppies moderate p41 band intensity was obvious at the time of acute Leptospirosis. One of them, surviving Leptospirosis, was tested 4 weeks later by Borrelia Western blot and showed a remarkable increase in band intensity in p41 as well as bands on p41/int. garinii and afzelii beside an increase in leptospiral antibody titer.

In both healthy beagles weak p41, p41/int. afzelii, and p41/int. garinii (IgM and IgG) resulted from Borrelia Western blot prior, and completely unchanged for 2 months, after leptospiral vaccination performed twice at a four-week interval. Leptospiral antibody titer in both beagles increased due to vaccination and no clinical or serological evidence of Leptospirosis was found prior to vaccination.

We conclude that leptospiral antibodies caused by natural infection cross-react in Borrelia Western blot in dogs and lead to an increase of band intensity with rising titer while leptospiral antibodies due to vaccination do not increase band intensity. We cannot rule out a leptospiral species-specific cross-reaction because *Leptospira spp.* from the naturally infected dogs were different to those contained in the vaccine. However p41 and p41/int. bands in Western blot of the two beagles prior to vaccination could be due to contact with non-pathogenic spirochetes of the oral cavity.

Summarizing p41 and p41/int. are no reliable marker for the diagnosis of canine Borreliosis because they are also detectable after contact to other pathogenic or non pathogenic naturally occurring spirochetes and should be excluded in interpretative patterns for Borrelia Western blot results.

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34 Variability of Protein Expression Pattern in *Borrelia burgdorferi* cultivated at increasing Concentrations of Penicillin and Doxycycline

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Compared with common bacterial pathogens, little is known about the possible mechanisms *Borrelia* may have generated to overcome the presence of antimicrobial agents. To gain better understanding of the interactions between antimicrobial agents and the Lyme disease spirochete, the effects of subinhibitory and minimal inhibitory concentrations of penicillin and doxycycline on the protein expression of *Borrelia* were investigated by proteome analysis.

The minimal inhibitory concentrations (MICs) of penicillin and doxycycline against *Borrelia burgdorferi* s.s. isolate LW2 as determined after 72 h by colorimetric microdilution susceptibility testing were 0.25 mg/l for both antibiotics. To analyse the antibiotic effect on the protein expression of *Borrelia*, 10 ml cultures (2.5×10^7 cells/ml in BSK II) were grown in the presence of penicillin G and doxycycline at concentrations ranging from 0.015 to 0.5 mg/l. Spirochetes were harvested after 24h and 48h of incubation and variations in protein expression were then further analysed by two-dimensional gel electrophoresis in combination with matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF).

In comparison with untreated controls exposure to penicillin G at 0.25 and 0.5 mg/l 15 protein spots identified in the penicillin-treated samples as well as 4 protein spots in the doxycycline-treated samples showed downward regulation at the MIC after 48h. A reduction in the synthesis of the three protein spots was obvious after the cells were exposed to both antibiotics. Interestingly, one protein showed significant over-expression after cultivation of *Borrelia* in the presence of doxycycline at the MIC.

The spots identified so far belong to proteins encoding genes that are localised on the borrelial chromosome. The proteins affected by the tested antibiotics are involved in many different metabolic pathways of the cell, e.g. in glycolysis, transport of carbohydrates, translation, transcription, cell division, and energy metabolism. Interestingly, a membrane-associated protein (Oms66) was found to be down-regulated in the presence of minimal inhibitory concentrations of penicillin.

In conclusion, *Borrelia* exposed to antimicrobial agents show a differential response with regard to their protein expression depending on the concentration and the class of the applied antibiotic. It is not yet clear whether these findings represent possible mechanisms of adaptation of the pathogen to circumvent the activity of antibiotics. This requires further analysis.

35 In vitro ketolides are more active than macrolides and azalides against the spirochete Borrelia burgdorferi

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Objective:
To date, antimicrobial agents belonging to the new class of the ketolides have not been tested *in vitro* against larger numbers of borrelia isolates derived from different clinical sources and geographic origin.

Materials and Methods:
Here we analysed the *in vitro* activity of new ketolides, ABT-773 and telithromycin, in comparison to that of four classical macrolides and one azalide derivative against 15 isolates of the *B. (B.) burgdorferi* sensu lato (s. l.) complex. In addition, we tested one *B. valaisiana* and one *B. bissettii* tick isolate. Minimal inhibitory concentrations (MICs), and minimal borreliacidal concentrations (MBCs) providing 100 % killing of the final inoculum were determined by a standardised methodology. Furthermore, time-kill experiments and electron microscope examinations of borreliae exposed to increasing concentrations of erythromycin, telithromycin, and ABT-773 were performed.

Results:
The rank order of potency on a µg/ml basis for the substances tested against *B. burgdorferi* was ABT-773 (MIC_{90}: 0.002 µg/ml, MBC_{90}: 0.25 µg/ml) > telithromycin (MIC_{90}: 0.007 µg/ml, MBC_{90}: 0.25 µg/ml) > azithromycin (MIC_{90}: 0.01 µg/ml, MBC_{90}: 0.5 µg/ml) and clarithromycin (MIC_{90}: 0.03 µg/ml, MBC_{90}: >0.5 µg/ml) > ceftriaxone (MIC_{90}: 0.03 µg/ml, MBC_{90}: 2 µg/ml) > roxythromycin (MIC_{90}: 0.06 µg/ml, MBC_{90}: >0.5 µg/ml) and erythromycin (MIC_{90}: 0.06 µg/ml, MBC_{90}: >0.5 µg/ml). Results of electron microscope analysis and time-kill studies clearly support enhanced *in vitro* activity of the ketolides on borreliae.

Conclusions:
Our findings emphasise the superior *in vitro* effectiveness of novel ketolide antibiotics ABT-773 and telithromycin in comparison to classical macrolides against *B. burgdorferi* under strictly standardised test conditions and the possible suitability of these substances for clinical trials on their performance in the treatment of Lyme disease.

36 Neuroborreliosis: Proton (1H) MR spectroscopy in 12 cases

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We report here on the results of a magnetic resonance spectroscopy (MRS) study in twelve neuroborreliosis patients. A PRESS sequence with parameters of TR=1500 ms, TE=35 ms, nex=192 was used. Eight cubic cm voxel was located in normal-appearing white matter of frontal lobe. Peaks indicating: N-acetylaspartate (NAA), choline (Cho), creatine (Cr), myoinositol (mI), lipids (Lip) and lactate (Lac) were identified and ratios of NAA/Cr, Cho/Cr, mI/Cr, Lip/Cr, Lac/Cr were calculated. A significant increase in Cho/Cr and Lip/Cr was noted. No differences were found among the mean levels of NAA/Cr and Lac/Cr but in 4 patients decreased N-acetylaspartate peak was observed; the mI/Cr ratio was slightly higher. Although the spectroscopic profile in neuroborreliosis patients is not specific MRS might be a useful tool in assessing tissue damage to the central nervous system of patients with Lyme disease.

37 Acute Encephalopathy in a Patient with a Chronic Cutaneous Form of Lyme Disease, Acrodermatitis Chronica Case Study

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A 48-year old woman, a farmer, exposed to tick bites, was admitted to the Neuroinfection Unit with symptoms of Neuroboreliosis. The patient had been diagnosed with Acrodermatis Chronica and positive Lyme titers few months prior to admission.

Chief complaints on admission: headache, dizziness, insomnia, memory problems, anhedonia, balance problem, blurred vision and diminished hearing. General examination: BP140/90. Pulse 78 Temp. 36.6^{O}C. Weight. 64kg, presents skin lesions around knees and ankles (Photos 1,2,3) Neurological examination: Tearful, depressed but alert and orientation in respect of time, place and person. No evidence of aphasia. Impaired short-term memory. Cranial Nerves: Visual fields normal. Pupils equally reactive to light, extraoccular movement intact, absent nystagmus. Face symmetrical but presented diminished sensation to pinprick on the left side of the face. Motor examination showed minimal left hemiparesis, DTR preserved with hyperreflexia on the left. Sensory examination showed left hemisensory deficits to primary sensory modalities. Romberg mildly positive, FTN and HTS impaired bilaterally. Gait: walks independently with mild ataxia.

Laboratory: Lyme titers IgM(-) and IgG(+++) by Abbott and Biomedica CSF: clear, colorless, TP 39.5 mg/dl, glucose 50mg/dl, CL 126, WBC 3/mm3, Lyme titers IgM (-) and IgG (+++) by Biomedica. Antibodies against virus of Encephalomeningitis ixodica and Treponema pallidum-negative.EEG-Normal, MRI of the brain including FLAIR sequence Normal, Head CT Normal: MR spectroscopy showed elevated Cho/Cr ration and decreased NAA/Cr ratio (Figure 1).

Patient was treated with Ceftraixon IV with minimal improvement.

38 Co-infection in ticks in selected habitats in Thuringia/Germany

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In order to identify the human pathogenic genospecies (*B. garinii*, *B. b*. sensu stricto and *B. afzelii*) in free living ticks, 121 *Borrelia* strains, obtained from infected *I. ricinus*, were analysed using species-specific primers, based on the OspA gene sequences of *B. b.* sensu lato. The PCR identification confirmed *B. garinii* as predominant species in 57 of 121 samples (47.1 %). *B. b.* sensu stricto was found in 23 samples (19.0 %), and 5 samples (4.1 %) were infected by *B. afzelii*.

Twenty-two isolates (18.2 %) could not be classified.

Rates of coinfection were higher in adults (17.1 %) than in nymphs (2.2 %). From the mixed isolates, 78.6 % were associated with *B. garinii* and *B. b.* sensu stricto, the most abundant genospecies in the investigated areas. Two isolates (14.3 %) bore *B. b.* sensu stricto/*B. afzelii* simultaneously and *B. garinii*/*B. afzelii* occurred together in only one isolate (7.1 %).

These results are in accordance with several studies in which the most frequent genospecies compose the majority of multiple infections in ticks.

On the other hand, greater heterogeneity of mixed infections in *I. ricinus* from the investigated habitats in comparison to the isolates could be suggested, especially as the identification for *B. valaisiana* was not carried out.

39 Borrelia quantification and strain differentiation by a novel single-run real-time PCR

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A LightCycler-based PCR protocol was developed which targets the ospA gene for the identification and quantification of the different *Borrelia burgdorferi* s. l. species in culture and in ticks, based on the use of a fluorescently labeled probe and an internally labeled primer. The detection limit of the PCR was 1 - 10 spirochetes. The melting temperature determined from the melting curve of the amplified product immediately after thermal cycling, allowed the differentiation of the three different *Borrelia burgdorferi* s.l. genospecies that are clinically relevant in Europe in the same PCR-run. This method [Rauter, JCM, 2002, 40: 36-43] represents a simplified approach to study the association of different *Borrelia* species in ticks, the risk of Lyme borreliosis and the putatively species-specific clinical sequelae.

To determine the reliability of the real-time PCR protocol, we studied the prevalence of *Borrelia burgdorferi* s. l. infection in *Ixodes ricinus* ticks. A total of 1055 ticks were collected by flagging vegetation in 5 different sites in the region of Konstanz (South Germany) and examined for the distribution of *B. burgdorferi* species by real-time PCR. The mean infection rate was 35 % (adults 40 %, nymphs 30 %). The predominant genospecies (18 % mixed infections) in the examined areas was *B. afzelii* (53 %), followed by *B. garinii* (18 %) and *B. burgdorferi* s. s. (11 %). 0.8 % of the infecting *Borrelia* could not be identified.

40 The new data on the genotyping of tick-borne Borreliae in the Asian part of Russia

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Tick-borne borreliosis (TBB) is a widespread group of infections transmitted by *Ixodes* ticks (in Eurasia mainly I.persulcatus and I.ricinus). The percentages of infected ticks *I.persulcatus* in Western Siberia based on immunofluorescent microscopy date ranges from 11.9 to 58 % in different territories. The study of isolates from *I. persulcatus* has revealed two genotypes of Borreliae - *B. garinii* and *B. afzelii*. The participation of other species of ticks in the circulation of tick-borne borreliae has not been reliably proved up to now although there are data identifying *Borrelia spp*. in ticks of the genus *Dermacentor* in Western and East Siberia (S. A. Rudakova, 1996; Suntzova O. V. et al., 1996). Genotyping based only on the nutritious environment of BSK-2 isolates cannot be recognized as the unique methodical approach for the confirmation of the circulation of various variants of *Borreliae* given the differences of cultural properties of various strains and genotypes and probability of presence uncultivated forms of these microorganisms which are not taken into account now In recent years new opportunities for the genotyping of *Borreliae* in individual copies of carriers have appeared; in principle this increases the opportunities for the study of natural foci.

The purpose of work was to study the genospecies structure of *Borreliae* in individual ticks in territories of Siberia with various types of the population of carriers and epidemic activity of the natural foci, including territories with no incidence of the main vector - *I. persulcatus*.

828 ticks of the genus *Ixodes, Haemaphysalis* and *Dermacentor*, including *I. persulcatus* - 408, *D. reticulatus* - 167, *D. marginatus* - 90, *H. concinna* - 163 were investigated.

Primers of gene p66 and locus rrfA-rrlB-5 '-CTGCGAGTTCGCGGGAGA-3' and 5 '-TCCTAGGCATTCACCATA-3 ' were used in PCR. Primers were synthesized on the Applied Biosystems Model 392. Positive results of PCR have been received in all four genera of ticks from all administrative territories. The most positive PSR results were found for *I. persulcatus* (31.5 %); the percentages of infected ticks of other species were much lower. *B. burgdorferi* sensu stricto was not identified, *B. afzelii* (28.4 %) and *B. garinii* (10.5 %) were detected in *I. persulcatus* and only in B. afzelii (4.8 % and 7.4 % respectively) in *Dermacentor* and *Haemaphysalis*.

41 *Borrelia burgdorferi* sensu stricto in biological samples in Portugal

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Abstract:
Lyme disease is a tick-borne disease with protean manifestations, including dermatological, rheumatological, neurological, and cardiac abnormalities. The best clinical marker for the disease is the initial skin lesion, erythema migrans, which occurs in 60 % to 70 % of infected adults and less frequently in children. In Portugal the first case of Lyme disease was identified in 1989 (Morais *et al*, 1989). After that, the presence of *Borrelia burgdorferi* (Filipe *et al*, 1990) was detected in the south of Portugal as well as other types of borrelia all over the country. The aim of this work was study the prevalence of *Borrelia sp*. in human samples, in ticks and in rodents. We study 110 human samples (CSF – cerebrospinal fluid), 66 ticks and 50 rodents. After the manipulation of the samples, the PCR – RFLP analyses of rrf (5S) – rrl (23S) intergenic spacer, the nucleotide sequencing of DNA and drawing on databases, it was possible to identify *Borrelia lusitaniae* in two human samples and *Borrelia valaisiana* in one human sample in Portugal. We identified one tick infected with *Borrelia lusitaniae*. We expected to find *Borrelia lusitaniae* because is the predominant borrelia organism in Portugal. In the rodents we didn't found any type of borrelia organism so this study, like others conducted in Portugal, supports the idea that the rodents are not the most important reservoirs of this agent in Portugal.

Other animals, like birds, are currently being studied and the evaluation of their role as potential reservoirs of *Borrelia burgdorferi* in mailand Portugal is now underway.

42 Comparison of plasmid profiles of *Borrelia burgdorferi* sensu lato strains

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Spirochetes from the complex *B. burgdorferi* sensu lato are unique in having linear chromosome and multiple linear and circular plasmids. The aim of the present study was to examine the plasmid profiles of strains isolated from Slovenian patients and to compare them with *Borrelia* species.

Borreliae were isolated form clinical material and cultivated for additional analysis in MKP medium at 33 ^{o}C. The strains were identified on species level by pulsed-field gel electrophoresis (PFGE) after restriction of chromosomal DNA with *Mlu*I. Plasmid profiles were also analysed by PFGE.

Of 363 *Borrelia* strains included in the present study, 198 were from patients with solitary erythema migrans (EM; 151 skin, 46 blood, and 1 CSF isolate), 41 from patients with multiple EM (MEM; 8 skin, 26 blood, and 7 CSF isolates), 13 from patients reporting having had EM (all isolates were from normal looking skin at the site of previous EM), 57 from patients with acrodermatitis chronica atrophicans (ACA; 56 skin and 1 blood isolate), 8 from patients with lymphocytoma (all were skin isolates), and 46 from patients with neuroborreliosis (NB; 1 skin, 3 blood, and 42 CSF isolates). 247/363 (68 %) strains were identified as *B. afzelii*, 109/363 (30 %) as *B. garinii*, and 7/363 (2 %) as *B. burgdorferi* sensu stricto.

In 306/363 (84 %) strains one big plasmid in the range 55-65 kb and 3-9 small plasmids in range 15-45 kb were found, while 57/363 (16 %) strains had “unusual” plasmid content, i.e. either plasmid dimer (95-110 kb) or multiple copies of a big plasmid (2-4 plasmids in the range 50-65 kb).

Out of 247 *B. afzelii* isolates 232 (94 %) strains had one big plasmid and several small plasmids, including 110/232 (47 %) strains with 5 small plasmids, 69/232 (30 %) with 6, 34/232 (15 %) with 4, 10/232 (4 %) with 7, 6 (3 %) with 3, 3/232 (1 %) with 8, and 1/232 with 9 small plasmids. “Unusual” plasmid content was found in only 15/247 (6 %) *B. afzelii* strains: 6/15 (40 %) strains revealed plasmid dimer but no big plasmid in the range 55-65 kb, 5/15 (33 %) showed dimer in addition to big plasmid, 3/15 (20 %) contained multiple copies of the big plasmid, and 1/15 (7 %) demonstrated two dimers and one big plasmid.

Sixty-seven out of 109 (61 %) *B. garinii* strains had one big plasmid and a number of small plasmids including 24/67 (36 %) strains with either 5 or 6 small plasmids, 8/67 (12 %) with 4, 7/67 (10 %) with 7, 3/67 (4 %) with 8, and 1/67 (1 %) with 3 small plasmids. “Unusual” plasmid content was established in 42/109 (39 %) *B. garinii* strains: 5/42 (12 %) strains showed plasmid dimer in addition to big plasmid, 1/42 (2 %) demonstrated plasmid dimer but lack big plasmid in the range 55-65 kb, while 36/42 (86 %) revealed multiple copies of the big plasmid.

All 7 *B. burgdorferi* sensu stricto strains had big plasmid and different number of small plasmids including 3 (43 %) strains with 5 small plasmids, and 1 (14 %) with either 3, 4, 6, or 7 small plasmids. No “unusual” plasmid content was found.

It is of interest that *B. garinii* strains more frequently showed an “unusual" plasmid profile than *B. afzelii* strains (42/109 versus 15/247, respectively; $p<0.0001$) and that in the subgroup of strains with “unusual" plasmid profile plasmid dimers were demonstrated more frequently in *B. afzelii* strains (12/15 versus 6/42, respectively; $p<0.0001$) while multiple copies of the big plasmid were established more frequently in *B. gariniii* strains (36/42 versus 3/15, respectively; $p<0.0001$). This study demonstrated not only heterogeneity of plasmid content within individual *Borrelia* species but also distinctions in plasmid profiles between different *Borrelia* species.

43 Characterization of *Borrelia burgdorferi* sensu lato strains by pulsed–field gel electrophoresis and arbitrarily primed PCR

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Between November 1998 and January 2001, we characterized 21 *Borrelia burgdorferi* sensu lato strains, which were isolated from patients with an illness consistent with Lyme borreliosis (LB). All patients lived in or near Belluno, Veneto, a region in north-east Italy where LB is endemic. All patients presented erythema migrans. Isolates were classified using pulsed-field gel electrophoresis (PFGE) and arbitrarily primed-PCR (AP-PCR). Twenty-seven borrelial strains were used as controls in the characterization; fifteen of them were local strains. Genomic DNAs were prepared using a slightly modified version of the procedure described by Taylor *et al*. (1991). Two restriction endonucleases, *Mlu*I and *Sma*I, were used to compare the different strains in PFGE. For arbitrarily primed PCR, fingerprinting was performed with the purified total genomic DNA extracted with Genomic–Tip 20G (Quiagen) using primers 1283, 1254, 1247 and AP13. PFGE allowed us to characterize each of the 21 isolates. Among them, 17 belonged to *Borrelia afzelii*, 3 to *Borrelia garinii*, and 1 to *Borrelia burgdorferi* sensu stricto. PFGE also revealed heterogeneity within the *Borrelia afzelii* and *Borrelia garinii* genomospecies. The AP-PCR confirmed the genotypic classification obtained with PFGE and the heterogeneity within strains belonging to the same genomospecies. However, there was only a partial correlation between PFGE and AP-PCR groupings. In conclusion, our findings show that PFGE and AP-PCR can be used as accurate and reliable methods i) to compare and classify *Borrelia* isolates and ii) for epidemiological studies. Our findings, which confirm our previous results, also contribute to understanding of the distribution of the *B. burgdorferi* sensu lato species in Italy. This is important for the better use of antigens in serology and for defining future vaccination policy.

44 Human toxemia after attachment of *Ixodes redikorzevi* (Acari: Ixodidae) females in Israel

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Ixodes redikorzevi Olenev is widely distributed over a huge territory including many countries of south-eastern Europe, the Caucasus, the Near and Middle East, and Central Asia. Its range is probably even larger because some countries in the Near and Middle East are insufficiently studied in this respect. Ticks of this species in Israel are traditionally identified as *I. r. theodori* (Warburton) although there is an opinion that it is too early to draw any final conclusion about the rank of different populations of the species. Numerous cases of female *I. redikorzevi* attacks on and attachment to humans followed by human toxicosis have been regularly described in Israel. The clinical symptoms include fever with high temperature (up to 39°C), severe pain at the site of tick attachment, vomiting and torticollis since most cases of attachment are in the neck area. The duration of tick attachment before its discovery and removal may continue for several days. The symptoms of human infestation by *I. redikorzevi* should be classified as tick-bite allergy or toxemia. There is no indication of tick attacks towards humans in other parts of the large *I. redikorzevi* range. All stages of this tick have been found on numerous small and middle-sized mammals and some birds, in Israel as well as other parts of the range. All examined ticks removed from people were unusually light or colourless (serum ticks) which is apparently connected with high lymph and tissue fluid content and with other non-erythrocytic materials in their meal. The same phenomenon was observed in Israeli ticks (females and nymphs) collected from mammals while specimens from birds had a normal brownish colour of various intensity. Serum ticks were also observed among *I. redikorzevi* collected from mammals in other parts of the range so this phenomenon seems not to be related to noxious substances secreted by ticks into the host body. The described behavioral and epidemiological patterns characterizing *I. redikorzevi* from Israel give a strong indication of their distinction from ticks of this species from the northern parts of their range.

45 Recent changes in abiotic variables for the development of *Ixodes ricinus* in Europe

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There is great concern about the effects of the forecast climate change on the population dynamics and distribution of some prominent tick species. The trend in recent years is the increase in average temperatures as well as the existence of winters with higher rainfall than normal. Several studies have been conducted into the expected effect of this climate on the long-term populations of the tick *Ixodes ricinus*. Models have been established to predict the habitat suitability of more or less wide territories according to different variables and using several statistical methods. However, no empirical studies about the effects of recent climate trends on abiotic suitability for that tick have been performed.

Here, I use a "climate envelope" model called DOMAIN which was developed to predict the fitness of habitat for some plant species, to evaluate the recent (1983-2001) changes in climate in Europe on a medium scale (8 km) and the impact on the habitat suitability for the populations of *I. ricinus*. This model does not need "absence data" in the training set and is, therefore, very useful for datasets where only "presence data" are available, as is the case here. Moreover, this model is not an indicator of incidence of tick-borne diseases, merely an appraisal of the probabilities of finding permanent tick populations and an indirect indicator of tick abundance. Remotely sensed imagery was used as the source for climate and vegetation parameters, available as composite pictures at 10-day intervals. The trend of abiotic variables has been studied using Fourier analysis of the 10-day series of images.

The general trend in this period has been, as expected, a small but progressive warming of the territory, together with wet winters in northern latitudes. However, this has been a dry period in much of the Mediterranean region, and colder than average in wide areas of Central Europe. The main consequence of these changes and the calculated trend is the sudden year-to-year changes of habitat fittingness for *I. ricinus* in marginal populations like those observed close to the Mediterranean region. In these areas, suitability changes markedly between consecutive years. However, the most prominent variations in the geographical distribution of the tick have been observed in the Scandinavian and Baltic Sea regions, close to the Gulf of Finland and Gulf of Riga areas. Warmer conditions over this wide area are providing ticks with a more suitable environment for development and survival. The overall consequence has been a "northward shifting" in latitude of suitable areas for *I. ricinus* populations.

46 Risk of transmission of *Borrelia afzelii* and unknown field-collected borreliae from *Ixodes ricinus* nymphs to the host after incomplete vector tick feeding

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Ixodes ticks are the main vectors of the causative agents of Lyme borreliosis. From the North American *B. burgdorferi* s.s. it is known that transmission to the host does usually not occur within the first 40–45 h of the blood meal. In contrast, it was found that unspecified European *B. burgdorferi* s.l. can be transmitted to the host by laboratory-reared *I. ricinus* nymphs within the first 24 h of feeding. It seems therefore necessary to determine the borrelia transmission dynamics in different agent-vector-combinations and specifically how common early transmission (i.e., within the first 24 h of feeding) may occur.

In the present study 5 different strains of *B. afzelii* were used to investigate their transmission dynamics in laboratory-reared, in vivo-infected *I. ricinus* nymphs (infection rates: >80 %). The same was done with *I. ricinus* nymphs field-collected in two Berlin forest areas (infection rates: >20 %) where four different genospecies had been detected in unfed *I. ricinus* nymphs (*B. afzelii* and *B. garinii* strongly dominating). The ticks were allowed to attach to tick-naive Mongolian gerbils in batches of up to 9 for 6 h, 12 h, 24 h, 36 h or a full blood meal. Field-tick batches without any *Borrelia*-infected feeding individuals were excluded. Some weeks later the infection status of each gerbil was examined by both Western blot and xenodiagnosis.

All gerbils fed on by ticks until repletion (n=22 gerbils) became infected and also infectious for feeding ticks. Whereas 74 % of the gerbils fed on by laboratory-reared *I. ricinus* nymphs for 36 h (n=34 gerbils) became infected and most of them also infectious for feeding ticks, 16 % of those gerbils fed on for $\leq$ 24 h (n=110 gerbils) became infected. Eighty-seven percent (n=15 gerbils) and 30 % (n=35 gerbils) of those gerbils fed on by batches of field-collected ticks with unknown borrelia infections for 36 and $\leq$ 24 h, respectively, became infected. This is in sharp contrast to the above-mentioned results from North America and may have considerable consequences for the risk of humans in Europe of becoming infected with *B. burgdorferi* s.l. by tick-bite.

47 The Rimov TBE vaccination project

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South Bohemia is a large-size nature focus of Tick-Borne Encephalitis (hereinafter TBE) in the Czech Republic. A certain part of the professional public still stands for the opinion that the people living in the focus of infection gain the natural protection in the course of their lives and are, therefore, protected against the infection and so the vaccination against TBE in these individuals is not necessary. The aim of this study was to determine prevalence of anti-TBE protective antibodies in long-time residents, and to increase public awareness and offer vaccination to the target population.

The study setting was a small town Rimov in the South Bohemian countryside, a hot spot area with history of many diagnosed cases of TBE in the past 35 years.

Patients and methods: All 531 inhabitants of the town were sent a study questionaire, with 70 % return rate, 280 residents (135 males, 48 %) were included in the study. All have agreed with blood sample collection and vaccination and signed informed consent form. The history of participants was documented by a questionaire, TBE clinical disease by a GP report, and the vaccination by the regional vaccination centre or GP report. Analyses were performed using the EPIDAT software. Immunozym FSME IgG Progen Immuno assay was used for serology testing (<63 VIE U/ml =negative, 63-126 = borderline and > 126 = positive).

Results: History of clinical TBE was reported in 17 people (6,07 % - 6 males, 11 females), 100 % of which had protective IgG levels (15 of them >600 IU/ml). Asymptomatic seroconversion was proven in 27 residents (9,64 %: 13 males, 14 females),100 % had protective IgG levels. The ratio of manifest cases of the disease to the inapparent ones is approximately 2 : 3. Total serological prevalence after experienced disease (both manifest and inapparent) is 16 %.

Forty three people – 15 % (17 males, 26 females) had been vaccinated, all tested with positive IgG levels. People vaccinated from 1989 to 1996 had not been revaccinated and their IgG levels remained protective (130 to >600).

Unprotected people were offered vaccination. Fifty two residents (mean age ± SD: 37.6 ± 20.7) declined vaccination even after beeing informed on their negative serology and susceptibility to the disease. They had been residing in the hot spot area for mean ± SD (range) 18.7 ± 17.4 (1 - 66) yrs. Some middle aged parents declined vaccination even for their children.

Conclusions : Majority of unvaccinated residents in this traditional hot spot area are susceptible to TBE. Surprisingly low vaccination rate in the given locality was increased by the authors' intervention from 15 % to 65 %. General vaccination programme is the only way how to prevent TBE in hot spot areas.