

Participation of Bats (Chiroptera, Mammalia) and Their Ectoparasites in Circulation of Pathogens of Natural Focal Infections in the South of Russia

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Abstract—To determine the species composition of ectoparasites, 65 individuals of 6 species of vesper bats (Chiroptera: Vespertilionidae) were examined. Altogether, 521 specimens of 11 species of arthropods (gamasid mites, soft ticks, and fleas) were collected, of which *Steatonyssus noctulus* and *Nycteridopsylla eusarca* were recorded for the first time from Rostov Province. As the result of laboratory studies of the bats and ectoparasites associated with them in Rostov Province, DNA of *Borrelia burgdorferi* s. l., genospecies *Borrelia afzelii* was detected in samples of *Pipistrellus pipistrellus*, *P. pygmaeus*, *P. kuhlii*, *Eptesicus serotinus*, *Carios vespertilionis*, *Steatonyssus periblepharus*; DNA of *Borrelia* spp. was detected in samples of *Macronyssus flavus*; DNA of *Ehrlichia* spp., in samples of *P. pipistrellus* and *P. kuhlii*; DNA of *Anaplasma phagocytophilum*, in samples of *Nyctalus noctula*. Participation of bats and their ectoparasites in the epizootic process of tularemia was recorded for the first time, by detection of *Francisella tularensis* DNA in samples of *P. pipistrellus*, *N. noctula*, *P. kuhlii*, and *Cimex ex gr. pipistrelli*. Markers of the Crimean-Congo hemorrhagic fever virus were discovered for the first time in samples of *N. noctula* and *P. pipistrellus*.

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Bats (Chiroptera) serve as hosts of various blood-sucking arthropods involved in circulation of bacterial, viral, and protozoan infection agents; thus, together with other groups of mammals, bats facilitate the spread of infections. The presence of bats in human settlements and buildings determines their epidemiological significance as a possible factor of transmission of dangerous diseases.

Extensive studies carried out globally in the recent decades have demonstrated active participation of bats and their parasites in the epizootic processes of over 260 pathogens, including those posing epidemiological risks: Ebola, Marburg, influenza, rabies viruses, bacteria of the genera *Rickettsia*, *Coxiella*, *Borrelia*, *Anaplasma*,

Ehrlichia, etc. (Botvinkin, 1988; Leroy et al., 2005; Walter et al., 2005; Calisher et al., 2006; Selnikova et al., 2006; Towner et al., 2009; Shchelkanov et al., 2012; Mühlendorfer, 2013; Melaun et al., 2014; Pfaender et al., 2014; Veikkolainen et al., 2014; Cui et al., 2015; Mora-telli and Calisher, 2015; Allocati et al., 2016; Dietrich et al., 2016; Reeves et al., 2016; Orlova and Kononova, 2018).

In Russia and the former Soviet republics, bats and soft ticks associated with them were found to participate in circulation of rabies, Issyk-Kul, Sokuluk, and Uzun-Agach viruses as well as bacteria of the genera *Bartonella*, *Brucella*, *Leptospira*, etc. (Botvinkin, 1988; Botvinkin et al., 2003; Selnikova et al., 2006; Alkhovsky

Table 1. Collection localities of bats and their ectoparasites in the south of Russia

No.	Locality	Coordinates	Landscape	Date, bat species, and number of ectoparasites collected
Rostov Province				
1.	Rostov-on-Don	47°16'N 39°49'E	Within city boundaries	23.XI.2015, <i>N. noctula</i> (14) 22.VII.2016, <i>P. kuhlii</i> (2) 24.III.2017, <i>N. noctula</i> (1) 13.VIII.2017, <i>P. kuhlii</i> (1) 28.VIII.2017, <i>P. kuhlii</i> (1) 7.IX.2017, <i>P. kuhlii</i> (1) 13.XI.2017, <i>P. kuhlii</i> (2)
2.	Azovsky District, Leninsky forestry	46°45'N 39°08'E	Planted forest stand	2.VII.2016, <i>P. pipistrellus</i> (10) 23.VII.2016, <i>E. serotinus</i> (2) 9.VII.2017, <i>P. pygmaeus</i> (3) 28.VI.2017, <i>P. pipistrellus</i> (9) 13.VIII.2017, <i>P. pipistrellus</i> (3)
3.	Sholokhovsky District, Veshenskaya	49°37'N 41°43'E	Floodplain vegetation	1–5.VI.2015, <i>P. kuhlii</i> (1), <i>P. nathusii</i> (8), <i>P. pipistrellus</i> (3)
Astrakhan Province				
4.	Astrakhan	46°20'N 48°02'E	Within city boundaries	29.VII.2017, <i>P. kuhlii</i> (24)
5.	Astrakhan State Nature Biosphere Reserve	45°34'N 47°54'E	Floodplain vegetation	2.VIII.2017, <i>P. kuhlii</i> (1)

et al., 2013, 2014; Lvov et al., 2014a, 2014b; Bai et al., 2017; Urushadze et al., 2017). The role of bats and their specific parasites in circulation of viral and bacterial pathogens has not been assessed earlier in the south of European Russia, except for a study of lyssaviruses detected in the brain samples of the common bent-wing bat *Miniopterus schreibersi* in the West Caucasus (Botvinkin et al., 2003).

We have studied the specific fauna of bat ectoparasites in Rostov and Astrakhan provinces, using the manual examination and brushing methods. In addition, bats and their ectoparasites from Rostov Province were studied in the laboratory in order to describe the possible circulation pathways and to reveal new components of the parasitic systems for natural focal infections. Our research was mostly focused on the synanthropic bat species which pose the greatest risk of transmitting infection to people, in particular the pipistrelles *Pipistrellus* spp. and the common noctule *Nyctalus noctula* (Schreber, 1774).

MATERIALS AND METHODS

The material was collected in 2015–2017; the collection localities are given in Table 1.

Ectoparasites were collected with needles and forceps and preserved in 70% ethanol. The mites and ticks were mounted on slides in Faure-Berlese medium, the fleas were cleared in 10% aqueous KOH solution and also mounted in Faure-Berlese medium (Whitaker, 1988). Identification was carried out under a Nikon Eclipse 50i microscope, using the available keys and other taxonomic publications (Theodor, 1967; Medvedev, 1996; Stanyukovich, 1997). Altogether, 612 arthropod specimens, including gamasid mites, soft ticks, fleas, and true bugs, were collected in order to determine the faunistic composition and perform laboratory tests. The mean intensity (MI) of parasitism was calculated as the mean number of ectoparasites on one infested host; the incidence (P) of parasitism was calculated as the percentage of infested host individuals.

In the course of epizootological monitoring, bats (brain samples) and their specific parasites collected in

Rostov-on-Don and in Azovsky District in the south of Rostov Province were tested for pathogens of ixodid tick-borne borrelioses (TBB), human monocytic ehrlichiosis (HME), human granulocytic anaplasmosis (HGA), tularemia, Crimean-Congo haemorrhagic fever (CCHF), West Nile fever (WNF), tick-borne encephalitis (TBE), hemorrhagic fever with renal syndrome (HFRS), and Batai fever (Table 3). Testing of field samples for the DNA of TBB, HME, HGA, and tularemia pathogens was carried out by PCR, using the following test kits manufactured by Isogen Laboratories: PCR diagnostic kits for DNA of *Borrelia* spp., *Borrelia burgdorferi* s. l. (*B. burgdorferi*, *B. garinii*, *B. afzelii*), *Borrelia afzelii*, *Anaplasma phagocytophilum*; AmpliSense TBEV, *B. burgdorferi* s. l., *A. phagocytophilum*, *Ehrlichia chaffeensis/E. muris*; GenPak DNA amplification kits for *Ehrlichia* spp., *E. muris*/Yamaguchi, and *Francisella tularensis* + *F. holarctica*. The viral antigens of CCHF, WNF, TBE, HFRS, and Batai fever were detected by enzyme-linked immunosorbent assay (ELISA) using the VectoCrimea-CHF-antigen and VectoTBE-antigen kits manufactured by Vector-Best, the Bio-Screen-WNV-AG, BioScreen-Batai-AG, and BioScreen-TBE-AG kits manufactured by Bioservice Biotechnology Company, and also the Hantagnost hantavirus diagnostic kit manufactured by Chumakov Federal Scientific Center for Research and Development of Immunobiological Products.

RESULTS AND DISCUSSION

The Fauna of Bat Ectoparasites in the Study Region

To determine the species composition of bat ectoparasites, 65 individuals of 6 species of vesper bats (Chiroptera: Vespertilionidae) were examined, and 521 specimens of acarines and insects were collected off them (Table 2).

Eptesicus serotinus Schreber, 1774, serotine bat

A single specimen (N1) of the gamasid mite *Steatonyssus periblepharus* Kolenati, 1858 was collected off one individual of this bat species (Leninsky forestry, 10.VIII.2016).

Nyctalus noctula (Schreber, 1774), common noctule

Altogether, 4 species of acarines and 2 species of insects were found on 6 noctules.

Spinturnix acuminatus (C.L. Koch, 1836): ♀ (Rostov-on-Don, 23.XI.2015); 3 ♀ (Rostov-on-Don, 10.VIII.2016).

Spinturnix myoti Kolenati, 1856: ♀ with intrauterine larva (Rostov-on-Don, 10.VIII.2016).

Macronyssus flavus (Kolenati, 1856): 3 ♀ (including 2 with intrauterine larva) (Rostov-on-Don, 23.XI.2015); 2 N1 (Rostov-on-Don, 10.VIII.2016); 19 ♀ (all with intrauterine larvae), 6 ♂, 12 N1 (Rostov-on-Don, 24.III.2017).

Steatonyssus noctulus Rybin, 1992: ♀, 3 ♂ (Rostov-on-Don, 10.VIII.2016); 2 ♂ (Rostov-on-Don, 24.III.2017). The species is recorded for the first time for Rostov Province.

Ischnopsyllus elongatus (Curtis, 1832): ♂ (Rostov-on-Don, 23.XI.2015).

Nycteridopsylla eusarca Dampf, 1908: 6 ♀, 3 ♂ (Rostov-on-Don, 23.XI.2015). The species is recorded for the first time for Rostov Province.

Pipistrellus nathusii (Keyserling, Blasius, 1839), Nathusius' pipistrelle

Altogether, 2 species of gamasid mites and 1 species of insects were found on 8 individuals of this bat.

Macronyssus kolenatii (Oudemans, 1902): ♀ with intrauterine larva, 2 ♂, 2 N1 (Veshenskaya, 1.VI.2015).

Steatonyssus periblepharus: 52 ♀ (including 13 ♀ with intrauterine eggs), 22 ♂, 98 N1 (Veshenskaya, 1.VI.2015).

Ischnopsyllus variabilis Wagner, 1898: 27 ♀, 3 ♂ (Veshenskaya, 1.VI.2015).

Pipistrellus pipistrellus (Schreber, 1774), common pipistrelle

Altogether, 2 species of gamasid mites and 1 species of insects were found on 8 individuals of this bat.

Macronyssus kolenatii: 3 ♀ (1 with intrauterine larva) (Leninsky forestry, 2.VII.2017); ♀ with intrauterine larva (Leninsky forestry, 9.VII.2017), N1 (Veshenskaya, 1.VI.2015).

Steatonyssus periblepharus: ♀, ♂, 9 N1 (Veshenskaya, 1.VI.2015); 5 ♀ (1 with intrauterine larva), 2 ♂,

Table 2. Ectoparasites recorded on bats in the south of Russia

Parasite species	Host species						Range
	<i>Eptesicus serotinus</i> n = 1	<i>Nyctalus noctula</i> n = 6	<i>Pipistrellus nathusii</i> n = 8	<i>Pipistrellus pipistrellus</i> n = 8	<i>Pipistrellus pygmaeus</i> n = 10	<i>Pipistrellus kuhlii</i> n = 32	
Acari: Ixodida: Argasidae							
<i>Carios vespertilionis</i>	—	—	—	—	—	4 1.3 9	4 Palaearctic, Afrotropical, Indo-Malayan, and Australian regions
Mesostigmata: Gamasina: Spintumicidae							
<i>Spinturnix acuminatus</i>	— 4 2 33	— — —	— — —	— — —	— — —	4	West-Central-Palaearctic subboreal
<i>S. myoti</i>	— 1 1 17	— — — —	— — — —	— — — —	— — — —	1	Palaearctic from Great Britain to the Far East, southwards to the Mediterranean including North Africa
Macronyssidae							
<i>Macronyssus flavus</i>	— 42 14 50	— — — —	— 5 1.3 50	— 5 1.3 50	— 5 1 50	— 1 1 3	42 Trans-Palaearctic subboreal
<i>M. kolenatii</i>	— — — —	— 5 1.3 50	— 5 1.3 50	— 5 1 50	— 5 1 50	— 1 1 3	16 West-Central-Palaearctic boreal-subboreal
<i>Steatonyssus noctulus</i>	— 6 3 33	— — — —	— — — —	— — — —	— — — —	— — — —	6 West-Central-Palaearctic subboreal
<i>St. periblepharus</i>	1 — 24.6 87	172 — 9.4 88	66 — 8 40	32 — 5 72	117 — 5 72	388	Trans-Palaearctic boreal-subboreal
Insecta: Siphonaptera: Ischnopsyllidae							
<i>Nycteridopsylla eusarca</i>	— 9 9 17	— — — —	— — — —	— — — —	— — — —	9	West-Palaearctic subboreal
<i>Ischnopsyllus elongatus</i>	— 1 1 17	— — — —	— — — —	— — — —	— — — —	1	Trans-Palaearctic subboreal
<i>Ischn. octactenus</i>	— — —	— — —	— — —	5 1.7 30	8 1.6 16	13	West-Palaearctic boreal-subboreal
<i>Ischn. variabilis</i>	— — — —	30 5 75	4 4 13	3 1 30	— — —	37	West-Palaearctic boreal-subboreal
Total	1 63 16 67	207 29.6 10.7 87	75 5.6 5.6 100	45 4.2 4.2 80	130 97	521	

For each parasite species, the first row is the number of specimens, the second row is MI, and the third row is P (%).

44 N1 (Leninsky forestry, 2.VII.2017); 2 ♀, 2 N1 (Leninsky forestry, 9.VII.2017).

Ischnopsyllus variabilis: 4 ♀ (Veshenskaya, 1.VI. 2015).

Pipistrellus pygmaeus Leach, 1825,
soprano pipistrelle

Altogether, 2 species of gamasid mites and 2 species of insects were found on 10 examined individuals of the soprano pipistrelle.

Macronyssus kolenatii: 3 ♀ (including 2 with intrauterine larvae), N1 (Leninsky forestry, 3.VII.2016); ♀ with intrauterine larva (Leninsky forestry, 9.VII. 2017).

Steatonyssus periblepharus: 4 ♀ (2 with intrauterine larvae), N1 (Leninsky forestry, 3.VI. 2016); ♀, ♂, 25 N1 (Leninsky forestry, 9.VII.2017).

Ischnopsyllus variabilis: 2 ♀, ♂ (Leninsky forestry, 3.VII.2016).

Ischnopsyllus octactenus (Kolenati, 1856): ♂ (Leninsky forestry, 28.VI.2017); ♀, ♂ (Leninsky forestry, 2.VII.2016); 2 ♀ (Leninsky forestry, 9.VII.2016).

Pipistrellus kuhlii (Kuhl, 1817), Kuhl's pipistrelle

Altogether, 1 species of soft ticks, 2 species of gamasid mites and 2 species of fleas were found on 32 examined individuals of this bat.

Carios vespertilionis Latreille, 1796: 3 L (Astrakhan, 29.VII.2017); L (Astrakhan Nature Biosphere Reserve, 2.VIII.2017).

Macronyssus kolenatii: ♀ (Rostov-on-Don, 13.VIII. 2017).

Steatonyssus periblepharus: 6 ♀, 3 ♂, 5 N1 (Veshenskaya, 1.VI.2015); 30 ♀, 4 ♂, 26 N1 (Astrakhan, 29.VII.2017); 3 N1 (Astrakhan Nature Reserve, 2.VIII.2017); ♀, 4 ♂, 3 N1 (Rostov-on-Don, 13.VIII. 2017); ♂, 31 N1 (Rostov-on-Don, 13.XI.2017).

Ischnopsyllus octactenus: 2 ♀ (Rostov-on-Don, 2.VII.2017); ♀, 3 ♂ (Rostov-on-Don, 13.VIII.2017); ♀ (Rostov-on-Don, 7.IX.2017); ♀ (Rostov-on-Don, 13.XI.2017).

Laboratory Testing of Bats and Their Ectoparasites

Five species of bats and 5 species of their ectoparasites were tested in order to determine their possible role in circulation of natural focal pathogens in Rostov Province (Table 3). A common noctule and Kuhl's pipistrelle were captured and examined for parasites in the east part of Rostov-on-Don city. Serotine bats were captured in a hunting tower located in a planted forest stand in Azovsky District, in the south of Rostov Province.

Common and soprano pipistrelles were captured in a mixed maternity colony discovered in another hunting tower in the same planted forest. The bats assemble there at the end of April and reproduce, so that by the end of June and the beginning of July the colony grows to 150–300 pipistrelles of both species. Young pipistrelles found shelter under the roof and under the bitumen felt lining of the tower. Various ectoparasites (gamasid mites, soft ticks, fleas, and bugs) were collected off the tower walls, from under the lining sheets, and off the bats captured in this colony.

All the examined bat species are common in the study region and abundant in some of its districts (Gazaryan et al., 2010).

Testing of bats and their ectoparasites for bacterial pathogens yielded the following results (Table 3).

DNA of bacteria of the genus *Borrelia* was detected in 18 out of 44 samples (40.9%) of bats and their ectoparasites. *Borrelia burgdorferi* s. l. of *Borrelia afzelii* genospecies prevailed among the positive samples: it was detected in 10 brain samples (52.6%) of the common pipistrelle, in 2 samples of the serotine bat, in larvae and adults of *C. vespertilionis* collected in the maternal bat colony, and also in 1 sample each of Kuhl's and soprano pipistrelles and the mite *S. periblepharus* collected off a common pipistrelle. DNA of *Borrelia* spp. was detected in a single sample of *Macronyssus flavus* collected off a common noctule. Participation of bats and their ectoparasites in circulation of *Borrelia* was demonstrated earlier (Hubbard et al., 1998; Socolovschi et al., 2012; Mühlendorfer, 2013; Cutler et al., 2016).

During testing of five species of bats for the presence of HME and HGA pathogens, DNA of *Ehrlichia* spp. was detected in brain samples of the common and Kuhl's pipistrelles (47.4% and 20.0%, respectively); DNA of

Table 3. Laboratory testing of bats and bloodsucking arthropods collected in Rostov Province, 2015–2017

Species	Number of bats and bloodsucking arthropods tested for markers of natural focal pathogens: number of specimens/samples/positive tests										
	TBB	Borrelia burgdorferi s. l.	Borrelia garinii	HME	HGA	tularemia	CCHF	WNF	TBE	HFRS	Batai fever
<i>Pipistrellus pipistrellus</i>	22/19/10	22/19/10	22/19/0	22/19/9	19/18/0	22/19/1	21/21/2	22/22/0	15/15/0	3/3/0	—
<i>Pipistrellus pygmaeus</i>	3/2/1	3/2/1	3/2/0	—	3/2/0	3/3/0	—	—	—	—	—
<i>Pipistrellus kuhlii</i>	7/6/1	7/6/1	7/6/0	6/5/1	7/5/0	7/5/2	7/6/0	—	6/5/0	—	4/4/0
<i>Nyctalus noctula</i>	7/7/0	7/7/0	7/7/0	5/5/0	7/7/1	7/7/1	15/15/2	2/2/0	6/6/0	—	5/5/0
<i>Eptesicus serotinus</i>	2/2/2	2/2/2	2/2/0	2/2/0	2/2/0	2/2/0	2/2/0	2/2/0	2/2/0	—	—
<i>Cimex ex gr. pipistrelli</i>	6/2/0	6/2/0	6/2/0	5/1/0	6/2/0	1/1/1	5/1/0	—	5/1/0	—	—
<i>Macronyssus flavus</i> from <i>N. noctula</i>	20/1/1	20/1/0	20/1/0	—	20/1/0	20/1/0	20/1/0	20/1/0	20/1/0	—	—
<i>Steatonyssus</i> <i>periblepharus</i> from <i>P. pipistrellus</i>	2/1/1	2/1/1	2/1/0	—	2/1/0	2/1/0	2/1/0	2/1/0	—	—	—
<i>Ischnosyllus</i> <i>octactenus</i> from <i>P. pipistrellus</i>	7/1/0	7/1/0	7/1/0	—	7/1/0	7/1/0	7/1/0	7/1/0	7/1/0	—	7/1/0
<i>Lipoptena fortiseta</i> * from deer	1/1/1	1/1/1	1/1/1	1/1/0	—	—	1/1/1	—	—	—	—

* The deer ked *Lipoptena fortiseta* was captured in a pipistrelle colony. Abbreviations of the pathogens are explained in the text.

Anaplasma phagocytophilum was detected in 1 out of 7 brain samples of the common noctule. Soft ticks associated with bats are known to be involved in circulation of *Ehrlichia* and *Anaplasma* (Socolovschi et al., 2012; Mühlendorfer, 2013; Lv et al., 2018).

During testing of our material for DNA of the tularemia pathogen, the presence of *F. tularensis* was for the first time detected in brain samples of the common pipistrelle (5.3%) and common noctule (14.3%), in 2 out of 5 samples of Kuhl's pipistrelle, and also in a single sample of the bug *Cimex ex gr. pipistrelli*. Analysis of the literature shows that *F. tularensis* has not been previously found in bats or their specific parasites.

It should be specially noted that a single specimen of the deer ked *Lipoptena fortisetaosa* Maa, 1965 (Diptera: Hippoboscidae) a parasite of large ungulates, was captured in the pipistrelle colony. Testing of this specimen revealed concurrent infection with *B. afzelii* and *F. tularensis*.

During ELISA testing for viral natural focal infections, markers of the Crimean-Congo haemorrhagic fever virus were for the first time detected in brain samples of the common noctule and common pipistrelle (9.5 and 13.3% of positive samples, respectively). According to the data of Müller and co-authors (2016), blood serum samples of bats were tested in Central Africa (Ghana, Gabon, the Congo), Central America (Panama), and West Europe (Germany); as a result, markers of the CCHF virus were detected in samples of African bats collected in Gabon and the Congo.

Testing of our material for other viruses (WNF, TBE, HFRS, and Batai) yielded negative results.

Thus, our research has revealed 11 species of parasites associated with 6 species of bats in the south of Russia; *Steatonyssus noctulus* and *Nycteridopsylla eusarca* were recorded for the first time in Rostov Province. The results of laboratory testing indicated possible circulation of the agents of ixodid tick-borne borrelioses, anaplasmosis, ehrlichiosis, tularemia, and Crimean-Congo haemorrhagic fever in bat populations and specific bat parasites in the southwest of Rostov Province. These findings are indirect evidence that bats and their ectoparasites may be involved in the maintenance of parasitic systems with specific circulation pathways, different from the usually considered schemes. It should

be noted that DNA of the tularemia pathogen was detected for the first time in samples from bats and true bugs. The discovery of *F. tularensis* DNA and CCHF viral antigen in brain samples of bats indicate a possible role of bats in pathogen preservation, as a new component of the corresponding parasitic systems. The interrelations of bats, their ectoparasites, and natural focal infection agents require further study.

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