

Wild animals as hosts for anthropophilic tick species in Serbia

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Institute for Medical Research, University of Belgrade



- Founded almost 70 years ago with the foundation of the Institute for Medical Research (Laboratory for Medical Arachnoentomology)
- Since 2009 – Center of excellence for food and vector borne zoonoses
- Research of ticks and tick-borne pathogens started on the Institute on 1981 (Dr. Marija Milutinović)

Area of research



- Arthropod vectors and vector borne pathogens
- Biology, ecology, physiology and genetics of vectors and vector borne pathogens
- Detection of vector borne diseases in animal and human populations
- Design and development of strategies for control and prevention of vector borne diseases

Team



- **Dr. sci. bio. Snežana Tomanović**, associate research professor
- **Sanja Ćakić**, PhD student
- **Darko Mihaljica**, PhD student
- **Ratko Sukara**, PhD student



Cooperation

- Faculty of Biology, University of Belgrade
- Faculty of Pharmacy, University of Belgrade
- Military medical academy, Belgrade
- Texas A&M University, College of Veterinary Medicine, Department of Veterinary Pathobiology, USA
- Institute for Microbiology and Immunology, Faculty of medicine, Ljubljana, Slovenia
- Laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine, WHO Collaborating Center for Research and Training in Mediterranean Zoonoses, Heraklion, Crete, Greece
- University of Veterinary Medicine, Vienna, Austria

Current research



- Focused on ticks-different aspects, mostly impact on human health
- 1. Tick fauna, tick-borne pathogens (TBP), parasite-host-pathogen relationships, enzootic cycles of tick borne pathogens
- 2. Markers of tick bite, anti-tick vaccines - antigenic determinants of tick saliva
- 3. TBP Local strains - Isolation, cultivation and characterization of tick-borne pathogens (*Borrelia burgdorferi sensu lato*)
- 4. Antimicrobial susceptibility of local strains

- Grants:
National grant
International grants (mobility - COST, bilateral projects...)

COOPERATION – people!



Human and animal pathology



🦋 Vectors—significant

Do not cause diseases it self, but enable spreading of the pathogens-causative agents of diseases

- 10% of described species transmit pathogenic microorganism
- Early XX century it was determined that ticks may act as vectors of human diseases
- 80s – recognised as vectors of Lyme diseases
- Hard ticks more significant vectors of human diseases than soft ticks



Lyme borreliosis



CCHF



Tularemia



Rickettsiosis

Human and animal pathology



- Ticks were recognised as parasites of humans in Ancient Greece
- today **over 30 species** are parasites of humans
- **Direct influence** (blood feeding) – limited
- Mechanical injuries (especially species with long mouth parts-*Ixodes*, *Hyalomma*, *Amblyomma*)
- Local irritation, anemia, allergic reactions,
- Paralysis (North America-*Dermacentor andersoni*, *D. variabilis*; South Africa-*Ixodes rubicundus*, *Rhipicephalus eversti eversti*; Australia-*I. holocyclus*)



Local irritation
(*Ixodes* sp.)



Mechanical injury
(*Amblyomma* sp.)



Paralysis
(*Ixodes holocyclus*)



Allergic reaction
(*Ixodes holocyclus*)

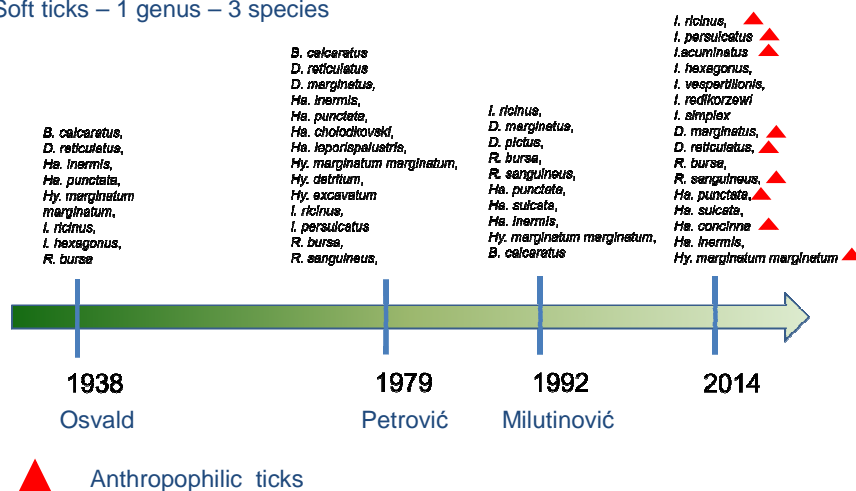
Studies on ticks in Serbia



Studies on ticks in Serbia commenced at the beginning of the XX century (mainly hard, exophilous tick species).

Hard ticks – 5 genera – 27 species

Soft ticks – 1 genus – 3 species



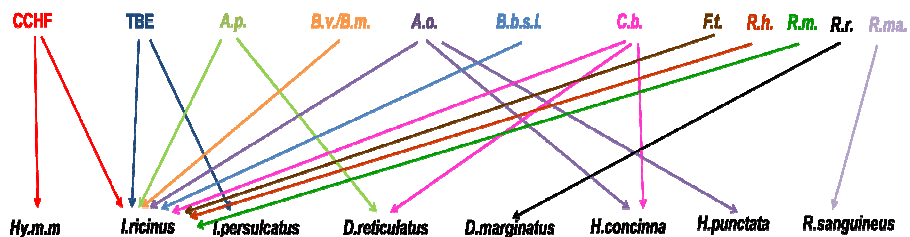
Studies of tick borne pathogens in Serbia



- Expansion of tick-borne pathogens in Europe
- Serbia is an endemic area for a number of tick-borne diseases (Lyme disease, CCHF, tularaemia, Q fever, human granulocytic anaplasmosis, TBE)
- Autochthonous cases of these diseases were continuously or periodically registered, but it is considered that the real epidemiological situation is underestimated.



Tick-pathogen relationships in Serbia

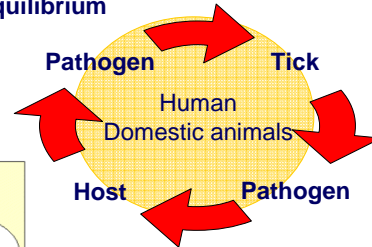
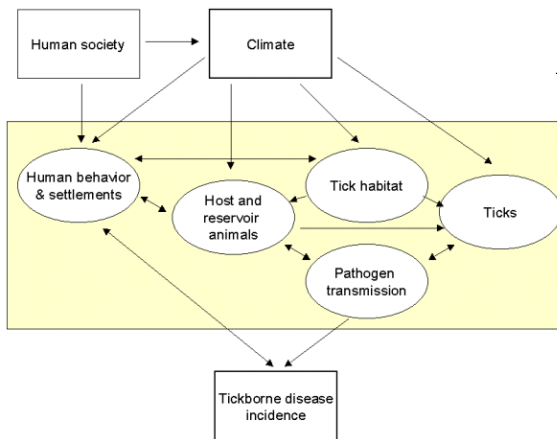


Relationship between viral and bacterial pathogen species and tick species in Serbia (Abbreviations: CCHF- Crimean-Congo Hemorrhagic Fever virus, TBE-Tick borne encephalitis virus, A.p.-*Anaplasma phagocytophilum*, B.v.-*Babesia venatorum*, B.m.-*B. microti*, A.o.-*Anaplasma ovis*, B.b.s.l.-*Borrelia burgdorferi sensu lato*, C.b.-*Coxiella burnetii*, F.t.-*Francisella tularensis*, R.h.-*Rickettsia helvetica*, R.m.-*R. monacensis*, R.r.- *R. raoultii*, R.ma.-*R. masiliae*)

Enzootic cycles and interrelationships



Coevolution
=
equilibrium

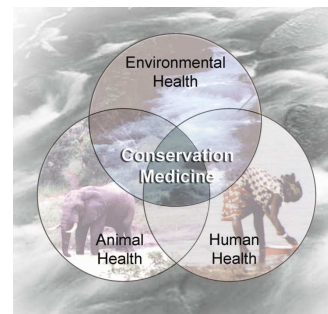


Conservation medicine-One health



- The concepts of **One health** and **Conservation medicine**
- Target species:
 1. Wild canids (golden jackal and red fox) – sinantrophic species
 2. European ground squirrel– nests/burrows on open habitats
 3. Bats – caves

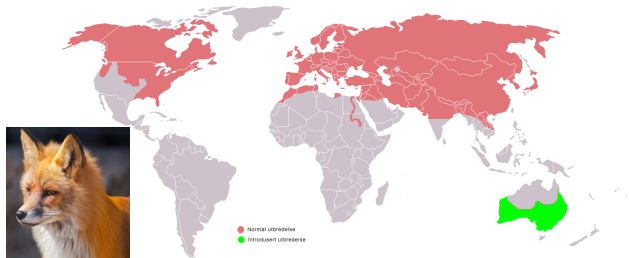
Sentinel species



Wild canids - red fox (*Vulpes vulpes*)



- Widely distributed species, inhabiting the whole territory of the state, adopted to live in different habitats
- The most abundant predatory species in our country
- Coming to the close proximity of human settlements-close contact with domestic or stay dogs
- In competition with golden jackals
- Hunting season throughout year
- Foxes play an important role in epidemiology of tick-borne diseases and in the maintenance of tick populations



Wild canids - red fox (*Vulpes vulpes*)



Study – PhD thesis Salem Juwaid

- During the period from 2010 to 2013
(2 winter seasons 2010/2011 and 2012/2013)
- 22 localities
- Hunted and roadkilled animals
- Data for each collected individual fox: sex, date of death and the most precise locality.
- Collected ticks were preserved in 70% ethanol until final identification
- Spleen samples
- Detection of pathogens in ticks and spleens



Wild canids - red fox (*Vulpes vulpes*)



- 67 red foxes - 41 ♂ 26 ♀
- Tick from 27 animals – 10 localities, 80 ticks (49 ♀ 18 ♂ 13n)
- 5 five ixodid species

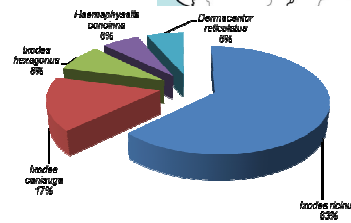
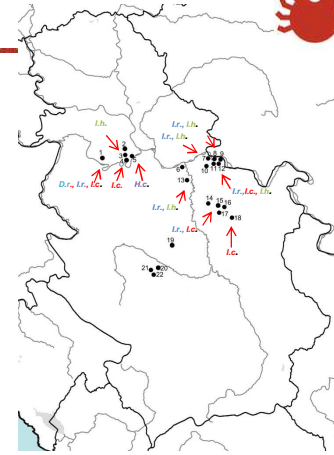
Ixodes ricinus - 38 ♀ 13 ♂ ▲

Ixodes canisuga - 2 ♀ 2 ♂ 9n

Ixodes hexagonus - 5 ♀ 2 ♂

Haemaphysalis concinna – 4n ▲

Dermacentor reticulatus - 4 ♀ 1 ♂ ▲



2nd International Symposium on Hunting, « Modern aspects of sustainable management of game populations » Novi Sad, Serbia, 17 – 20. October, 2013.

Original scientific paper UDC: 685.363.1:599.742.17

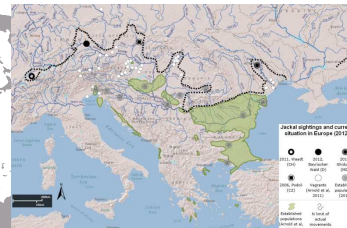
TICK SPECIES (ACARI: IXODIDAE) OF RED FOXES (*VULPES VULPES*) IN SERBIA

Tomarović, S.; Radović, Z.; Čabrl, S.; Mirošević, D.; Stokan, R.; Penezić, A.; Burazović, J.; Čerović, D.

Wild canids – golden jackal (*Canis aureus*)



- High spreading potential and sinantropic preferences.
- Dynamic population size and range
- **Europe** – declination (beginning XX cent.) → recolonization → rapidly increasing populations, widening ranges toward Central and Eastern Europe
- **In Serbia** - neared extinction World War II (poisoning). Only two relic populations in Srem and eastern Serbia near Bulgarian borders. Beginning of the 1980s, started to spread quickly along the rivers (Timok, Morava, Danube, and Sava) and to increase in number -fusion of the two relic populations and widening of the range. Local populations - the highest density at the Balkan Peninsula.
- An opportunistic species, capable of using a wide range of food sources, come close to human settlements
- In competition with red fox
- The increase in densities and the widening of populations have not been accompanied by research on ectoparasites and pathogens of interest both to animals and to humans.

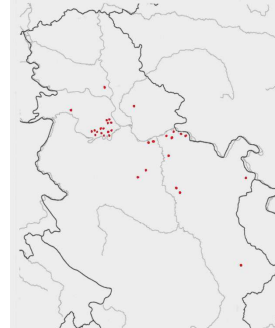


Wild canids – golden jackal (*Canis aureus*)



Study – PhD thesis Ratko Sukara

- During the period from 2010 to 2016
- 33 localities
- Hunted and roadkilled animals
- Data for each collected individual fox: sex, date of death and the most precise locality.
- Collected ticks were preserved in 70% ethanol until final identification
- Spleen samples
- Detection of pathogens in ticks and spleens



Bats



- With approximately 1200 species - around quarter of all mammalian species on Earth.
- Bats are hosts to a large number of ectoparasites, including ticks, which can act as vectors of zoonotic agents.
- Three hard tick species have adapted to live exclusively on bats (*Ixodes simplex* mainly on *Miniopterus schreibersii*, *I. kopsteini* on mastiff bats (*Tadarida* sp.), *I. vespertilionis* parasitizes different bat species).
- in Europe *I. simplex* and *I. vespertilionis* are recorded, together with the recently described species *I. ariadnae*, collected from caves and bats in Hungary.
- Increasingly recognized as reservoirs of emerging, mostly zoonotic, viral and bacterial pathogens.
- In comparison to other mammalian hosts, ticks parasitizing bats have been largely understudied, especially in the central part of the Balkan Peninsula, where the last data from the field research date from almost 25 years ago.



Figure 3. *Ixodes vespertilionis* - larva *



Figure 4. *Ixodes simplex* - larva *

Horová et al. Parasites & Vectors (2015) 8:47
DOI 10.1185/s12875-015-0486-4



SHORT REPORT

Open Access

Contributions to the morphology and phylogeny of the newly discovered bat tick species, *Ixodes ariadnae* in comparison with *I. vespertilionis* and *I. simplex*

Sándor Horová^{1*}, Jendř Konečný^{1,2}, Agnieszka Estrada-Peña³, Isabel G. Fernández de Mesa³, Snežana Tomonović⁴ and Joël de la Fuente^{5*}

Horová et al. Parasites & Vectors (2015) 8:47
DOI 10.1185/s12875-015-0486-4



RESEARCH

Open Access

High degree of mitochondrial gene heterogeneity in the bat tick species *Ixodes vespertilionis*, *I. ariadnae* and *I. simplex* from Eurasia

Sándor Horová^{1,2*}, Agnieszka Estrada-Peña³, and Horová^{1,2}, Oliver Plešinger⁴, Berna Avcı⁵, Andrej G. Mikulak⁶, Abdu Talab⁷, Snežana Tomonović⁸, Jolana Brázdová⁹, Nilsa Takao¹⁰, Tereza Čížková¹¹, Peter Engel¹², Yuqing Tian¹³, Martina Čížková¹⁴, Isabel G. Fernández de Mesa¹⁵, José de la Fuente¹⁶, Kamen Todorov¹⁷, Takanori Yamashita¹⁸ and Al Takano¹⁹



Bats



Study – PhD thesis Jelena Burazerović

- Balkans (Serbia, Montenegro, Bosnia and Herzegovina, and Former Yugoslav Republic of Macedonia -FYROM).
- April – October 2013 – 2015.
- 21 locality, from 149 bats belonging to 7 species
- Ticks were identified to the species level using morphological keys (Pomerancev 1950 , Arthur 1956).
- Molecular analysis of the cytochrome oxidase subunit I (COI) gene as a complementary.



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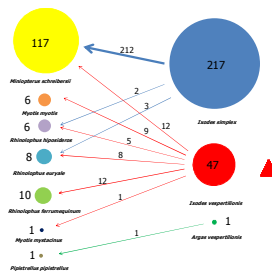
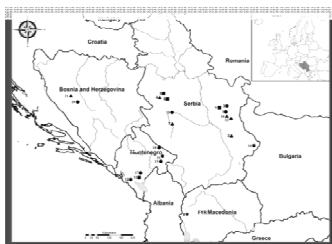
Ticks (Acari: Argasidae, Ixodidae) parasitizing bats in the central Balkans

Authors: Authors and affiliations

J. Burazerović, S. Čuček, S. Mihajlović, R. Sukara, D. Čović, S. Tomasević

265 ticks – 3 species

- *Ixodes simplex* -217 (24♀, 105 nymphs, 78 larvae)
- *Ixodes vespertilionis* - 47 (5 ♀, 24 nymphs and 18 larvae) ▲
- *Argas vespertilionis* (1 larva)



European ground squirrel (*Spermophilus citellus*)



- Medium size rodent species
- Inhabits open habitats with short-grass (steppe, dry meadows, pastures)
- Lives in colonies, in long complex burrow systems, with each individual having its own burrow system
- Behavioral characteristics make *S. citellus* exquisitely suitable host for ticks in the mentioned ecosystems.
- *Spermophilus citellus* is distributed in Southeastern and Central Europe, and its range is divided in two main geographic areas by the Carpathian Mountains.
- IUCN Red List of Threatened Species as vulnerable since 2008.



European ground squirrel (*Spermophilus citellus*)



•summer months (July-early September) 2007-2013, as a part of continuous population monitoring in Vojvodina (northern part of Serbia).

- 12 localities
- 1009 ticks were collected from 151 animals
- Morphological identification of ticks was performed using standard taxonomic key
- Molecular analysis of the cytochrome oxidase subunit I (COI) gene as a complementary.

2 species

- Ixodes laguri* – 796 (102♀, 2♂, 666n, 26l)
- Haemaphysalis concinna* – 213 (213n)

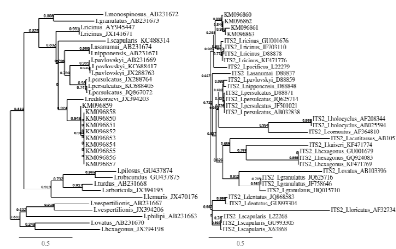
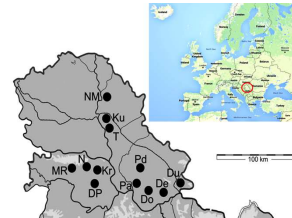


Fig. Phylogenetic analysis based on COI (a) and ITS2 (b) sequences of *Ixodes* sp. ticks obtained in this study (accession numbers KM096850-KM096863) and those previously deposited in the GenBank

Ongoing research – tick borne pathogens in ticks and reservoirs



- **Molecular** detection (ticks, different tissues)

- *Babesia* spp.,
- *Hepatozoon* spp.
- *Borrelia* spp.
- *Rickettsia* spp.,
- *Anaplasma* /*Ehrlichia* spp.
- *Coxiella burnetii*,
- *Francisella* spp.,
- *Bartonella* spp.
- *Leishmania*
- *Bartonella*

} Golden jackals

Red fox, golden jackal – ticks, tissue
bats, ground squirrel - ticks

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Research Article
Presence of *Leishmania* and *Brucella* Species in the Golden Jackal *Canis aureus* in Serbia

Darko Cirović,¹ Dimosthenis Chalkiadis,² Suzana Yovanović,² Ratko Sukara,² Aleksandra Penić,¹ Yannis Tselentis,^{3,4} and Anna Paraskevi¹



Thank you