Eur. J. Epidemiol. 0392-2990
May 1991, p. 229-236

EUROPEAN JOURNAL OF EPIDEMIOLOGY

Vol. 7, No. 3

ECOLOGICAL QUESTIONS CONCERNING RICKETTSIAE[1]

J. ŘEHÁČEK*[2] and I.V. TARASEVICH**

*Institute of Virology, Slovak Academy of Sciences, Dúbravská cesta 9, 842 46 Bratislava, C.S.F.R.

**Gamaleya Institute of Epidemiology and Microbiology, U.S.S.R., Academy of Medical Sciences, 123D98, Moscow, U.S.S.R.

Key words: Rickettsiae - Ecology - Vectors - Host animals

The past ten years were characterized by the appearance of several "new" transmissible spotted fever group (SFG) rickettsioses, e.g. Israeli, Japanese and Astrakhan fevers. The factors responsible for their establishment probably include the introduction of chemicals from industry, agriculture and the timber industry into natural habitats. Such factors may influence the pathogenicity of these rickettsiae. In this case, in addition to the human influence, the mechanism of the circulation of the agents under natural conditions of both abiotic (climate, etc.) and biotic (flora and fauna) components may play a decisive role. The modern management of breeding domestic animals, indoor and outdoor maintenance, seasonal migrations, new animal foods, stress, etc., can be important factors affecting the biological properties of the Q fever agent. Nonpathogenic rickettsiae, rickettsia-like symbionts and other microorganisms circulating in nature may also influence the pathogenic rickettsiae. Studies on their interrelationships in hosts and vectors may markedly contribute to the understanding of the circulation of pathogenic rickettsiae in nature. Recognition of factors causing the appearance of new rickettsial agents or differences in pathogenicity of rickettsial strains is important not only for the prognosis of rickettsial diseases but also for the *prognosis of other* infectious diseases.

The last two decades of the 20th century are characterized by an increase in social and economic activity and by their influence on processes in nature. For example, the clearing of woods for pastures, heavy metallic and chemical industry, applications of various pesticides in agriculture and forest areas, water pollution, pollution from automobiles, etc., are negative factors which may, to a certain degree, contribute to the appearance of various diseases of humans and animals. This appears to resemble a "reflecting defensive answer of nature" to these human insults on the fundamental elements of natures existence. It is difficult to postulate whether these new conditions assisted or directly contributed to the appearance of new diseases with rickettsial etiology. Several new rickettsial organisms, namely the SFG's have been recently discovered but their appearance seems to be attributed more to the better understanding of rickettsiae as organisms and to the improvement of methods employed for their recognition, identification and characterization. From this recent data, the strains of the SFG rickettsiae that cause in Israel a disease which differs symptomatically, namely by the absence of "tache noire", from other rickettsioses of this group (20) have been published. Most cases of this disease occur in the summer and primarily in children; possibly the life style of young children playing outdoors enables early exposure, probably via ticks, to the causative agent (63). Another example is the finding of SFG rickettsiae in human infections in Japan, (29), and the detection of antibodies to SFG rickettsiae in humans (24, 53, 64) in several regions of the country as well as by the isolation of rickettsia from patients with febrile

[1] Presented at the 4th International Symposium on Rickettsiae and Rickettsial Disease, Pieštany, C.S.F.R., 1-6 October, 1990.

[2] Corresponding author.

exantematous illness (39, 58). Following the current taxonomy based on antigenic analyses, the taxonomic name *Rickettsia japonica* was proposed (59) Although the vector of this rickettsia still remains to be determined (54), the reservoir is probably the large Japanese field mouse, *Apodemus speciosus* (37).

Several years ago "Astrakhan fever" appeared in the delta of the Volga river in the U.S.S.R. It is believed that the etiologic agent of the disease is a rickettsia of the SFG. However, the infectious agent as well as the vector, supposedly the tick *Rhipicephalus sanguineus,* have not yet been verified (3, 7, 23, 30).

New data on the presence and importance of rickettsial infections have been reported from the People's Republic of China. This country has been for many years "terra incognita" for rickettsial diseases, however, given this data from the last few years it appears that at least five different rickettsial diseases occur there. In addition to the well known diseases, such as scrub typhus, murine typhus, epidemic typhus and Q fever, one or more SFG rickettsioses probably occur in this country. Antibodies to the SFG rickettsiae found in humans, domestic animals and free living animals, occasionally in a high percentage, suggest that the SFG rickettsiae are spread throughout China. The SFG rickettsiae have also been isolated from patients, ticks and rodents (15, 16). The isolated strains were initially reported to be two antigenically distinct groups, which would have suggested new SFG species of rickettsiae. However, these strains were later identified as *Rickettsia sibirica* (17, 62).

To understand the process of a rickettsial infection, its appearance, epidemiology and prophylaxis, the fundamental data concerning its ecology are obviously desirable. We emphasize the circulation of a rickettsia in an area which has not specific definitive available pattern. To explain the course of a zoonosis all fundamental factors in the area under study have to be taken into account, i.e. the fauna of local domestic and free-living vertebrates as hosts and those species of probable ectoparasitic arthropods as vectors as well as data on local environmental conditions. The last component is of principal value. It comprises both biotic (fauna and flora) and abiotic (climate, mean seasonal temperature, average daily solar radiation, elevation, etc.) factors and it determines decisively the process of a rickettsiosis in nature. It also determines, for instance, whether a new agent introduced via birds into a habitat will find in this new milieu convenient conditions for its "realization". Research on the circulation of *R. sibirica* and *C. burnetii* in different geographic locations with various climatic conditions in the U.S.S.R., determined by the presence of antibodies in humans and various animals, demonstrated that the incidence of agents with regard to the infection of humans is directly related to the "richness" of biocoenoses. For instance, the natural foci of tick typhus as shown by seropositivity in humans gave the following results: in southern taiga 0.3%, forest-steppes 0.9% and steppes 1.2% (60). Occurrence of Rocky Mountain Spotted Fever in relation to climatic, geophysical and ecologic factors in south-eastern U.S.A. for 1970-1980 demonstrated a bimodal incidence of this disease with peak onsets of cases in mid-May and mid-July in the oak-hickory-pine, oak-hickory, and Appalachian oak zones of climax vegetation during 1970-1974, while in 1975-1980 a single peak of cases in mid-June was observed in the oak-hickory-pine zone. Bimodality persisted in both remaining zones. The first peak of illness predominated in northern cooler areas, and the second peak, in southern, warmer areas (25).

In general, the primary foci of rickettsioses involve zoonoses of small mammals. Secondary foci develop under the influence of human activity, and domestic or synanthropic animals become the main source for human infection. In addition to natural conditions, human activity in nature plays a very important role in the development or disappearance of a rickettsial infection. For example, the change of pasture maintenance of cattle to its confined stable breeding, the change of the pastures into fields or the modification of wild shrubby pastures to grassy pastureland can result in deticking of the habitats and the eradication of coxiellosis from such areas. In contrast, the urbanization of Siberia facilitating a closer contact of man with nature and more frequent contact of people with ticks infected with *R. sibirica*, results in an increase of tick typhus morbidity and higher levels of antibodies to the causative agent in humans (50). However, human activity with animal involvement in the background comprises a very wide spectrum of different ways for the dissemination of rickettsioses. As an example, one of the most extensive epidemics of Q fever involving 415 confirmed cases occurred in the Val de Bagnes, Valais, Switzerland, during the autumn of 1983. This outbreak started three weeks after 12 flocks of sheep descended from the alpine pastures to the valley. As a result, 21% of the population of the villages in the lower part of the valley along the road followed by the sheep contracted Q fever during this period (14).

Human activity also resulted in the establishment in anthropogenic foci where in addition to domestic animals, pets namely cats and dogs, play an important role. Dogs were shown as indicators of the presence of rickettsiae in various areas (18, 31). Dogs, for instance, screened in N. Carolina, U.S.A. for the SFG rickettsiae antibodies demonstrated a prevalence rate of 15% for exposure to *R. montana,* 11% for *R. rhipicephali* and 5% for *R. rickettsii* (10). Dogs are highly sensitive to various species of rickettsiae. Previous studies involving various species of rickettsiae and different routes of administration revealed a dose-related correlation with severity of clinical signs ranging from mild to fatal. The clinical symptoms as shown, for example, with the infection of *R. rickettsii* included lethargy, anorexia, fever, ocular discharges and diarrhea (11). Vacationers in Mediterranean countries where the brown dog tick, *Rhipicephalus sanguineus*, occurs abundantly are often

accompanied by dogs that are mainly responsible for transferring infectious ticks with *R. conorii* from that area to their homes. Although not indigenous in several central and western European countries these ticks may survive and develop, especially indoors, and establish a focus of fever boutonneuse which may persist for a long time, as shown in several countries in Central Europe (41). These findings support a suggestion that dogs, mainly stray animals, may serve as enviromental sentinels for establishing the geographic prevalence of SFG rickettsioses foci (10). It is also suggested that the high rates of positive results of fever boutonneuse detected in rural and urban populations are the consequence of bites by *R. sanguineus* ticks carrying *R. conorii.* These ticks are frequently observed not only on dogs but also on the floors, walls and other parts of buildings where dogs live and can spread to adjacent structures (fences, trees, etc.). In Spain, in the province of Salamanca 93% of the dogs have antibodies against *R. conorii*, 73.5% of the population tested positive and in small villages in the surrounding area 82% of the population was seropositive (22). However, not only dog ticks but also dog fleas, namely of the genus *Ctenocephalides*, may be involved in the circulation of *R. conorii* among dogs and, in a limited number of cases, may infect a man. This may explain, at least in part the inconsistent presence of the "tache noire" in patients with fever boutonneuse. In 20.8% of *Ctenocephalides* sp. fleas collected from stray dogs in Palermo, Sicily, the presence of organisms displaying fluorescence with *R. conorii* antisera was detected (32).

In the past it was assumed that cats were not an important link in the ecology or rickettsiae in the field. However, this opinion has changed rapidly during the last few years due to observations of Q fever epidemics in Canada. Cats are most popular pet after the dog, especially in villages where the household without cats is the exception rather than the rule. The closest contact between animal and man perhaps occurs between humans and cats. Previously it was shown that cats inoculated subcutaneously with *C. burnetii* developed fever, lack of appetite and lethargy. The agent was present in the blood of some animals for one month and in the urine for two months following infection (42). It is possible that cats become infected during carnivorous activity. Q fever epidemics and cases can be associated with exposure to products of feline parturition. In Canada an outbreak of Q fever occurred in 1987 among members of an urban poker playing group who were exposed to a parturient cat (27). Another outbreak of Q fever affected 16 of 32 employees at a truck repair plant in Nova Scotia. This outbreak was probably due to aerosols from clothing contaminated by contact with newborn kittens (33). Contact with parturient cats infected with *C. burnetii* also resulted in an outbreak of Q fever among residents of Baddeck, Victoria County, Nova Scotia (34).

The role of free living animals in the ecology of rickettsiae has been investigated many times. We would like to present some recent data concerning the involvement of lagomorphs in the ecology of these microorganisms. Lagomorphs as a source of Q fever infection for man was demonstrated in Canada where 45% of showshoe hares caught in the area where a Q fever patients snared rabbits had antibodies to *C. burnetii* (35). Seropositivity to spotted fever antigens in wild rabbits in Costa Rica was 55% and *R. rickettsii* was isolated from *Haemaphysalis leporispalustris* ticks parasitizing on them (18). In Italy, 78.9% of rabbits *Oryctolagus cuniculus* living in a protected zone of Tuscany were seropositive to the SFG rickettsiae, *R. conorii* and *R. slovaca.* Seroconversion towards *R. conorii* was also observed in guinea pigs inoculated with homogenates of *Rhipicephalus pusillus* ticks taken off the wild rabbits (13). *Lepus europaeus* imported from eastern Europe (country not specified) into free hunting territories in Italy were shown to be positive (16.9%) to *C. burnetii* antigen (51). This data is in agreement with those from Czecho-Slovakia, where Řeháček et al. (47) reported a 5.3% seropositivity to *C. burnetii* in specimens from 263 animals from six different areas in Bohemia. This was confirmed by a 2.8% seropositivity reported from a more comprehensive study of 2180 hares hunted in southern Bohemia (61).

From the broad spectrum of animals which may play a role in the circulation of rickettsiae in nature let us also to consider birds. They are susceptible to these pathogens and they are able to disseminate them in their bodies, in excretions or via infectious ticks feeding on their blood. Feeding of birds, their habitats, residence, seasonal wandering and migrations should also be taken into account. In studying the biology of pigeons' infection with chlamydiae in Bratislava, in winter, we found them to be seropositive (almost 10%) for *C. burnetii.* These birds were supposed to be typical city residents, however, the source of their infection was found to be a cooperative farm about 30 km from Bratislava where the birds collected their daily food, i.e. corn, from silage holes. The holes were at this time settled with rats who completed their food needs in stables occupied with *C. burnetii* infected cattle. Thus the town population of pigeons contributed indirectly to the discovery of a Q fever focus in the surroundings of Bratislava. Whether these birds introduce coxiellae to city dwellers is another question (45).

Let us now to present some ideas concerning vectorship. The relation between agent and vector is established by evolution. According to Pavlovsky's ideas, vectors are separated into many categories according to their capacity to transmit an agent. In our opinion, they should be first classified as the basic vectors, essential for the transmission, maintenance vectors, i.e. vectors being unavoidable, obligatory for the presence of an agent in nature, maintaining its original biological properties and the others - distributing vectors, i.e. vectors disseminating the infection by different routes. An example of the first category of vectors are ticks of the *Dermacentor* genus for most rickettsiae of the SFG, the tick *R. sanguineus*

for *R. conorii* and body lice for *R. prowazekii.* Without the association of these vectors with these rickettsiae the proper rickettsial diseases do not persist permanently and, if they develop through other vectors they exist only for a limited time period. The second group of vectors, the distributing types, include an unlimited number of arthropod species. These vectors become infected either by feeding on rickettsemic blood or other body fluids of vertebrates, or through contact with their body surfaces. They disseminate the agent by all known ways, e.g. by biting, mostly through contaminated mouth parts or regurgitation, by contact of hosts with their contaminated body, for instance during scratching, by ingesting infectious bodies by the hosts, by contact of the food of host animals with their contaminated bodies, i.e. excretions, etc. Relationships between most species of rickettsiae and their probable vectors have been characterized and discussed (43, 44), however, there exist species of rickettsiae where the role of vectors has been shown but the data about their relationships are still not explained. For example, until about 1980 it had been generally accepted that infected fleas do not pass any known pathogens to their progeny. But this is not true as it has now been found that *R. typhi* is transmitted transovarially in *Xanopsylla cheopis* fleas (6). However, data on the physiological relationships of rickettsia to organs, tissues and cells of its vectors are scarce. There exist only a few papers in this field of research, one on oxygen uptake and CO_2 elimination in ticks infected with *C. burnetii* and *R. sibirica* and another on changes in amino acid composition in ticks infected with the same agents (8). Also, data on the influence of rickettsiae on the viability of different stages of ticks and of the influence of the infection on the structure and function of single organs and tissues are not yet available. Little data have been accumulated about this problem, for example, a limited cytopathic effect in the salivary glands and ovarian tissues of *R. sanguineus* ticks massively infected with *R. rhipicephali* (21).

Another problem concerns the influence of the vector on various properties of rickettsiae. It seems that a vector may influence the pleomorphism of a rickettsia, its virulence, antigenic properties, etc. This question does not only concern arthropods but also includes other aspects such as vertebrate hosts and environmental conditions which may have an effect on the properties of a pathogen through vectors. We propose to carry out experiments on the maintenance of rickettsia in selected arthropods and vertebrates under various environmental conditions, which would be very important. Such studies might contribute to the elucidation of basic ecological relationships between the microorganisms and their host animals. They may be important for practical reasons since, for example, an appropriate change in the environment could result in the eradication of a vector and subsequently in the disappearance of pathogens from a particular area.

Another area concerning vectors in relation to rickettsiae is the community of microorganisms which may affect the rickettsiae. Very few studies have been carried out in this field (44, 46). The significance of such studies is illustrated by the paper by Burgdorfer et al. (12), concerning a nonpathogenic rickettsia-like organism named "East side agent" in *Dermacentor andersoni* ticks collected on the eastern side of the Bitterroot Valley in Montana, U.S.A. This agent occupies cells and tissues of some tick organs and prevents an invasion of the ovaries by pathogenic *R. rickettsii*, resulting in the inability of the tick to transmit rickettsia transovarially. Other investigations have shown that no coxiellae were seen in the brain of *Dermacentor reticulatus* ticks experimentally inoculated with only coxiellae, but in the presence of *Rickettsiella phytoseiuli,* coxiellae attacked the cortical layer of synganglion (48). Furthermore, *C. burnetii* experimentally cultivated in *D. reticulatus* ticks, which were shown to be naturally infected with an arbovirus, revealed a marked alteration in its ultrastructure (49). Examination of ticks in Europe for infection with microorganisms revealed various rickettsial species, rickettsia-like organisms, arboviruses and probably also indigenous tick viruses, various species of bacteria, bacterium-like organisms, borreliae, protozoan parasites, etc. What the relationships among these microorganisms in an arthropod milieu is unknown and we think it would be appropriate to investigate. Also, their relationship to vertebrate hosts may be decisive for the outbreak of an infection as, for example, the infection of *R. akari* in man. This rickettsia probably circulates normally in the house mouse population in an enzootic cycle. When mice become infected with lymphocytic choriomeningitis virus and die, show fever in acute infection or have changes in skin respiration and in texture of the tissue, the mites leave to seek the nearest suitable host, i.e. man (26).

In the last decade there was reported for haematophagous arthropods a phenomenon on vertebrate host antibody penetration through gut walls into the haemolymph (1, 19, 36). It appeared that the crossing of IgG occurred into the haemocoel of various arthropods, e.g. flies, ticks and fleas, however, the significance of the regulation of pathogens, namely rickettsiae within arthropods under the influence of these antibodies, has not yet been examined on more comprehensive material. In the flea, *R. typhi* invades the epithelial gut lining and propagates therein; rickettsiae are released into the gut lumen where they are exposed to the ingested antibodies. Host antibody to *R. typhi* was detected on the surface of rickettsiae not only in the gut content but also in the flea haemolymph. Such antibody-coated *R. typhi* in *X. cheopis* fleas, once transmitted to the host either mediates enhancement of uptake and destruction by the host macrophages or facilitates their clearance from the host (40). Prefeeding of fleas on immune hosts 3 - 5 days before being given an infected blood meal did not influence the acquisition of rickettsiae and their subsequent infection (4). Maintenance of infected fleas on immune hosts had

no significant effect on the establishment and subsequent growth of rikettsiae within the fleas, however, infected fleas maintained for 19-22 days on immune hosts failed to transmit *R. typhi* to baby rats (5). The phenomenon on antibody penetration through gut into haemocoel evidently also occurs in ticks (9, 56), however, their interaction with rickettsiae present there has not been investigated.

There has been a surge of activity in the SFG rickettsiae in the last decade in several parts of the world. For various historical, technical and other reasons the identification of significantly different strains of SFG rickettsiae as species has caused many problems. Thus strains related to *R. rickettsii* exhibiting considerable differences in virulence and antigenic composition have been isolated in various regions of the U.S.A. (2). However, intensive studies by investigators at the Rocky Mountain Laboratory in Hamilton, Montana, have shown a vast diversity of nonpathogenic SFG rickettsiae in ticks. At present serological surveys do not distinguish infection with one SFG rickettsia from that with another. Therefore, new techniques were developed to identify rickettsial agents that are serologically difficult to differentiate. Employing tools from the molecular biology revolution, i.e. technology of DNA: DNA homology, restriction nuclease analysis, immunoblot analysis of surface proteins and lipopolysaccharides, etc., promise to supply tools by which existing strains could be differentiated. The cloned DNA-protein analysis considerably speeds and simplifies identification of isolates and should facilitate a better understanding of the contribution of rickettsial biotypes and nonpathogenic rickettsia to the ecology and epidemiology of rickettsial diseases. Analysis of surface proteins of human isolates obtained in Western Sicily and from ticks *R. sanguineus* in the same region by SDS-PAGE electrophoresis showed that at least two species other than *R. conorii* are present in this region (57).

Our knowledge of rickettsiae and rickettsioses, namely of the SFG, in South America, South Africa and Australia is very poor. We suppose that the isolation and characterization of strains belonging to this group of rickettsiae might contribute to the explanation of the evolution of tick-borne rickettsioses and might be also helpful for the explanation of the development of these parts of the world.

In recent years, however, there has been a growing recognition of the occurrence of more serious and chronic infections, in Q fever, such as hepatitis with focal granulomas and endocarditis. Recent studies suggest that the *C. burnetii* strains associated with endocarditis harbour a plasmid not found in non-endocarditis strains. This marker may help to determine if endocarditis-causing strains originate from some unique or specific sources. Many new cases of endocardial involvement have been reported in the last few years. Although *C. burnetii* infection has a worldwide distribution, the highest number of cases has been reported from Australia, Great Britain and Spain (55), however, no clear epidemiologic or ecologic data exist for the source of these infections.

Until the present no *C. burnetii* strains exhibiting low virulence have been isolated with the exception of a strain exerting low virulence for mice, which was isolated from a patient with acute Q fever in Japan (38). An interesting feature in coxiellosis has been observed in the last decade in the C.S.F.R., it is the discrepancy between the high seropositivity in cattle and low, negligible or absent morbidity in humans. Under adverse environmental conditions and the unusual resistance of the agent, which is capable of surviving under humid or dry conditions and remain infectious for months, it is still not clear why the disease does not manifest itself more frequently in man (28). Do rickettsial strains with attenuated virulence circulate in the region?

Although our knowledge of rickettsioses as zoonoses has grown tremendously during the last years and now has been enriched by advanced techniques of molecular biology, many problems of the relationship between vectors, their hosts and rickettsiae still need more data for explanation. Recognition of factors causing the appearance of new rickettsial agents or differences in pathogenicity of their strains is important to reveal not only the prognosis of rickettsial diseases but also the prognosis of other infectious diseases.

REFERENCES

1. *Ackerman S., Clare F.B., McGill T.W.* and *Sonenshine D.E.* (1981): Passage of host serum components, including antibody, across the digestive tract of *Dermancentor variabilis* (Say). - J. Parasitol. *67:* 737-740.

2. *Anacker R.L., List R.H., Mann R.E.* and *Wiedbrauk D.L.* (1986): Antigenic heterogeneity in high - and low-virulence strains of *Rickettsia rickettsii* revealed by monoclonal antibodies. - Infect. Immun. *51:* 653-660.

3. *Androsova S.V., Nezhneva V.N., Pogatkin A.K., Kabin V.V., Bondarenko N.M.* and *Zhuravlov V.I.* (1989): Contribution to the question on disease of unclear etiology in the Astrakhan region. - Tez. dokl. nauchn. konf. Astrakhan *1:* 57-58 (in Russian).

4. *Azad A.F.* (1986): Acquisition and persistence of murine typhus infection in *Xenopsylla cheopis* after feeding on immune rats. - Pages 158-162 in D. Borovsky and A. Spielman, eds., Host regulated developmental mechanisms in vector arthropods. University of Florida - IFAS, Vero Beach, Florida.

5. *Azad A.F.* and *Emala M.A.* (1987): Supression of *Rickettsia typhi* transmission in fleas maintained on murine typhus-immune rats. - Am. J. Trop. Med. Hyg. *37:* 629-635.

6. *Azad A.F., Traub S.* and *Bagar S.* (1985): Transovarial transmission of murine typhus rickettsiae in *Xenopsylla cheopis* fleas. - Science *227:* 543-545.

7. *Balaeva N.M.* and *Ignatovich V.F.* (1989): Serological investigations concerning the tick typhus disease in the Astrakhan region. - Vopr. rikketsiol. *4:* 168-172 (in Russian).

8. *Balashov Yu. S.* and *Daiter A.B.* (1973): Haematophagous arthropods and rickettsiae. - Ed. Nauka, Leningrad, p. 1-249. (in Russian).

9. *Ben-Yakir D.* (1989): Quantitative studies of host immunoglobulin G in the hemolymph of ticks (Acari). - J. Med. Entomol. *26:* 243-246.

10. *Breitschwerdt E.B., Moncol D.J., Corbett W.T., MacCormack J.N., Burgdorfer W., Ford R.E.* and *Levy M.G.* (1987): Antibodies to spotted fever-group rickettsiae in dogs in North Carolina. - Am. J. Vet. Res. *48:* 1436-1440.

11. *Braitschwerdt E.E., Walker D.H., Levy M.G., Burgdorfer W., Corbett W.T., Hurlbert S.A., Stebbins M.E., Curtis C.C.* and *Allen D.A.* (1988): Clinical, hematologic, and humoral immune response in female dogs inoculated with *Rickettsia rickettsii* and *Rickettsia montana.* - Am. J. Vet. Res. *49:* 70-75.

12. *Burgdorger W., Hayes S.P.* and *Mavros A.J.* (1981): Nonpathogenic rickettsiae in *Dermacentor andersoni:* A limiting factor for the distribution of *Rickettsia rickettsii.* - *In:* Rickettsiae ad Rickettsial Disease, Academic Press p. 585-594.

13. *Ciceroni L., Pinto A., Rossi C., Khoury C., Rivosecchi L., Stella A.* and *Cacciapuoti B.* (1988): Rickettsiae of the spotted fever group associated with the host-parasite system *Oryctolagus cuniculi/Riphicephalus pusillus.* - Zbl. Bakt. Hyg. A *269:* 211-217.

14. *Dupuis G., Petite J., Péter O.* and *Vouilloz M.* (1987): An important outbreak of human Q fever in a Swiss Alpine valley. - Int. J. Epidemiol. *16:* 282-287.

15. *Fan M.Y., Wang J.G., Jiang Y.X., Zong D.G., Lenz B.* and *Walker D.H.* (1987a): Isolation of a spotted fever group rickettsia from a patient and related ecologic investigations in Xinjiang Uygur Autonomous Region of China. - J. Clin. Microbiol. *25:* 628-632.

16. *Fan M.Y., Walker D.H., Yu. S.R.* and *Liu Q.H.* (1987): Epídemiology and ecology of rickettsial diseases in the People's Republic of Cina. - Rev. Inf. Dis. *9:* 823-840.

17. *Fan M.Y., Xue J.Y* and *Walker D.H.* (1988): Antigenic analysis of Chinese strains of spotted fever group rickettsiae by protein immunoblotting. - Am. J. Trop. Med. Hyg. *39:* 497-501.

18. *Fuentes L.* (1986): Ecological study of Rocky Mountain Spotted Fever in Costa Rica. - Am. J. Trop. Med. Hyg. *35:* 192-196.

19. *Fujisaki K., Kamio T.* and *Kitaoka S.* (1984): Passage of host serum components, including antibodies specific for *Theileria sergenti,* across the digestive tract of argasid and ixodid ticks. - Ann. Trop. Med. Parasitol. *78:* 449-450.

20. *Gross E.M.* and *Yagupsky P.Y.* (1987): Israeli rickettsial spotted fever in children. - Acta Tropica *44:* 91-96.

21. *Hayes S.F.* and *Burgdorger W.* (1979): Ultrastructure of *Rickettsia rhipicephali,* a new member of the spotted fever group rickettsiae in tissues of the host vector *Rhipicephalus sanguineus.* - J. Bacteriol. *137:* 605-613.

22. *Herrero-Herrero J.I., Ruiz-Beltrán R., Martin-Sánchez A.M.* and *Garcia E.J.* (1989): Mediterranean spotted fever in Salamanca, Spain. Epidemiological study in patients and serosurvey in animals and healthy human population. - Acta Tropica *46:* 335-350.

23. *Kabin V.V., Vetlugina K.F., Androsova S.V., Vasilkova O.L., Nezhneva V.N.* and *Vetkovskaya V.A.* (1989): New disease of a rickettsial etiology in the Astrakhan region. - Tez. dokl. nauch. konf. Astrakhan *1:* 56-57. (in Russian).

24. *Kaiho I., Tokieda M., Ohtwara M., Uchiyama T.* and *Uchida T.* (1988): Occurrence of rickettsiosis of spotted fever group in Chiba prefecture of Japan. - Japan. J. Med. Sci. Biol. *41:* 69-71.

25. *Kaplan J.E.* and *Newhouse V.F.* (1984): Occurrence of Rocky Mountain Spotted Fever in relation to climatic, geophysical, and ecologic variables. - Am. J. Trop. Med. Hyg. *33:* 1281-1282.

26. *Krinsky W.L.* (1983): Does epizootic lymphocytic choriomeningitis prime the pump for epidemic rickettsialpox? - Rev. inf. Dis. *5:* 1118-1119.

27. *Langley J.M., Marrie T.J., Covert A., Waag D.M.* and *Williams J.C.* (1988): Poker players penumonia. An urban outbreak of Q fever following exposure to a parturient cat. - New England J. Med. *319:* 354-356.

28. *Lisák V., Vošta J.* and *Řeháček J.* (1989): The incidence of *Coxiella burnetii* and *Chlamydia psittaci* in cattle reared in Southern Bohemia. - Vet. Med. *34:* 403-410. (in Slovak).

29. *Mahara F., Koga K., Sawada S., Taniguchi T., Shigemi F., Suto T., Tsuboi Y., Ooya A., Koyama H., Uchiyama T.* and *Uchida T.* (1985): J. Jap. Assoc. Infect. Dis. *59:* 1165-1171. (in Japanese).

30. *Makarova V.A.* and *Tarasevich I.V.* (1989): Preliminary results of investigations of a disease of spotted fever group in the Astrakhan region. - Vopr. rikkesiol. *4:* 75-77. (in Russian).

31. *Mansueto S.* and *Vitale G.* (1984): Antibodies to *Rickettsia conorii* in dogs in western Sicily. - Trans. Roy. Soc. Trop. Med. Hyg. *78:* 681-682.

32. *Mansueto S., Vitale G., Lavagnino A., Di Rosa S.* and *Merulla R.* (1989): Rickettsia of the spotted fever

group in dog fleas (*Ctenocephalides spp.*). in Western Sicily. - Ann. Trop. Med. Parasitol. *83:* 325.

33. *Marrie T.J., Langille D., Papukna V.* and *Yates L.* (1989): Truckin pneumonia – an outbreak of Q fever in a truck repair plant probably due to aerosols from clothing contaminated by contact with newborn kittens. - Epidem. Inf. *102:* 119-127.
34. *Marrie T.J., MacDonald A., Durant H., Yates L.* and *McCormick L.* (1988): An outbreak of Q fever probably due to contact with a parturient cat. - Chest *93:* 98-103.
35. *Marrie T.M., Williams J.C., Schlech III W.F.* and *Yates L.* (1986): Q fever penumonia associated with exposure to wild rabbits. - Lancet February *22:* 427-429.
36. *Minoura H., Chinzei Y.* and *Kitamura S.* (1985): *Ornithodoros moubata:* Host immunoglobulin G in tick hemolymph. - Exp. Parasitol. *60:* 355-363.
37. *Morita C., Yamamoto S., Tsuchiya K., Yoshida Y., Yabe T., Kawabata N.* and *Fukui M.* (1990): Prevalence of spotted fever group rickettsia antibody in *Apodemus speciosus* captured in an endemic focus in Miyazaki prefecture, Japan. - Jpn. J. Med. Sci. Biol. *43:* 15-18.
38. *Oda H.* and *Yoshie K.* (1989): Isolation of *Coxiella burnetii* strain that has low virulence for mice from a patient with acute Q fever. - Microbiol. Immunol. *33:* 969-973.
39. *Okada T., Tange Y* and *Kobayashi Y.* (1990): Causative agent of spotted fever group rickettsiosis in Japan. - Inf. Immun. *58:* 887-892.
40. *Osterman J.V.* (1985): Rickettsiae and hosts. - Acta Virol. *29:* 166-173.
41. *Péter O., Burgdorfer W., Aeschlimann A.* and *Chatelanat P.* (1984): *Rickettsia conorii* isolated from *Rhipicephalus sanguineus* introduced into Switzerland on a pet dog. - Z. Parasitenkd. *70:* 265-270.
42. *Randhawa A.S., Dieterich W.H., Jolley W.B.* and *Hunter C.C.* (1974): Coxiellosis in pound cats. - Feline Practice *4:* 37-38.
43. *Řeháček J.* (1989): Ecological relationships between ticks and rickettsiae. - Eur. J. Epidemiol. *5:* 407-413.
44. *Řeháček J.* and *Daiter A.B.* (1989): Ixodid ticks and rickettsiae *In:* Rickettsioses, proceedings of scientific papers of Pasteur Institute in Leningrad *66:* 68-88 (in Russian).
45. *Řeháček J., Kocianová E.* and *Brezina R.* (1984): The possible significance of urban populations of the pigeons *Columba livia f. domestica* in propagation of *Coxiella burnetii* and *Chlamydia psittaci* in Bratislava. - Biológia (Bratislava) *39:* 293-300 (in Slovak).
46. *Řeháček J., Kováčová E., Čiampor F., Grešíková M.* and *Tarasevich I.V.* (1987): Experimental double infection with *Coxiella burnetii* and tick-borne encephalitis virus in *Dermacentor reticulatus* ticks. - Acta Virol. *30:* 65-74.
47. *Řeháček J., Vošta J., Tarasevich I.V., Brezina R., Yablonskaya V.A., Plotnikova L.F., Fetisova N.F.* and *Hanák P.* (1977): Rickettsioses studies. 3. Natural foci of rickettsioses in south Bohemia. - Bul. WHO *55:* 455-461.
48. *Řeháček J.* and *Šutáková G.* (1989a): Interaction between *Dermacentor reticulatus* cells and *Coxiella burnetii* in vivo. - Acta Virol. *33:* 465-473.
49. *Řeháček J.* and *Šutáková G.* (1989b): Virus-like particles in *Dermacentor reticulatus* ticks. - Acta Virol. *33:* 577-581.
50. *Rudakov N.V., Tofanjik N.V., Kordubaylov A.A., Gorbunov N.S., Strigina N.P., Kanyscheva V.F., Chervyakov V.I.* and *Yuchanova T.V.* (1989): Occurrence of foci of endemic rickettsioses in the territory of western Siberia. - Vopr. Rikketsiol. *4:* 116-119. (in Russian).
51. *Scholl F., Lillini E.* and *Aleandri M.* (1987): Serological investigation against *Coxiella burnetii* in imported hares *Lepus europaeus.* - Acta Medit. di Patol. Infett. e Trop. *6:* 323-324.
52. *Šutáková G.* and *Řeháček J.* (1990): Mixed infection of *Rickettsiella phytoseiuli* and *Coxiella burnetii* in *Dermacentor reticulatus* female ticks: electron microscopy study. - J. inv. Pathol. *55:* 407-416.
53. *Suto T.* (1985): Evidence of spotted fever rickettsial infection in Japan as demonstrated by the indirect immunoperoxidase test. - Microbiol. Immunol. *29:* 1243-1246.
54. *Tada N., Fujita T., Mahara F., Tada T.* and *Huang W.H.* (1988): Surveys of natural cycle of spotted fever pathogens in Japan. - Proc. Sino-Japanese Symp. Parasitic Zoonoses. p. 185-192.
55. *Tellez A., Sainz C., Echevarria C., de Carlos S., Fernandez M.V., Leon P.* and *Brezina R.* (1988): Q fever in Spain: Acute and chronic cases, 1981-1985. - Rev. inf. Dis. *10:* 198-202.
56. *Tracey-Patte P.D., Kemp D.H.* and *Johnston L.A.Y.* (1987): *Boophilus microplus*: passage of bovine immunoglobulins and albumin across the gut of cattle ticks feeding on normal or vaccinated cattle - Res. vet. sci. *43:* 287-290.
57. *Tringali G., Intonazzo V., Sferlazzo A., Perna A.* and *Argento A.* (1987): Epidemiology of boutonneuse fever in western Sicily; analysis of the surface proteins of tick-borne spotted fever group rickettsiae isolated in western Sicily. - Abstract from Rickettsilogy: The present and the future. Conference held in Palermo 21-28 June 1987, p. 89.
58. *Uchida T., Tashiro F., Funato T.* and *Kitamura Y.* (1986): Isolation of a spotted fever group rickettsia from a patient with febrile exanthematous illness

in Shikoku, Japan. - MiKcrobiol. Immunol. *30:* 1323-1326.

59. *Uchida T., Yu X., Uchiyama T.* and *Walker D.H.* (1989): Identification of a unique spotted fever group rickettsia from humans in Japan. - J. inf. Dis. *159:* 1122-1126.

60. *Voroncova T.A.* (1989): Geographical variants of immunological structure of inhabitants in mixed foci of tick encephalitis and endemic rickettsioses in the European territory of the U.S.S.R. - Zh. Micr. Epid. Imminuol. *12:* 45-50 (in Russian).

61. *Vošta J., Hanák P., Řeháček J., Brezina R.* and *Grešíková M.* (1981): The field hare (*Lepus europaeus Pallas*, 1778) as a reservoir of zoonoses. - Folia venatoria *10-11:* 163-177. (in Czech).

62. *Wang J.G.* and *Walker D.H.* (1987): Identification of spotted fever group rickettsiae from human and tick sources in the People's Republic of China. - J. inf. Dis. *156:* 665-669.

63. *Yagupsky P., Sarov B.* and *Sarov I.* (1989): A cluster of cases of spotted fever in a Kibbutz in southern Israel. - Scand. J. Infect. Dis. *21:* 155-160.

64. *Yamamoto S., Kawabata N., Uchiyama T.* and *Uchida T.* (1987): Evidence for infection caused by spotted fever group rickettsia in Kyushu, Japan. - Japan. J. Med. Sci. Biol. *40:* 75-78.