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New research directions of bats in Azerbaijan - bats as a potential reservoir of some zoonotic diseases

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Bats - the only flying mammals, accounting for 25% (33 species) of the Azerbaijani mammalian fauna. Those have also been focused on by the scientific community, zoologists, veterinarians, and virologists for the recent 20 years because of their assumed or confirmed role in the spillover of several new zoonotic diseases. Such studies have not been carried out so far in Azerbaijan and the situation with the study of other bats diseases is not satisfactory. Thus, the national studies on bats on ecto-, endo- and blood-parasites require at least an update, dating back to the 70s of the last century. This paper provides an overview of the potential or confirmed reservoir role of bats in a number of pathogens, the importance of research in this area and, in particular, the first research attempts in coronary studies conducted in Azerbaijan within the frame of Western Asia Bat Research Network.

Keywords: *Bats, zoonotic diseases, parasites, sampling site, colonies, Western Asia*

INTRODUCTION

Bats have attracted the attention of the scientific community over the last 20 years due to the possibility of being a natural reservoir of some viruses (especially coronaviruses) and have become the subject of relevant multi-disciplinary research projects (Kuzmin et al., 2011). The epidemiological side of bats, especially probability of them as potential natural reservoir of viral diseases has never been studied in Azerbaijan. In addition to general faunal and zoological researches, the study of bats from different ecological groups and species as a natural reservoir in the spread or spill over of ecto, endo- parasites, as well as viral diseases is of great importance. As part of a regional multidisciplinary study in Western Asia, investigations being conducted in Azerbaijan will determine the role of local bat fauna representatives on the regional epidemiological map of bat borne diseases.

MATERIALS AND METHODS

As a first step, bats were obtained by dedicated mist (japan) nets at the entrances of shelters (anthropogenic and natural habitats) during evening fly out and morning return into the roosts via application of standard techniques (Kunz et al., 1996). In some cases, individuals were captured from summer shelters manually or by using special catchers-butterfly nets. Each individual caught was first identified up to the species level, age and sex of animal were determined by generally accepted methods.

A blood sample (from one of the large vessels of tail), an oral swab, and rectal swab (in absence of excrement) were then taken from each individual and fixed in an RNA-Later solution (Fig. 1). Smaller wing punches were also taken for further DNA analysis for species having controversial taxonomy. All samples were stored at -80° C (in cruioshipper with nitrogen solution) and then transferred into an ultra-cold refrigerator for further storage until lab analysis execution.

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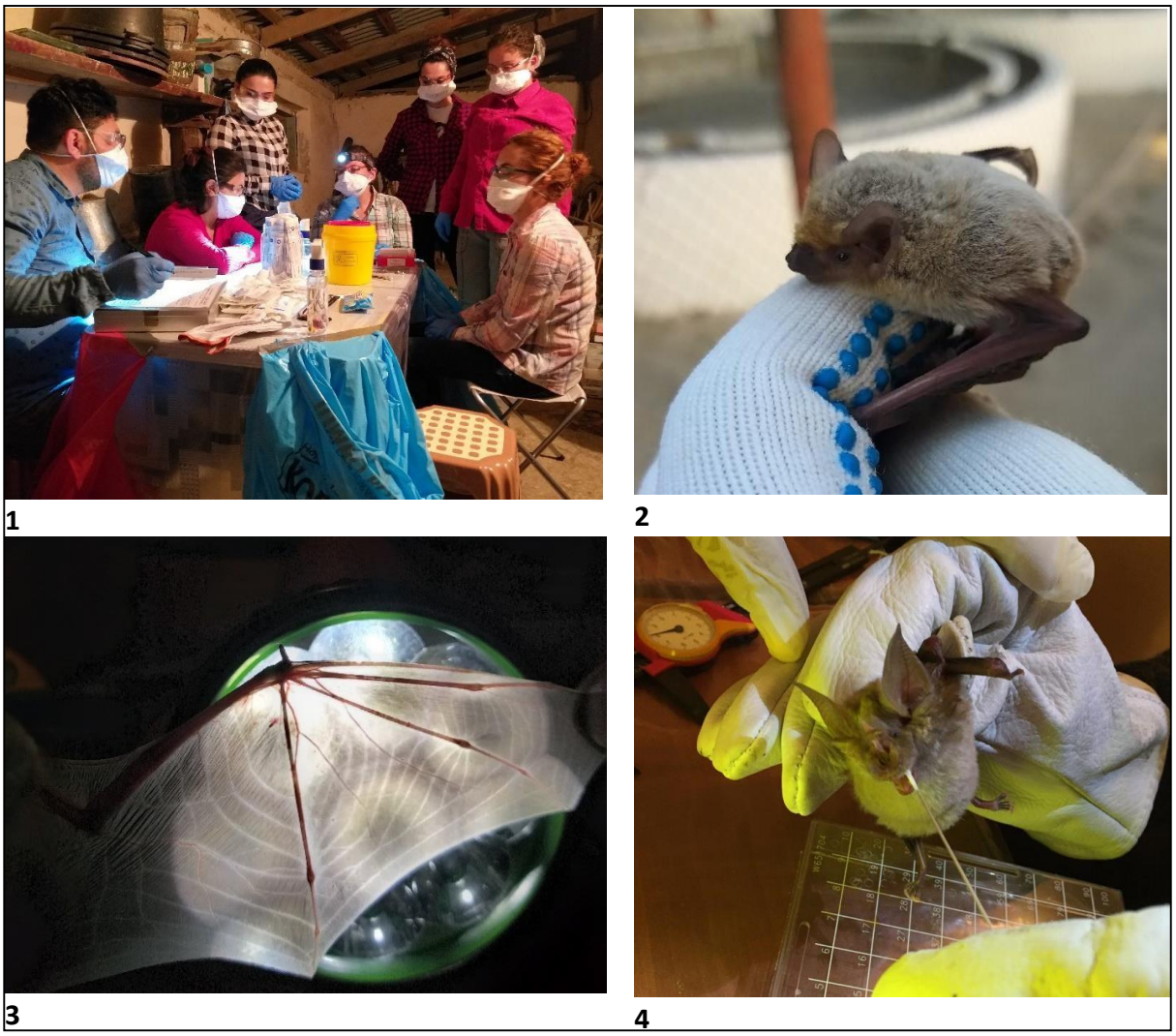

Fig. 1. Sampling: (1– team, 2 – *Pipistrellus kuhlii*, 3 – age determination via wing bones, 4 – oral swab taking from *Rhinolophus ferrumequinum*).

To determine ectoparasites of bats, beetles and other insects from the fur of individuals were collected and put into special solutions, blood films (peripheral blood smear) were prepared from collected blood samples and fixed in 70% methanol, excrements were fixed in alcohol solution (Dryden et al., 2005; Houwen, 2020).

Blood parasites and ectoparasites will be identified by the specialists of the Institute of Zoology, viral (coronoviruses) analysis will be carried out in the Georgian NCDC laboratory by application of appropriate methods. According to the protocol, 90 individuals were obtained from each sampling site (bat colony), and upon completion of sampling the bats were released.

Three sampling sites: 1. Greater Caucasus (Gakh city), 2. Lankaran Natural Province (Fishermen's settlement near the Kyzylagach reserve) and 3. Caspian lowland (Neftchala district, Mayak settlement) were visited over the period of 2019-2020 for bats' samples collection (Fig. 2).

RESULTS AND DISCUSSION

Among the diseases of bats in Azerbaijan, their parasitic fauna has been studied the most. In 1966, research on 700 individual bats of 15 species revealed 24 helminth species (Shakhtakhtinskaya et

al., 1971; Sadikhov, 1978). The most common helminth-infected species were *Rhinolophus ferrumequinum* (greater horseshoe bat) and *Nyctalus noctule* (noctule bat). Blood parasites were found in some local bat species (Zeyniyev, Rakhmatulina 1990) and infection was recorded in 70.3% of 30 individuals belonging to eight species. Four types of blood parasites: *Trypanasoma, Babesia, Grahamella, Micrafilaria* were registered and it was confirmed that the frequency of invasion was low in infected individuals, and in some cases, individual parasites were found.

Weak coccidial infections in bats have also been reported (Musayev, 1976; Musayev, Gauzer 1971). *Eimeria vespertilii* Musayev and Veysov, 1961; *E.zakirica* Musayev, 1967, and *E. mehelyii* sp.n species are described in *Rhinolophus mehelii*.

Ectoparasites have been better studied in bats (Dubovchenko, 1969; Mulyarskaya, 1978; Hadjiyev et al., 1982). From 1,572 individuals of 11 species, 97.3% were found to be infected with 90 species of ectoparasites, of which 91% were ticks, 36.2% were blood-sucking flies, 19.1% were fleas, and 1.7% were lice. A richer ectoparasite fauna has been revealed among the more densely colonized bats. The majority of ectoparasites (97.2%) were specific to bats only and were not similar to the ectoparasitic fauna of birds and rodents. According to Dubovshenko (1976), more than 20 of the 90 ectoparasite species in bats of Azerbaijan might be carriers or reservoirs of infectious and invasive pathogens.

Unfortunately, the potential role of bats in the spread of viral diseases in Azerbaijan has not been assessed. Although rabies has been recorded in bats for more than a century, the carrier of some pathogens that have emerged in recent years (lissaviruses, henipaviruses, coronaviruses) have been confirmed to be bats, while others (filoviruses) are still being assumed to be bats. New pathogens are being recorded in bats.

Rabies is caused by lissaviruses of the *Rhabdoviridae* family and is characterized by the highest mortality rate. For 10 of the 11 species of lissavirus, bats play a major reservoir role (Kuzmin et al., 2011). While the classical rabies virus (RABV) circulates only in American bats, more different types of lissavirus (EBLV-2 and EBLV-2) are known in Europe from 3 bat species (*Eptesicus serotinus, Myotis daubentonii, M. dasycneme*), two of which are widespread in our country. *E. serotinus* is a common species and is closely related to manmade structures, as well as forming mixed colonies with *P. kuhlii*, the most common type of synanthropic bat.

Filoviruses are known as Marburg (MARV) and Ebola (EBOV) viruses. The geography of the first disease is assosiated with Africa and Sahara, and although the virus was recorded only in fruit-eating bats in 2001-2005, the MARV virus was later reported in Congo, in bats of *Miniopterus* and *Rhinolophus* genuses. Representatives of both genused are included into bat auna of Azerbaijan.

Severe Acute Respiratory Syndrome (SARS), which has been known to cause coronaviruses (CoV) since 2002, has also been reported in bats (and other mammals). Later, SARS-like viruses were found in bats in Europe, South America, Australia and Africa. Alpha and beta coronoviruses, known from bats of the Vespertilionoidae family, have been reported to be genetically similar to the coronovirus described from China. The basis of the fauna of Azerbaijani bats are the species of the family Vespertilionoidae (25 out of 33 species).

The geographical position of Azerbaijan has led to the diversity of bats, and has created a basis for the combination of different bat species with different zoogeographical origin (Fig. 3). From 33 local bat species, 41% are Trans-Paleoarctic, 35% East Asian arid and temperate, 15% European, and 9% Turan desert and semi-desert species (Rakhmatulina, 2005). Studying of bats and assosiated diseases (in migrant and sedentary species) is even more important in such an environment. Particularly, it is important to take preventive measures against viral diseases that may be dangerous to human health.

Bearing in mind the above mentioned note, the first field research on the study of coronoviruses in bats was launched in Azerbaijan in 2019 within the West Asian Bat Network (WAB-Net) project, and the second part of the research was completed in August and September of 2020. It is planned to inspect bats from 3 more new sampling sites during summer-autumn season of 2021. In accordance with protocol requirements, 90 individual bats were obtained from each sampling site, relevant rectal, oral swabs, excrement, blood were collected as well as wing punches (DNA samples) were taken after which the animals were finally released.

In September 2019, two species (*P. kuhlii* - 88 individuals and *P. nathuisii* - 2 ind.) from a sampling site in Mayak settlement of Neftchala region, in August 2010, 4 species (*P. kuhlii* - 29 ind., *P. pipistrellus* - 40 ind., *P. nathuisii* - 20 individuals and *Eptesicus serotinus* - 1 individual) from a sampling site at Balikchylar settlement of Lankaran region, and in September 2020, 4 more species (*P. pipistrellus* - 1 ind., *Myotis emarginatus* - 47 ind., *Rhinolophus ferrumequinum* - 37 ind. and *Rh. hipposideros* - 5 ind.) from sampling site in Gakh town were inspected (Table 1).

The target of the field works is bats from different ecological groups, both for synanthropic species associated with manmade structures and natural shelters (caves, rocks, tree hollow etc.). The research will be continueing in the coming years and will cover bat species from different natural provinces of the country. As similar studies will be conducted in the South Caucasusian and a number of Western Asian countries by the end of 2021, the role of Azerbaijani bats in the epidemiological situation of coronaviruses in the region will be clarified. Laboratory tests will continue until the end of 2021, and the results will be published and posted.

In order to look for ecto- and endo (blood)-parasites of bats, blood films (peripheral blood smear) were prepared from 50 bat individuals blood samples, including the species *Myotis alcatoe* (2 individuals) and *Pipistrellus kuhlii* (8 individuals) captured in Sitalchay settlement of Khizi region and Chayli village of Gobustan in addition to 7 species (270 individuals) of bats obtained for viral investigations. 200 smears were prepared on the basis of individual blood samples, ectoparasites from 30 individual bats were isolated and collected for further identification.

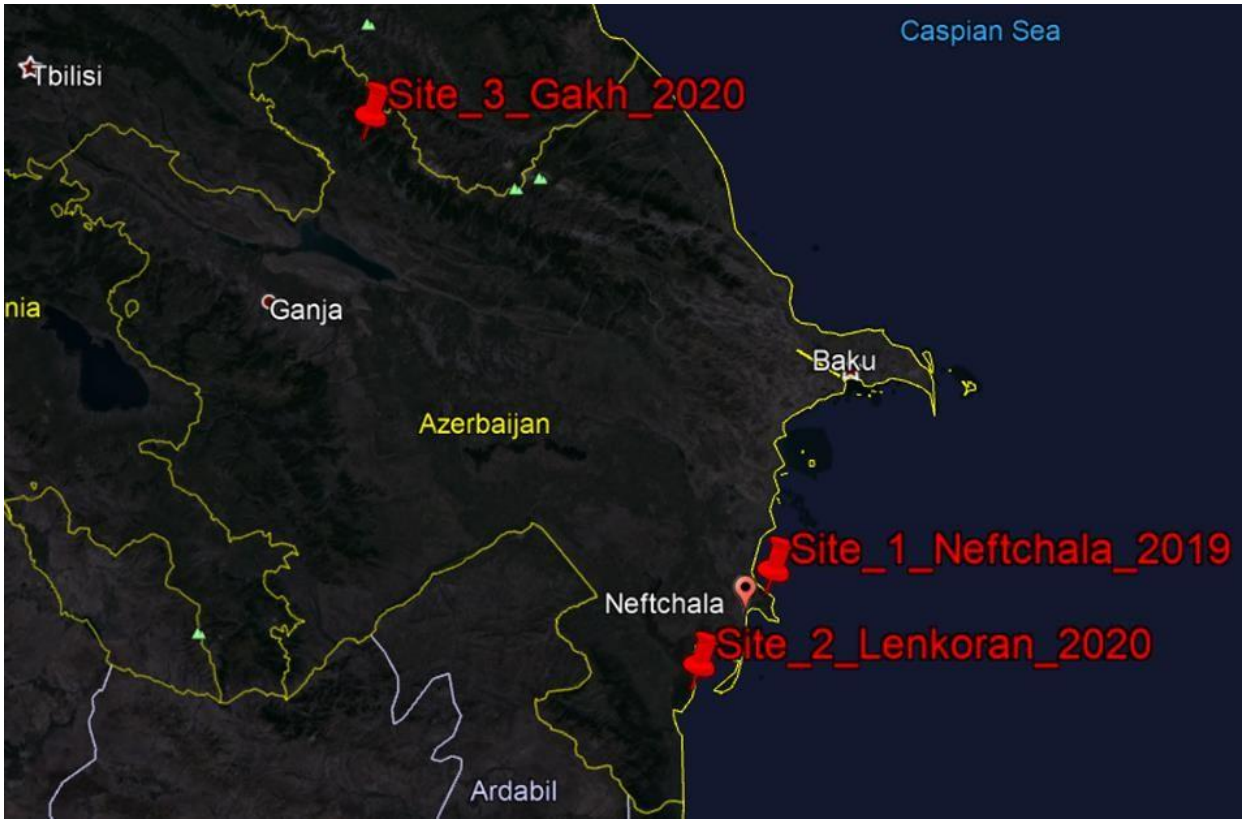

Fig. 2. Sampling sites of bats in Azerbaijan (period of 2019-2020).

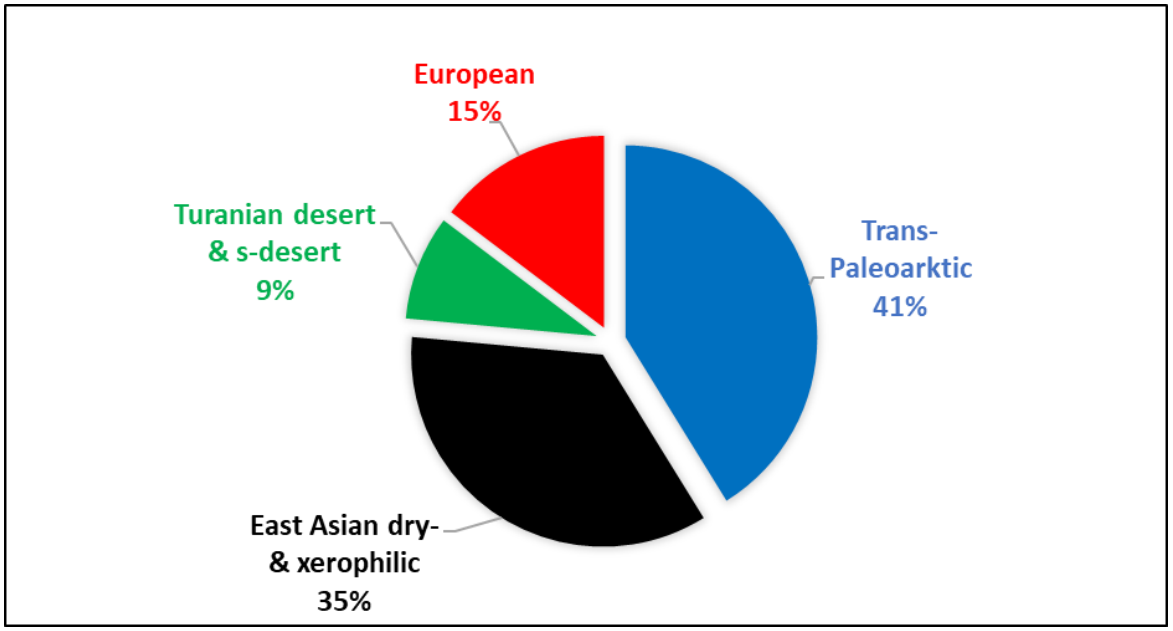

Fig. 3. Zoogeographical origin of bats in Azerbaijan.

Tab.1. Bats species and number of individuals captured for further analysis over the period of 2019-2020.

Bat species		Site 1: Neftchala	Site 2: Lenkoran	Site 3: Gakh
Scientific name	Common name	Number of individuals		
1. *Pipistrellus kuhlii*	*Kuhl's pipistrelle*	88	29	0
2. *P. pipistrellus*	*Common pipistrelle*	0	40	1
3. *P. nathusii*	*Nathusi'spipistrelle*	2	20	0
4. *Eptesicus serotinus*	*Serotine bat*	0	1	0
5. *Myotis emarginatus*	*Geoffroy's bat*	0	0	47
6. *Rhinolophus ferrumequinum*	*Greater horseshoe bat*	0	0	37
7. *Rh. hipposideros*	*Lesser horseshoe bat*	0	0	5

These studies aslo revealed new summer and winter roosts of greater and lesser horshore bats (*Rh.ferrumequinum* and *Rh.hipposideros*), as well as Geoffroy's bat (*Myotis emarginatus*) in the Greater Caucasus, summer colony of alcato bat (*Myotis alcatoe*) in Khyzi and Gobustan region. These are the new addendums onto the distribution maps of bats in Azerbaijan

CONCLUSIONS

1. The first- and second-year bat sampling studies for inspection of bats as potential natural reservoir of coronaviruses have been completed in Azerbaijan.
2. In total, 270 individuals from seven species of bats were obtained from three natural geographic provinces. Blood samples, oral and rectal swabs (or excrement), and wing punches (DNA samples) were obtained from each individual for the cases if extra taxonomic identification will be required, respectively.
3. In parallel, samples for ecto-, endo-, blood parasites of bats captured have been collected for further cameral analysis, which will refresh and fulfil the aged (50-60 years old) data and much more local bat species will be inspected. Local bat species were last studied in the 1960s. Ecto-parasites of only

11 species, blood parasites of eight bat species, helminths of 15 species of bats were investigated.

4. For the first time, new winter and summer roosts of four bat species (greater and lesser horseshoe bats, Geoffroy's bat and Alcato bat) were revealed in the Greater Caucasus.

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Azərbaycanda yarasaların yeni tədqiqat istiqamətləri - yarasalar bir sıra zoonoz xəstəliklərin potensial rezervuari kimi

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Azərbaycan məməlilər faunasının 25%-i (33 növ) yeganə uçan məməlilər olan yarasalar sinfinin payına düşür. Yarasalar həm də bir sıra yeni aşkar olunan zoonoz (virus) xəstəlikərin yayılmasındakı roluna görə xüsusliə son 20 ildə elmi ictimaiyyətin, zooloq, veterinar, virusuloqların diqqət mərkəzində olub müvafiq kompleks tədqiqat layihələrinin obyektlərinə çevirlimişlər. Bu kimi tədqiqat işləri ölkəmizdə həyata keçirliməyib və yarasaların digər xəstəliklərinin öyrənilməsi vəziyyəti də qənaətbəxş deyildir. Beləki, yarasaların ekto-, endo- və qan parazitləri üzrə respublikada aparılmış araşdırmaları keçən əsrin 70-ci

illərinə təsadüf etdiyindən yenilənmə tələb edir. Məqalədə yarasaların bir sıra virus xəstəliklərinin potensial və ya təsbit olunmuş rezervuar rolunun əhəmiyyəti, bu sahədə araşdırmaların vacibliyi və xüsusilə korona-virusların öyrənilməsi sahəsində həyata keçirilən ilkin işlər haqqında məlumat verilir.

Açar sözlər: *Yarasalar, zoonoz xəstəlikləri, parazitlər, nümunəgötürmə sahələri, koloniyalar, Qərbi Asiya*

Новое направление исследований летучих мышей в Азербайджане - летучие мыши как потенциальный резервуар ряда зоонозных заболеваний

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Летучие мыши, являющиеся единственными летающими млекопитающими, составляют 25% фауны млекопитающих Азербайджана (33 вида). В течение последних 20 лет летучие мыши были в центре внимания научного сообщества: зоологов, ветеринаров и вирусологов, особенно из-за их роли в распространении ряда недавно открытых зоонозных (вирусных) болезней. Подобные исследования в нашей стране не проводились, и ситуация с изучением других болезней летучих мышей остается неудовлетворительной. Таким образом, исследования экто-, эндо- и кровяных паразитов летучих мышей в стране требуют обновления, так как относятся к 70-м годам прошлого века. В статье представлена информация о потенциальной или установленной резервуарной роли летучих мышей в ряде вирусных заболеваний, важности исследований в этой области и, в частности, о начальной работе, проводимой для изучения коронавирусов.

Ключевые слова: *Летучие мыши, зоонозы, паразиты, пункты отбора проб, колонии, Западная Азия*