Bundesinstitut
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VIth International Potsdam Symposium on Tick-borne Diseases (IPS-VI)

Scientific Programme

Thursday, April, 26th, 2001

10.30	**Registration**
12.30 - 12.45	**Opening**
	SPECIAL ISSUES ON TBE Moderators: P. Kimmig, Ch. Kunz
12.45 - 13.00	*Ch. Kunz* (Vienna, Austria): Vaccination against TBE in Austria: The success story continues
13.00 - 13.15	*R. Kaiser* (Pforzheim, Germany): TBE in Germany and the clinical course of the disease
13.15 - 13.30	*K. Kristiansen* (Bornholm, Denmark): TBE in Denmark – in particular on Bornholm
13.30 - 13.40	**Discussion**
13.40 - 14.20	**Poster Session and Coffee Break**
	CHARACTERIZATION OF NATURAL TBD FOCI: DIFFERENT APPROACHES Moderators: J. Gray, S. Randolph
14.20 - 14.40	*S. Randolph* (Oxford, U.K.): Predictions of risk of tick-borne diseases
14.40 - 15.00	*U. Kitron* and *M. Guerra* (Urbana, Atlanta, USA): Characterization of foci of TBD
15.00 - 15.10	**Discussion**
15.10 - 15.25	*L. Gern, J.-L. Perret, F. Gremion, E. Guigoz, O. Rais, and Y. Moosmann* (Neuchâtel, Switzerland): A 5 year survey on the prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* ticks, on tick density and clinical cases of Lyme Borreliosis in an endemic area in Switzerland
15.25 - 15.40	*R. Oehme, K. Hartelt, S. Moll, H. Backe*, and *P. Kimmig* (Stuttgart, Germany): Foci of TBD in Southwest Germany
15.40 - 15.55	*T. Krech* (Kreuzlingen, Switzerland): TBE foci in Switzerland

15.55 - 16.10	*J. Süss, Ch. Schrader, U. Abel, A. Bormane, A. Duks,* and *V. Kalnina* (Berlin, Heidelberg, Germany; Riga, Latvia): Characterization of TBE foci in Germany (and Latvia)
16.10 - 16.25	*M. Labuda, E. Elecková, M. Licková,* and *A. Sabó* (Bratislava, Trnava, Slovakia): TBE virus foci in Slovakia
16.25 - 16.40	*X. Han, M. Aho, S. Vene, M. Peltomaa, A. Juseviciene, P. Leinikki, A. Vaheri,* and *O. Vapalahti,* (Helsinki, Finland; Stockholm, Sweden; Kaunas, Lithuania): TBE virus foci in Finland
16.40 - 16.55	*M. Haglund and the ISW-TBE Meningitis Study Group* (Kalmar, Sweden)*:* Occurence of TBE in areas previously considered non-endemic: preliminary results from an ISW-TBE study
16.55 - 17.10	**Discussion**
17.10 - 17.30	**Coffee Break**
	TICK-BORNE DISEASES IN ANIMALS Moderators: G. Stanek, J. G. Thalhammer
17.30 - 17.45	*M. W. Leschnik, G. C. Kirtz,* and *J. G. Thalhammer* (Vienna, Austria) TBE in dogs
17.45 - 18.00	*P.-F. Humair* (Neuchâtel, Switzerland): Birds and *Borrelia*
18.00 - 18.10	*A. Kaiser, A. Seitz*, and *O. Strub* (Mainz, Germany) Lyme borreliosis in passerine birds
18.10 - 18.25	*G. Stanek* (Vienna, Austria): Horses and *Borrelia*
18.25 - 18.40	*K. Stöbel, A. Schönberg,* and *C. Staak* (Berlin, Germany): A new non-species dependent ELISA for the serological diagnosis of Lyme borreliosis in zoo animals
18.40 - 18.50	**Discussion**
18.50 - 19.05	*E. Olsson-Engvall,* and *A. Egenvall* (Uppsala, Sweden): Granulocytic ehrlichiosis in dogs and horses
19.05 - 19.20	*J. S. Liz* (Neuchâtel, Switzerland): Ehrlichiosis in *Ixodes ricinus* and wild mammals
	BABESIOSIS
19.20 - 19.35	*J. Gray, L.-V. v. Stedingk*, and *M. Granström* (Dublin, Ireland; Stockholm, Sweden): Zoonotic Babesiosis
19.35 - 20.00	**Discussion and Poster Session**
20.00	**Symposium dinner** in the canteen of the BgVV
22.00	**Bus transfer to the SORAT Hotel**

Friday, April 27th, 2001

Lyme borreliosis I

CLINICAL MEDICINE, DIAGNOSTICS AND THERAPY
Moderators: R. Kaiser, B. Wilske

9.00 **Some remarks**

9.05 - 9.20 *B. Wilske* (Munich, Germany):
Microbial diagnosis in Lyme borreliosis

9.20 - 9.35 *R. Lange* (Berlin, Germany):
Diagnostic strategy for the detection of Lyme borreliosis

9.35 - 9.55 *K.-P. Hunfeld, P. Kraiczy,* and *V. Brade* (Frankfurt/M., Germany):
In vitro Susceptibility of *Borrelia burgdorferi* against recently developed antimicrobial agents – Implications for new therapeutic approaches in Lyme disease?

9.55 – 10.10 *R. Kaiser,* and *C. Saadé* (Pforzheim, Germany):
Treatment of Lyme borreliosis – State of the art

10.10 – 10.30 **Discussion**

10.30 – 11.15 **Poster Session and Coffee Break**

Lyme borreliosis II

IMMUNOLOGY/VACCINATION
Moderators: M. Simon, D. T. Dennis

11.15 - 11.45 **Keynote lecture:**

D. T. Dennis (Fort Collins, USA):
Lyme Disease Vaccine: The United States' Experience

11.45 – 12.10 *V. Brade, C. Skerka, M. Kirschfink, P.F. Zipfel,* and *P. Kraiczy* (Frankfurt/M., Jena, Heidelberg, Germany):
Immune evasion of *Borrelia burgdorferi* - Failure of sufficient killing of the pathogens by complement and antibody

12.10 - 12.35 *M.M. Simon,* and *R. Wallich* (Freiburg, Heidelberg, Germany):
Vaccination against L.b. in Europe: a special problem for man and dogs?

12.35 – 12.55 *R. Wallich,* and *M.M. Simon* (Heidelberg, Freiburg, Germany):
New strategies to treat established *B.b*. infections

Lyme borreliosis III

ECOLOGY

12.55 - 13.10 *K. Kurtenbach, S. De Michelis, H.-S. Sewell, S. Etti, S. M. Schäfer, E. Holmes, R. Hails, M. Collares-Pereira, M. Santos-Reis, K. Hanincová, M. Labuda, A. Bormane,* and *M. Donaghy* (Oxford, U.K.; Lisbon, Portugal; Bratislava, Slovakia; Riga, Latvia):
The key role of selection and migration in the ecology of Lyme borreliosis

FREE TOPICS
Moderator: V. Brade

13.10 – 13.25 *I. Uspensky* and *I.I. Uspensky* (Jerusalem, Israel):
Artificially created colonies and abundant populations of the brown dog tick, *Rhipicephalus sanguineus*, in and near human dwellings

13.25 – 13.45 *M. Modlmaier, R. Kuhn, O.-R. Kaaden,* and *M. Pfeffer* (Munich, Mainz, Germany):
Transmission studies of an European Sindbis virus in the floodwater mosquito *Aedes vexans*

13.45 – 14.00 **Discussion**

14.00 **Closing remarks**

16.00 **Workshop ISW-TBE**
invited participants only

Vaccination against TBE in Austria: The Success Story Continues

Ch. Kunz
Institute of Virology, University of Vienna, Austria

In the pre-vaccination era Austria had the highest incidence of TBE of all European countries. In some years up to almost 700 hospitalized cases were recorded, although our diagnostic methods were not as potent as they are today and sampling was far from complete. Thus, there was considerable underreporting and in a number of years patients with overt TBE must have exceeded the 1000 cases mark.

After the Austrian vaccine became commercially available in 1976 vaccination attempts were mainly directed at containing the disease in high-risk groups such as forest workers and farmers. However, although TBE virtually disappeared in these professional groups, morbidity rates remained at about the same level in the general population. The turn only came after the initiation of a mass vaccination campaign starting in 1981.

This measure gradually increased the vaccination coverage from 6 % in 1980 to more than 80 % by the end of 2000 exceeding 90 % in some of the high risk areas, for example Carinthia. After the year 1988 less than 200 cases and after 1997 less than 100 cases were seen annually. In 1999 and 2000 with 41 and 60 cases, respectively, the lowest incidence ever was recorded in Austria. In Carinthia, where the average annual incidence of TBE previously amounted to 140 cases and vaccination coverage now is about 95 %, only 2 patients were hospitalized because of TBE in 2000. Vaccination of children proved to be extremely effective in controlling the disease. Whereas in the pre-vaccination era almost 25 % of the patients were 0-14 years of age, in the last 4 years only 4.5 % of the cases were in that age group.

The dramatic decline of TBE is not shared by other European countries, including Austria's neighbours. This is particularly striking when the incidence of TBE in Austria and the Czech Republic is compared. In the latter 490 cases of TBE were reported in 1999 and 709 in 2000. In that country even in high-risk areas such as Southern Bohemia (population about 1 million, 234 cases in the year 2000) less than 10 % of the population has undergone vaccination, a situation as it existed in Austria at the beginning of the 80ies. Our experience indicates that this low vaccination coverage does not suffice to significantly lower the incidence of TBE.

TBE in Germany and the clinical course of the disease

R. Kaiser
Neurologische Klinik, Städt. Klinikum Pforzheim

Tick-borne encephalitis (TBE), which is caused by a homonymous virus of the enveloped virus family *Flaviviridae*, occurs in nearly all countries of Central Europe. In Germany, TBE only occurs in certain regions of Bavaria, Baden-Württemberg, and South Hesse, where it is endemic. During the last ten years in these „Lands", approx. 1400 patients fell ill from infection with the TBE-virus. Detailed epidemiological and clinical data were available from 850 patients. Sixty seven percent of the patients recalled a previous tick-bite. More than two thirds of infections occurred between June and August. Most of the patients were infected during leisure time. Men fell ill twice as frequently as women. A diphase course of disease was seen in 74% of patients.

TBE mostly manifested as meningitis (47%) or meningoencephalitis (42%) and less frequently as encephalomyelitis (11%). Patients with meningitis mostly complained of severe headaches, fever and vomiting. Patients were treated in hospital on average for 10 days. After discharge from hospital most patients suffered from a reduced ability to endure stress for a further 4 to 6 weeks. Patients with meningoencephalitis were affected more severely. Most of them had a pronounced disturbance of consciousness, with somnolence and coma occurring more frequently than delirium. The most conspicuous feature of TBE was ataxia, occurring in about one third of patients with meningoencephalitis. On average, patients with meningoencephalitis were in hospital for 15 days. At check-ups 6 months to 5 years after the acute phase of illness most patients reported a reduced ability to endure stress for one to four months after discharge from hospital. Individual patients still revealed a slight impairment of co-ordination when examined neurologically up to five years after the acute phase of the illness. The most serious complication of TBE is myelitis, which always occurs in association with brain-stem encephalitis. Patients were impaired by mono-, para-, or tetrapareses. On average, patients with myelitis were treated in hospital for 4 weeks. At their check-ups most of these patients showed no recovery from paresis even 3 years after onset of disease. Of all 850 patients, 7% needed intensive care and assisted ventilation. Specialist rehabilitation for several weeks to months was necessary in 18%. Long term prognosis was favourable in 35% of all patients. Temporary deficits (hypacusis, ataxia, paresis) were seen in 38% and persistent impairments in 27% of patients. Mortality was about 1%.

Diagnosis of TBE is established by a recent stay in an endemic area, a tick bite - if recalled -, a prodromal stage with influenza like symptoms, the clinical symptoms of meningitis and - optional – encephalitis or myelitis, and the demonstration of TBE-specific IgM- and IgG-antibodies in serum. Analysis of the cerebrospinal fluid (CSF) indicates inflammation of the nervous systems (pleocytosis, damage of the blood-CSF-barrier, intrathecal synthesis of immunoglobulins). As there is no specified treatment active immunisation is recommended for all persons who live in areas where TBE is endemic and who expose themselves to ticks.

TBE in Denmark - in particular on Bornholm

K. Kristiansen

Medical public health officer, Bornholm

TBE in Denmark – in particular on Bornholm

In Denmark tick-borne encephalitis viruses are found only on the island of Bornholm, located about 100 kilometres east of the rest of Denmark. Until 1999 no cases of TBE were diagnosed on Bornholm for a period of forty years. In the rest of Denmark one single case was reported in 1984, and it was related to travel activity. TBE simply was not part of the customary diagnostic considerations.

Rediscovery of TBE

In 1998 a forty-one year old man from Bornholm was to hospitalized with a diagnosis of meningo-encephalitis. The cause was not found. A year later this person was still suffering from fatigue and failings of memory. His general practitioner, who had incidentally learned about TBE during a course, recognized that these symptoms might be after-effects of TBE. The GP examined his patient for antibody against TBE-viruses. The finding was fully consistent with an undergone TBE-infection the previous year. The man had not been travelling. With this finding TBE was 'rediscovered' on Bornholm.

Previous studies of TBE on Bornholm

It is not surprising that TBE was not found on Bornholm for many years. One only finds what one is looking for, and for some reason or other no examinations for TBE were carried out. But it does seem strange that no such examination was carried out since it was made evident forty years ago that TBE was prevalent on Bornholm. In the late 1950'ies one of my predecessors, medical public health officer Svend Kofoed, had a special interest in tick-borne meningo-encephalitis and he prompted the Statens Seruminstitut to investigate further into this. As a result consultant Eyvind Freundt from 1958 to 1962 studied a large number of animal and human sera for antibodies against TBE-viruses(1) Freundt also attempted to isolate virus but didn't succeed.

Through examination of case records from the County Hospital of Bornholm 10 years ago, Freundt found 79 patients admitted with a diagnosis of acute meningo-encephalitis. Among the 79 he selected 12 for a retrospective serological study. Blood samples showed that antibodies were present in 8 of these persons. 6 of them were forest workers or farmers. On the basis of seasonal occurrence, clinical course and spinal fluid findings Freundt concluded that the illness of a maximum number of 39 patients out of the 79 might have been caused by TBE virus, accordingly a maximum of 39 cases during a period of ten years. The population of Bornholm is approximately 45,000 persons.

Furthermore Freundt examined 545 cases of meningo-encephalitis from the rest of Denmark, and not one of these sera contained TBE antibody. Blood samples were collected from 40 forest workers from Bornholm, and 12 of these, or 30 per cent, contained TBE antibody. Three of the forest workers had symptoms of meningo-encephalitis and were included in the cases from the County Hospital. Among 508 adult residents of Bornholm antibodies were found in 1,4 per cent of all, 2 per cent among the men and 1 per cent among the women. 305 Danish blood donors who had never been to Bornholm, were all found completely sero-negative.

Sera from deer were also examined by Freundt. Among 29 samples antibodies were found in 83 per cent. In comparison antibodies were not found in any of the samples collected from 257 deer from the rest of Denmark. This was the picture in the late fifties after which TBE was more or less forgotten in Denmark until 1999.

How is the situation today?

In 1998 two cases were discovered on Bornholm. In 1999 three cases occurred and again in 2000 another three cases. In Denmark there is no obligation to notify the central authorities of cases of TBE. As, however, all samples are supposedly sent to the same place, the Statens Seruminstitut, we have reason to believe our information is complete.

Present set of problems and studies

Since 1976 a vaccine against TBE has been commercially available. With TBE rediscovered on Bornholm it has become of interest to clarify how a Danish recommendation should be formulated concerning a possible vaccination programme. For this purpose a series of studies have been started:

Meningo-encephalitis with unknown cause 1994-1999

The Statens Seruminstitut keeps samples of spinal fluid for 5 years. This made it possible for senior registrar Kirsten Lauersen from the County Hospital of Bornholm to examine samples of spinal fluid from patients who over the past five years have been admitted with a diagnosis of meningo-encephalitis with unknown cause. The survey is still unpublished, but the preliminary results indicate that there has been one or two cases of TBE every year among the residents of Bornholm.

Examination of forest workers on Bornholm

The forest workers have all been aware of the existence of TBE on Bornholm. Consultant Freundt's survey hadn't been forgotten by everybody. I don't know the background, but at a meeting in the local works council the forest workers had expressed a wish to have their blood examined for TBE and *Borrelia*. Accordingly the forest supervisor turned to me in 1999 to have an examination carried out.

It turned out that 5 out of 32 workers had antibodies against TBE-virus, that is 16 per cent. The average age of the workers with antibody was 52 years, for the rest the average age was 48 years. Consultant Freundt mentions only the age of those with antibody where the average age was 48 years.

Incidentally 3 of the 5 workers with antibodies against TBE-virus also had antibodies against *Borrelia*, that is 60 per cent where only 33 per cent of those without antibody against TBE-virus had Borrelia-antibody. According to the workers who were TBE-positive they had not been seriously ill and none had complications. The State Forest Department afterwards gave all workers an offer of free vaccination. Until now 7 of the 27 who didn't have antibody against TBE, have been vaccinated.

Examination of blood donors resident on Bornholm – prevalence

The study is in the process of being approved by the Scientific-ethical Committee. Blood samples from 750 donors will be tested to find antibodies against TBE-virus. The donors will also be asked to fill in a questionnaire about exposure to tick-borne diseases at work and at leisure. The results will probably be available in the autumn of 2001.

Study of ticks – number and incidence of virus

In June 2000 Per Mostrup Jensen and his staff from the Royal Danish Veterinary and Agricultural University were on Bornholm. They collected about 4,000 ticks, which were subsequently examined for TBE-virus by PCR-technique. The study has not yet been published, but TBE-virus was found in about two per cent of the ticks.

Study of deer – Bornholm and the rest of Denmark.

If funds can be raised, it is the intention to repeat the study undertaken by Freundt of deer all over the country. Obviously this study is of more interest in other places than Bornholm.

Interim conclusion:

TBE cannot be characterized as a serious general problem in Denmark, but a vaccination programme for risk groups on Bornholm might be considered.

Literature

Freundt EA. Endemisk forekomst på Bornholm af centraleuropæisk virus meningo-encephalitis, overført af skovflåter. Ugeskr Læger 1963; 125: 1098-04.

Predictions of Risk of Tick-borne Diseases

S. E. Randolph
Department of Zoology, University of Oxford.

To make predictions about the distribution and abundance of vector-borne diseases, our group in Oxford uses an integration of two complementary approaches:

- The statistical approach is based on identifying correlations between the spatial patterns of environmental conditions and patterns of vector and disease distribution.
- The biological approach develops models of the processes underlying the observed patterns, whose rates are usually determined by the key environmental factors identified through the statistical approach.

The essential part of any prediction is the test of its validity. Predictions about the present may be tested against existing observations, although these observations, which often form the basis for the predictions in the first place, may not be entirely accurate. Using multi-temporal satellite imagery, we have predicted the present distribution of tick-borne encephalitis (TBE) foci. It corresponds with the recorded foci with 85% accuracy. The remaining false negatives (false predictions of absence) and false positives (false predictions of presence) are particularly interesting as they focus our attention on new questions. What more do we need to understand to improve the accuracy? Are the predictions warning us of inaccurate records to date, or the potential spread of disease into new areas?

Predictions about the future cannot be tested so simply. A population model for *Ixodes ricinus*, near to completion, will allow us to predict the impact of climate change on geographically variable patterns of tick seasonal dynamics. This has recently been identified as a key determinant of the presence of endemic cycles of TBE virus. Such a biological model will improve on the statistical methods, currently our only option, that gave the following results. Using present and future climate scenarios instead of satellite imagery, we have captured the present distribution of TBE and predicted that the range may shift northwards and contract under the forecast conditions of climate change. The "present" is based on average environmental conditions from 1982-1993 (from satellites) or 1960-1990 (climate models). The annual incidence of TBE in central Europe and the Baltic States has changed significantly since then; the trends correspond with our predictions for the future along the southern and northern edges of the range, but not within the core of the range. This alerts us to non-climatic causes, such as political and sociological changes that may have resulted in increased rates of human infection with TBE virus.

Characterization of Foci of Tick-borne Diseases

U. Kitron[1], and M. Guerra[2]

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Tick-borne disease (TBD) transmission foci need to be characterized in space and time, and are often discontinuous on both scales. An active TBD focus is dependent on the fulfillment of three conditions: tick survival, pathogen survival and opportunities for human exposure. Given vast differences between soft-bodied and hard-bodied ticks and between one, two and three host ticks, this talk will focus on the *Ixodes ricinus* complex. The essentials for tick survival include food sources (hosts for immature and adult stages), adequate population densities for reproduction (maintaining $Ro \geq 1$), protection from environmental extremes for eggs, questing larvae, nymphs & adults, and successful diapause of larvae and adults. The pathogen survival kit includes sufficient densities of ticks and suitable reservoir hosts, and opportunities for transmission between them in order to maintain infection.

Opportunities for human exposure depend on sufficient number of encounters between ticks and humans. Tick survival depends on both abiotic (soil, topography) and biotic (vegetation, vertebrate hosts) factors. Pathogen survival depends on a combined high enough rate of horizontal transmission and vertical transmission (transovarial, trans-stadial) to make up for loss due to transmission to humans and other dead-end hosts. Opportunities for human exposure depend on human demographics, socio-economic factors and patterns of leisure and occupational activities. Diversity and population dynamics of reservoir hosts and their suitability as hosts for ticks and pathogens play a role in determining the degree to which all three conditions are met. Because tick foci need to be characterized on a range of spatial and temporal resolutions, data for such characterization include a variety of surveillance data, field and laboratory experimental data, and results of statistical and mathematical analysis and modeling. The application of new tools from molecular biology, geographic information system and satellite imagery and of appropriate analytical tools allow for detection of unknown foci and prediction of new ones. A long-term multi-scale study of *Ixodes scapularis* and Lyme disease in the north-central U.S. is presented. Diverse surveillance methods of ticks, rodents, deer, canines and humans were coupled with environmental characterization *in situ* to create a habitat profile for Lyme disease ticks. Incorporating various digitized databases, a statistical model was used to develop a risk map for tick distribution in the region. The resulting model is tested against the known tick distribution and in predictions of invasion and establishment of new foci.

A 5 Year Survey on the Prevalence of *Borrelia burgdorferi* sensu lato in *Ixodes ricinus* Ticks, on Tick Density and Clinical Cases of Lyme Borreliosis in an Endemic Area in Switzerland

L. Gern, J.-L. Perret, F. Gremion, E. Guigoz, O. Rais, and Y. Moosmann
Institute of Zoology, University of Neuchâtel, Neuchâtel, Switzerland

Informations concerning tick density and the prevalence of the different *Borrelia burgdorferi* sensu lato (sl) species in tick populations are very important for the understanding of Lyme Borreliosis (LB) epidemiology and prevention. The goals of this study were to relate *I. ricinus* tick population and its infection with *B. burgdorferi* sl to the incidence of LB in the human population of Neuchâtel (Switzerland) over a period of 5 years. Neuchâtel was choosen because of its geographical situation - constrained between a lake and a wooded hillside which is known as an habitat for *B. burgdorferi* sl infected *I. ricinus* ticks and because of the frequent contacts of the local population with the wooded hillside.

A total of 15,532 ticks were collected by flagging the low vegetation from March 1996 to December 2000. Ticks could be collected from vegetation every month, even in winter. Tick questing density greatly varied during and between years with a maximum questing tick density in spring. The higher questing tick density was observed in spring 1997 with 207 nymphs and 34 adults per 100 m^2.

A total of 3,237 ticks (2,086 nymphs and 1,151 adults) were examined for *Borrelia* infection using IF (including estimation of spirochaete number/tick) and by *Borrelia* isolation. Among years the prevalences of *Borrelia* using IF in nymphs and adults were significantly different. Usually adults presented a higher prevalence of infection that nymphs except in 1999. Estimation of the number of spirochaetes/tick showed that the annual prevalence of highly infected adults and nymphs varied among years. No correlation could be observed between the prevalence of infection in ticks and the proportion of highly infected ticks. *B. burgdorferi* ss, *B. afzelii*, *B. garinii*, *B. valaisiana* and *B. lusitaniae* were isolated from ticks collected in Neuchâtel.

LB incidence in the local human population was estimated from data collected from physicians who sent patient serum to our laboratory for a LB serology. Physicians were retrospectively asked to fill up a questionnaire for each patient presenting a positive serological result in order to confirm the clinical diagnosis of LB. During 1996-1999, 188 patients were retrospectively diagnosed as having LB. ECM was the most frequent clinical manifestation observed, followed by neurological problems, and the highest incidence was observed in 1997 with 147 cases/100,000 inhabitants.

Foci of Tick-borne Diseases in Southwest Germany

R. Oehme, K. Hartelt, S. Moll, H. Backe, and P. Kimmig
State Health Office Baden-Württemberg, Germany

Of the tick-borne infections found in south-western Germany, Lyme disease and tick-borne encephalitis (TBE) are the most significant while ehrlichiosis, Q-fever and babesiosis are of secondary importance. Blood samples taken from ca. 4,000 forestry workers from throughout Baden-Württemberg were tested for antibodies to TBE virus, *Borrelia burgdorferi* sensu lato and *Ehrlichia* spp. (genogroup *E. phagocytophila*). Prevalence rates of antibodies to TBE virus ranged from 0 % to 20 %. The highest rates were found in the southwest of Baden-Württemberg, an area in which the most clinical cases have been recorded. However rates ranging from 10 % to 20 % were also found in other areas which are not recognised as risk areas due to the low number of clinical cases (Ludwigsburg, Schwäbisch Hall). Our results show that TBE occurs throughout Baden-Württemberg.

The prevalence of *Borrelia burgdorferi* sensu lato antibodies in Baden-Württemberg ranged from 10 % to 40 % with the main foci only partially overlapping those of TBE. Prevalence rates of *Ehrlichia* antibodies ranged from 5 % to 15 % extending in a band from the southwest to a main focus in the northeast of the state. Investigations into the prevalence of infection of ticks with TBE virus, *Borrelia burgdorferi* sensu lato and *Ehrlichia* spp. (genogroup *E. phagocytophila*) were carried out using PCR. The main foci of TBE infections in ticks were found to lie in the southwest of Baden-Württemberg, with rates of 0.3 % (Kirchzartener Becken), 1.4 % (Kinzigtal), 2.0 % (Simonswäldertal), 2.2 % (Elztal), Bodman [Lake Constance] (1.2 %) and in Sipplingen [Lake Constance] (2.3 %). Lower values were found in the Stuttgart area: Stuttgart Botnang (0.5 %), Großbottwar (0.8 %), Bietigheim (0.3 %) and Pforzheim (0 - 0.5 %). Investigations of ticks for the presence of *Borrelia burgdorferi* sensu lato were carried out mainly in the Stuttgart area. Average infection rates for nymphs and adults were about 15 % with adults ranging to 40 % and nymphs between 5 % and 12 %. Maximum infection rates for larvae were 1 %.

DNA from the intergenic spacer region between 5S and 23S rRNA genes was amplified by PCR and was then hybridized with DIG-labeled oligonucleotide probes, which were specific for *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and group VS116. *B*. *burgdorferi* sensu stricto was found in 109 (9.9 %), *B. garinii* in 242 (21.9 %), *B. afzelii* in 407 (36.8 %) and group VS116 in 152 (13.7 %) cases. Double infections were found in 71 (6.4 %) and the presence of three species were found in 9 (0.8 %) ticks. 116 (10.5 %) isolates could not be classified.

Investigations of infection rates of ticks with *Ehrlichia* spp. (genogroup *E. phagocytophila*) were carried out in the Rems-Murr area (Berglen), on the plain of the upper Rhine (Lahr) and in the Lake Constance area (Sipplingen). The infection rates were 3.1 %, 2.7 % and. 2.6 % respectively. In about 1% of ticks there was a double infection with *Borrelia burgdorferi* sensu lato. The significance of Ehrlichia infections in Europe remains unknown due to the lack of documented clinical cases.

TBE Foci in Switzerland

T. Krech
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Objective

To give information about the situation in Switzerland.

Methods

Tick-borne encephalitis (TbE) in Switzerland has to be reported to official organizations, such as the state health departments and the federal health department. The registered cases are published weekly in the bulletin of the "Bundesamt für Gesundheit (BAG)". A map with geographical places of infection is published by the BAG regularly. Furthermore we collect epidemiological data in our diagnostic setting servicing part of eastern Switzerland. Some data derive from an epidemiological study conducted in 1995 by P. Baumberger, T. Krech and B. Frauchiger (Schweiz. Med. Wochenschr. 1996;126: 2072-2077) and from another study conducted in the principality of Liechtenstein (T. Krech, C. Aberham, G. Risch, Ch. Kunz: 1992; 122: 1242-1244). The data of a more recent publication are also included (M. Schwanda et al. Schweiz. Med. Wochenschr. 2000;130: 1447-1455).

Results

TBE is endemic in areas of northern Switzerland and the principality of Liechtenstein. The number of yearly reported new infections in Switzerland ranges between 60 and 120 cases approximately, most of them with neurological manifestations. This is an incidence of about 0.5 - 1.5 per 100,000 inhabitants per year. The number is probably an underestimation, since unrecognized moderate infections under the clinical picture of flu-like symptoms might be more common than disease with neurological manifestations.

In the last 10 years a prominent increase of the number of reported cases occurred in Switzerland from 30 to 70 cases per year to 60 to 120 cases per year. This increase was mainly caused by the emergence of TBE in the cantons of Thurgau, Aargau and St. Gallen, all localised in the north – eastern part of Switzerland. In the canton Thurgau with 223.000 inhabitants we observed between 1990 and 2000 a sharp increase from one to 26 cases per year of registered TbE-cases. This is an incidence of up to 12 cases per 100,000 inhabitants per year. Thus, the canton Thurgau became the most dangerous region in Switzerland for TBE. Our earlier observations revealed only 3 cases in the canton Thurgau between 1970 und 1980, not a single case was reported between 1980 and 1990. In 1996 we diagnosed 6, in 1997 15, in 1998 10, in 1999 26, and in 2000 12 cases, almost all of them requiring hospitalisation. In 1998, 2 cases required intensive care, in 1996 we had one patient who was ventilated for neurological reasons for more than 3 months, in 1999 we had one death caused by TBE virus.

Beside of the long known endemic areas of the regions of Schaffhausen, Thun and Biel-Ins, several more focuses have been detected during the last 20 years. These are situated for instance around the lake of Zürich or in the region of Chur. In the canton Thurgau, new cases could be located east of the presently known foci. This could be one explanation for the higher incidence during the last years. However, these new microfoci could also be the result of an increased awareness by the medical staff, since it often turns out that the foci have been known in the local population for longer times. Also the fact, that gardens and parks are turned into wild biotopes with bushes, long grass and humid biotopes could add to the spread and emergence of new foci. These new microfoci could add to the incidence of cases, because vaccination is generally not offered to the population in these areas.

The vaccination policy has now been adapted in the canton Thurgau to the new situation by recommending the vaccine to everybody who goes out to the nature regularly.

Conclusions

Beside of the known larger epidemic areas there are several TBE - microfoci in Switzerland and there are still more and more microfoci recognized. Despite active vaccination for persons at risk the incidence of reported TBE-cases has increased during the last 20 years, now being between 60 and 120 cases per year, some of them being very severe. Still no TBE foci have been recognized in western und southern Switzerland so far.

Characterization of TBE foci in Germany (and Latvia)

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Knowledge concerning the prevalence of the tick-borne encephalitis virus (TBEV) in wild living tick populations is very important for the understanding of the epidemiology of the disease and for the immuno prophylaxis strategy.

In Germany high and low risk areas of TBE exist. In the years 1997-2000 543 autochthonous clinical TBE cases were recorded, of which 140 and 373 were reported in the high risk areas of Bavaria and Baden-Wuerttemberg, and 22 and 8 in the low risk areas in Hesse (Odenwald) and Rhinland-Palatinate, respectively. In correspondence to these case reports we have measured the virus prevalence in free living ticks in these four risk areas and compared these findings with the situation in high risk areas in Latvia. In Latvia in the years 1997-2000 2797 clinical TBE cases were recorded. For the studies in Germany, a total of 18.408 *I. ricinus* ticks (15.680 nymphs and 2.728 adults) were collected by flagging and examined for TBEV, in Latvia 525 *I. ricinus* ticks (350 adults and 175 nymphs) and 281 *I. persulcatus* ticks (adults only).

Information on annual and seasonal differences of the TBEV prevalence in natural TBE foci is not available in Germany. According to our published model (Zentralbl. Bakteriol. 289 (1999) 564-578), we have continued the study to measure the virus prevalence. Starting in 1997, we have performed annual measurements (in every May and September) of the virus prevalence in ticks in high risk areas of Bavaria (8 foci) and Baden-Wuerttemberg (5 foci). A total of 16.410 ticks (13.920 nymphs and 2.490 adults) were examined for TBEV.

The ticks were tested for the presence of TBEV-RNA using a sensitive, nested-RT-PCR. The virus prevalence in the Bavarian foci of the whole tick population ranged from 0 to 2.0 % during these four years. For the adults prevalence rates between 0 and 5.3 % and for the nymphs rates between 0 and 1.4 % were found..

In the high risk areas of Baden-Wuerttemberg, in the Black forest, the virus prevalence of the whole tick population ranged from 0.2 to 3.4%. For the adults prevalence rates were between 0 and 4.8 % and for the nymphs between 0.2 and 3.4%. Using the same model, we have also tested the low risk areas in the Odenwald (840 nymphs, 160 adults) and in Rhinland-Palatinate (920 nymphs, 78 adults). Ticks were collected in those areas where most TBE cases were registered. The virus prevalence in the Odenwald were 0 % in adults and 0.5 % in nymphs, whereas in ticks from Rhinland-Palatinate we have not found any positive PCR signal.

In the high risk TBEV area there was a slight correlation between the number of cases and the virus prevalence in ticks. Sequence data of the PCR products have shown that all strains in Germany were closely related to the central European virus prototype Neudoerfl. In *I.ricinus* ticks collected in Riga county, the following virus prevalence was found: in females 2.4 %, in males 3.7 %, and in all adults 3.0 %, in nymphs 2.4 % and in the total *I.ricinus* tick population 2.8 %.

The virus prevalence in *I. persulcatus*, collected in the eastern parts of Latvia was 6 % in females, 4 % in males and 5 % in all adults. All the PCR products were sequenced and a phylogenetic tree was constructed. Studies in natural foci of TBE in Latvia have shown that in *I. ricinus* the central European virus subtype exists too whereas in *I. persulcatus* both strains have been found. These strains are related to the Siberian cluster with its prototype Vasilchenko, as well as to the central European virus subtype with its prototype Neudoerfl.

Tick-borne Encephalitis Virus Voci in Slovakia

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Tick-borne encephalitis (TBE) virus occurs in endemic foci over a huge territory of Europe including Slovakia. TBE virus as a typical arbovirus relies on two types of hosts for its survival: ticks act as both virus vectors and reservoir hosts, and vertebrates amplify the virus infection by acting as a source of infection for feeding ticks. Longitudinal monitoring of TBE virus in ticks and vertebrate hosts including humans over a period of 40 years resulted in the identification of certain areas of Slovakia where TBE virus is endemic. These are concentrated to the western, southern, and eastern parts of the country. There is no evidence that the size and location of the natural TBE foci have changed significantly during the last decades.

The environmental conditions in a focus are given by the fact that the non-parasitic phases of the life cycle of *Ixodes ricinus*, the principal tick vector of TBE virus in Europe, do not survive in areas where the relative humidity of the microclimate falls below 80% for prolonged periods, or in areas prone to flooding. Distribution of TBE foci in Slovakia has been associated with the annual 8°C isotherm, with a mean annual rainfall of 800 mm and with plant communities of thermophilic growth within mixed oak and black-locust forests encountered in the subcarpathian subprovince of the West Carpathian province or in the uninundated areas of the Danube River basin in the Pannonian zoogeographic province.

Tick bite is, in general, the main route of acquiring TBE virus infection. Less frequent is the alimentary way by drinking the row goat and sheep milk. In Slovakia, the largest outbreak of TBE after drinking inadequatly temperature treated goat milk was registered in Rožňava (southern Slovakia) in 1951, followed subsequently by sporadic cases and local family outbreaks. Numbers of diagnosed hospitalised cases of TBE in Slovakia vary from less than 20 to almost 100 cases annually.

TBE virus foci in Finland

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In Finland, with approximately 20 cases of TBE annually, the known endemic areas are situated mainly in the archipelago and coastal regions, with highest incidence in the Åland islands, where the TBEV seroprevalence was found to be 5% in helathy blood donors.
We screened *Ixodes ricinus* panels collected in 1996-1997 from two endemic areas for the presence of TBEV RNA. Two distinct RT-PCR methods were applied, and both were shown to have an approximate detection limit of 10 focus forming doses FFD/100 μl. One out of 20 pools (a total of 139 ticks) from Helsinki Isosaari Island and one out of 48 pools (a total of 450 ticks) from Åland were positive with both methods, while the remaining pools were negative. The observed overall TBEV frequency (0.34%) in ticks in endemic areas of Finland, was similar to the low incidence found by virus isolation in mice in the 1960s (0.5%) and the endemic foci have remained rather stable.
We could still detect TBEV RNA in 0.02% of a positive pool of ten nymphs suggesting that the viral RNA load within a nymph was in the range of 200 000 FFD. Sequence analysis did not show geographical clustering of the Finnish strains, suggesting an independent emergence of different TBE foci from the south, possibly by migratory birds. Furthermore, no TBEV RNA positive ticks were found in *I. ricinus* panels consisting of 130 pools (726 ticks) from Helsinki city parks or 41 pools (197 ticks) from the Võrmsi Island in Estonia.
In addition, we have screened 1500 human sera from Lithuania and found a TBEV seroprevalence of 3% in the population. [Han et al, J Med Virol 63 (2000)]

Occurrence of TBE in Areas previously Considered non-endemic: Preliminary Results from an ISW-TBE Study.

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The ISW-TBE (International Scientific Working Group on Tick-Borne Encephalitis) was instituted in 1999 as a collaboration between colleagues from the majority of countries where TBE is known to be endemic. The main aims of this collaboration is to create a fruitful network to further develop and coordinate the work within the field of TBE, including aspects such as awareness of the disease within society and medical community, analysis of risk of travelers, development of practical guidelines concerning general information and recommendations for vaccination, funding for collaborative research projects, and the so-called Meningitis Study.

The 'Meningitis Study' is a joint collaboration between colleagues in Austria, Belgium, France, Italy, the Netherlands, Sweden and Switzerland. The aim of this ISW-TBE study is to clarify if there are endemic foci of TBE in previous non-recognised areas. The study is retrospective and includes departments of infectious diseases and microbiological/virological laboratories located in geographical areas of interest. The study participants will locate patients treated during recent years for viral CNS-infections of unknown aetiology. Stored sera will be screened, after informed consent, for TBE IgM and IgG antibodies with the use of commercial ELISA-kits. Positive results will be confirmed at a central laboratory by ELISA and neutralization test. Seropositive individuals will be informed and epidemiological and clinical data will be collected by use of a standardized questionnaire. This questionnaire contains questions concerning the geographical origin of the TBEV infection, vaccination status, including other flaviviruses, and some condensed clinical data.

The included geographical areas were chosen in regions presently considered to be TBE non-endemic. In some parts of Europe there is a tradition not to look for TBE cases among patients with viral CNS infections. The reasons for this may vary but may be due to a conviction that TBE is non-existent in the area, the lack of causal treatment for TBE, or that TBE is, incorrectly, considered as a quite harmless infection where serological diagnosis is not necessary from a clinical perspective. Accordingly, economical arguments against screening have also been used.

An epidemiological background will be presented covering the Nordic countries, illustrating how epidemiological data can be generated to locate "new" endemic foci. Preliminary data from the ISW-TBE Meningitis Study will be presented and "new" endemic areas will be identified.

Tick-borne-encephalitis (TBE) in dogs

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Tick-borne-encephalitis (TBE) is caused by a *flavivirus* and transmitted by ticks. It is known in dogs for nearly 30 years and the number of TBE cases is increasing. In addition to fever cerebrocortical, thalamic, and brainstem symptoms occur simultaneously. Not all TBE-infections in dogs do lead to clinical signs but peracute/lethal as well as subacute and chronic courses have been reported. TBE is a seasonal disease, depending on climate related tick activity. Infected ticks are spreading the virus over central Europe with a tendency to expand to new endemic areas in western Europe.

Epidemiology

Tick-borne-encephalitis (TBE) is a viral disease (zoonosis) caused by a neurotropic *arbovirus* (Süss et al., 1994). TBE-virus, genus *flavivirus*, family *flaviviridae* is a frequent cause of meningitis, meningoencephalitis, meningoencephalomyelitis or meningoradiculoneuritis in man in central Europe (Kaiser et al., 1997; Kunz, 1994).

In humans TBE was described for the first time in 1931 by Schneider in Austria. In 1937 a virus was isolated and identified to cause TBE and classified as *flavivirus*. At that time ticks (*Ixodidae*) were identified as vectors of this disease (Kunz,1994).

TBE is a seasonal disease, depending on the activity of ticks, and is proved to be biphasic. The first cases of TBE in dogs often occur in early spring, whereas the number of infections in dogs reaches its peak during June – July, abates slightly during the hot, dry summer months and again shows rising incidence in October. The activity dependence of the ticks is explainable by climate data like air - and ground temperature and, air humidity (Kirtz, 1999). Distribution of TBE-infection in dogs expands over the European continent. New endemic areas for TBE in man in France, Germany and, Sweden underline the tendency of western expandation of TBE infected ticks (Kirtz et al., 2001). Because of our societies mobility, and the fact that we take our dogs along when we travel, TBE in dogs seems to be a good indicator for the spread of infections.

In central Europe the main responsible vector is *Ixodes ricinus*. Because these ticks feed on man and animals they transmit blood– and tissue parasites such as the TBE-virus, rickettsia, spirochetes, bacteria and filaria.

Natural hosts are rodents (mice, rats), ruminants (deer), carnivores (fox, badger) and birds, as well as sheep, cattle and, dogs (Greene, 1990; Stanek, 1994). Infected ticks are carrying the virus for their life time but are not affected by it (Kahl, 1994). A vertical transmission (transovarial) and a transstadial transmission within the tick population has been shown (Binn, 1987; Kahl, 1994). Despite these facts the infection rate of *Ixodes ricinus* with the TBEV in central Europe is only 0,1 – 5% (Blaskovic, 1958; Kunz, 1994). In Switzerland a seroprevalence of IgG antibodies against the TBE-virus in dogs of 3,6-5% was reported (Matile et al., 1981), in Switzerland and Germany 23% (Müller, 1997), and in Austria 24% (Kirtz et al., 1998).

Based on a large series of tests in an Austrian dog population (n=545) no age-, breed-, or gender related disposition for TBE could be detected (Kirtz, 1999). Although Ixodes ricinus is considered the main vector for transmission of TBE, transmission thought by milk from infected ruminants to humans has been described (Gresikowa et al., 1975; Kohl et al., 1996)

Clinical signs

In 1972 Gresikowa et al. identified the TBE-virus to cause neurological symptoms in dogs (Gresikowa et al., 1972a, Gresikowa et al.,1972b, Wandeler et al., 1972). A few cases have been reported in dogs in the recent years (Tipold, 1997; Reiner and Fischer, 1998).

Clinical symptoms can be observed after an estimated incubation time of 5 to 9 days and include fever, apathy, anorexia and neurological symptoms. Altered behaviour and consciousness, reduced muscle tone of the limbs and seizures indicate acute thalamic and cerebrocortical lesions, whereas vestibular strabismus, nystagmus, loss of sensitivity in the head area, facialpareses and inability to swallow point to diffuse brainstem lesions. Hyperalgesia in the neck is common with meningeal inflammation. In rare cases spinal cord lesions lead to reduced spinal reflexes (Kirtz et al. 1999; Reiner and Fischer, 1998; Tipold, 1997). The neuropathological and immunohistochemical findings of central nervous system lesions and distribution of TBE-virus in affected dogs confirm the reported clinical symptoms (Weissenböck et al., 1998).

Four different courses of TBE infection in dogs have been reported. Half of the seropositive dogs do not develop clinical signs. The peracute course leads to death within one week. In some of these cases antibodies are even not detectable. Similar findings described in canine distemper were explained by a depressed or delayed humoral immunological response (Tipold et al., 1999).

When the affected dog survives the first week, the prognosis becomes remarkable better. In an acute course of illness clinical signs improve after one to three weeks and disappear often without any sequelae. A chronic course has been described where affected dogs recover from their neurological deficits to a normal status within one to six months. A high percentage of these patients show an adequate immune response by developing high antibody titer (Kirtz, 1999).

Diagnosis and treatment

Physiotherapy and symptomatic therapy is useful because no causal therapy has been developed until now. Use of steroids is controverse because its application seems to prolong viraemia in early stages of the disease (Tipold et al., 1993) while therapy with corticosteroids in convalescent dogs had a positive effect on the course of disease (Reiner and Fischer, 1998). Until now there is no vaccine for dogs available. The decreasing number of fatal TBE-infections in man due to vaccination programs shows the importance to intensify our effort in developing a TBE-vaccine for dogs.

Whenever neurological symptoms resulting from central nervous system lesions are combined with fever, one has to take inflammatory (infectious and non infectious) disease into consideration.

The tentative diagnosis of TBE in a dog is made when clinical symptoms and specific case history (tick-exposition, endemic area) are present. Possible differential diagnosis to TBE are distemper, rabies, toxoplasmosis, neosporosis, pseudorabies, borreliosis, bacterial meningitis and, granulomatous meningoencephalitis. For differentiation serological and cytological tests of blood and cerebrospinal fluid (CSF) are useful and necessary. During viraemia the virus is detectable by PCR in blood, cerebrospinal fluid, and tissue of infected dogs.

Detection of TBE-antibodies has become available by indirect ELISA and serum neutralisation test.

Birds and *Borrelia*

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Lyme borreliosis is caused by spirochaetes of the complex *Borrelia burgdorferi* sensu lato: 3 genospecies (*B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*) are recognized as pathogenic and 7 additional genospecies (non-pathogenic or without known pathogenicity) are associated with this complex. These spirochaetes are mainly vectored by ticks of the *Ixodes ricinus* complex, which have a large distribution in the Northern hemisphere: *I. ricinus* in Europe, *I. persulcatus* in Eastern Europe and Asia, *I. scapularis* in Eastern and mid-Western North America and *I. pacificus* in Western North America. These tick species are known to feed on a large variety of vertebrate species including mammals, birds and reptiles.

B. burgdorferi sensu lato circulate in nature through a maintenance cycle involving competent tick vectors and competent vertebrate reservoirs. Strong evidence of reservoir competence of various species of small mammals has been reported in Europe, North America and Japan. On the other hand, the involvement of birds, which are frequent hosts for subadult ticks of the *I. ricinus* complex, has remained controversial for a long time. Infected ticks were certainly collected from various avian species in Europe, North America and Japan but the presence of spirochaetes in bird-feeding ticks does not imply that birds are reservoirs of *B. burgdorferi* sensu lato. The isolation of spirochaetes from the skin of *Turdus* sp. revealed the infection status of the passerines but was not a proof of reservoir competence either. However, the presence of 2 genospecies, *B. garinii* and *B. valaisiana*, in bird skin biopsies from different individuals suggests that a specific association exists between birds and particular *Borrelia* genospecies.

Tick xenodiagnosis, which is the most valuable method to evaluate the reservoir competence of a host, has finally been performed on blackbirds, *Turdus merula*, in Switzerland and pheasants, *Phasianus colchicus*, in the UK. Both avian species have been demonstrated to transmit spirochaetes to naive ticks. And as for bird skin biopsies, only *B. garinii* and *B. valaisiana* were characterized in xenodiagnostic ticks. This observation emphasizes the hypothesis that a specific association exists between birds and certain genospecies of *B. burgdorferi* sensu lato.

But ticks of the *I. ricinus* complex are not the only vectors of *B. burgdorferi* sensu lato. Spirochaetes of Lyme borreliosis are also maintained in seabird colonies. In these isolated environments present in both the Northern and Southern hemispheres, *B. burgdorferi* sensu lato is transmitted between the seabird tick, *I. uriae*, and seabirds. Characterization of spirochaetes has revealed that *B. garinii* is the genospecies involved in this secondary maintenance cycle.

In addition to their role in the maintenance of *B. burgdorferi* sensu lato, birds may contribute to the short- and long-distance dispersal of *Borrelia* and *Borrelia*-infected ticks.

All these observations demonstrate that the involvement of birds in Lyme borreliosis is greater than firstly thought.

Lyme borreliosis in passerine birds

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In order to explore the competence of birds as reservoirs for Lyme disease spirochaetes, we determined the presence of *Borrelia* DNA in tick larvae which can naturally be found attached to the bird. Birds were caught at several study sites along the Rhine valley in SW Germany between August 1999 and March 2001. A total of 987 *Ixodes ricinus* larvae were collected from 225 birds in 20 host species. There is strong evidence that several of these bird species are possible avian reservoir hosts for Lyme disease spirochaetes based upon the tick infestation pattern (infestation rate, larva-nymph ratio, host density, and behavioural aspects, respectively). Four passerine species out of five selected have not been subject to detailed reservoir competence analysis and previous results were based on a very small sample size (nightingale *Luscinia megarhynchos,* dunnock *Prunella modularis*, chiffchaff *Phylloscopus collybita*, reed warbler *Acrocephalus scirpaceus*). In these species, we studied the prevalence of Lyme disease in ticks and in one species in ticks and blood samples.

We used the nested polymerase chain reaction method (PCR) to amplify DNA and analysed one larva per bird. Oligonucleotid primers specific for the *osp*A gene of *Borrelia burgdorferi* sensu lato were used. *Borrelia* DNA could be detected in six out of nine larval ticks from the nightingale, in one out of eleven ticks from the dunnock, and in three of nine ticks from the chiffchaff. None of the ticks removed from robins *Erithacus rubecula* in winter were found to be *Borrelia* positive. Blood samples of nightingales caught during the breeding period were *osp*A-gene-positive in 39 birds (53%). To verify the results, samples of PCR-amplified *Borrelia osp*-DNA for *I. ricinus* were subject to nucleotid sequence analysis. The results of PCR detected *Borrelia* DNA are analysed regarding the bird's life history and individual characteristics (sex, age, condition, and migratory status).

Horses and Borrelia

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Background

Grazing animals are continuously exposed to tick bites. Consequently, one may expect that horses will also become infected with the various pathogens carried by ticks including *Borrelia burgdorferi* sensu lato. Whether they may develop clinical disease due to this pathogen is controversial. However, since the discovery of the agent, reports are available on single cases of clinical Lyme borreliosis in horses in the USA. We were interested to learn about the dynamics of the humoral immune response by comparing blood samples drawn at the period of highest tick activity and again nine months later.

Methods

Blood samples were drawn from 311 horses of the Spanish Riding School in Vienna, the stud farm Piber and stud farms in Kärnten, Niederösterreich, Salzburg and Steiermark. Of about 60% of horses a second blood samples could be drawn after about nine months. Antibodies to *Borrelia burgdorferi* sensu lato were determined by immunoblot (Westernblot) using five different genospecies as antigens (*B. afzelii*, *B. burgdorferi* sensu stricto, *B. garinii*, *B. lusitaniae*, and *B. valaisiana*). Additionally, all sera were screened for antibodies by a hemagglutination assay (Labor Diagnostika).

Results

The Lippizaner horses from the stud farm Piber and the Spanish Riding School in Vienna constitute about 79% of all horses investigated. The remaining horses divide among six stud farms with numbers of horses ranging from five to 19 animals. The sex distribution was almost equal (ca 51% males), the age of the animals ranged from one week to 33 years.

At the time of blood withdrawal all animals were clinically without signs and symptoms of disease. About 15% of the horses had a history of disease including dermatitis, arthritis, and abortus. None of these manifestations could be directly associated with Lyme borreliosis.

The quality and number of bands achieved with each borrelia genospecies used in this study and determined for each serum specimen was correlated with that of the second sample and with the age and the geographical location of the stud farm. The highest relative number of bands was regularly achieved within the first year of life and with *B. afzelii* and *B. garinii*. In older animals changes in the blot patterns between first and second sample were not especially pronounced. By considering three criteria for the interpretation of Lyme borreliosis immunoblots in humans (EUCALB, MIQ, in house) about 33% of horses experienced a blot confirmed borrelia infection.

Conclusions

The results indicate that a certain proportion of horses become infected with *Borrelia burgdorferi* sensu lato. The infecting agents are almost exclusively strains of the genospecies *B. afzelii* and *B. garinii* whereas blot patterns with the other genospieces are apparently due to cross reactivity. The humoral immune response of horses against Lyme borrelia appears to be protective since there were no animals seen during the investigation period which presented with clinical disease.

A New non-species dependent ELISA for the serological diagnosis of Lyme borreliosis in zoo animals

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Although Lyme Borreliosis (L.b.) is regarded as one of the most important zoonotic diseases, very few research reports have documented examinations on free-ranging wild animal populations and no study in zoo animals has been published in Europe. One of the problems connected with the diagnosis of L.b. in wild animals is often the lack of species specific secondary antibodies for serological tests.

For our study concerning the exposure of zoo animals to *Borrelia (B.) burgdorferi* s.l. and the occurrence of L.b. in various German zoos, a modified non-species dependent ELISA was developed. Specific IgG antibodies against *B. burgdorferi* s.l. were detected by peroxidase labelled protein A or protein G conjugates. For this, both conjugates were tested for their binding affinities to 160 different wild animal species representing 25 families out of 7 orders. With 5 exceptions, all tested species reacted with either protein A or protein G, and 47 species reacted with both of the conjugates. A difference in the ability and intensity of binding were not only observed between the two conjugates, but also between individuals of the same species. Although in general, variations within one species and even among species of the same family were finally negligible. In combination with an easy method for the long-term preservation of antigen-coated microtitre plates, the ELISA developed for this study could essentially facilitate serological examinations regarding L.b. in wild animal sera.

Overall, the results indicate that commercially available protein A and protein G conjugates could be useful alternatives to species specific secondary antibodies in various diagnostic assays on sera of a wide range of wild mammals and, therefore, should be more often considered as versatile diagnostic tools in wildlife studies.

Granulocytic Ehrlichiosis in Dogs and Horses

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Granulocytic ehrlichiosis in dogs and horses has been diagnosed with increasing frequency in Swedish dogs and horses since 1990. By nucleotide sequence analysis of the 16S rRNA gene, it has been shown that Swedish canine and equine isolates are identical and closely related to *E. phagocytophila* and *E. equi*. With respect to the 16S rRNA gene sequence, the Swedish animal *Ehrlichia* is also identical to the agent causing human granulocytic ehrlichiosis (HGE).

In both dogs and horses, acute granulocytic ehrlichiosis is characterised as a febrile illness with inappetence and fatigue as the most prominent signs. In addition, horses often develop distal limb edema and ataxia, and dogs lameness and gastrointestinal symptoms. Sometimes petechiae can be seen on mucus membranes. Microscopic ehrlichial inclusions can be seen in stained blood smears of circulating blood granulocytes and a profound thrombocytopenia is usually found in the early course of disease. The diagnosis is in this stage based on demonstration of ehrlichial inclusions in blood granulocytes. Antibodies are produced and significant levels can usually be detected within a week. For serology, an indirect fluorescent antibody (IFA) test with a commercially available *E. equi* antigen is used.

Animals with long-standing diffuse signs of disease, along with a raised antibody titre to *E. equi*, are often suspected of having 'chronic' granulocytic ehrlichiosis. Owners refer to a wide variety of clinical signs such as mild lameness, fatigue, stiffness, inappetence, unwillingness to perform, and demand serological testing. If the animal is seropositive, this is taken as an indication that the clinical signs are caused by an ongoing *Ehrlichia* infection and the animal is treated with antibiotics. However, the existence of chronic granulocytic ehrlichiosis in dogs and horses, meaning persistent infection together with clinical manifestations has been questioned and has not been proven yet.

In order to expand our understanding of granulocytic ehrlichiosis in dogs and horses, different studies have been undertaken. PCR-based assays for detection of *Ehrlichia* DNA in blood and tissue samples have been developed. In experimental inoculation studies of dogs and horses, a state of persistent infection has been demonstrated in some animals, but it could not be associated with any signs of illness. Serological studies on naturally infected animals, have shown that titres decrease to low levels or disappear around six to eight months after the acute disease. The overall seroprevalences in both Swedish dogs and horses have been shown to be around 17%. Using logistic regression on the dog data has shown that during the study period (1991 to 1994), the proportion of seropositive dogs increased with age and season. Multivariable modelling of the horse data showed that age, racing activity, geographic region, season, serologic titre to *B. burgdorferi* s. l., and pasture access were risk factors for seropositivity. The proportion of healthy horses with positive titers was not statistically different from unhealthy horses.

Ehrlichiosis in *Ixodes ricinus* and wild mammals

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Granulocytic *Ehrlichia* species (GE) are obligate intracellular bacteria which have been well established as tick-borne pathogens of veterinary importance and have recently emerged as a threat to public health. Final species designation for the human granulocytic ehrlichiosis (HGE) agent awaits description, but it is closely related or conspecific with two well-known agents of veterinary diseases, *Ehrlichia equi*, the agent of the equine granulocytic ehrlichiosis, and *Ehrlichia phagocytophila*, the agent of the tick-borne fever in ungulates. Since 1995, serological evidence of HGE has been demonstrated in several European countries. Well-documented cases of HGE were described in Slovenia, and more recently in the Netherlands and Sweden.

In the USA, GE have often been associated with *Ixodes scapularis* and *Ixodes pacificus* ticks, which may serve as the primary vectors. The white-footed mouse (*Peromyscus leucopus*) and the white-tailed deer (*Odocoileus virginianus*) are implicated as natural reservoirs. But in Europe, very little is known about animal reservoirs and the ecology of GE. Nevertheless, several studies, using specific PCR for the 16S rRNA gene, have shown that *Ixodes ricinus* ticks are infected with GE.

GE infection was demonstrated in *I. ricinus* ticks and wild mammals in Switzerland. Six ticks (1.4 %) of 417 *I. ricinus* collected by flagging vegetation contained ehrlichial DNA. A total of 201 small mammals from five species: wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), earth vole (*Pitymys subterraneus*), bank vole (*Clethrionomys glareolus*), and common shrew (*Sorex araneus*) were trapped. The analysis of *I. ricinus* collected on 116 small mammals showed that nine *C. glareolus* and two *A. sylvaticus* hosted infected tick larvae. In these rodents, GE infection was also detected in blood, spleen, liver and ear samples. Further examinations of 190 small mammals, without ticks or with non-infected ticks, showed the presence of ehrlichial DNA in spleen and other tissues from six additional *C. glareolus*, two *A. flavicollis* and one *S. araneus*. Serological analysis in chamois (*Rupicapra rupicapra*) and roe deer (*Capreolus capreolus*) showed that 66.9 % (89/133) of roe deer and 51.3 % (20/39) of chamois had antibodies against GE. Ehrlichial DNA was amplified by PCR in 19 of 103 (18.4 %) blood samples from roe deer.

Partial 16S rRNA gene sequences from ticks and mammals showed a high degree of homology with GE isolated from humans. In contrast, *groESL* heat shock operon sequence analysis showed a strong divergence between samples derived from rodents, and from roe deer or questing ticks or from other published *Ehrlichia* sequences.

This study suggests that these wild mammal species, common hosts of *I. ricinus* ticks, are likely to be natural reservoirs for GE, and that GE heterogeneity exists in mammal and tick populations.

Zoonotic Babesiosis

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Babesiosis is a well-known tick-borne disease of animals and is attracting increasing attention as an emerging zoonosis. The first recorded case occurred in 1957 in Yugoslavia and manifested as a fulminant infection with fatal outcome in a splenectomised farmer. The causative organism, *B. divergens,* which is transmitted by *Ixodes ricinus,* is a common cause of bovine babesiosis in Europe and more than 20 further human cases have been confirmed, always in splenectomised individuals. This rare disease often ends fatally, though effective drugs are now available. Since the late 1960's at least 200 cases caused by a rodent parasite, *B. microti,* have occurred in North America, mostly in unsplenectomised individuals. More recently, other *Babesia* spp. have caused human disease in California, Washington State and Missouri.

Although *B. microti* also occurs in Europe, human cases have not been confirmed. This has been explained by the suggestion that the principle vector is the rodent tick, *I. trianguliceps,* which does not bite humans. However, *I. ricinus,* the vector of several other zoonoses, has also been implicated as a vector of *B. microti* and this has been confirmed recently in transmission experiments. Furthermore, an American strain of zoonotic *B. microti* was transmitted by *I. ricinus,* which suggests that this human-biting tick is capable of transmitting strains of *B. microti* from widely separate geographical areas, and may transmit several European strains. Transmission of *B. microti* to humans in Europe is suggested by serological surveys and by one unpublished study reporting that this parasite may give rise to untypical symptoms in Lyme borreliosis cases.

Microbiological diagnosis in Lyme borreliosis

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Lyme borreliosis is a multisystem disease involving many organs as skin, nervous system, joints and heart being caused by at least three species, *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* in Europe. Except in cases characterised by pathognomonic clinical manifestations, the diagnosis of Lyme borreliosis usually requires confirmation by means of a microbiological diagnostic assay. Mainly antibody detection methods are used for this purpose which are counting among the most frequently requested serological tests in microbiological laboratories. Detection of the causative agent by culture isolation and nucleic acid techniques is confined to special indications, e.g. to clarification of clinically and serologically ambiguous findings. Application of these methods shall be reserved to laboratories specialised in this type of examination.

Antibody detection methods

Serological examination should follow the principles of a rational stepwise diagnosis: 1. A serological screening assay and 2. in the event of a positive or equivocal result a confirmatory assay (two step approach). A sensitive ELISA (at least second generation) is recommended, which – in case it is reactive – should be confirmed by the immunblot. If a whole cell-lysate is used as antigen, diagnostic bands must be defined by monoclonal antibodies, in case of recombinant antigens identification of diagnostic bands is much more easier. Commercial recombinant antigen immunblots are better standardised than the conventional ones, the lower sensitivity might be overcome in the future by addition of further immunorelevant antigens (i.e. p58, additional Osp17 homologues). For the conventional blot strains expressing variable antigens (OspC, Osp17) should be used (i.e. strain PKo). Interpretation criteria for the immunblot recommended by the DGHM are published the MiQ 12 Lyme-Borreliosis (1). Determination of the CSF/serum index should be performed in case of neuroborreliosis.

Detection of the causative agent

Culture for borreliae is performed only in few specialized laboratories (i.e. NRZ for Borreliae). Cultures from clinical material (skin, other biopsies, CSF) must be controlled up to 6 weeks or more. Recently an isolate was obtained not before 8 weeks of incubation from an ACA patient. Interestingly this strain is different from the 10 hitherto described *B. burgdorferi* s.l. species, resembling a new variant isolated from an Erythema migrans in Holland. Nucleic acids techniques are increasingly used – often by investigators without sufficient experience. Primers must be designed in order to detect different species and subspecies of *B. burgdorferi* s.l. The OspA gene has been frequently used. Recommended specimens for the PCR are the same as for culture; urine and blood are not recommended. If available, synovial fluid from patients suspicious for Lyme arthritis should be investigated. In two independant studies it was found that the causative agents of Lyme arthritis are heterogeneous in Germany, comprising all three humanpathogenic species and several *B. garinii* subspecies. The predominance of *B. afzelii* in specimens from human skin as well as the heterogeneity of borreliae in CSF has been shown by analysis of isolates as well as PCR amplicons. Data on strains from patients are important for development of a European vaccine.

1. Wilske, B.; Zöller,L.; Brade, V.; Eiffert, M.; Göbel, U.B.; Stanek, G.; and Pfister, H.-W. MIQ 12 Lyme-Borreliose, Urban & Fischer Verlag, München Jena (2000) 1-59

Diagnostic strategy for the detection of Lyme borreliosis

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Furthermore 25 years after the first description of the pathogen agent *Borrelia burgdoferi* the diagnosis of Lyme borreliosis based primarily on clinical criteria. But multiple differential diagnoses have to be considered and laboratory confirmation based on various test procedures is required until now. For screening assays the methods of immunofluorescence assay (IFA), enzyme linked immunoassay (EIA), or indirect hemagglutination assay (IHA) are most frequently in use. Since the last ten years a stepwise diagnostic procedure in USA and Europe is recommended. The use of serological screening assay as the first diagnostic step, followed by a confirmatory assay only in the case of a positive or borderline result of the screening assay is now "state of the art". Most frequently a sensitive enzyme linked immunoassay followed by a sensitive and specific immunoblot technique. But the quality of the indirect serological tests and most important the interpretation criteria of the commercial tests vary considerably. The choice of Borreliae strains, antigen preparation, production conditions, test procedures and the interpretation criteria of the test results vary in a wide range. The immunoblot used as a confirmatory assay, must fulfill highest quality requirements but in practice under routine laboratory conditions, these aspects are often not fulfilled. The major reason for this "lack" is that Lyme borreliosis is no sexual transmitted disease and there no official approval is necessary for the commercial suppliers until now. An other more and more important aspect is the introduction of molecular biological assays in the routine laboratory. Numerous authors published many articles about the usefulness of the polymerase chain reaction as a diagnostic tool for diagnosing *Borreliae* in human specimen. However, a routine method has not been established until yet. Furthermore no standardised method for DNA isolation from different human specimen has been developed so far. For this reason PCR method is until now no routine method and their application should be restricted to few laboratories with experiences in the field of lyme borreliosis.

Summarized it is important to inform the clinicians about the limitations of the used tests and it would be very helpful to organize an european or worlwide consensus conference to built up accepted rules for the diagnosis of Lyme Borreliosis.

In vitro Susceptibility of *Borrelia burgdorferi* against recently Developed Antimicrobial Agents – Implications for new Therapeutic Approaches in Lyme Disease?

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In Germany, the average number of individuals that become newly infected with *Borrelia burgdorferi*, the causative agent of Lyme disease, is estimated at 30,000 to 60,000 per year. Lyme disease represents a disorder of potentially chronic proportions, and relatively little is known on the *in vivo* pharmacodynamic interactions of antimicrobials with borreliae. So far, evidence-based drug regimens for the effective treatment of Lyme disease have not been established definitively. Therapeutic failures have been reported to occur, however, for almost every suitable antibiotic agent currently available. The reported incidence of delayed cures and resistance to treatment ranges from 6 % in stage I and II up to 70 % in stage III of the disease. Thus, resistance to treatment and a protracted course of the disease continue to pose problems for clinicians in the management of patients suffering from chronic Lyme disease. The overall natural resistance of the pathogen against antibiotic substances and the optimum duration of antimicrobial chemotherapy, therefore, have to be evaluated in greater detail.

To analyse the *in vitro* effectiveness of new antimicrobials against the *B. burgdorferi* complex, in our studies a novel standardised colorimetric microdilution method is applied to investigate the minimal inhibitory concentrations (MICs) of the tested antimicrobials against *B. burgdorferi.* MICs were read after 72 hours of incubation. Moreover, time-kill studies are performed by application of conventional cell counts in combination with dark-field microscopy and the minimal borreliacidal concentrations (MBCs) were determined by subculture experiments. Our recent studies provide evidence for a possible heterogeneity of the different borrelial genospecies in respect to their antibiotic susceptibilities for antimicrobials like the penicillins and streptogramins. Moreover, we can demonstrate for the first time that besides ß-lactams, macrolides, and tetracyclines which are recommended for stage-dependent therapy of Lyme borreliosis, spectinomycin, the streptogramins and recently introduced substances like new quinolones, and the ketolide family of antimicrobial agents also show enhanced *in vitro* activity against borreliae*.* This are novel and so far unpublished data.

Moreover, new quinolones and ketolides probably remain active against both, intracellularly sequestered borreliae (due to high intracellular activity) and L-forms (due to inhibitory effects independent from cell wall biosynthesis). Some of these antimicrobial agents, if effective *in vitro* and *in vivo*, also may prove to be useful agents in the therapy of certain manifestations of Lyme disease. Furthermore, these antimicrobial agents may represent very effective therapeutic alternatives on account of their oral availability, favorable pharmacodynamic profiles, and high tissue levels in cases where ß-lactames or tetracyclines cannot be administered without detrimental side-effects. Our *in vitro* data hence the possible suitability of new antimicrobial agents either alone or in combination with well-established compounds for the stage-dependent therapy of Lyme disease. Accordingly, the potential role of new antimicrobials for the therapy of Lyme disease should be evaluated further by *in vivo* studies and clinical trials.

Treatment of Lyme borreliosis – State of the Art

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The diagnosis of Lyme borreliosis is based on characteristic clinical symptoms and serological findings. Treatment with antibiotics is indicated if both criteria are present.

A number of antibiotics has been investigated to eliminate *Borrelia burgdorferi sensu lato* in vitro and to cure clinical symptoms. However, data from in vitro and in vivo studies do not correlate: Cephalosporines of the third generation (Ceftriaxone and Cefotaxime) and Makrolides (Erythromycine, Roxithromycine Azithromycine) show very low minimum inhibitory concentrations (MIC < 0.5) and therefore should be highly effective in vivo. MIC concentrations > 0.5 have been reported for Doxycycline, Tetracycline, Amoxicillin, Ampicillin, Penicillin and Cefuroxime. *Borrelia burgdorferi* sensu lato has been reported to be resistant to Aminoglykosides and Co-trimoxazole.

Clinical studies indicate that Cephalosporines, Cefuroxime, Doxycycline, Penicillin G and Amoxicillin are very useful, but Makrolides are less effective in vivo. Patients with early Lyme disease (Erythema migrans, lymphadenosis cutis benigna, carditis with 1st of 2nd degree heart block, acute arthritis, meningitis, cranial-nerve palsy, acute radiculopathy) should be treated with oral antibiotics (Amoxicillin, Doxycycline, Cefuroxime) for 14 to 21 days. Patients with 3rd degree heart block, recurrent arthritis after oral regime, acrodermatitis chronica atrophicans, borrelial encephalomyelitis or chronic neuroborreliosis should be treated with parenteral antibiotics (Cephalosporines, Penicillin G) for 14 to 21 days. Treatment for 28 days is justified only in acute and chronic arthritis. There are no convincing data to recommend any treatment for longer than 28 days in patients with Lyme borreliosis. Efficacy of treatment should be monitored by resolution of clinical symptoms and not by changes in antibody concentrations. Corticosteroids are useful to lower clinical symptoms, but not to cure the infection.

Lyme Disease Vaccine: The United States' Experience

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In December, 1998, the United States Food and Drug Administration (FDA), approved a lipidated recombinant *Borrelia burgdorferi* outer surface protein A (rOspA) vaccine against Lyme disease (LYMErix^{tm}, SmithKline Beecham Pharmaceuticals). This followed a large randomized, controlled field trial demonstrating the product's safety and efficacy (76 % protection after 3 doses). Licensed for use in persons aged 15 – 70 years, LYMErix is labeled for intramuscular injection of 0.5 ml (30 µg) on a three-dose schedule of 0.1, and 12 months, with second and third doses administered several weeks preceding the peak *B. burgdorferi* transmission period. The Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) published recommendations on LYMErix use, based on an individual's assessment of risk, categorized by geographic location and whether exposures to tick infested habitats were expected to be frequent or prolonged. Persons at high or moderate geographic risk were advised that they "should" or "may wish to" consider vaccine use depending on anticipated habitat exposure. Recommendations regarding booster doses were deferred pending evaluation of immunogenicity data. A theoretical pathogenic effect of rOspA vaccination was noted based on a molecular mimicry between the dominant T-cell epitope of OspA and human leukocyte function associated antigen 1 (hLFA-1). Persons with treatment-resistant Lyme arthritis were recommended not to be vaccinated because of the known relationship of this condition and OspA immune reactivity. Field trials of LYMErix in children have been conducted, but data have not yet been considered by FDA or ACIP.

From January 1999 to August 15, 2000, approximately 1,45 million doses of LYMErix were distributed, targeted to populations living, working, or recreating in highly endemic states and counties. Decisions on vaccine administration have been made by the private sector, either by the individual health care provider or by health management organization policy. Federal, state and local health authorities have provided guidance on vaccine use, but public programs have not assumed responsibility for distributing vaccine or setting policies on its use.

Analysis of Vaccine Adverse Event Reporting System (VAERS) data for the first 19 months postlicensure identified only 66 serious events (0.0004 % of all doses distributed). Both VAERS data and post-licensure studies by the vaccine manufacturer disclosed no unexpected patterns of adverse events by age, gender, dose number, and time to onset. However, press reports describing public concern over possible vaccine-related arthritis are thought to have had a negative impact on vaccine use. A case-control study has been initiated by FDA to examine further the question of vaccine induced arthropathy. Two population-based case-control studies are in progress to determine vaccine efficacy, and prevention effectiveness of vaccination will be examined in CDC-sponsored community-based prevention projects.

Future vaccine considerations include the following: 1) optimal vaccine schedules, including timing of booster doses; 2) recommendations for vaccine use in children; 3) strategies targeting vaccine to populations at greatest risk. 4) assessment of the impact of vaccination on disease incidence, and determination of cost-effectiveness of vaccine use;5) continued surveillance for serious adverse effects.

Immune Evasion of *Borrelia burgdorferi* - Failure of Sufficient Killing of the Pathogens by Complement and Antibody

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The innate immune system and in particular the complement system as a first line of defense play a key role for the elimination of microorganisms after entrance in the human host. Like other pathogens *Borreliae* must develop strategies to overcome host defense systems. By investigating serum susceptibility of *Borreliae* we found that mainly *B. afzelii* isolates were serum-resistant while the majority of *B. burgdorferi* s.s. isolates expressed an intermediate serum-sensitive phenotype. In contrast, *B. garinii* isolates were effectively killed by complement and therefore classified as serum-sensitive. Up to now we identified five proteins expressed on the outer surface of *Borreliae* which interact directly with the two main complement regulatory proteins FHL-1/reconectin and factor H belonging to the alternative complement pathway. These borrelial proteins were termed CRASP´s (complement regulators-acquiring surface proteins). Binding of FHL-1/reconectin and factor H occured to serum-resistant and intermediate serum-sensitive borrelial isolates but not to serum-sensitive isolates which lack these proteins. We conclude from our results that the control of complement activation on the borrelial surface by the interaction of CRASP´s with complement regulatory proteins represents an important mechanism of immune evasion of borrelial isolates belonging to the genospecies *B. afzelii* and *B. burgdorferi* s.s..

Analysing the humoral adaptive immune response of patients we detected sera which killed serum- (NHS-) resistant *Borreliae*. Borreliacidal activity was observed most frequently with sera of patients with stage III disease. The killing of NHS-resistant isolates by these immune sera always required antibodies and complement. Bactericidal activity, however, was not detected in all immune sera of the different disease stages, although specific anti-Borrelia antibodies were present according to serological test results. This observation suggested that not all borrelial antigens are able to induce a borreliacidal immune response. In an extensive analysis of 24 immune sera we identified up to 12 antigens including OspC with the greatest potential for the induction of borreliacidal antibodies. The borreliacidal potential of anti-OspC antibodies was directly tested on an OspC-expressing borrelial wild-type isolate and the corresponding OspC-lacking variant. In these studies only the wild-type isolate expressing OspC on its surface was found positive for the lytic complement complex indicating the great importance of this antigen for the control of the infection. Additional studies are required to identify further "protective" antigens among these 12 proteins which are privileged candidates for infection control according to our studies with patient immune sera (supported by the DFG Br446/11-1 and Zi342/5).

Vaccination against Lyme Borreliosis in Europe: a Special Problem for Man and Dogs?

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Both man and dogs are only background figures in the zoonotic cycle of *Borrelia burgdorferi* (*B.b*.), but are susceptible to infection and/or disease. Because of the known species-specific interaction between hosts and pathogens it is to be expected that the course of *B.b.* infection will not be identical for man and dogs. However, recent studies indicate that both species develop similar kinds of immune responses and common clinical symptoms, such as arthritis, upon natural infection. Moreover, it seems that protective immunity, as conveyed by antibodies (Ab) to the outer surface protein A (OspA) follow the same principles in both species. Thus, it is to be expected that the special problems associated with the development of a suitable human vaccine for Europe also holds true for dogs.

A vaccine based on OspA was shown to induce highly protective Ab responses in man and dogs and to protect both species against infection with members of *B.b.* sensu strictu (*B.b.* s.s.). A respective vaccine formula was recently approved for use in humans and dogs in the USA. The potential of this vaccine is weakened by at least two shortcomings. First, it is unfit for use in Europe because it does not induce cross-protective immunity against the other two pathogenic *B.b.* species (*B. afzelii, B, garinii*) existing on this continent in addition to *B.b.* s.s. However, a trivalent formula containing OspA proteins derived from each of the three *B.b.* species was recently shown to induce full protective immunity to any European *B.b.* strain in mice and is presently tested in clinical trials. Second, the vaccine is inappropriate for therapeutic use. This is because *B.b.* express OspA solely in the tick, but not in mammalian hosts and thus escape anti-OspA Ab in this environment. Recent studies showed that *B.b.* express other Osps, including OspC, during infection in mice and that passive transfer of anti-OspC Ab leads to resolution of disease symptoms and eradication of *B.b.* These results encourage further efforts into the development of suitable prophylactic and therapeutic vaccines which benefit both the master and his dog.

New Strategies to Treat Established *B. burgdorferi* Infections

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Lyme disease, caused by the spirochete *Borrelia burgdorferi (B.b.),* is the most common tick-borne infectious disease in humans in Europe and the US. The multisystem illnes is fraught by a number of serious problems such as a) unpredictable onset and manifestation of disease symptoms, b) limitation of diagnostic tools, c) unreliability of antibiotic therapy d) insufficient immune control of disease and e) persistence of spirochaetes. Vaccination with recombinant outer surface protein A (OspA) from *Borrelia burgdorferi* provides excellent antibody-mediated protection against challenge with the pathogen in animal models and in humans. However, the bactericidal antibodies are ineffective in the reservoir host, since OspA is expressed by spirochaetes only in the vector, but rarely, if at all, in mammals. By immuno screenings of an expression library of *B. burgdorferi* strain ZS7 with sera from experimentally infected mice we have now isolated three novel genes, *zs3*, *zs6* and *zs8*. All three genes are located together with *ospA/B* on the linear plasmid lp54 but unlike *ospA/B* are expressed by spirochaetes during infection, at least temporarily. The respective gene products are poorly immunogenic in mice but elicit antibodies in humans. We show that recombinant preparations of ZS3 and ZS8 but not ZS6 induce functional antibodies in mice capable to protect immunodeficient recipients against subsequent experimental infection. These findings suggest that ZS3 and ZS8 represent potential candidates for a 'second generation' vaccine to prevent and/or cure Lyme disease.

The Key Role of Selection and Migration in the Ecology of Lyme Borreliosis

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It is discussed controversially whether *Borrelia burgdorferi* sensu lato, a genetically diverse bacterial species-complex, is maintained in nature by distinct transmission cycles involving multiple hosts. Here, several lines of evidence are presented, indicating that *B. burgdorferi* s.l. is ecologically differentiated through selection. Questing adult *Ixodes ricinus* ticks were collected across central, western and south western Europe in the years 1998-2000 and analysed for infection with *B. burgdorferi* s.l. using PCR, followed by DNA-DNA hybridisation and nucleotide sequencing of multiple loci. Statistical analysis of the frequency distribution of genospecies in individual ticks showed that the combination of *B. garinii* and *B. valaisiana* was significantly more frequent than expected, whereas co-infections of *B. afzelii* with any other genospecies were significantly less frequent than expected under neutralist assumptions. Whilst displaying some polymorphism, most genotypes of *B. garinii* detected in this study showed close phylogenetic relationships with genotypes of *B. garinii* identified in avian-tick transmission cycles from western Europe, but not with OspA serotype 4 strains. *B. afzelii*-specific alleles, on the other hand, clustered together with strains previously shown to be associated with small rodents. The mutual exclusion of bird- and rodent-associated *Borrelia* genotypes in the individual ticks analysed in this study can only be explained by the operation of selection and not by geographic segregation. Selection is corroborated by transmission studies using natural hosts, which showed that *B. burgdorferi* s.l. genotypes are not only differentially acquired by feeding ticks, but also differentially retained/eliminated in ticks. Previous studies have shown that the sensitivity (resistance) patterns of *Borrelia* strains to the complement system of avian and mammalian species precisely match the transmission patterns observed so far. Selection of *Borrelia*, therefore, is most likely mediated by host complement, possibly operating in the midgut of the feeding tick. Thus, host complement appears to be the major determinant of host association. We have predicted earlier that host association of spirochaetal genotypes will manifest itself in different rates of migration. In fact, phylogeographic analyses validate this prediction, with high rates of migration observed for bird-associated genotypes. Altogether, it is emerging that the ecology of Lyme borreliosis is largely host-driven and that selection and migration are major forces shaping the population structures of *B. burgdorferi* s.l..

Artificially Created Colonies and Abundant Populations of the Brown Dog Tick, *Rhipicephalus sanguineus*, in and near Human Dwellings

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People often alter their neighbourhoods creating an additional epidemic danger due to arthropod vectors. Pets may be of great importance in such transformations. We describe here how dogs, which are the main hosts of the brown dog tick *Rhipicephalus sanguineus*, influence epidemiological features of human habitats in some areas of Israel. Three cases were under consideration. (a) Typical domestic colonies were observed several times in Jerusalem just inside apartments in many-story buildings, where dogs are kept indoors. A single adult female tick, fully engorged on a dog, that drops off in a human dwelling initiates a colony. (b) Pseudo-domestic colonies, or micropopulations of ticks were reported from small yards or gardens near the dwellings where dogs live in kennels. (c) An artificially created field population of *R. sanguineus* was observed in a farm in the central part of Israel. Dogs were guarding the farm along its perimeter. In both the last cases (b) and (c) dogs collect ticks when they move freely, and ticks are also attracted to dogs from some distance and actively attack them. In all three cases, the development of the ticks on the dogs changes from the normal 3-host cycle to a 2-host cycle when larvae do not leave the dogs after feeding but molt into nymphs which feed on the same host.

In case (a) engorged nymphs leave the host and migrate upwards over the inside or outside walls of the house, often forming clusters in small cracks or depressions inside the walls. As a rule, on seeing nymphs the inhabitants take control measures against the ticks trying to terminate the further development of the colony. In cases (b) and (c) ticks are found all over the kennels and in small holes in the soil next to the kennels, as well as in the permanent resting sites of the dogs. Their abundance in these sites is extremely high, as is the number of ticks on each dog (gorging nymphs and adults). Unfed adults from observed colonies or populations have a significantly larger scutal index (and, hence, weight after molting) than unfed ticks collected in the control field site. In Israel, ticks living in nature have one generation per year. Ticks observed on the farm, as well as ticks in kennels, develop faster (because of the 2-host cycle and immediate availability of the hosts) and a significant part of their population has two generations per year. *R. sanguineus* is the main vector and reservoir of *Rickettsia conorii*, the causative agent of Mediterranean spotted fever, as well as the vector of *Ehrlichia canis*, the causative agent of canine ehrlichiosis. Domestic colonies and abundant populations of *R. sanguineus* near human dwellings create an additional risk to humans who can be attacked by infected ticks in and around their homes. Such a concentration of ticks close to humans might be the cause of some cases of illness recorded from Israeli towns.

Transmission Studies of an European Sindbis Virus in the Floodwater Mosquito *Aedes vexans*

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Sindbis viruses (Genus Alphavirus, family *Togaviridae*) are well known throughout many parts of Afrika, Asia, Australia and Europe. The virus is thought to cycle between *Culex* mosquitoes and passerine birds in nature. Man mostly becomes accidentially infected while "invading" this transmission cycle at the natural focus. Besides various *Culex* spp., *Aedes* spp. have been claimed to serve as bridging vectors for human infections. The respective disease in humans is characterized by fever, rash, myalgia and long-lasting arthralgia. Interestingly, this disease is rather common in most of the Scandinavian peninsula and neighboring parts of former Russia, and virus isolations are documented around the Mediterranean. In Germany, however, Sindbis viruses were never found although there are two major flyways used by migrating birds. This study aimed to test the vector competence of the most abundant floodwater mosquito, *Aedes vexans*, for Sindbis virus. A colony of *Ae. vexans*, derived from the Kühkopf area at the river Rhine were used for primary infection studies via intrathoracal inoculation. At day 5/6 post inoculation, viral titres increased about a thousandfold. After this peak, viral replication slowly declined to an average of a 15-fold of the original virus uptake (40 PFU) after three weeks (end of experiment).

For oral infection studies an artificial feeding device containing Sindbis virus-spiked bovine blood was used. Twelve hours after the blood meal, mean virus titres were 320 PFU per headless mosquito, but no virus was re-isolated from head squashes. Over the next 12 hours, the mean viral titres further decreased to 32 PFU/carcass in 50 % of this group while out of the remaining half no virus was detectable. After fourteen days none of the 36 mosquitoes examined harbored detectable virus. Thus, we conclude that the colonized *Ae. vexans* show a midgut infection barrier for the Sindbis virus tested and thus are unlikely to play a role in virus transmission - given the presence of viraemic birds roosting in Germany during migration.