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„Seroprevalence of sandfly-borne viruses in Austrian
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1 Introduction

1.1 An introduction to Virology

To date, more than 7,000 viral genomes have been sequenced, with 2284 species officially assigned by the International Committee of Taxonomy of Viruses (Fauquet 2008, King et al. 2011). Viruses are obligate intracellular, host-dependent infectious particles, constraining of either RNA or DNA, proteins and a lipid layer. Inside of the host cell, viruses have the ability to reproduce, a major feature of living organisms. A significant difference to cellular organisms is their mode of replication. After host cell entry, viruses break up into different components, replicate and assemble afterwards (Kann 2010). Viruses can infect every type of prokaryotic and eukaryotic cell, potentially causing enormous damage to the affected cell. However, viruses are considered to play major roles in the evolution of the three domains of life (Forterre 2006). In the recent past, viruses have found to be useful as a source for pesticides, anti-cancer agents or as gene vectors (Carter & Saunders 2007).

The beginnings of virology can be dated back to the 19th century, when scientists first assumed the presence of infectious agents other than bacteria. These objects were called viruses (latin: mucus, poison) and considered as invisible, not filterable and not culturable. Eventually, Ernst Ruska managed to illustrate *Vaccinia* virus, which was used for the smallpox vaccine, by electron microscopy in 1938. Initiating a new era in virology, he received the Nobel Price for Physics in 1986 for the invention of the electron microscope (Ruska et al. 1939, Robinson 1986, Hof & Dörries 2005, Riedel 2005).

1.1.1 The origin and evolution of viruses

Viruses are assumed to be numerically the most frequent biological units on earth, yet evolutionary processes are still not entirely clear (van Regenmortel & Mahy 2010). Assuming that all living cells originate from LUCA (last universal common ancestor), a universal tree of life can be drawn. The origin

of viruses is much more complex and no such tree can be deduced (Forterre & Prangishvili 2009). Several hypotheses have been postulated on the origin of viruses. Traditional views are the evolution of the viruses from genes from pro- and eukaryotes, or the origin from an ancient viral world, previous of cellular life. A modern hypothesis describes the reduction to cellular life forms to RNA viruses (Gilbert 1986, Woese 1987, Forterre 2005, Koonin et al. 2006). Satisfying answers have not been found yet, while Carter & Saunders (2007) assume several origins as a result of the huge diversity of replication procedures, genome types and virion structures, Forterre (2006) suggests an evolution of the DNA viruses from the RNA viruses. With short generation times, a high number of offspring and their comparatively simple structure, viruses are optimal units for research on evolutionary processes (Modrow et al. 2010).

1.1.2 Virus components and structure

Infectious virus particles vary from 16 nm (Circovirus) to more than 300 nm (Mimivirus) in size and consist of several components (Figure 1). Viruses strictly contain either RNA or DNA, which can be present as single strand or double strand. Single strand nucleic acids can vary in polarity, +, - or +/- (ambisense) (Modrow et al. 2010). Viruses differ considerably in their genome sizes. While hepatitis delta virus has 1.7 kilobases (kb), the Mimivirus genome consists of over 1000 kilobases (kb) (Carter & Saunders 2007). Proteins contribute significantly to the mass of a virus and are coded by the viral nucleic acids produced by the host cell. The structural proteins build up the stable envelope and the regulatory proteins are in charge of transcription and replication of genes. Some viruses are additionally packed with enzymes and functional analog host proteins, essential for the survival of a particle in the host cell. Additionally, certain viruses are enveloped by a

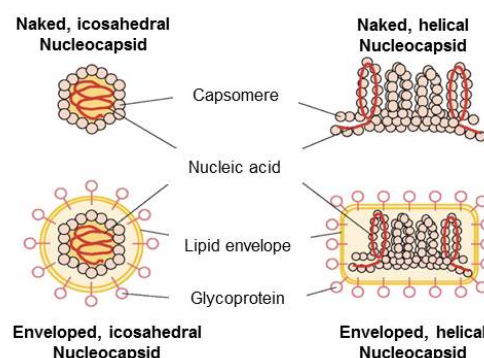


Figure 1. Basic structure of a virus.
Modified from Hof & Dörries 2005.

double lipid layer originating from the host cell. Glycoproteins are embedded, containing a cytoplasmatic, transmembrane and extracellular part. Capsids are built of multiple subunits, the capsomeres, and form, together with nucleic acids, the nucleocapsid. The assembly of the nucleic acid is differentiated into helical or cubic (Hof & Dörries 2005).

1.1.3 Classification

In early classifications, viruses were defined and separated from bacteria by their physicochemical features (Hof & Dörries 2005). Different classification systems have been established, suitable

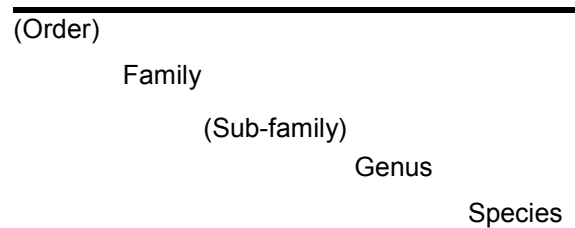


Figure 2. Hierarchy of recognized viral taxa.

for various scenarios. An anthropomorphic scheme is the classification by disease, important for health issues but excluding those not causing diseases. Other systems are based on the basis of the host organism, on virus particle morphology, viral nucleic acids or on the basis of phylogeny (Dimmock et al. 2016) (Table 2). In 1966, a first attempt to universally classify viruses was made at the International Congress of Microbiology in Moscow. Since then, the International Committee of Taxonomy of Viruses (ICTV) has been in charge of the classification and nomenclature of viruses (King et al. 2011). The first report was published by Wildy (1971). The concept of virus species as the lowest taxon in a branching hierarchy was introduced in the 7th report of Virus Taxonomy, by Van Regenmortel et al. (2000) (Figure 2).

The 9th report of virus taxonomy includes 2284 virus species, 349 genera, 19 subfamilies, 87 families and 6 orders. Additionally, a chapter including a huge number of unassigned viruses is available

Table 1. Virus Taxonomy. Orders and families written in black represent the virus taxonomy of the 9th ICTV report by King et al. (2011). Red written text represents changes in the newest ICTV online taxonomy release 2017.

Order	Families
Bunyavirals	9
Caudovirales	3
Herpesvirales	3
Ligamenvirales	2
Mononegavirales	8
Nidovirales	4
Picornavirales	5
Tymovirales	4
Unassigned	65/84

(King et al. 2011). The newest report is available online, as the 10th report (ICTV Online 10th Report 2016).¹

Table 2. Classification of viruses by nature of their genomes. The column “families” shows the number of assigned and unassigned families in the respective order. The column “Arbovirus” shows the distribution of arboviruses. The last line shows subviral agents, which are no viruses and do not include any arboviruses.

Order	Families	Arbovirus
DNA viruses		
dsDNA viruses	25	Asfaviridae
ssDNA viruses	7	-
DNA and RNA reverse transcribing viruses	5	-
RNA viruses		
dsRNA viruses	8	Reoviridae
Negative-stranded ssRNA viruses	9	Rhabdoviridae Orthomyxoviridae Bunyaviridae
Positive-stranded ssRNA viruses	27	Flaviviridae Togaviridae
Unassigned viruses	82	-
The subviral agents: Viroids, satellites and agents of spongiform encephalopathies (prions)	4	Viroids Satellites Vertebrate Prions Fungi Prions

Although a classification scheme by species, genus and family is used in virology, the concept differs from those in zoology, botany or bacteriology. A classical phylogenetic system in virology is not possible because of the complex evolution of viruses. Similar viruses can have independent origins, with a majority of virus groups considered to be polyphyletic (Aspöck & Dobler 2010).

¹ Virus taxonomy in this thesis follows the most recent published, 9th virus taxonomy report by King et al. (2011). The newest ICTV online 10th report, which however is not yet published, suggests changes at different taxonomic levels. Changes within the order level are represented in Table 1.

1.2 Arthropod-borne (Arbo) viruses

Arboviruses represent a group of viruses without phylogenetic relatedness, characterized by three criteria: multiplication in a certain hematophagous arthropod, as well as in a vertebrate host and the transmission from vector to host through the salivary duct during a blood meal. The currently known 500 species of arboviruses belong to several virus families (Table 3). Generally, arboviruses occur on all continents, with an increase in the number of species correlated with a decrease in distance to the equator (Aspöck & Dobler 2010).

Table 3. Virus families including arboviruses.

Family	Characterization	Arbovirus genus	Arthropod host
Asfaviridae	dsDNA-iruses, spherical, enveloped, 170-190 nm	Asfivirus	Ceratopogonidae
Reoviridae	dsRNA-viruses, naked, icosahedral, 60-80 nm	Orbivirus	Ceratopogonidae Phlebotominae Culicidae Ixodidae
		Coltivirus Seadornavirus	Ixodidae Culicidae
Rhabdoviridae	Neg. ssRNA-viruses, not segmented, enveloped, 100-430 x 45-100 nm	Vesiculovirus	Culicidae Phlebotominae
		Ephemerovirus	Culicidae Phlebotominae
Orthomyxo-viridae	Neg. ssRNA-viruses, segmented, spherical or filamentous, enveloped, 80-120 nm	Thogotovirus	Ixodidae
Bunyaviridae	Neg. ssRNA-viruses, spherical, enveloped, 80-120 nm	Orthobunyavirus	Culicidae Ixodidae
		Nairovirus	Ixodidae Argasidae
		Phlebovirus	Phlebotominae Culicidae Ixodidae
Flaviviridae	Pos. ssRNA-viruses, spherical, enveloped, 40-50 nm	Flavivirus	Ixodidae Culicidae
Togaviridae	Pos. ssRNA-viruses, not segmented, spherical, enveloped, 70 nm	Alphavirus	Culicidae

A great challenge for arboviruses is the replication in highly distant phylogenetic taxa, invertebrates and vertebrates in particular. However, this

lifestyle also goes along with several benefits, such as high mobility through winged arthropods and protection from environmental factors, resulting in a vast distribution range (Hanley & Weaver 2008). Of the known arboviruses around 100 are pathogenetic to humans, prominent representatives being the Chikungunya virus, Yellow fever virus, Japan-Encephalitis virus and Dengue viruses, with the highest number of arboviral infections worldwide (Preiser 2010).

1.2.1 History of arbovirology

First records of “plagues” transmitted by biting and bloodsucking arthropods date to Homer (~750 BC), Aristophanes (~450-380 BC) or Aristoteles (384-322 BC), who were all writing about the nuisance of certain hematophagous arthropods (Mullen & Durden 2009). Although the causative agents were unclear, Dengue fever are known to have caused high mortality in the tropics in the 16th century and Yellow fever in the 17th and 18th century (Shope & Meegan 1997). The year 1877 can be marked as the start of modern medical entomology, with Sir Patrick Manson describing the transmission of *Wuchereria bancrofti* by a bloodsucking insect (Mullen & Durden 2009).

In 1933, British scientists isolated a virus from a human for the first time, namely H1N1. This virus, known to have caused the “Spanish” influenza, infected ~500 million people worldwide, resulting in 50 million deaths in a pandemic in 1918-1919 (Smith et al. 1933, Taubenberger 2006). The first arbovirus isolation in Europe was successful in 1949, when a strain of CEE (Tick-borne encephalitis) was isolated from *Ixodes ricinus*. In 1959, an arbovirus, Tahyna virus, was isolated from mosquitoes in Europe for the first time, marking the beginning of extensive arbovirus research in Central Europe. In the following decades six arbovirus species, namely Tahyna virus, Batai virus, Lednice virus, West Nile virus, Usutu virus and Sindbis virus, have been isolated in Central Europe (Bardos & Danielova 1959, Aspöck 2012).

Over the next decades, knowledge and identified species increased dramatically, as a result of improved diagnostic and laboratory techniques. Today more than 545 arbovirus species have been described, with more

than 150 known to cause diseases in humans, still, information on transmission cycles, hosts and vectors is partially scarce (Shope & Meegan 1997, Cleton et al. 2012).

1.2.2 Arbovirology in Austria

After the first isolations of arboviruses in Czechoslovakia in 1949 and during the following years, first studies on arboviruses were also performed in Austria, mainly on TBE virus, by Hans Moritsch (Aspöck 2012). During the next decades, a team of Austrian scientists led by Christian Kunz, intensified work on a TBE vaccine, which was successfully launched in 1971 and subsequently further optimized (Barrett et al. 2003). At the same time, Aspöck (1965) proposed arboviral research as a subdiscipline of entomology. The first isolation of a mosquito-transmitted virus, *Tahyna virus*, in Austria was accomplished by Aspöck & Kunz (1966). Shortly after, several strains of Calovo virus were isolated from *Anopheles* species in the Burgenland (Aspöck & Kunz 1968). Extensive and successful work on medical entomology and arbovirology was performed throughout the second half of the 20th century in Austria, with complex methods for the detection, isolation and identification of viruses. The rise of molecular techniques, simplified and accelerated the work, however main questions on vector capacity, circulation of viruses and medical importance remained (Aspöck 2012). Today climate change, globalization and newly introduced arthropod species keep research on vectors, hosts and pathogens highly important (Aspöck 2016).

1.2.3 Vectors & hosts

Several arthropods have been identified as vectors for arboviruses. Hard ticks (Ixodidae), soft ticks (Argasidae), mosquitoes (Culicinae and Anophelinae), sandflies (Phlebotominae) and midges (Ceratopogonidae) are considered to be frequent vectors. Mites (Acari), bugs (Heteroptera), fleas (Siphonaptera), black flies (Simuliidae) and horseflies (Tabanidae) are known to host arboviruses, however, their vector capacities have not been

completely identified though (Aspöck & Dobler 2010). More than 14,000 species of arthropods in 400 genera are hematophagous (Ribeiro 1995). Arboviruses can be taken up during a blood meal by the bloodsucking arthropods, but not all species support virus multiplication (Burgdorfer & Varma 1967).

The vast majority of arboviruses are part of zoonotic transmission cycles, consequently not requiring a human host (Karabatsos 1985). Usually, humans are infected accidentally and can be considered as dead-end hosts (Gubler 1998). A rare exception are the Dengue viruses, which are maintained by a human-mosquito cycle exclusively (Gubler 1997). Common hosts for arboviruses are several mammals and birds, additionally reptiles and amphibians could play a subsidiary role. The suitability to function as a host depends on various factors, the most important ones being the viral load and the duration of viraemia (Aspöck & Dobler 2010).

1.2.4 Transmission cycles

The infection cycle usually starts with the uptake of a virus by an arthropod during a blood meal on an infected host (Burgdorfer & Varma 1967). After the virus uptake, gut epithelia are infected and the virus multiplies and is released to the hematocele from which salivary glands and oviducts can be infected. Besides this typical horizontal way of infection, arboviruses are known to also be transmitted vertically, namely transovarially, by being passed to the eggs and transstadially from larval stages to the adults (Aspöck & Dobler 2010) (Figure 3). However, vertical transmission typically leads to a lower virulence of the virus, compared to horizontal transmission (Agnew & Koella 1997, Clayton & Tompkins 1994). Probably every arbovirus, with a few potential exceptions, is maintained by vertical transmission for a certain number of cycles, but is dependent on horizontal transmission to keep up viraemia (Hanley & Weaver 2008).

Under certain circumstances, arboviruses can also be transmitted differently. Transmission during lactation has been described, as well as infection of humans by drinking raw milk. Infection after contact with wounded viremic animals has also been described (Dobler & Aspöck 2010). Ticks may even

be infected while sucking blood simultaneously with an infected individual, on the same host (Aspöck & Dobler 2010). The fitness of the arthropod host depends on the mode of transmission and the virus and arthropod species involved in the transmission cycle (Lambrechts & Scott 2009). RNAi and various innate immune responses, such as toll, immunodeficiency factor (Imd), and Janus kinase (JAK)- signal transduction and activators of transcription (STAT) pathways operate against arbovirus infections in mosquitoes (Cheng et al. 2016).

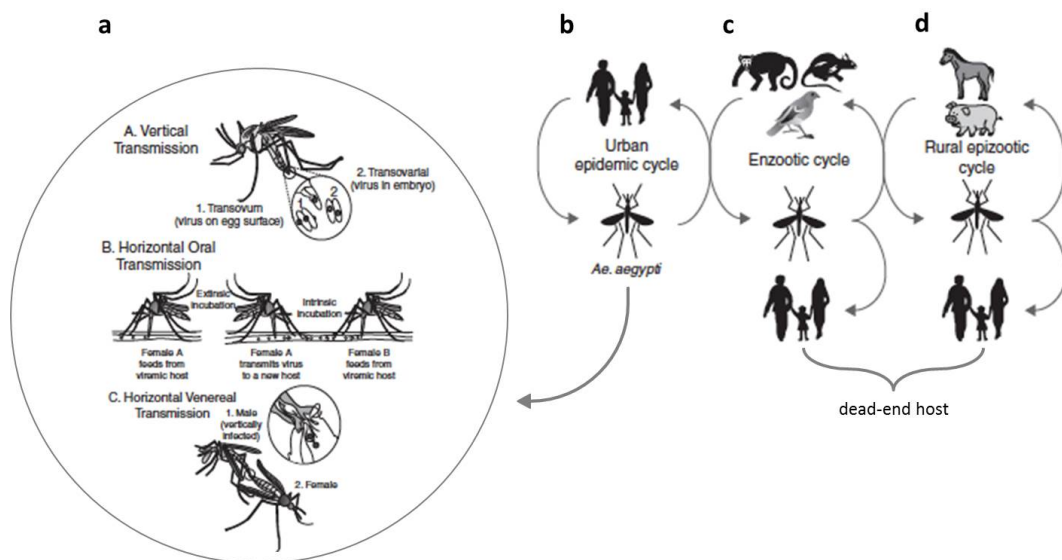


Figure 3. Arboviral transmission cycles. Different modes of transmission are shown. By vertical transmission, pathogens can be passed to the offspring (a-A). During a bloodmeal a host organism can be infected and pathogens can be taken up by another arthropod (a-B). Horizontal transmission occurs in several ways. Usually arboviruses have zoonotic cycles, but certain viruses (Dengue fever) are adapted to a direct human-arthropod life cycle (b). Mostly, small mammals, birds or non-human primates function as hosts. Humans are infected as false targets with short viraemia ending up as dead-end hosts (c). An infection of domestic animals can increase the chance of humans being infected (d). Modified from Hanley & Weaver 2008.

1.2.5 Medical relevance & treatment

Many infected humans show minor symptoms or even remain asymptomatic (Preiser 2010). Still, 200,000 people are infected with Yellow fever virus every year, causing 30,000 deaths. More than 50 million cases of Dengue fever virus infections are recorded every year, with 500,000 people hospitalized and 20,000 deaths (WHO 2009a, WHO 2009b, Guzman 2010 & WHO 2012). Symptoms include febrile illness with headaches and polyarthrititis (Dengue-fever), hemorrhagic fevers (Crimean-Congo

hemorrhagic fever virus) and affection of the central nervous system (TBE virus). Until now, only three vaccines, namely against tick-borne encephalitis (TBE) virus, Japan-Encephalitis (JE) virus and Yellow-fever virus are available (Aspöck & Dobler 2010).

1.3 The Bunyaviridae

The Bunyviridae comprise five genera of viruses, Tospovirus, Orthobunyavirus, Phlebovirus, Nairovirus and Hantavirus (Table 4) and are, with over 350 serotypes, amongst the largest groups of RNA viruses (Elliott & Schmaljohn 2013, Plyusnin et al. 2011)². A common feature of all five species is a tri-segmented ssRNA genome, with a large, medium and small segment (Kolakofsky & Hacker 1991). Bunyaviridae have a helical capsid and the enveloped virion has a spherical structure with 80-120 nm of diameter. The L segment (large) encodes a RNA-dependent RNA polymerase, the M segment (medium) encodes two glycoproteins, responsible for virus attachment and cell entry, whereas the S segment (small) encodes the nucleocapsid protein (Pettersson & von Bonsdorff 1987, Contigiani et al. 2017).

In the last decades, numerous “new” pathogens have been identified, such as SARS coronavirus, MERS coronavirus, both classified as “most dangerous” by the WHO or Severe fever with thrombocytopenia (SFTS) virus (Shigeta & Yamase 2005, Yu et al. 2011, Zaki et al. 2013). Many newly discovered infectious diseases are viral zoonoses. Bunyaviridae are considered to include various emerging virus groups, which have not been examined in detail and cause several severe diseases in humans and other animals, cattle most importantly (Vaheri & Vapalahti 2011).

² The classification in this section follows the 9th ICTV taxonomy report, chapter Bunyaviridae by Plyusnin et al. (2011). However, major changes within this family are in the not yet published 10th report of virus taxonomy by the ICTV. The family Bunyaviridae has been restructured and is now classified as a new order, the Bunyavirales. The genera Tospovirus, Nairovirus and Hantavirus have been elevated to the taxonomic level of families, Tospoviridae, Nairoviridae and Hantaviridae. The genus Orthobunyavirus has been classified to a newly introduced family Peribunyaviridae. The genus Phlebovirus has been classified to the new family, Phenuiviridae. Additionally, new families have been added, Feraviridae, Fimoviridae, Jonviridae and Phasmaviridae.

Table 4. Classification of the Bunyaviridae, with its five genera. Viruses within Tospovirus are transmitted by arthropods, but infect plants and viruses within Hantavirus circulate between rodents without a vector, thus both genera do not belong to the group of arboviruses. Three genera of the bunyaviridae fulfill the three criteria for arboviruses (Orthobunyavirus, Phlebovirus, Nairovirus). Modified from Léger & Lozach.

Genus	Hosts		Vectors
Tospovirus	plants		thrips
Orthobunyavirus		arthropods	mosquitoes, culicoid flies, ticks
Phlebovirus	mammals		phlebotomine flies, mosquitoes, ticks
Nairovirus			ticks, culicoid flies, mosquitoes
Hantavirus		rodents	

1.4 The genus Phlebovirus

Altogether, 37 member viruses, within 9 antigenic complexes have been assigned to the genus Phlebovirus, with 32 additional unassigned members, subdivided into two antigenic groups, the Sandfly fever group and the Uukuniemi group (Plyusnin et al. 2011, Elliott & Schmaljohn 2013) (Table 5)³. As biochemical and genetic data is scarce, taxonomy is based on antigenic relationships (Palacios et al. 2011). Geographically distributed in North and South America, Europe, Africa, Asia and Tasmania (Australia), phleboviruses are primarily transmitted by sandflies, but also by mosquitoes and ticks to small parts (Tesh 1988, Aspöck & Dobler 2010, Wang et al. 2014). The distribution of this virus group over the Americas, Europe, Africa and Asia has been known for decades, as reviewed by Tesh (1988), whereas the first documentation of the appearance in Australia was made more recently. Catch-me-cave and Precarious point, two new Phlebovirus serotypes, were isolated from ticks associated with king penguins on Macquarie Island south of Australia by Major et al. (2009). Another new virus, the Hunter Island Group virus (HIGV), was isolated from ticks on

³ The online ICTV report 2017 includes a 10th Phlebovirus species, the SFTS (Severe fever with thrombocytopenia) phlebovirus. Additionally, all species names have been changed, e.g. Bujaru virus to Bujaru phlebovirus.

Albatross Island in Tasmania, Australia (Wang et al. 2014). The distribution of the respective virus species is linked to its vector range. Phleboviruses have their main distribution in tropical to subtropical regions, but they also occur in temperate regions to some extent (Tesh 1988). Because genetic data of many phleboviruses is scarce, taxonomy is based on their antigenic relationships, evaluated by Cross-complementation fixation (CF) and Plaque reduction neutralization test (Nichol et al. 2005).

Table 5. Taxonomy of the genus Phlebovirus (Plyusnin et al. 2011). The genus Phlebovirus contains 37 assigned member viruses within 9 species (serocomplexes), divided into the Sandfly fever group (black) and the Uukuniemi group (light grey). Red written text represents changes in the newest ICTV online taxonomy release 2017.

Current species name	Proposed species name	Assigned viruses
Bujaru virus	Bujaru phlebovirus	2
Candiru virus	Candiru phlebovirus	6
Chilibre virus	Chilibre phlebovirus	2
Frijoles virus	Frijoles phlebovirus	2
Punta Toro virus	Punta Toro phlebovirus	2
Rift Valley fever virus	Rift valley fever phlebovirus	3
Salehabad virus	Salehabad phlebovirus	2
Sandfly fever Naples virus	Sandfly fever Naples phlebovirus	5
SFTS virus	SFTS phlebovirus	-
Uukuniemi virus	Uukuniemi phlebovirus	13
Unassigned		32

1.4.1 Molecular biology of the genus Phlebovirus

As members of the Bunyaviridae, all phleboviruses are spherical, enveloped virions and possess a tri-segmented negative-stranded RNA genome. The tripartition consists of a large (L), medium (M) and small (S) segment. The S segment conducts an ambisense coding strategy, a special feature differentiating this genus from all other Bunyaviridae. The L protein, a viral RNA-dependent RNA polymerase and a polypeptide precursor to the glycoproteins, is encoded by the L and M segment. Whereas, the S segment encodes two proteins, the nucleoprotein N and the non-structural protein Ns (Bouloy 2011, Elliott & Brennan 2014). The RNA segments do not exhibit a proofreading activity, resulting in high mutation rates, a potentially enhanced virulence and a tendency to rapidly form new species. The tri-segmentation supports genome reassortment, in the case of two co-infecting virus species (Alkan et al. 2013) (Figure 4).

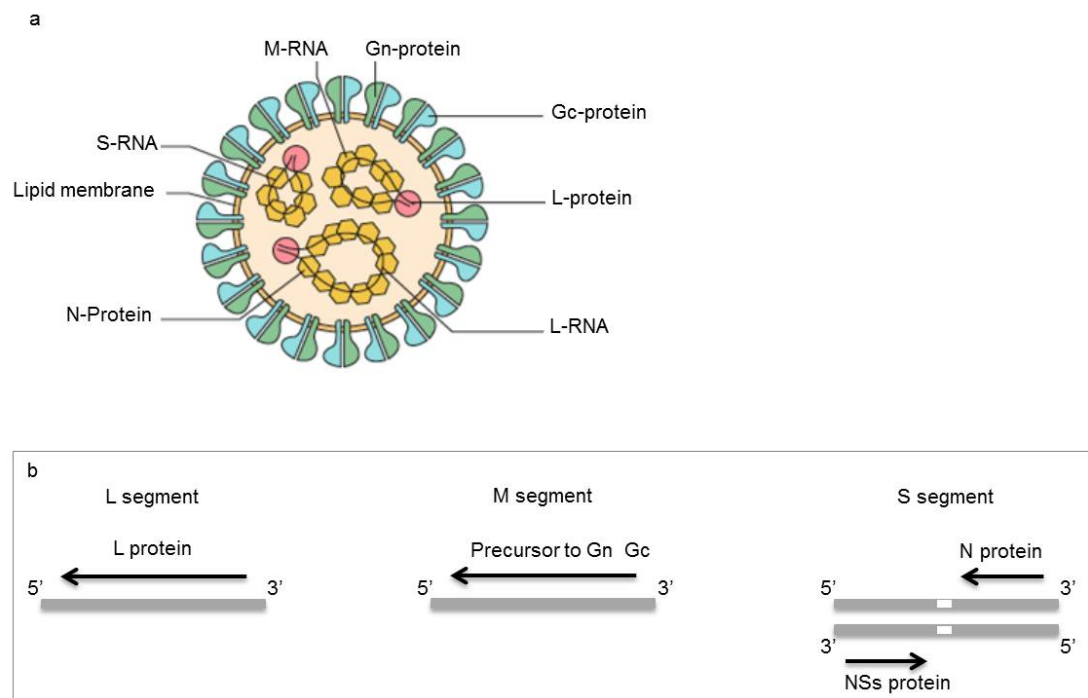


Figure 4. Structure of a Bunyavirus (a). Glycoproteins Gn and Gc form heterodimers. L, M and S-segment are connected to the N-protein. The L-protein possibly binds to the viral promoter, built by the aligned 3' and 5' ends of the RNAs. Genome organization of a phlebovirus (b). L and M segment are coded in a negative sense, the S segment in an ambisense way. Modified from Günther 2010.

1.4.2 Epidemiology

The 9 serocomplexes of the genus Phlebovirus are largely distributed, following the distribution of their respective vectors. Some of these are transmitted by either sandflies, mosquitoes or ticks, whilst the principal vectors of a few members are still unknown (Palacios et al. 2011).

Bujaru virus and Candiru virus are distributed in South America, Chilibre virus and Frijoles virus are circulating in Panama, Central America, with only the two members of the Candiru complex known to cause disease in humans. Sandflies have been identified to function as vectors for Chilibre and Frijoles virus, whereas the vectors for the two other species have not been identified yet (CDC 2017, Vasconcelos et al. 2017, Elliott & Brennan 2014, Palacios et al. 2011). The members of Punta Toro virus circulate in Panama and Colombia and can cause febrile illness in humans, with sandflies of the genus *Lutzomyia* acting as vectors (Palacios et al. 2015). Salehabad virus contains two assigned member viruses, Salehabad virus

(SALV) and Arbia virus (ARBV) isolated from sandflies in Iran and Italy, respectively (Verani et al. 1988, King et al. 2011, Palacios et al. 2013). Up to date, this serocomplex has not been associated with human diseases. A novel strain, Adria virus (ADRV), was isolated from sandflies in Albania in 2005 and then from the blood of a child in Greece in 2009. This virus is suggested to be a member of the Salehabad serocomplex, but has not been assigned yet, by the ICTV (Anagnostou 2011, Papa et al. 2011, Palacios et al. 2013). Rift valley fever virus (RVFV) was the first Phlebovirus species discovered in 1930, when a large number of sheep suffered from abortion and died in the Rift Valley in Kenya (Daubney et al. 1931). To date, RVFV is geographically distributed over Africa and has spread to the Arabian Peninsula and Madagascar (Gerdes 2004). RVFV is mainly transmitted by mosquitoes, however one member virus, Icoaraci virus (ICOV), is known to be transmitted by mosquitoes and phlebotomines. While mosquitoes play a major role for the infection of large herds of grazing animals, humans are primarily infected through the contact with infected tissue and body fluids, or the consumption of raw milk (Pepin et al. 2010, King et al. 2011). Uukuniemi virus represents the second Phlebovirus group, Uukuniemi group, with all member viruses transmitted by ticks (Plyusnin et al. 2011). Uukuniemi virus was first isolated in 1960 from ticks in southern Finland and has since then been isolated in countries of Central and Eastern Europe and Central Asia (Saikku & Brummer-Korvenkontio 1973, CDC 2017). Other members of this virus species, Oceanside virus, Precarious Point virus and Tunis virus were isolated from ticks in Oregon (USA), Tasmania (Australia) and Tunis (Tunisia), respectively and thus show a vast geographic range (Thomas et al. 1973, St George et al. 1985, Chastel et al. 1994). In 2011, Severe fever with thrombocytopenia syndrome virus (SFTSV) was isolated from patients with severe symptoms, such as fever, thrombocytopenia, leukocytopenia and multi-organ dysfunction. Ticks were identified as vectors, when RNA of the respective virus was extracted (Yu et al. 2011). Additionally, a human-to-human transmission through the exposure to blood was demonstrated by Liu et al. (2012). The SFTSV serocomplex is presented as a tentative 10th phlebovirus species in the recent 10th online report of the ICTV (2017).

Sandfly fever Naples virus (SFNV) was isolated from diseased soldiers in World War II in 1944 and later also isolated from sandflies of the genus *Phlebotomus* (Sabin 1951, Verani et al. 1980). SFNV shows a large geographic distribution range from Mediterranean countries in Europe and North Africa to Asia, as far as Bangladesh (Ayhan et al. 2017a). To date, the SFNV serocomplex comprises five assigned member viruses and several unassigned tentative viruses (King et al. 2011). Toscana virus (TOSV) shows a very close genetic relatedness and was isolated from sandflies in Central Italy in 1971 (Verani et al. 1980). Its geographic distribution is currently assumed to be mainly the Mediterranean European region and the farthest northern countries of Africa (Ayhan et al. 2017a). Another important unassigned Phlebovirus is the Sandfly fever Sicilian virus, initially isolated from soldiers in Egypt during World War II and subsequently in Sicily from US Army soldiers and showing a broad distribution similar to SFNV (Sabin 1951, Ayhan et al. 2017a). Symptoms of phleboviral infections caused by SFNV and SFSV are identical and were first described by Alois Pick (1886) among Austrian troops in the Balkan. Sandflies were identified as vectors and the disease was named “Papatacci fever” or “three-day fever” (Doerr 1909). TOSV is known to potentially cause neurological symptoms such as meningoencephalitis (Charrel et al. 2005). A new Sicilian-like phlebovirus, Cyprus virus (CYPV) was isolated from Greek soldiers in 2002 after a large outbreak of febrile illness (Papa et al. 2006).

The vector of SFSV is *Phlebotomus* (*Phlebotomus*) *papatasi* Scopoli, 1786, whereas the vector for CYPV has not been identified yet. However *P. papatasi* is suggested to be a potential vector (Tesh et al. 1977, Depaquit et al. 2001). SFNV has been isolated from *Phlebotomus* (*Laroussius*) *perniciosus* Newstead, 1911, *Phlebotomus* (*Laroussius*) *perfiliewi* Parrot, 1930 and *P. papatasi* in Italy, Serbia and Egypt, respectively (Verani et al. 1980, Gligic et al. 1982, Schmidt et al. 1971). TOSV was first isolated from *P. perniciosus* and *P. perfiliewi* in Italy (Verani et al. 1980, Verani et al. 1988). More recently, TOSV RNA was detected in *Sergentomyia* (*Sergentomyia*) *minuta* Rondani, 1843 in France, however the role of this species as a potential vector infecting humans is unclear, since *S. minuta* typically feeds on reptiles (Charrel et al. 2006). Additionally, TOSV RNA was described in

Phlebotomus (Laroussius) tobbi Adler, Theodor & Lourie, 1930, by Ergunay et al. (2014), in Northern Cyprus.

Partially, high seroprevalence rates in humans and animals are reported in countries where phleboviruses are circulating. A seroprevalence of 77.2% for TOSV antibodies was observed in forestry workers in Tuskany, Italy, while the control group showed 22.7% seropositivity (Valassina et al. 2003). In domestic animals seroprevalences of 11.5% for TOSV in France, up to 64% for TOSV in Spain and 20%, 32% and 57% for TOSV, SFSV, SFNV in Cyprus, respectively, have been detected (Brisbarre et al. 2011, Navarro-Mari et al. 2011, Eitrem et al. 2009). In Eastern Europe and the Balkans, seroprevalence rates of 15.6% for SFSV, 57.6% for SFNV and 37.5% for TOSV were shown in Croatia (Tesh et al. 1976, Punda-Polić et al. 2012a). In Bosnia and Herzegovina a seroprevalence of 10.3% for TOSV was observed (Hukić & Salimović-Besić 2009). In the Kosovo, a seroprevalence of 9.6% and 27.9% for SFSV and SFNV, respectively was observed (Tesh et al. 1976). More recently, Venturi et al. (2011) revealed a seroprevalence of 5.5% for TOSV and SFNV in the Kosovo. To date, no studies on seroprevalence rates in Syria and the Lebanon have been published.

1.4.3 Medical relevance and treatment

Symptoms of SFNV and SFSV are similar. After an incubation period of 3-5 days, sudden high fever, headache, photophobia, myalgia, malaise and retro-orbital pain for two to three days can be observed. (Sabin 1951, Bartelloni & Tesh 1976). TOSV infections are considered to be mostly asymptomatic. In some cases, a self-limiting febrile illness without the involvement of the central nervous system (CNS) is developed. After an incubation period of up to two weeks, fever, nausea, myalgia, neck rigidity and poor levels of consciousness can be observed. However, in some cases neurological symptoms, such as meningitis, meningoencephalitis and encephalitis occur, especially in Mediterranean countries (Cusi et al. 2010, Charrel 2005, Sanbonmatsu-Gámez et al. 2009). Other severe cases have been documented, such as persistent personality changes and aggressiveness (Serata et al. 2011), a comatose state and seizures (Kuhn et

al. 2005) or a case of lethal encephalitis (Bartels et al. 2012). Cyprus virus, typically causes a three day self-limiting febrile illness (Papa et al. 2006, Konstantinou et al. 2007).

To date, no approved vaccines or antiviral drugs for phleboviral infections are available. In a preliminary study, Savellini et al. (2008), achieved 100% protection against infection with a lethal TOSV strain by immunizing with a combination of N and Gc proteins. However, the efficacy of a broad-range vaccine is questioned, because the huge phleboviral genome diversity makes cross protection between strains difficult (Alkan et al. 2013). Treatment of Phlebovirus infections is currently only symptomatically (Depaquit et al. 2010). Selenazole was shown to be effective against Sandfly fever Sicilian virus (SFSV) (Kirsi et al. 1983), preventive treatment with Ribavirin during a SFSV infection was also efficient (Huggins 1989) and British troops in Afghanistan were treated with doxycycline (Bailey et al. 2011). Alternative approaches are the use of insecticides and repellents. Insecticide treatment largely depends on the sandfly species and is more efficient against anthroponotic sandflies. Insecticide-treated bed nets only have a limiting effect on the exposure to sandflies (Gidwani et al. 2011, Alkan et al. 2013).

1.4.4 Relevance to the Armed Forces

The exposition of military personnel to sandflies and phleboviruses has been of great concern for many decades. During the Great Eastern Crisis, the Austrian troops on the Balkan suffered from a febrile illness, clinically described by Pick in 1886. German and British troops were affected by sandfly fever in the Mediterranean, North Africa and the Middle East during World War II (Sabin 1951). Several cases of febrile illnesses among military troops have been published. Swedish United Nations soldiers were affected by sandfly fever in Cyprus (Eitrem et al. 1990) and in 2002 44% of a Greek Army troop suffered from a self-limiting febrile illness and anti-Phlebovirus antibodies were detected (Papa et al. 2006). Various Phlebovirus strains were isolated from diseased soldiers of Soviet troops in Afghanistan (Gaïdamovich et al. 1989). Seroprevalence rates of up to 70% were

observed in military personnel in Pakistan (Bryan et al. 1996). As many as 92.9% of U.S. Army soldiers suffering from fever, headache, malaise and myalgia were positive for Sandfly fever Sicilian Virus (SFSV) in Iraq (Ellis et al. 2008). These cases show, that the introduction of immunologically naïve military personnel, to sandfly endemic areas, may pose a major risk for acquiring a symptomatic Phlebovirus infection (Alkan et al. 2013).

1.5 Sandflies

Sandflies (Phlebotominae), belong to the family Psychodidae within the class Insecta. More than 800 sandfly species have been described. Sandflies have a wide distribution and can be divided into Old World and New World species (Figure 6). Three genera, with approximately 375 species are present in the Old World and Australia, inhabiting different regions in Europe, Africa and Asia. In the New World, three genera with approximately 434 species inhabit the Nearctic and Neotropic (Akhoundi et al. 2016). Sandfly populations may occur as far as 50°N to the north and 40°S to the south and can inhabit a high altitudinal range, from 300 meters below to 3200 meters above sea level (Lane 1993, Artemiev 1980, Maroli et al. 2013). Different pathogens including *Leishmania* spp., bacteria and arboviruses (mainly Phlebovirus, Vesiculovirus and Orbivirus to some extent) are transmitted by sandflies. Approximately 70 species belonging to the genera *Phlebotomus* and *Lutzomyia* are of medical relevance (Dobler & Aspöck 2010b).



Figure 5. *Phlebotomus papatasi* (female) after blood uptake. Maroli et al. (2013).

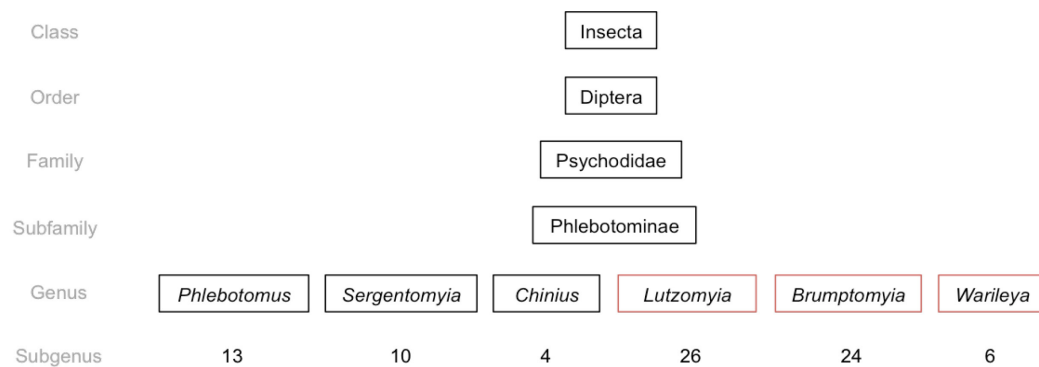


Figure 6. Taxonomy of sandflies. The black box color at the genus level represents the Old World genera, whereas a red box color represents the New World genera. Taxonomy adopted from Akhoundi et al. 2016.

1.5.1 Biology and life cycle

Adult sandflies are up to 5 mm in length, the thorax, abdomen and the large wings being densely packed with hair-like structures (Figure 5). This contributes to a noiseless but weak flight ability, particularly at high wind speed or rain. Usually the flight range is restricted to approximately 500 meters or less from the breeding site and right above the ground (Rutledge & Gupta 2009). In Sub-tropic regions, breeding sites mainly consist of humid crevices, animal burrows and shelters or caves, whereas leaf litter, trunks of trees or canopies are occupied in the Neotropics. Adult sandflies feed on various sugar sources, such as honey dew, nectar or fruit juices. Additionally, the telmophagous females bite and suck blood for egg development. Males do not possess mandibles and do not bite (Rutledge & Gupta 2009, Loos-Frank & Lane 2017). The metamorphosis includes four stages, eggs, larvae (four instars), pupae and adults (Figure 7). Around 100 or less eggs are laid into warm, moist and organic-rich breeding sites after the blood meal. Only a few species do not require a blood meal prior to their first oviposition. Temperature-dependent metamorphosis to the larval stage takes 7-10 days

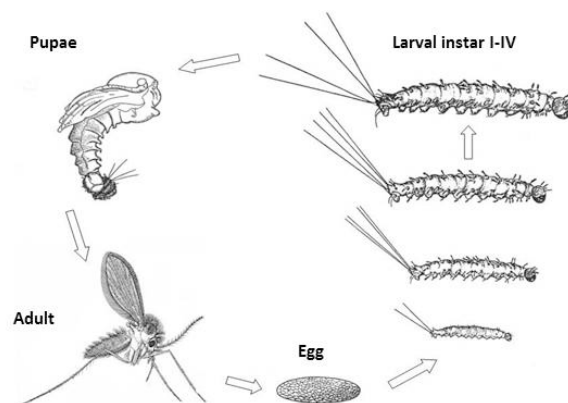


Figure 7. Life cycle of the Phlebotominae. Modified from Lawyer & Perkins 2004.

and another three weeks for the emergence of the pupae. The whole cycle takes approximately 70 days. A diapause in the egg stage or the fourth larval instar is possible (Walochnik & Aspöck 2010, Maroli et al. 2013).

1.5.2 Vector capacity

Sandflies are known to be vectors of the protozoans *Leishmania* spp., the α -proteobacterium *Bartonella bacilliformis* and of arboviruses (Ready et al. 2013).

Leishmania spp. (Kinetoplastida, Trypanosomatidae) are transmitted by sandflies of the genus *Phlebotomus* in the Old World and of *Lutzomyia* in the New World. Depending on the *Leishmania* species transmitted, different clinical forms of the disease can occur including visceral, cutaneous, mucocutaneous and diffuse cutaneous leishmaniasis. Leishmaniasis is endemic in Latin America, Africa, the Indian Subcontinent, the Middle East, Southeast Asia and the Mediterranean region and among the world's most important vector borne diseases. Worldwide, approximately 12 million people are infected, 350 million are at risk of being infected and two million new infections occur ever year. Several mammals and humans can function as reservoirs (Ready 2010, Walochnik & Aspöck 2010).

Carrion's disease (Oroya fever or bartonellosis) caused by *Bartonella bacilliformis*, has a high fatality rate among humans and is transmitted by the sandfly species *Lutzomyia (Verrucarum) verrucarum* Townsend, 1913. The disease is mainly restricted to the Peruvian Andes where *L. verrucarum* is present, however a distribution to the Amazon region is assumed. Two clinical forms of the disease, a fatal and a non fatal, are known. Seroprevalences of over 50% have been observed in Peru. Small mammals and humans have been suggested to function as reservoirs, but not proven (Ihler 1996, Chamberlin et al. 2002, Hambruch et al. 2004).

Altogether viruses of three families are transmitted by sandflies, namely Chandipura virus (Rhabdoviridae), Changuinola virus (Reoviridae) and most importantly Phleboviruses (Bunyaviridae). Chandipura virus was isolated from patients with febrile illness and from sandflies in India (Bhatt & Rodrigues 1967, Dhanda et al. 1970). Changuinola virus was isolated from a

febrile patient and from sandflies in Panama. Additionally, antibodies have been detected in opossums (Karabatsos 1985, Monath & Guirakhoo 1996). Phleboviruses have been isolated from sandflies, mosquitoes and mammals including humans. Virus RNA has been detected in various vertebrates, but their roles as reservoirs have not been clarified yet and may be considered dead-end hosts (Linthicum et al. 1984, Tesh 1988, Alkan et al. 2013). Several modes of transmission have been observed in sandflies. Transovarial (vertical) transmission is suggested to play an important role and has been observed several times. Laboratory infected sandflies can transmit Toscana virus to their F1 progeny, however transovarial transmission seems to depend on sandfly species (Ciufolini et al. 1985, 1989, 1991). Tesh and Modi (1987) observed a decline of virus infection rates in subsequent generations and suggested that the maintenance cannot be exclusively dependent on this mode of transmission. Other modes, such as venereal (horizontal) transmission from infected males to uninfected females during mating and the maintenance of TOSV during diapausing, was observed in the laboratory (Tesh et al. 1992). Detailed data on hosts, reservoirs and vector capacities of medically important sandfly species is still lacking. *Phlebotomus papatasi*, *P. perniciosus*, *P. ariasi* and *P. perfiliewi* are the main vectors for phleboviruses in Europe and the Middle East (Depaquit 2010).

A medically important issue is the overlapping vector capacity of sandflies for both *Leishmania* spp. and phleboviruses, which accounts to a possible risk for co-infection with both pathogens. A significant correlation between seropositivity for *L. infantum* and TOSV was shown in Marseille, France (Bichaud et al. 2011), whereas in Portugal a significant association between *L. infantum* and SFSV infections was observed (Maia et al. 2017). In Northern Cyprus, *Phlebotomus tobbi* was identified maintaining both, *L. infantum* and TOSV (Ergunay et al. 2014). These findings show, that the elucidation of the vector capacities of sandflies and the role of vertebrates including humans as reservoir hosts has to be a major task in future research.

1.5.3 The impact of climate change and globalization on sandfly distribution in Europe

Since 2.5 million years, alternating glacial and interglacial events occur, lasting up to 100.000 years and 20.000 years, respectively. Approximately 15.000 years ago, the last glacial period ended and set the start for the development of the current biodiversity on earth (Aspöck 2008). With the beginning of the present interglacial period, animals in Europe started to remigrate to unsettled areas from refugial centers, mainly located in the Mediterranean basin (Aspöck 2010). Within these huge migration events, various species, including arthropods, populated new areas, possibly hosting diverse types of pathogens. Several reasons for the rather recent emergence of many pathogens have been suggested. Some may have simply been overlooked until the second half of the 20th century, some have spread recently through anthropogenic activity. Other reasons are the distribution by vectors such as migrating birds, or the natural colonization as a result of changing ecological conditions in certain areas. The rise of the temperature by climate change is by far the most important factor for ectothermic arthropods. Another important factor is the spread of arthropods and diseases through globalization and thus human migration events (Aspöck & Walochnik 2014).

Over 20 sandfly species are distributed over the Mediterranean basin and were considered as strictly Mediterranean faunal species. In 1999, the first record of a sandfly species north of the Alps, *Phlebotomus (Transphlebotomus) mascittii* Grassi, 1908, was made in Germany. These findings were first linked to climate change, but then considered as overlooked populations and remigrated from refugial centers in the Holocene (Naucke et al. 2000, Aspöck et al. 2008). Still, climate change could be the driving force for the recent enlargement of inhabited areas (Naucke et al. 2011). Medlock et al. (2014) predicted an increasing risk for the establishment of sandfly species along the Atlantic coast of Europe and parts of Germany, Austria and Switzerland. The associated risk of the introduction of new pathogens is possible but has to be assessed individually, because several other important factors such as the replication rate of the pathogen,

the biting rate of the respective vector, the duration of viraemia and vector and host availability also play a role.

1.5.4 Sandflies in Austria

The possible occurrence of sandflies in Austria because of suitable climatic conditions had already been discussed by Aspöck et al. in 2006. The first detection of sandflies in Austria was reported by Naucke et al. in 2011. 24 individuals of the species *Phlebotomus mascittii* were caught during fieldwork in July 2009 and 2010 in Carinthia. Further observations of *P. mascittii* were reported in three federal states of Austria including Lower Austria, Styria and the Burgenland (Poepl et al. 2013, Obwaller et al. 2014). The early findings of sandflies in Europe were suggested to be a result of climate change, but now the fact that *P. mascittii* is found in small populations with tight ecological niches, such as tunnels, dwellings or animal sheds is widely accepted as the main reason why these populations, that are considered to be endemic have been overlooked for so many years (Grimm et al. 1993, Aspöck et al. 2008, Naucke et al. 2008a).

The vector capacity of *P. mascittii* has not been entirely clarified yet. The possibility of its functioning as a vector for *L. infantum* had already been proposed by Pesson et al. (1985) and Naucke et al. (2008b). More recently an individual of *P. mascittii* was tested positive for the infection with *L. infantum* in Italy (Zanet et al. 2014). In Austria, one individual of *P. mascittii* was tested positive for *L. infantum*, as was a dog within the sampling area (Obwaller et al. 2016). However, the vector capacity of *P. mascittii* for phleboviruses remains unclear.

1.6 Purpose of the study

The Austrian Armed forces were established in 1955, starting worldwide missions in 1960. The main objectives of the Austrian army are securing the Austrian border and disaster management (Oebh 2017a). Internationally, the Austrian Armed forces operate to preserve peace in several areas of conflict, cooperating with the United Nations (UN) and regional organizations, such as the OSCE, the EU or the NATO (Oebh 2017b). Operations have been accomplished in parts of Europe, Africa and the Middle East. 300 Austrian soldiers are stationed in Bosnia and Herzegovina, 407 in the Kosovo and 181 in the Lebanon, with headquarters in Sarajevo, Pristina and Naqura, respectively (Oebh 2017c). The troops stationed in the Golan, Syria, were removed in 2013, after almost 40 years of support (Oebh 2017d).

During their missions in foreign countries, Austrian soldiers are at potential risk of exposure to various pathogens, such as parasites, bacteria or viruses, depending on the operational area. National cross-sectional studies on seroprevalences of antibodies against *Leishmania* spp. and Hepatitis E among Austrians participating in UN missions have been conducted (Poepl et al. 2012, Lagler et al. 2014). However, the risk of exposure to phleboviruses among Austrian military personnel is unknown.

This study provides first data on seroprevalences of antibodies against phleboviruses in Austrian military personnel and participants in missions.

The first aim was to assess the seroprevalences of sandfly-borne viruses in Austrian soldiers returning from their missions abroad. For comparison, sera of soldiers investigated before their missions were included in the study.

The second aim was to find out which phlebovirus species are involved and whether there are differences in overall positivity and in species compositions between the respective operational areas.

The third aim was to assess risk factors associated with phlebovirus infections and evaluate possible prophylactic measures.

2 Material & Methods

2.1 Serum samples

Altogether, 1524 sera were provided by the Austrian Armed Forces.

2.1.1 Soldiers returning from missions

The samples were aliquots of serum samples taken from Austrian soldiers for a routine medical examination after their return to Austria from their missions in Bosnia and Herzegovina, the Kosovo, Syria and the Lebanon, respectively (Table 6). The samples from Syria, the Lebanon and BIH were collected in June and July 2013, the samples from the Kosovo between March 26 to 27th and April 2 to 3rd, 2015. All soldiers were asked to fill out a detailed questionnaire (Table 7). Serum samples were kept at -20°C until use.

Table 6. Number of serum samples in this study.

	BIH*	Kosovo	Syria	Lebanon	Control samples	Total
Provided samples	61	261	101	63	1025	1524
Tested samples	61	261	101	63	500	986

*BIH: Bosnia and Herzegovina.

2.1.2 Soldiers before missions (control group)

As a reference group (control samples), serum samples taken from soldiers before their respective missions were included in this study (Table 6). However, these soldiers had partly been on one or several military mission before. All these serum samples were kept at -20°C until use.

Of the total 1025 samples, 500 were randomly picked by using the Excel function “=RAND()”. Means and standard deviations of the age of both, the control sera (n=1051, mean=28.86, sd=9.22) and the random sample (n=500, mean=28.28, sd=8.85), were determined in a Shapiro-Wilk Normality Test and showed no normal distribution for both samples. After a logarithmic transformation of the data, a second Shapiro-Wilk Normality test was

performed, but also this revealed no normal distribution. Consequently, a Mann-Whitney-Wilcoxon Test was performed and revealed no significant difference in means of age ($p=0.33$), and the random sample was proved valid for further studies (R development core team 2008).

Table 7. Information obtained from the patients with a questionnaire.

BIH, Kosovo, Syria & Lebanon	Control sera
Age	Age
Sex	Sex
Federate state	Federate state
Status of employment: professional, militia	Status of employment: professional, militia
Duration of mission	Prior missions
Vacation during mission	Vacation
Symptoms: fever, limb pain	Leishmaniasis
Prior missions	
Activities: Running, Mountaineering, Cycling, Football	
Tick bite	
Animal contact: Dog, cat, rodent	
Pets: Dog, cat	
Leishmaniasis	

2.1.3 Ethics

This study was approved by the Ethics committee of the OeBH. Informed written consent was obtained from all participants.

2.2 Indirect immunofluorescence assay

An indirect immunofluorescence test (Euroimmun, Lübeck, Germany) with subsequent fluorescence microscopy was used for antibody detection strictly following the recommendations of the manufacturer (Table 8). All serum samples were tested at an initial dilution of 1:10 and positive samples were diluted subsequently to evaluate the respective titers (Table 9). A positive and a negative control were added for every incubation, as recommended (Figure 8). The operation areas BIH, Syria, the Lebanon and the control samples were screened for IgG antibodies against SFSV, CYPV, SFNV and TOSV. The Kosovo samples were only screened for IgG antibodies against SFSV, TOSV and RVFV, because at the time no other test kit was available. Fluorescence microscopy was performed with a Nikon Eclipse E 800 (Nikon Instruments, Amstelveen, Netherlands) microscope. Slides were inspected with 400-fold magnification (Nikon Plan Fluor, 40x/0.75, Ph2 DLL) at a

wavelength of 420-490 nm. For microphotography a Nikon 1 J1 camera (Nikon Instruments, Amstelveen, Netherland) and the NIS-Elements Basic Research software (Nikon Instruments, Amstelveen, Netherland) were used (Figure 9).

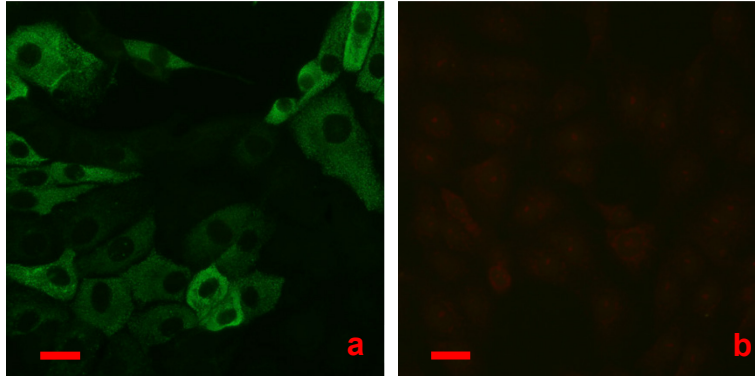


Figure 8. Positive control (a) and negative control (b). Antibodies against Sandfly fever viruses fluoresce in the cytoplasm of infected cells. Not all cells are infected (a). The negative control does not show fluorescence (b). Scalebar 10 μ m.

Table 8. Workflow for antibody detection.

Working step	Description
Preparation	Thaw sera and vortex briefly. Pipet 90 μ l PBS into micro tube (1.5 ml), add 10 μ l of serum (desired ratio 1:10) and vortex briefly.
Pipet	Pipet 30 μ l of sample medium on Biochip for every sample (Fig. 5).
Incubation	Put slides into humid chamber and incubate for 30' at 37°C.
Wash	Drip off samples from slide and wash with PBS, while shaking with 60 rpm for 5'. Remove slide from PBS, drip off and remove PBS from Biochip spaces.
Pipet	Mix conjugate (Fluorescein) with blue dye (ratio: 8 drops per 100 μ l conjugate). Pipet 20 μ l of Conjugate-mix on each Biochip.
Incubation	Put slides into humid chamber and incubate for 30' at 37°C.
Wash	Drip off samples from slide and wash with PBS, while shaking with 60 rpm for 5'. Remove slide from PBS, drip of and remove PBS from Biochip spaces.
Cover	Put 5 drops of Glycerol on slide and cover with coverslip.
Analyze	Analyze samples under microscope.

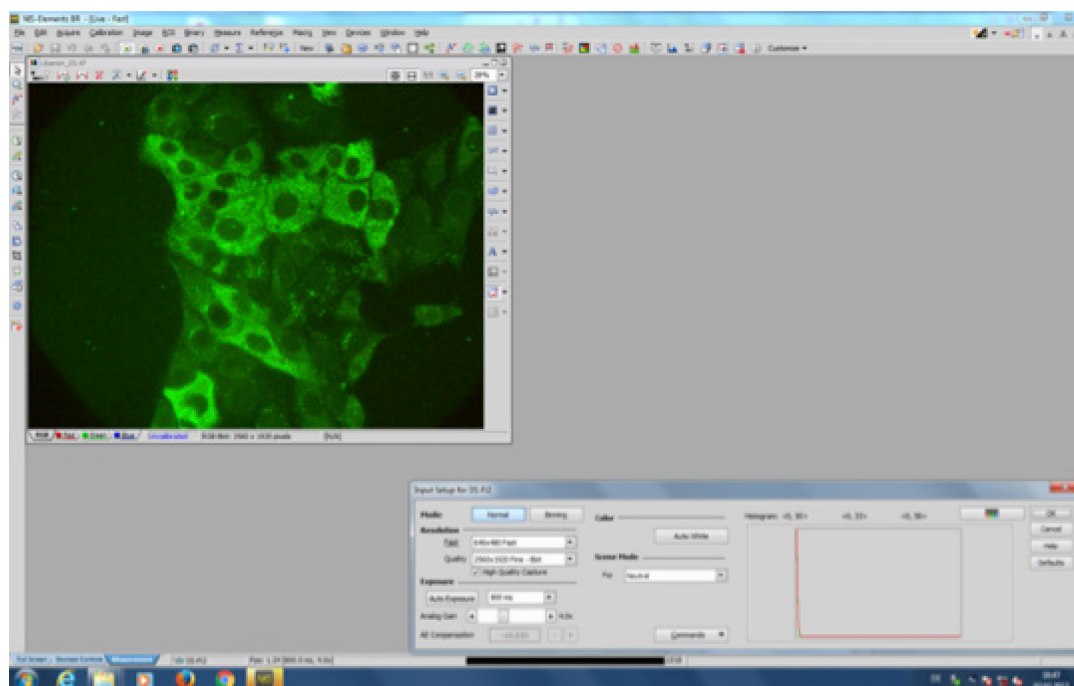


Figure 9. Screenshot of NIS software while editing a microphotography of a positive sample.

2.2.1 Titers and differentiation of cross-reactions

Titers and possible cross-reactions were evaluated as described in Table 4 by serial dilutions. Samples were diluted 1:10, 1:100 and 1:1000. If a clear differentiation was not possible, a dilution of 1:500 was added (Table 9). Potential cross-reactions between SFNV, SFSV, CYPV and TOSV can be differentiated by the comparison of titers (Table 10). Fluorescence intensity was evaluated by microscopy.

Table 9. Workflow for serial dilutions as recommended by EUROIMMUN.

Dilution	Scheme of dilution
1:10	100 µl PBS + 11,1 µl sample (undiluted)
1:100	100 µl PBS + 11,1 µl 1:10 diluted sample
1:500	50 µl PBS + 11,1 µl 1:100 diluted sample
1:1000	100 µl PBS + 11,1 µl 1:100 diluted sample

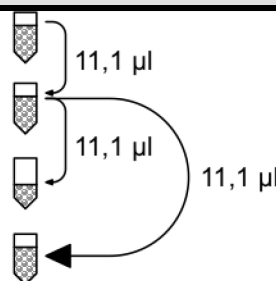
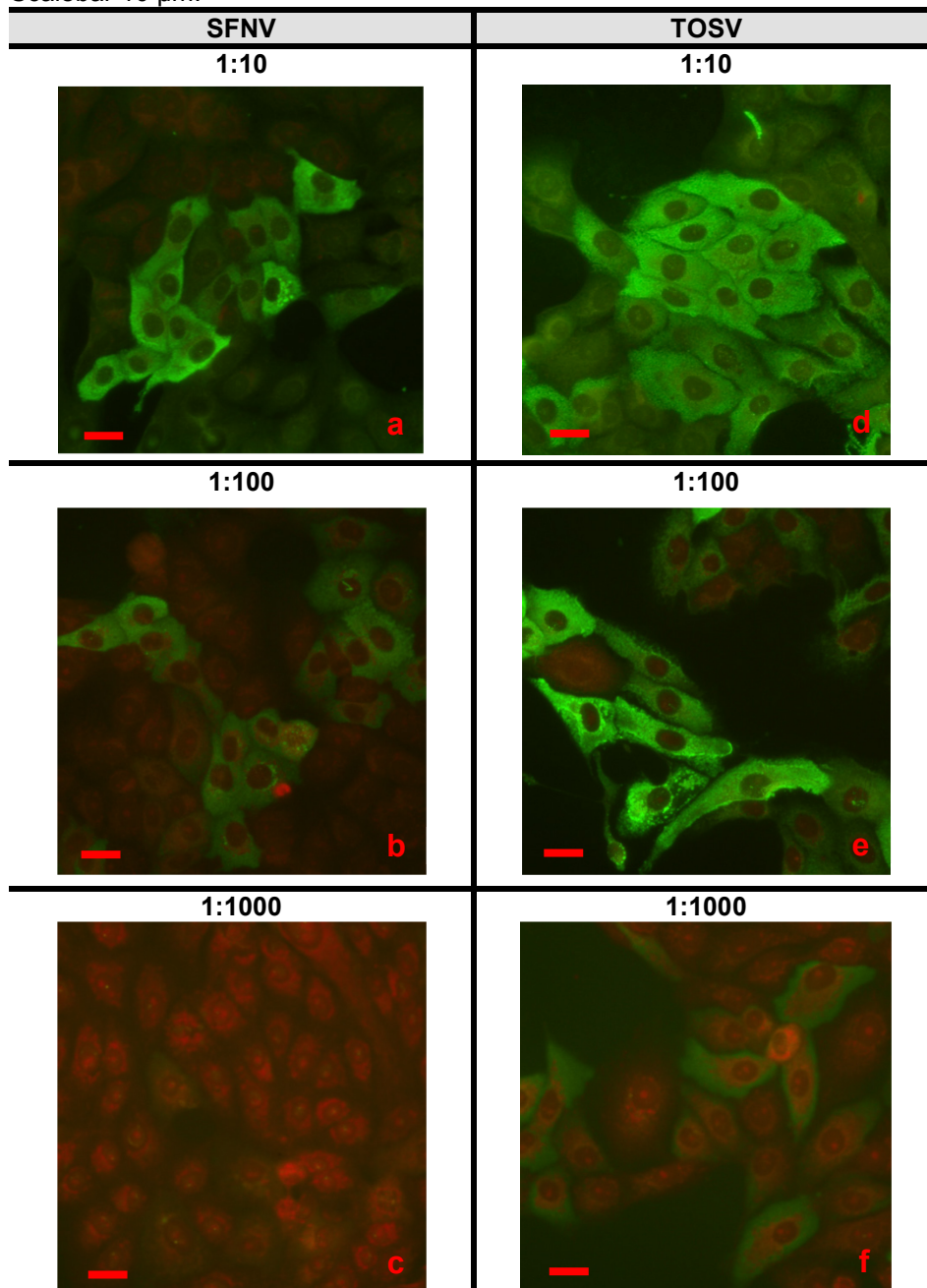


Table 10. Example of a serial dilution to evaluate titers and differentiate possible cross-reactions between SFNV and TOSV. The left column shows a SFNV-positive sample at dilutions: 1:10 (a), 1:100 (b) and 1:1000 (c). The right column shows a TOSV-positive sample at dilutions 1:10 (d), 1:100 (e) and 1:1000 (f). At titers of 1:10 and 1:100 this sample is positive for both, SFNV and TOSV, (a,b,d,e). At 1:1000, there is only reactivity with TOSV, indicating the antibodies to be specific for for TOSV (f) and non-specific for SFNV (e). Scalebar 10 μ m.



2.2.2 Variance in fluorescence

Negative samples may present a certain variability in cell morphology (Figure 10). Depending mainly on the overall immune status of the patient, the immunoreactivity of the sera can vary in its characteristics. Non-specific immunoreactivities can be the result of the presence of anti-nuclear antibodies against mitochondria, various antigens, core components, or an autoimmune disease.

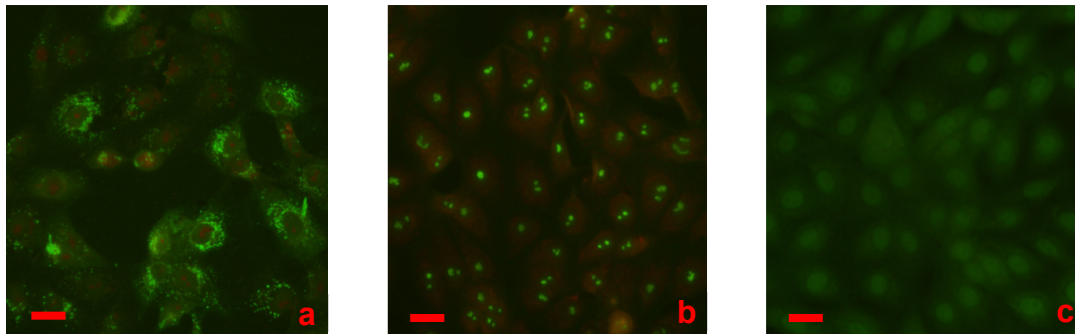


Figure 10. Examples of non-specific immunoreactivities. Cytoplasm is stained partly granular (Figure 10a), only nuclei are stained (Figure 10b), all cells fluoresce (Figure 10c). Scalebar 10 μ m.

2.3 Anti-*Leishmania* antibody testing

A RIDASCREEN *Leishmania* AB (K7121) test (R-Biopharm AG, Darmstadt, Germany) was used for anti-*Leishmania* antibody detection, strictly following the manufacturers' instructions (Table 11). This test is an enzyme immunoassay (EIA) for the qualitative detection of antibodies against *Leishmania* spp. in human serum. Altogether, 261 sera from soldiers returning from the Kosovo were analyzed. Serum samples were thawed and briefly vortexed. The EMIT test kit was brought to room temperature (20–25°C). Then, the respective number of wells was placed on the sampling plate and two positive controls and two negative controls were added. Photometric measurement was accomplished with an Anthos ht II Photometer (ANTHOSGroup GmbH, Starnberg, Deutschland) at 450/620 nm.

A sample index was evaluated and results were categorized as negative (<0.9), borderline (0.9-1.1) and positive (>1.1) based on the manufacturer-established cut-offs.

Table 11. Workflow for anti-*Leishmania* spp. antibody detection.

Working step	Description
Dilute	Dilute 10 µl serum with 490 µl sample buffer and vortex briefly (diluent) 1:50
Incubate	Cover up and incubate for 15' at room temperature
Washing	Dilute washing buffer (WASH) with distilled water 1:20 Empty microwell plate Fill up rows with 300 µl and empty again Repeat 5 times
Conjugate	Add two drops of conjugate (CONJUGATE) to each slot
Incubate	Cover and incubate for 15' at room temperature
Washing	Dilute washing buffer (WASH) with distilled water 1:20 Empty microwell plate Fill up rows with 300 µl and empty again Repeat 5 times
Dye	Add 1 drop of substrate (SUBSTRATE) and 1 drop of chromogen (CHROMOGEN) to each slot
Incubate	Cover up and incubate for 15' at room temperature
Stop	Add 1 drop of stop reagent (STOP) to each slot. Evaluate with photometer (see photometric measurement).

2.4 Statistics

Numerical data is presented as mean and standard deviation. A Mann-Whitney-Wilcoxon test was performed to evaluate differences between operational areas. Categorical data was analyzed with a Fisher's exact test, using Phlebovirus seropositivity, Phlebovirus species and titers as the predictor variables. Odds ratios (OR) with exact 95% Confidence Intervals (CI) were estimated. A two-sided p-value <0.05 was considered statistically significant. Microsoft Excel 2011 for Mac and the R environment for Mac were used for data analysis (R core team 2008).

3 Results

3.1 Age and sex distribution

3.1.1 Overall age and sex distribution

Of the 986 study participants, 976 were male and 10 were female. The minimum age was 18, whereas the maximum age was 63. The median age was 27 (mean=30.4, sd=9.9).

3.1.2 Comparison between operational areas and control group

Of the 486 participants returning from the operational areas, 482 were male and 4 were female. The minimum age was 19 and the maximum age was 63, with a median age of 29 (mean=32.6, sd=10.5). The mean age of the soldiers returning from the operational areas was significantly higher than the control group (p-value<0.001). The control group (CG) comprised of 500 participants, with 494 males and six females. The minimum age was 18 and the maximum age 60, with a median age of 25 (mean=28.3, sd=8.9).

3.1.3 Operational areas

Of the 61 participants returning from BIH, males exclusively, the minimum age was 19 and the maximum was 44. The median age was 24 (mean=25.25, sd=5.78), being significantly lower than the CG (p-value=0.05), the Kosovo (p-value<0.001), Syria (p-value<0.001) and the Lebanon (p-value<0.001).

Of the 261 participants returning from the Kosovo, 258 were male and three were female, while the minimum age was 20, the maximum age was 61. The median age was 28 (mean=31.9, sd=9.9) and was significantly higher than in the control group (CG) (p-value<0.001), but significantly lower than Syria (p-value<0.001) and the Lebanon (p-value=0.04).

Returning soldiers from Syria comprised of 101 participants, males exclusively. The age ranged from a minimum of 20 to a maximum of 63

years. The median age of 37 (mean=37.6, sd=11.45), was significantly higher than in the CG (p-value<0.001), but similar to the Lebanon (p-value=0.07).

Of the 63 participants returning, from the Lebanon, 62 were male and one female. The minimum age was 21 and the maximum was 60. The median age of 32 (mean=34.21, sd=10.22) was significantly higher than in the CG (p-value<0.001) (Figure 11).

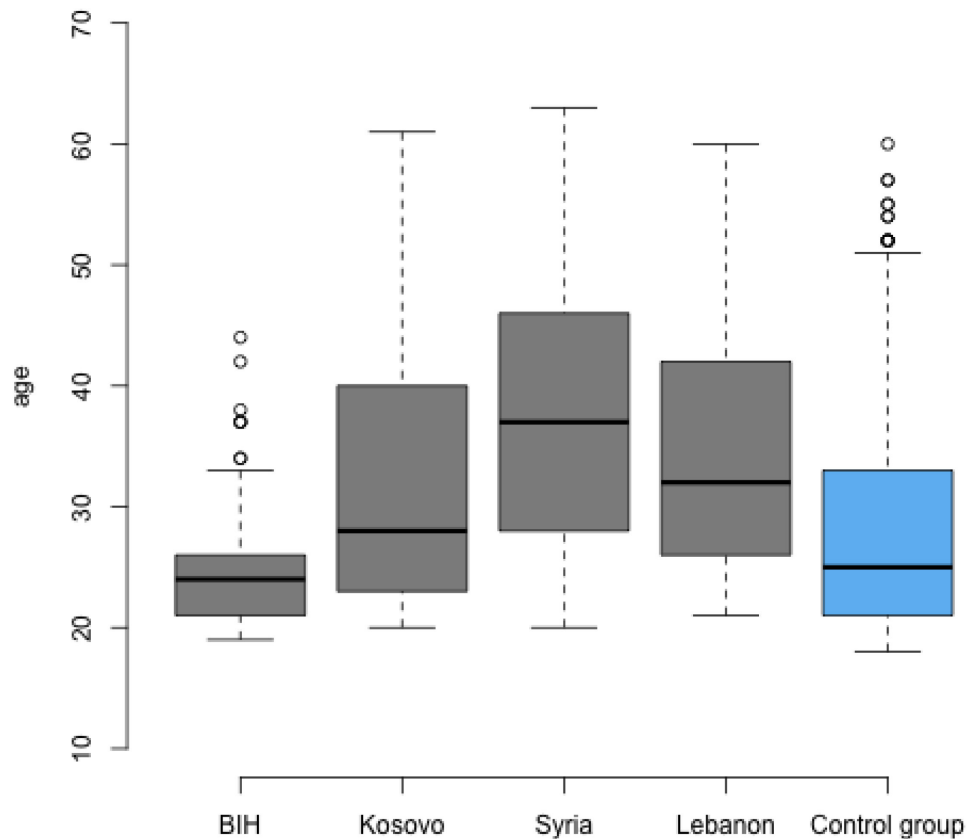


Figure 11. Boxplot of the age of participating soldiers.

3.2 Seroprevalences

3.2.1 Overall seroprevalences

Altogether, 146 sera (14.8%) showed a positive reaction against Phlebovirus antigen.

3.2.2 Soldiers returning from operational areas vs. control group

The overall seroprevalence of the soldiers returning from operational areas was 18.3% (89/486) and was significantly higher than in the CG (p-value=0.002). The age group 20-29 showed the highest seroprevalence (20.40%) being significantly higher than the respective age group in the CG (OR=2.1, 95% CI=1.3-3.5, p-value=0.003) (Table 12). In the control group (CG), 11.4% were tested positive for Phlebovirus antibodies (57/500) (Figure 12). The highest seroprevalence (14.58%) was observed in the age group 30-39 (Table 12).

3.2.3 Seroprevalences in the returning soldiers

In soldiers returning from BIH, seropositivity was 11.48% (7/61) (Figure 12). No significant difference compared to the CG was detected (OR=1.008, 95% CI=0.37-3.27). A decreased (but not significant) seropositivity compared to the Kosovo was observed (OR=0.5, 95% CI=0.2-1.2) (Figure 5). Antibodies were detected in the age group 20-29 exclusively, showing a seropositivity of 14.30% (Table 12).

In soldiers returning from the Kosovo, a seropositivity of 20.69% (54/261) was observed (Figure 12). The seroprevalence was significantly higher than in the control group (OR=2.03, 95% CI=1.32-3.11, p-value<0.001). The highest seroprevalence (23.02%) was detected in the age group 20-29 (Table 12). Of 261 soldiers returning from the Kosovo, 36 were positive (13.8%) and 19 were borderline (7.3%) for *Leishmania* spp. antibodies.

Serum samples from soldiers returning from Syria showed a positivity of 18.81% (19/101) being significantly higher compared to the CG (OR=1.8, 95% CI=0.96-3.26, p-value=0.05) (Figure 12). An increased (but not

significant) seroprevalence compared to the soldiers returning from the Lebanon was observed (OR=1.4, 95% CI=0.6-3.8). The highest seroprevalence (24.00%) was observed in the age group 40-49 (Table 12). The seroprevalence in soldiers returning from the Lebanon was 14.29% (9/63), with no significant difference compared to the CG (OR=1.3, 95% CI=0.53-2.83, p-value=0.53) (Figure 12). Again, the highest seroprevalence (23.08%) was observed in the age group 40-49 (Table 12).

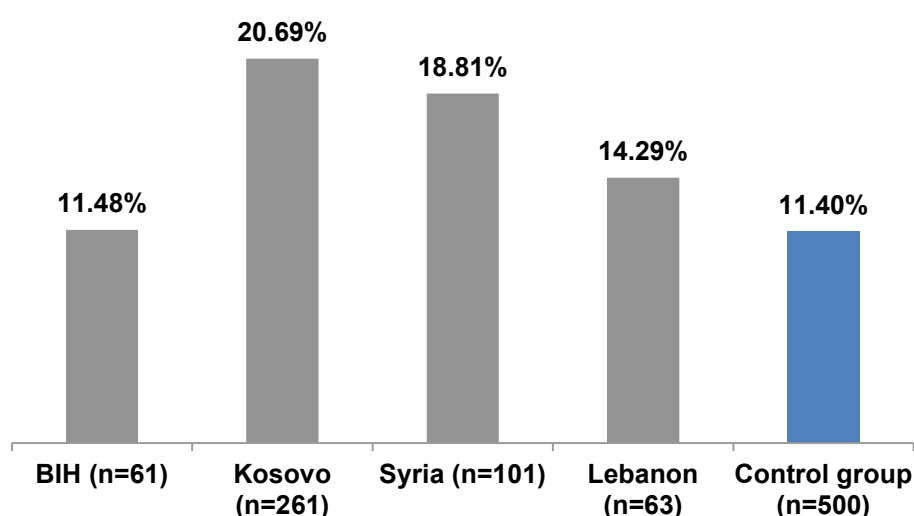


Figure 12. Seroprevalences by operational areas.

Table 12. Seroprevalences by age.

Age groups	positive/total (%)	Odds ratio (95% CI)	p-value
Operational areas (total)*			
<20	0/2 (0.00%)	-	-
20-29	51/250 (20.40%)	2.1 (1.3-3.5)	0.003
30-39	12/95 (12.63%)	0.85 (0.3-2.1)	0.8
40-49	20/99 (20.20%)	2.8 (0.9-11.9)	0.09
50-59	6/36 (16.67%)	1.5 (0.23-16.8)	1
>59	0/4 (0.00%)	-	-
BIH⁺			
<20	0/2 (0.00%)	-	-
20-29	7/49 (14.29%)	reference	-
30-39	0/8 (0.00%)	-	-
40-49	0/2 (0.00%)	-	-
50-59	-	-	-
>59	-	-	-

Kosovo

<20	-	-	-
20-29	32/139 (23.02%)	reference	
30-39	9/49 (18.37%)	0.8 (0.3-1.8)	0.6
40-49	11/59 (18.64%)	0.8 (0.3-1.8)	0.6
50-59	2/13 (15.38%)	0.6 (0.06-3.0)	0.7
>59	0/1 (0.00%)	-	-

Syria

<20	-	-	-
20-29	8/34 (23.53%)	reference	
30-39	1/22 (4.55%)	0.16 (0.003-1.4)	0.07
40-49	6/25 (24.00%)	1.03 (0.3-4.0)	1
50-59	4/18 (22.22%)	0.9 (0.2-4.3)	1
>59	0/2 (0.00%)	-	-

Lebanon

<20	-	-	-
20-29	4/28 (14.29%)	reference	
30-39	2/16 (12.50%)	0.9 (0.07-7.0)	1
40-49	3/13 (23.08%)	1.8 (0.2-12.7)	0.7
50-59	0/5 (0.00%)	-	-
>59	0/1 (0.00%)	-	-

Control group

<20	5/44 (11.36%)	reference	
20-29	32/294 (10.88%)	0.95 (0.3-3.3)	1
30-39	14/96 (14.58%)	1.3 (0.4-5.1)	0.8
40-49	4/48 (8.33%)	0.7 (0.1-3.6)	0.7
50-59	2/17 (11.76%)	1.03 (0.09-7.2)	1
>59	0/1 (0.00%)	-	-

Control group (without prior operation)

<20	5/44 (11.36%)	reference	
20-29	23/230 (10.00%)	0.87 (0.3-3.1)	0.79
30-39	5/36 (13.89%)	1.25 (0.26-6.0)	0.75
40-49	0/9 (0.00%)	-	-
50-59	0/2 (0.00%)	-	-
>59	-	-	-

*Seroprevalences were compared with the respective age group of the control group.

*Seroprevalences of the age groups in the operational areas were compared with the reference value in the respective area.

3.3 Diversity of phleboviruses

3.3.1 Overall diversity

Of 146 positive sera, 85 showed antibodies against TOSV (58.22%), 41 against SFSV (28.08%), 3 against CYPV (2.05%) and 3 against SFNV (2.05%). In 4 participants the possible cross-reaction between TOSV and SFNV (2.74%) could not be clarified and in 5 participants the possible cross-reaction between SFSV and CYPV (3.42%) could not be clarified. A double infection was observed in 5 participants (3.42%). While soldiers returning from BIH showed the lowest Phlebovirus diversity, participants of Syria and the Lebanon showed the highest. Phlebovirus diversity was also high in the control group (Figure 13).

3.3.2 Soldiers returning from operational areas vs. control group

Altogether, 89 sera of soldiers returning from operational areas revealed antibodies against phleboviruses. Antibodies against all four Phlebovirus species tested were observed. 46 sera were positive for TOSV (51.69%), 34 for SFSV (38.20%), three for CYPV (3.37%) and one for SFNV (1.12%). Additionally, one cross-reaction between TOSV and SFNV (1.12%) and two cross-reactions between SFSV and CYPV (2.25%), as well as two double infections (2.25%) were detected (Figure 6). The rate of positivity for SFSV was significantly higher in the returning soldiers (OR=4.4, 95% CI=1.7-12.8, p-value<0.001) compared to the CG. The age group 20-29 showed the highest Phlebovirus diversity (Table 13). In the control group, 57 sera revealed anti-Phlebovirus antibodies, namely 39 against TOSV (68.42%), 7 against SFSV (12.28%) and 2 against SFNV (3.51%). Three cross-reactions between TOSV and SFNV (5.26%), as well as three between SFSV and CYPV (5.26%) could not be clarified. Three double infections (5.26%) were observed. In the CG, no serum showed antibodies against CYPV. The positivity against TOSV was significantly higher than the one against SFSV and SFNV, respectively (p-value<0.001). The highest diversity was observed in the age group 20-29 (Table 13).

3.3.3 Diversity in the different operational areas

The soldiers returning from BIH showed antibodies against two different phleboviruses, namely, five against TOSV (71.43%) and two against CYPV (28.57%), respectively. The highest rates for both, TOSV and CYPV, compared to the CG and the other operational areas. Again, the age group 20-29 exhibited the highest rates of both TOSV and CYPV. No seropositivity was observed in the other age groups (Figure 13).

Of the 54 positive samples from soldiers returning from the Kosovo, 24 were positive against TOSV (44.44%) and 30 against SFSV (55.56%). The rate of antibodies against SFSV was significantly higher than in the CG (OR=8.7, 95% CI=3.2-27.1, p-value<0.001). The highest TOSV positivity was observed in the age group 50-59, whereas the highest SFSV positivity was observed in the age group 30-39 (Table 13).

Among soldiers returning from Syria, 19 were positive. Of these, 13 showed antibodies against TOSV (68.4%), one against SFSV (5.3%), one against CYPV (5.3%), two samples could not be differentiated and are considered as cross-reactivity SFSV/CYPV (10.5%) and two samples showed a double infection (10.5%). The highest positivity for TOSV (36.84%) was found in the age group 20-29. Positivity for CYPV (5.26%) was only observed in this age group. The positivity rate for TOSV was significantly higher than the one for SFSV and CYPV (OR=34.5, 95% CI=3.8-1725.6, p-value<0.001), respectively.

Of the sera of soldiers returning from the Lebanon, nine were positive, four for TOSV (44.4%), three for SFSV (33.3%), one for SFNV (11.1%) and one cross-reacted between TOSV/SFNV (11.1%). Only the age group 20-29 exhibited positivity for three different phleboviruses, namely TOSV, SFSV and SFNV. Thus, this age group showed the highest diversity (Table 13).

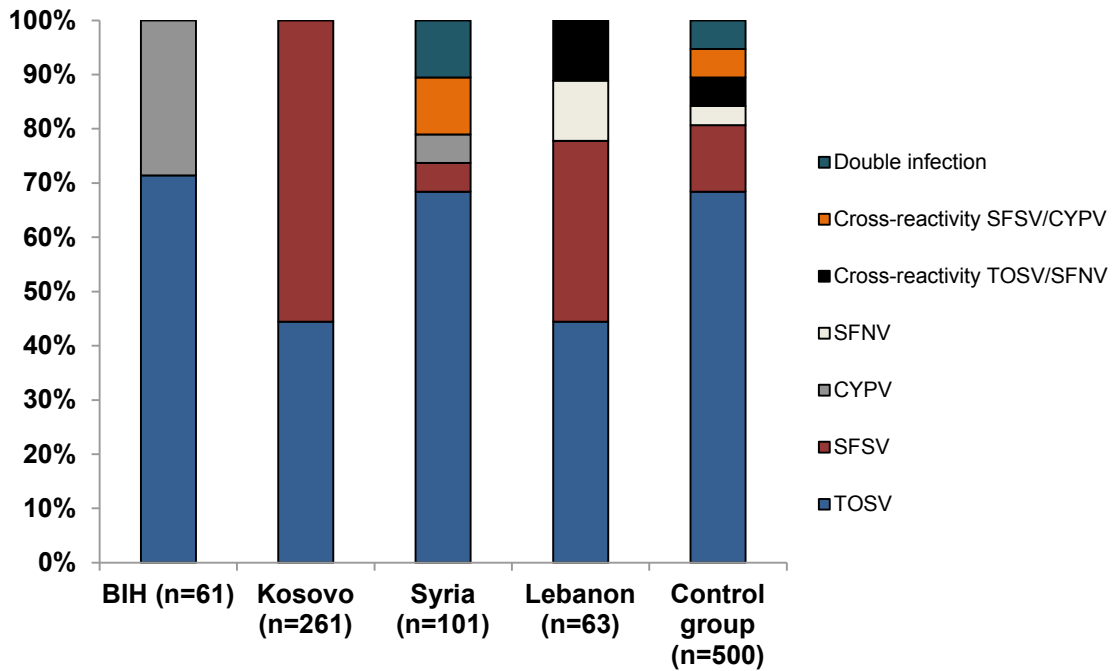


Figure 13. Diversity of phleboviruses in participating soldiers.

Table 13. Diversity of phleboviruses by age.

Age group	TOSV positive/total (%)	SFSV positive/total (%)	CYPV positive/total (%)	SFNV positive/total (%)	CROSS TOSV/SFNV positive/total (%)	CROSS SFSV/CYPV positive/total (%)	DOUBLE positive/total (%)
Operational areas (total)							
<20	-	-	-	-	-	-	-
20-29	30/250 (12.00%)	16/250 (6.40%)	3/250 (1.20%)	1/250 (0.40%)	1/250 (0.40%)	0/250 (0.00%)	0/250 (0.00%)
30-39	5/95 (5.26%)	7/95 (7.37%)	0/95 (0.00%)	0/95 (0.00%)	0/95 (0.00%)	0/95 (0.00%)	0/95 (0.00%)
40-49	8/99 (8.08%)	11/99 (11.11%)	0/99 (0.00%)	0/99 (0.00%)	0/99 (0.00%)	1/99 (1.01%)	0/99 (0.00%)
50-59	3/36 (8.33%)	0/36 (0.00%)	0/36 (0.00%)	0/36 (0.00%)	0/36 (0.00%)	1/36 (2.78%)	2/36 (5.56%)
>59	-	-	-	-	-	-	-
total	46/486 (9.47%)	34/486 (7.00%)	3/486 (0.62%)	1/486 (0.21%)	1/486 (0.21%)	2/486 (0.41%)	2/486 (0.41%)
Bosnia and Herzegovina							
<20	0/2 (0.00%)	-	0/2 (0.00%)	-	-	-	-
20-29	5/49 (10.20%)	-	2/49 (4.08%)	-	-	-	-
30-39	0/8 (0.00%)	-	0/8 (0.00%)	-	-	-	-
40-49	0/2 (0.00%)	-	0/2 (0.00%)	-	-	-	-
50-59	-	-	-	-	-	-	-
>59	-	-	-	-	-	-	-

Results

total	5/61 (8.20%)	-	2/61 (3.28%)	-	-	-	-
Kosovo[*]							
<20	-	-	-	-	-	-	-
20-29	17/139 (12.23%)	15/139 (10.79%)	-	-	-	-	-
30-39	2/49 (4.08%)	7/49 (14.29%)	-	-	-	-	-
40-49	3/59 (5.08%)	8/59 (13.56%)	-	-	-	-	-
50-59	2/13 (15.38%)	0/13 (0.00%)	-	-	-	-	-
>59	0/13 (0.00%)	0/13 (0.00%)	-	-	-	-	-
total	24/261 (9.20%)	30/261 (11.49%)	-	-	-	-	-
Syria							
<20	-	-	-	-	-	-	-
20-29	7/34 (20.59%)	0/34 (0.00%)	1/34 (2.94%)	-	-	0/34 (0.00%)	0/34 (0.00%)
30-39	1/22 (4.55%)	0/22 (0.00%)	0/22 (0.00%)	-	-	0/22 (0.00%)	0/22 (0.00%)
40-49	4/25 (16.00%)	1/25 (4.00%)	0/25 (0.00%)	-	-	1/25 (4.00%)	0/25 (0.00%)
50-59	1/18 (5.56%)	0/18 (0.00%)	0/18 (0.00%)	-	-	1/18 (5.56%)	2/18 (11.11%)
>59	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)	-	-	0/2 (0.00%)	0/2 (0.00%)
total	13/101 (12.87%)	1/101 (0.99%)	1/101 (0.99%)	-	-	2/101 (1.98%)	2/101 (1.98%)
Lebanon							
<20	-	-	-	-	-	-	-
20-29	1/28 (3.57%)	1/28 (3.57%)	-	1/28 (3.57%)	1/28 (3.57%)	-	-
30-39	2/16 (12.50%)	0/16 (0.00%)	-	0/16 (0.00%)	0/16 (0.00%)	-	-
40-49	1/13 (7.69%)	2/13 (15.38%)	-	0/13 (0.00%)	0/13 (0.00%)	-	-
50-59	0/5 (0.00%)	0/5 (0.00%)	-	0/5 (0.00%)	0/5 (0.00%)	-	-
>59	0/1 (0.00%)	0/1 (0.00%)	-	0/1 (0.00%)	0/1 (0.00%)	-	-
total	4/63 (6.35%)	3/63 (4.76%)	-	1/63 (1.59%)	1/63 (1.59%)	-	-
Control group							
<20	3/44 (6.82%)	1/44 (2.27%)	-	0/44 (0.00%)	1/44 (1.75%)	0/44 (0.00%)	0/44 (0.00%)
20-29	23/294 (7.82%)	3/294 (1.02%)	-	2/294 (0.68%)	2/294 (0.68%)	0/294 (0.00%)	2/294 (3.51%)

Results

30-39	9/96 (9.38%)	3/96 (3.13%)	-	0/96 (0.00%)	0/96 (0.00%)	2/96 (2.08%)	0/96 (0.00%)
40-49	3/48 (6.25%)	0/48 (0.00%)	-	0/48 (0.00%)	0/48 (0.00%)	1/48 (2.08%)	0/48 (0.00%)
50-59	1/17 (5.88%)	0/17 (0.00%)	-	0/17 (0.00%)	0/17 (0.00%)	0/17 (0.00%)	1/17 (5.88%)
>59	-	-	-	-	-	-	-
total	39/500 (7.80%)	7/500 (1.40%)	-	2/500 (0.40%)	3/500 (0.60%)	3/500 (0.60%)	3/500 (0.60%)

Control group (without prior operation)

<20	3/44 (6.82%)	1/44 (2.27%)	-	0/44 (0.00%)	1/44 (2.27%)	0/44 (0.00%)	0/44 (0.00%)
20-29	16/230 (6.96%)	2/239 (0.87%)	-	2/230 (0.87%)	2/230 (0.87%)	0/230 (0.00%)	1/230 (0.43%)
30-39	3/36 (8.33%)	1/36 (2.78%)	-	0/36 (0.00%)	0/36 (0.00%)	1/36 (2.78%)	0/36 (0.00%)
40-49	0/9 (0.00%)	0/9 (0.00%)	-	0/9 (0.00%)	0/9 (0.00%)	0/9 (0.00%)	0/9 (0.00%)
50-59	0/2 (0.00%)	0/2 (0.00%)	-	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)
>59	-	-	-	-	-	-	-
total	22/321 (6.85%)	4/321 (1.25%)	-	2/321 (0.62%)	3/321 (0.93%)	1/321 (0.31%)	1/321 (0.31%)

*Samples tested only for TOSV and SFSV seropositivity.

3.4 Titers

3.4.1 Overall titers

Overall, 146 samples were positive and antibody titers ranged from 1:10 to 1:1000, 63.7% showing 1:10, 17.12% 1:320, 13.7% 1:100 and 5.48% 1:1000. The overall highest titers were detected in the Kosovo and the Lebanon, namely against TOSV and SFSV. High titers were also detected in the control group.

3.4.2 Returning soldiers vs. control group

In soldiers returning from the operational areas, titers ranged from 1:10 to 1:1000. The majority of the positive sera revealed titers of 1:10. Again, the age group 20-29 showed the highest percentage of sera with a titre of 1:10. The highest titers were detected against TOSV (3.37%) and SFSV (1.12%) (Table 14). In the control group (CG), 57 positive sera were positive. Titers also ranged from 1:10 to 1:1000. Also here, the majority of sera showed a titre of 1:10, namely against TOSV. This Phlebovirus species was the predominant one in this group (47.37%) and also the one with the highest titers (1:1000) (OR=11.6, 95% CI=3.6-50.3, p-value<0.001). The highest titers (1:1000) were observed in the age groups 30-39 and 40-49, respectively.

3.4.3 Operational areas

In soldiers returning from BIH, titers ranged from 1:10 to 1:100, 57.14% showing 1:10 and 42.86% showing 1:100 (Figure 14). Specific antibodies were observed for the age group 20-29 exclusively. The highest positivity was observed for TOSV at 1:100 and was significantly higher than the CG (OR= 12.4, 95% CI=1.3-129.4, p-value=0.01) and the Kosovo (OR=8.8, 95% CI=1.0-78.0, p-value=0.02) (Table 14).

In soldiers returning from the Kosovo, titers ranged from 1:10 to 1:1000, 74.07 % showing 1:10, 12.96% showing 1:100, 11.11% showing 1:320 and

1.85% showing 1:1000 (Figure 9). Titers of 1:1000 were only observed against TOSV (1.85%) in the age group 20-29 (Table 14).

In soldiers returning from Syria, titers ranged from 1:10 to 1:320, 57.89% showing 1:10, 26.32% showing 1:320 and 15.79% showing 1:100 (Figure 14). The highest positivity at a titer of 1:10 was observed against TOSV. Also here, the age group 20-29 showed the highest positivity at a titer of 1:10 compared to the other age groups. Soldiers returning from Syria revealed the highest percentage of positivity at the titer of 1:320 (4.95%) compared to the CG and the other operational areas.

In soldiers returning from the Lebanon, titers ranged from 1:10 to 1:1000, 33.33% showing 1:10, 33.33% showing 1:100 and 33.33% showing 1:1000 (Figure 14). This group had the highest percentage of sera with a titer of 1:1000, being significantly higher than the CG (OR=6.3, 95% CI=0.8-48.8, p-value=0.05) and Syria (OR=23.9, 95% CI=1.6-1400.1, p-value=0.008) (Table 14).

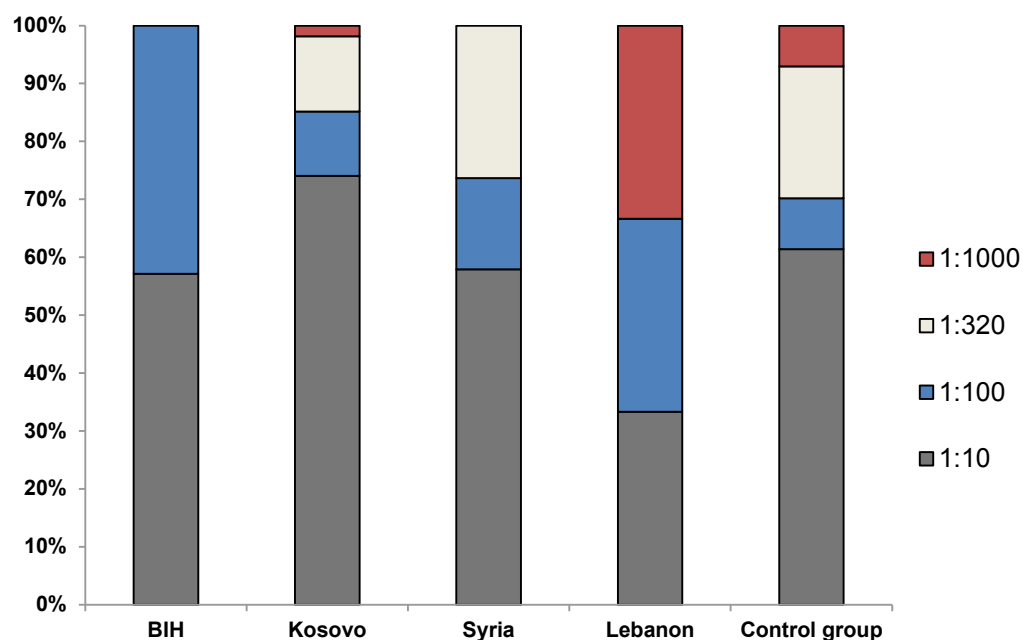


Figure 14. Comparison of titers between operational areas. Data is presented as percent of positive tested samples of the respective operational area.

Table 14. Antibody titers by age.

	1:10 positive/total (%)	1:100 positive/total (%)	1:320 positive/total (%)	1:1000 positive/total (%)
Operational areas (total)				
<20	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)
20-29	35/250 (14.00%)	9/250 (3.60%)	6/250 (2.40%)	1/250 (0.40%)
30-39	9/95 (9.47%)	1/95 (1.05%)	1/95 (1.05%)	1/95 (1.05%)
40-49	12/99 (12.12%)	5/99 (5.05%)	1/99 (1.01%)	2/99 (2.02%)
50-59	2/36 (5.56%)	0/36 (0.00%)	4/36 (11.11%)	0/36 (0.00%)
>59	0/4 (0.00%)	0/4 (0.00%)	0/4 (0.00%)	0/4 (0.00%)
total	58/486 (11.93%)	15/486 (3.09%)	12/486 (2.47%)	4/486 (0.82%)

Bosnia and Herzegovina

<20	0/2 (0.00%)	0/2 (0.00%)	-	-
20-29	4/49 (8.16%)	3/49 (6.12%)	-	-
30-39	0/8 (0.00%)	0/8 (0.00%)	-	-
40-49	0/2 (0.00%)	0/2 (0.00%)	-	-
50-59	-	-	-	-
>59	-	-	-	-
total	4/61 (6.56%)	3/61 (4.92%)	-	-

Kosovo

<20	-	-	-	-
20-29	24/139 (17.27%)	2/139 (1.44%)	5/139 (3.60%)	1/139 (0.72%)
30-39	8/49 (16.33%)	0/49 (0.00%)	1/49 (2.04%)	0/49 (0.00%)
40-49	7/59 (11.86%)	4/59 (6.78%)	0/59 (0.00%)	0/59 (0.00%)
50-59	1/13 (7.69%)	0/13 (0.00%)	1/13 (7.69%)	0/13 (0.00%)
>59	0/1 (0.00%)	0/1 (0.00%)	0/1 (0.00%)	0/1 (0.00%)
total	40/261 (15.33%)	6/261 (2.30%)	7/261 (2.68%)	1/261 (0.38%)

Syria

<20	-	-	-	-
20-29	5/34 (14.71%)	2/34 (5.88%)	1/34 (2.94%)	-
30-39	1/22 (4.55%)	0/22 (0.00%)	0/22 (0.00%)	-
40-49	4/25 (16.00%)	1/25 (4.00%)	1/25 (4.00%)	-
50-59	1/18 (5.56%)	0/18 (0.00%)	3/18 (16.67%)	-
>59	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)	-
total	11/101 (10.89%)	3/101 (2.97%)	5/101 (4.95%)	-

Lebanon

<20	-	-	-	-
20-29	2/28 (7.14%)	2/28 (7.14%)	-	0/28 (0.00%)

30-39	0/16 (0.00%)	1/16 (6.25%)	-	1/16 (6.25%)
40-49	1/13 (7.69%)	0/13 (0.00%)	-	2/13 (15.38%)
50-59	0/5 (0.00%)	0/5 (0.00%)	-	0/5 (0.00%)
>59	0/1 (0.00%)	0/1 (0.00%)	-	0/1 (0.00%)
total	3/63 (4.76%)	3/63 (4.76%)	-	3/63 (4.76%)

Control group

<20	4/44 (9.09%)	0/44 (0.00%)	1/44 (2.27%)	0/44 (0.00%)
20-29	20/294 (6.80%)	3/294 (1.02%)	8/294 (2.72%)	1/57 (0.34%)
30-39	8/96 (8.33%)	2/96 (2.08%)	2/96 (2.08%)	2/96 (2.08%)
40-49	2/48 (4.17%)	0/48 (0.00%)	1/48 (2.08%)	1/48 (2.08%)
50-59	1/17 (5.88%)	0/17 (0.00%)	1/17 (5.88%)	0/17 (0.00%)
>59	0/1 (0.00%)	0/1 (0.00%)	0/1 (0.00%)	0/1 (0.00%)
total	35/500 (7.00%)	5/500 (1.00%)	13/500 (2.60%)	4/57 (0.80%)

Control group (without prior operation)

<20	4/44 (9.09%)	0/44 (0.00%)	1/44 (2.27%)	0/44 (0.00%)
20-29	13/230 (5.65%)	3/230 (1.30%)	7/230 (3.04%)	0/230 (0.00%)
30-39	3/36 (8.33%)	0/36 (0.00%)	2/36 (5.56%)	0/36 (0.00%)
40-49	0/9 (0.00%)	0/9 (0.00%)	0/9 (0.00%)	0/9 (0.00%)
50-59	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)	0/2 (0.00%)
>59	-	-	-	-
	20/321 (6.23%)	3/321 (0.93%)	10/321 (3.12%)	0/321 (0.00%)

Table 15. Antibody titers against Phlebovirus species.

Phlebovirus	1:10 positive/total (%)	1:100 positive/total (%)	1:320 positive/total (%)	1:1000 positive/total (%)	total positive/total (%)
Operational areas (total)					
TOSV	25/89 (28.09%)	11/89 (12.36%)	7/89 (7.87%)	3/89 (3.37%)	46/89 (51.69%)
SFSV	27/89 (30.34%)	4/89 (4.49%)	2/89 (2.25%)	1/89 (1.12%)	34/89 (38.20%)
CYPV	3/89 (3.37%)	0/89 (0.00%)	0/89 (0.00%)	0/89 (0.00%)	3/89 (3.37%)
SFNV	1/89 (1.12%)	0/89 (0.00%)	0/89 (0.00%)	0/89 (0.00%)	1/89 (1.12%)
CROSS TOSV/SFNV	1/89 (1.12%)	0/89 (0.00%)	0/89 (0.00%)	0/89 (0.00%)	1/89 (1.12%)
CROSS SFSV/CYPV	1/89 (1.12%)	0/89 (0.00%)	1/89 (1.12%)	0/89 (0.00%)	2/89 (2.25%)
DOUBLE	0/89 (0.00%)	0/89 (0.00%)	2/89 (2.25%)	0/89 (0.00%)	2/89 (2.25%)
Bosnia and Herzegovina					
TOSV	2/7 (28.57%)	3/7 (42.86%)	-	-	5/7 (71.43%)
CYPV	2/7 (28.57%)	0/7 (0.00%)	-	-	2/7 (28.57%)

Kosovo

TOSV	14/54 (25.93%)	4/54 (7.41%)	5/54 (9.26%)	1/54 (1.85%)	24/54 (44.44%)
SFSV	26/54 (48.15%)	2/54 (3.70%)	2/54 (3.70%)	0/54 (0.00%)	30/54 (55.56%)

Syria

TOSV	9/19 (47.37%)	2/19 (10.53%)	2/19 (10.53%)	-	13/19 (68.42%)
SFSV	0/19 (0.00%)	1/19 (5.26%)	0/19 (0.00%)	-	1/19 (5.26%)
CYPV	1/19 (5.26%)	0/19 (0.00%)	0/19 (0.00%)	-	1/19 (5.26%)
CROSS SFSV/CYPV	1/19 (5.26%)	0/19 (0.00%)	2/19 (10.53%)	-	2/19 (10.53%)
DOUBLE	0/19 (0.00%)	0/19 (0.00%)	1/19 (5.26%)	-	1/19 (5.26%)

Lebanon

TOSV	0/9 (0.00%)	2/9 (22.22%)	-	2/9 (22.22%)	4/9 (44.44%)
SFSV	1/9 (11.11%)	1/9 (11.11%)	-	1/9 (11.11%)	3/9 (33.33%)
SFNV	1/9 (11.11%)	0/9 (0.00%)	-	0/9 (0.00%)	1/9 (11.11%)
CROSS TOSV/SFNV	1/9 (11.11%)	0/9 (0.00%)	-	0/9 (0.00%)	1/9 (11.11%)

Control group

TOSV	27/57 (47.37%)	3/57 (5.26%)	7/57 (12.28%)	2/57 (3.51%)	39/57 (68.42%)
SFSV	4/57 (7.02%)	2/57 (3.51%)	1/57 (1.75%)	1/57 (1.75%)	8/57 (14.04%)
CYPV	-	-	-	-	-
SFNV	2/57 (3.51%)	0/57 (0.00%)	0/57 (0.00%)	0/57 (0.00%)	2/57 (3.51%)
CROSS TOSV/SFNV	1/57 (1.75%)	0/57 (0.00%)	2/57 (3.51%)	0/57 (0.00%)	3/57 (5.26%)
CROSS SFSV/CYPV	1/57 (1.75%)	0/57 (0.00%)	1/57 (1.75%)	1/57 (1.75%)	3/57 (5.26%)
DOUBLE	0/57 (0.00%)	0/57 (0.00%)	2/57 (3.51%)	0/57 (0.00%)	2/57 (3.51%)

Control group (without prior operation)

TOSV	14/33 (42.42%)	2/33 (6.06%)	6/33 (18.18%)	0/33 (0.00%)	22/33 (66.67%)
SFSV	3/33 (9.09%)	1/33 (3.03%)	1/33 (3.03%)	0/33 (0.00%)	5/33 (15.15%)
CYPV	-	-	-	-	-
SFNV	2/33 (6.06%)	0/33 (0.00%)	0/33 (0.00%)	0/33 (0.00%)	2/33 (6.06%)
CROSS TOSV/SFNV	1/33 (3.03%)	0/33 (0.00%)	2/33 (6.06%)	0/33 (0.00%)	3/33 (9.09%)
CROSS SFSV/CYPV	0/33 (0.00%)	0/33 (0.00%)	1/33 (3.03%)	0/33 (0.00%)	1/33 (3.03%)
DOUBLE	0/33 (0.00%)	0/33 (0.00%)	0/33 (0.00%)	0/33 (0.00%)	0/33 (0.00%)

3.5 Risk factors

3.5.1 Overall risk factors

Significant associations were observed between anti-SFSV antibodies and a vacation during the respective mission (OR=2.4, 95% CI=1.2-5.1, p-value=0.01), between anti-TOSV antibodies and dog contact during the respective mission (OR=1.7, 95% CI=1.04-2.7, p-value 0.03) and between anti-SFNV antibodies and *Leishmania* spp. antibodies (OR=19.2, 95% CI=1.0-1134.4, pvalue=0.03) (Table 17). Moreover, significant correlations were detected between a titer of 1:1000 and previously completed missions (OR=8.8, 95% CI=1.1-397.1, p-value=0.01), particularly, previous missions to Syria (OR=5.0, 95% CI=0.8-25.7, p-value=0.05) and to BIH (OR=6.3, 95% CI=1.2-34.1, p-value=0.02). Also, a significant correlation between a titer of 1:100 and dog contact during the mission (OR=2.7, 95% CI=1.0-7.4, p-value=0.04) was revealed (Table 18).

Table 16. Overall risk factors.

Risk factors	OR	95% CI	p-value
Missions			
Prior mission	0.96	0.7-1.4	0.9
Kosovo	0.8	0.5-1.2	0.3
Syria	1.4	0.8-2.4	0.2
Bosnia	1.3	0.8-2.2	0.25
Vacation			
Vacation	1.4	1.0-2.1	0.06
Outdoor activities			
Outdoor activities	1.3	0.75-2.3	0.5
Animal contact			
Dog	1.4	0.9-2.11	0.14
Pets			
Pet	0.9	0.7-1.4	0.79
<i>Leishmania</i> ELISA			
Overall antibodies	1.0	0.6-1.6	1
Positive antibodies	1.1	0.6-2.0	0.76
Borderline antibodies	0.97	0.4-2.0	1

Table 17. Correlations between Phlebovirus species and risk factors.

	TOSV OR/ 95% CI (p-value)	SFSV OR/ 95% CI (p-value)	CYPV OR/ 95% CI (p-value)	SFNV OR/ 95% CI (p-value)	Double infection OR/ 95% CI (p-value)
Prior mission					
Prior mission	0.95/ 0.6-1.5 (0.9)	1.07/ 0.54-2.1 (0.87)	0.6/ 0.01-11.9 (1)	-	1.2/ 0.09-17.2 (1)
Kosovo	0.9/ 0.5-1.5 (0.7)	0.8/ 0.3-1.8 (0.7)	1.5/ 0.03-28.1 (1)	-	0.97/ 0.02-12.2 (1)
Syria	1.4/ 0.65-2.6 (0.36)	1.7/ 0.6-4.02 (0.2)	-	-	2.7/ 0.05-33.9 (0.38)
BIH	1.4/ 0.7-2.5 (0.3)	0.9/ 0.3-2.2 (1)	-	-	6.2/ 0.45-86.4 (0.1)
Vacation					
Vacation during mission	1.0/ 0.6-1.6 (1)	2.4/ 1.2-5.1 (0.01)	2.2/ 0.1-127.7 (0.6)	2.2/ 0.1-127.7 (0.6)	1.1/ 0.08-14.9 (1)
Outdoor activities					
Sports	0.93/ 0.5-1.8 (0.8)	3.7/ 0.9-32.0 (0.07)	0.37/ 0.02-21.7 (0.4)	0.37/ 0.02-21.7 (0.4)	-
Animal contact					
Dog	1.7/ 1.04-2.7 (0.03)	0.5/ 0.2-1.1 (0.07)	0.9/ 0.02-17.3 (0.9)	0.9/ 0.02-17.3 (0.9)	1.8/ 0.13-24.9 (0.6)
Pet					
Pet	0.98/ 0.6-1.6 (1)	0.9/ 0.5-1.8 (0.8)	0.6/ 0.01-11.1 (1)	2.3/ 0.12-136.5 (0.6)	0.4/ 0.007-4.8 (0.6)
Leishmania ELISA					
Overall antibodies	0.7/ 0.34-1.4 (0.4)	1.5/ 0.6-3.1 (0.4)	2.6/ 0.04-49.2 (0.4)	10.3/ 0.5-506.1 (0.07)	-
Positive antibodies	0.8/ 0.3-1.9 (0.8)	1.7/ 0.6-4.1 (0.27)	-	19.2/ 1.0-1134.4 (0.03)	
Borderline antibodies	0.8/ 0.25-2.1 (0.8)	0.8/ 0.2-3.5 (0.8)	6.7/ 0.1-130.3 (0.2)	-	-

Table 18. Correlations between titers and risk factors.

	1:10 OR/ 95% CI (p-value)	1:100 OR/ 95% CI (p-value)	1:320 OR/ 95% CI (p-value)	1:1000 OR/ 95% CI (p-value)
Prior mission				
Prior mission	0.8/ 0.6-1.5 (0.9)	0.8/ 0.3-2.2 (0.8)	0.7/ 0.3-1.7 (0.4)	8.8/ 1.1-397.1 (0.03)
Kosovo	0.8/ 0.5-1.4 (0.5)	0.97/ 0.3-2.9 (1)	0.4/ 0.07-1.3 (0.16)	1.8/ 0.3-9.1 (0.4)
Syria	1.5/ 0.8-2.8 (0.2)	0.4/ 0.01-2.7 (0.7)	1.1/ 0.2-3.8 (0.8)	5.0/ 0.8-25.7 (0.05)

BIH	0.9/ 0.4-1.7 (0.9)	1.1/ 0.2-3.8 (0.8)	2.0/ 0.6-5.3 (0.15)	6.3/ 1.2-34.1 (0.02)
Vacation				
Vacation during mission	1.4/ 0.9-2.2 (0.13)	1.6/ 0.6-4.7 (0.37)	0.8/ 0.3-2.02 (0.7)	3.3/ 0.6-33.2 (0.16)
Outdoor activities				
Sports	1.04/ 0.6-2.1 (1)	1.7/ 0.4-15.0 (0.76)	2.1/ 0.5-19.0 (0.4)	1.3/ 0.2-58.4 (1)
Animal contact				
Dog	1.3/ 0.7-2.1 (0.3)	2.7/ 1.0-7.4 (0.04)	1.0/ 0.3-2.8 (1)	0.6/ 0.01-4.5 (1)
Pet				
Pet	0.8/ 0.5-1.3 (0.4)	0.9/ 0.3-2.5 (1)	1.3/ 0.5-3.05 (0.7)	1.2/ 0.2-6.2 (1)
<i>Leishmania</i> ELISA				
Overall antibodies	1.2/ 0.6-2.04 (0.66)	0.56/ 0.06-2.4 (0.76)	0.97/ 0.2-2.9 (1)	0.7/ 0.02-5.7 (1)
Positive antibodies	1.2/ 0.5-2.4 (0.7)	1.1/ 0.1-4.5 (1)	1.3/ 0.25-4.5 (0.7)	-
Borderline antibodies	1.3/ 0.5-2.8 (0.5)	-	0.6/ 0.01-3.5 (1)	1.9/ 0.04-15.2 (0.4)

3.5.2 Risk factors in returning soldiers vs. the control group

In participants from all operational areas, significant correlations were detected between anti-Phlebovirus antibodies and running (OR=2.35, 95% CI=1.2-4.8, p-value<0.01) and between anti-Phlebovirus antibodies and *Leishmania* spp. positive antibodies (OR=2.1, 95% CI=0.97-4.2, p-value=0.05) (Table 19). Also significant associations were observed between anti-TOSV antibodies and symptoms (OR=2.14, 95% CI=1.1-4.1, p-value=0.02) (Table 20). Significant correlations were observed between a titer of 1:10 and running (OR=2.8, 95% CI=1.2-7.6, p-value=0.01) and *Leishmania* spp. antibodies (OR=2.05, 95% CI=1.01-3.09, p-value=0.04). A titer of 1:320 was significantly associated with fever (OR=3.8, 95% CI=0.8-14.7, p-value=0.05) (Table 21). In the control group a significant association between anti-Phlebovirus antibodies and a prior mission in BIH was

observed (OR=2.8, 95% CI=1.09-7.36, p-value=0.02) as well as between a double infection and a prior mission in BIH (OR=31.6, 95% CI=1.6-1879.6, p-value=0.01) (Figure 15, Table 19). A titer of 1:1000 was significantly associated with a prior mission completed in BIH (OR=15.9, 95% CI=1.11-225.5, p-value=0.02) (Table 21).

Factors

Missions

Prior mission

Kosovo

Syria

BIH

Vacation

Vacation during mission

Outdoor activities

Sports

Animal contact mission

Dog

Pets

Pet

Leishmania ELISA

Overall antibodies

Positive antibodies

Borderline antibodies

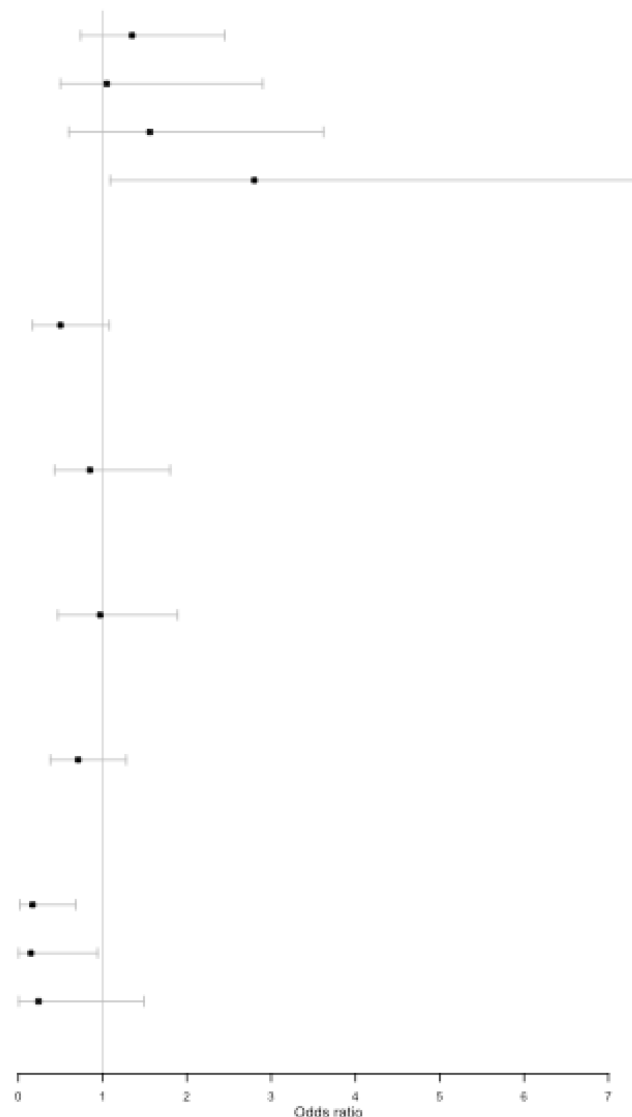


Figure 15. Forestplot of risk factors for Phlebovirus seropositivity in the control group. Dots represent odds ratios and horizontal lines represent 95% CIs.

Table 19. Risk factors, returning soldiers vs. control group

Risk factors	Operational areas (total)	Control group	Control group (without prior operation)
	OR/ 95% CI(p-value)	OR/ 95% CI(p-value)	OR/ 95% CI(p-value)
Missions			
Prior mission	0.7/ 0.4-1.1(0.08)	1.35/ 0.74-2.45(0.31)	-
Kosovo	0.6/ 0.3-1.1(0.07)	1.05/ 0.5-2.9(0.87)	-
Syria	1.3/ 0.2-2.6(0.5)	1.56/ 0.6-3.62(0.35)	-
Bosnia	0.8/ 0.4-1.5(0.6)	2.8/ 1.09-7.36(0.02)	-
Symptoms			
Symptoms	1.3/ 0.7-2.1(0.37)	-	-
Fever	1.5/ 0.7-2.9(0.3)	-	-
Limb pain	0.85/ 0.25-2.3(1)	-	-
Vacation			
Vacation during mission	1.8/ 0.96-3.5(0.06)	0.5/ 0.17-1.08(0.09)	0.29/ 0.03-1.22(0.09)
Outdoor activities			
Sports	1.8/ 0.7-5.2(0.25)	0.85/ 0.43-1.8(0.6)	0.93/ 0.38-2.5(0.83)
Running	2.35/ 1.2-4.8(0.005)	-	-
Mountaineering	1.2/ 0.7-2.03(0.45)	-	-
Cycling	1.02/ 0.5-1.9(1)	-	-
Football	1.03/ 0.18-3.9(1)	-	-
Animal contact			
Animal contact	1.07/ 0.65-1.7(0.8)	-	-
Dog	0.99/ 0.6-1.6(1)	0.97/ 0.47-1.89(1)	1.34/ 0.24-4.9(0.72)
Cat	1.3/ 0.7-2.2(0.35)	-	-
Rodent	0.97/ 0.02-8.1(1)	-	-
Pets			
Pet	1.2/ 0.73-1.9(0.48)	0.71/ 0.38-1.28(0.26)	0.47/ 0.2-1.06(0.07)
Leishmania ELISA			
Overall antibodies	1.8/ 0.99-3.23(0.04)	0.17/ 0.02-0.68(0.004)	0.3/ 0.03-1.22(0.09)
Positive antibodies	2.1/ 0.97-4.2(0.05)	0.15/ 0.004-0.94(0.03)	0.27/ 0.006-1.74(0.23)
Borderline antibodies	0.37/ 0.6-3.4(0.37)	0.24/ 0.006-1.49(0.16)	0.38/ 0.009-2.51(0.49)

Table 20. Correlations between Phlebovirus species and risk factors.

	TOSV OR/ 95% CI (p-value)	SFSV OR/ 95% CI (p-value)	CYPV OR/ 95% CI (p-value)	SFNV OR/ 95% CI (p-value)	Double infection OR/ 95% CI (p-value)
Operational areas (total)					
Prior mission					
Prior mission	0.6/ 0.3-1.2 (0.16)	0.8/ 0.3-1.6 (0.48)	0.4/ 0.007-8.3 (0.6)	-	-
Kosovo	0.65/ 0.3-1.4 (0.3)	0.6/ 0.2-1.5 (0.3)	1.2/ 0.02-23.5 (1)	-	-
Syria	1.1/ 0.4-2.8 (0.8)	1.6/ 0.5-4.2 (0.3)	-	-	-
BIH	1.1/ 0.5-2.4 (0.7)	0.6/ 0.2-1.6 (0.4)	-	-	-
Symptoms					
Symptoms	2.14/ 1.1-4.1 (0.02)	0.4/ 0.11-1.03 (0.05)	-	-	-
Fever	1.9/ 0.8-4.3 (0.15)	0.7/ 0.13-2.3 (0.8)	-	-	-
Limb pain	1.9/ 0.55-5.5 (0.2)	-	-	-	-
Vacation					
Vacation during mission	1.9/ 0.8-5.3 (0.15)	1.3/ 0.5-3.6 (0.7)	0.7/ 0.03-39.0 (0.58)	-	-
Outdoor activities					
Sports	1.26/ 0.4-5.02 (0.81)	4.1/ 0.7-170.2 (0.24)	0.23/ 0.01-13.9 (0.28)	-	-
Running	1.76/ 0.78-45 (0.22)	2.8/ 0.96-11.16 (0.07)	0.71/ 0.04-41.97 (1)	-	-
Mountaineering	1.07/ 0.52-2.12 (0.87)	1.4/ 0.6-3.01 (0.44)	1.1/ 0.02-21.27 (1)	-	-
Cycling	0.7/ 0.26-1.65 (0.56)	1.5/ 0.6-3.4 (0.37)	-	-	-
Football	1.38/ 0.15-6.3 (0.66)	0.88/ 0.02-6.1 (1)	-	-	-
Animal contact					
Animal contact	1.9/ 0.96-3.7 (0.06)	0.38/ 0.15-0.87 (0.01)	0.56/ 0.01-10.9 (1)	-	-
Dog	1.9/ 0.96-3.6 (0.06)	0.43/ 0.16-1.0 (0.05)	-	-	-
Cat	1.2/ 0.55-2.4 (0.6)	0.75/ 0.27-1.8 (0.7)	1.5/ 0.03-28.6 (1)	-	-
Rodent	1.9/ 0.04-17.8 (0.45)	-	-	-	-

Results

Pet					
Pet	1.26/ 0.65-2.4 (0.53)	1.1/ 0.5-2.35 (0.86)	0.6/ 0.01-11.85 (1)	-	-
Leishmania ELISA					
Overall antibodies	1.4/ 0.6-3.03 (0.41)	1.8/ 0.7-4.3 (0.15)	2.4/ 0.04-47.36 (0.4)	-	-
Positive antibodies	1.8/ 0.64-4.4 (0.19)	2.1/ 0.7-5.7 (0.13)	-	-	-
Borderline antibodies	1.2/ 0.29-3.5 (0.77)	1.2/ 0.2-4.13 (0.74)	6.2/ 0.1-121.1 (0.2)	-	-

Control group

Prior mission

Prior mission	1.4/ 0.7-2.9 (0.3)	1.35/ 0.2-8.1 (0.7)	-	-	3.6/ 0.2-213.7 (0.5)
Kosovo	1.2/ 0.5-2.7 (0.55)	1.4/ 0.1-8.9 (0.65)	-	-	1.8/ 0.03-34.4 (0.5)
Syria	1.7/ 0.6-4.5 (0.26)	1.5/ 0.03-12.8 (0.5)	-	-	4.6/ 0.08-89.0 (0.27)
BIH	1.8/ 0.4-5.7 (0.29)	-	-	-	31.6/ 1.6-1879.6 (0.01)

Vacation

Vacation during mission	0.18/ 0.02-0.7 (0.007)	0.6/ 0.01-5.0 (1)	-	3.6/ 0.05-283.5 (0.39)	1.8/ 0.03-34.9 (0.5)
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Outdoor activities

Sports	0.7/ 0.3-1.7 (0.4)	1.5/ 0.2-71.9 (1)	-	0.3/ 0.003-20.2 (0.37)	-
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Animal contact

Dog	1.4/ 0.6-2.9 (0.4)	-	-	-	1.5/ 0.03-29.1 (1)
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Pet

Pet	0.7/ 0.4-1.5 (0.4)	0.4/ 0.04-2.7 (0.45)	-	1.1/ 0.01-85.3 (1)	0.5/ 0.01-10.4 (1)
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Leishmania ELISA

Overall antibodies	0.13/ 0.003-0.8 (0.01)	-	-	5.4/ 0.07-422.1 (0.29)	-
Positive antibodies	-	-	-	9.7/ 0.1-767.6 (0.18)	-
Borderline antibodies	0.37/ 0.009-2.3 (0.5)	-	-	-	-

Table 21. Correlations between risk factors and titers, returning soldiers vs. control group.

	1:10 OR/ 95% CI (p-value)	1:100 OR/ 95% CI (p-value)	1:320 OR/ 95% CI (p-value)	1:1000 OR/ 95% CI (p-value)
Operational areas (total)				
Prior mission				
Prior mission	0.6/ 0.34-1.1 (0.09)	0.56/ 0.2-1.8 (0.3)	0.86/ 0.23-3.3 (1)	2.6/ 0.2-137.2 (0.63)
Kosovo	0.6/ 0.28-1.2 (0.17)	0.6/ 0.11-2.26 (0.6)	0.48/ 0.05-2.8 (0.5)	2.44/ 0.18-33.9 (0.58)
Syria	1.39/ 0.56-3.08 (0.4)	0.51/ 0.01-3.5 (1)	0.65/ 0.02-4.64 (1)	7.4/ 0.53-103.96 (0.07)
BIH	0.53/ 0.21-1.18 (0.13)	0.9/ 0.16-3.35 (1)	1.8/ 0.39-6.9 (0.31)	3.58/ 0.26-49.9 (0.21)
Symptoms				
Symptoms	0.8/ 0.4-1.5 (0.54)	2.1/ 0.6-6.8 (0.16)	2.4/ 0.6-9.2 (0.2)	2.4/ 0.17-39.9 (0.6)
Fever	0.99/ 0.36-2.4 (1)	1.12/ 0.12-5.1 (0.7)	3.8/ 0.8-14.7 (0.05)	2.4/ 0.05-30.8 (0.4)
Limb pain	0.8/ 0.15-2.7 (1)	1.1/ 0.02-7.4 (1)	1.3/ 0.03-9.8 (0.55)	-
Vacation				
Vacation during mission	1.7/ 0.8-3.8 (0.2)	1.3/ 0.35-7.4 (1)	1.7/ 0.35-15.8 (0.74)	-
Outdoor activities				
Sports	1.7/ 0.8-3.8 (0.2)	-	1.3/ 0.2-56.9 (1)	0.35/ 0.03-18.6 (0.36)
Running	2.8/ 1.2-7.6 (0.01)	5.1/ 0.8-217.7 (0.13)	1.06/ 0.26-6.2 (1)	0.35/ 0.03-4.9 (0.28)
Mountain	1.4/ 0.75-2.6 (0.3)	0.5/ 0.1-2.1 (0.4)	1.1/ 0.24-4.2 (1)	2.2/ 0.16-30.8 (0.6)
Cycling	1.05/ 0.5-2.13 (0.86)	0.6/ 0.07-2.8 (0.75)	1.45/ 0.23-5.3 (0.7)	1.34/ 0.03-16.9 (1)
Football	1.06/ 0.11-4.8 (1)	2.2/ 0.05-16.2 (0.4)	-	-
Animal contact				
Animal contact	0.84/ 0.5-1.5 (0.58)	2.3/ 0.7-8.7 (0.19)	1.6/ 0.43-6.5 (0.56)	0.4/ 0.007-4.7 (0.63)
Dog	0.9/ 0.5-1.6 (0.78)	1.7/ 0.53-5.6 (0.4)	1.05/ 0.26-3.9 (1)	0.5/ 0.01-6.1 (0.65)
Cat	0.93/ 0.45-1.8 (1)	2.01/ 0.6-6.5 (0.23)	2.2/ 0.53-8.04 (0.2)	0.98/ 0.02-12.4 (1)
Rodent	-	-	8.4/ 0.17-85.2 (0.14)	-
Pet				
Pet	0.9/ 0.5-1.7 (0.89)	0.8/ 0.24-2.6 (0.8)	6.3/ 1.3-60.5 (0.01)	1.2/ 0.09-17.1 (1)

Results

Leishmania ELISA

Overall antibodies	2.05/ 1.01-3.9 (0.04)	0.74/ 0.08-3.37 (1)	1.64/ 0.28-6.8 (0.44)	1.6/ 0.03-20.5 (0.53)
Positive antibodies	1.9/ 0.75-4.3 (0.15)	1.5/ 0.16-6.75 (0.65)	3.25/ 0.55-13.6 (0.1)	-
Borderline antibodies	2.2/ 0.8-5.3 (0.07)	-	-	4.1/ 0.08-52.7 (0.27)

Control group

Prior mission

Prior mission	1.5/ 0.67-3.11 (0.35)	1.2/ 0.1-10.6 (1)	0.53/ 0.1-2.1 (0.4)	-
Kosovo	1.3/ 0.52-3.0 (0.5)	2.4/ 0.2-21.1 (0.3)	0.3/ 0.01-2.0 (0.32)	1.2/ 0.01-2.0 (1)
Syria	1.6/ 0.5-4.5 (0.37)	-	1.7/ 0.17-8.0 (0.38)	3.03/ 0.06-38.6 (0.35)
BIH	2.16/ 0.3-14.07 (0.19)	-	2.9/ 0.3-14.07 (0.19)	15.9/ 1.11-225.5 (0.02)

Vacation

Vacation during mission	0.46/ 0.12-1.35 (0.2)	-	0.3/ 0.01-2.02 (0.32)	3.6/ 0.26-50.05 (0.21)
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Outdoor activities

Sports	0.6/ 0.26-1.44 (0.19)	0.27/ 0.04-4.6 (0.38)	0.48/ 0.45-135.47 (0.48)	-
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Animal contact

Dog	1.09/ 0.4-2.5 (0.84)	0.75/ 0.02-7.66 (1)	0.9/ 0.16-3.56 (1)	-
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Pet

Pet	0.74/ 0.34-1.6 (0.48)	1.6/ 0.19-19.7 (0.68)	0.32/ 0.06-1.25 (0.09)	1.08/ 0.08-15.06 (1)
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Leishmania ELISA

Overall antibodies	0.15/ 0.004-0.93 (0.03)	-	0.44/ 0.01-3.04 (0.7)	-
Positive antibodies	0.28/ 0.01-1.74 (0.24)	-	-	-
Borderline antibodies	-	-	1.23/ 0.03-8.8 (0.6)	-

3.5.3 Risk factors depending on operational areas

3.5.3.1 Risk factors in soldiers returning from Bosnia and Herzegovina

No significant associations, neither between anti-Phlebovirus antibodies and risk factors (Figure 16, Table 22), nor between Phlebovirus species and risk factors (Table 23) or titers and risk factors (Table 24), were observed for BIH.

Factors

Missions

Prior mission

Kosovo

Symptoms

Symptoms

Limb pain

Vacation

Vacation during mission

Outdoor activities

Sports

Running

Cycling

Football

Animal contact mission

Animal contact

Dog

Cat

Pets

Pet

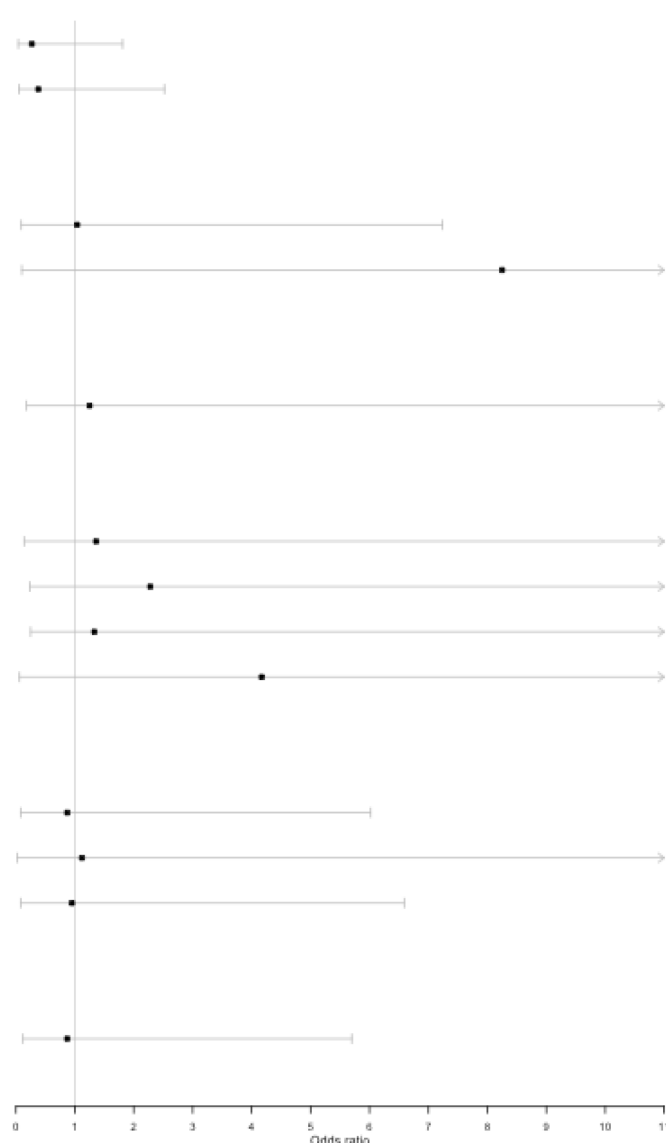


Figure 16. Forestplot of risk factors for Phlebovirus seropositivity in Bosnia and Herzegovina. Dots represent odds ratios and horizontal lines represent 95% CIs.

3.5.3.2 Risk factors in soldiers returning from the Kosovo

A significant correlation was detected between anti-SFSV antibodies and running (OR=4.15, 95% CI=0.99-37.1, p-value=0.05) and a strong trend towards a general association between Phlebovirus antibodies and running was observed (OR=2.47, 95% CI=0.98-7.5, p-value=0.06) (Figure 17, Table 22). Moreover, a trend towards an association between TOSV and observed symptoms was detected (OR=2.48, 95% CI=0.9-6.5, p-value=0.06) (Table 23).

Factors

Missions

Prior mission
Kosovo
Syria
BIH

Symptoms

Symptoms
Fever
Limb pain

Vacation

Vacation during mission

Outdoor activities

Sports
Running
Mountaineering
Cycling
Football

Animal contact mission

Animal contact
Dog
Cat
Rodent

Pets

Pet

Leishmania ELISA

Overall antibodies
Positive antibodies
Borderline antibodies

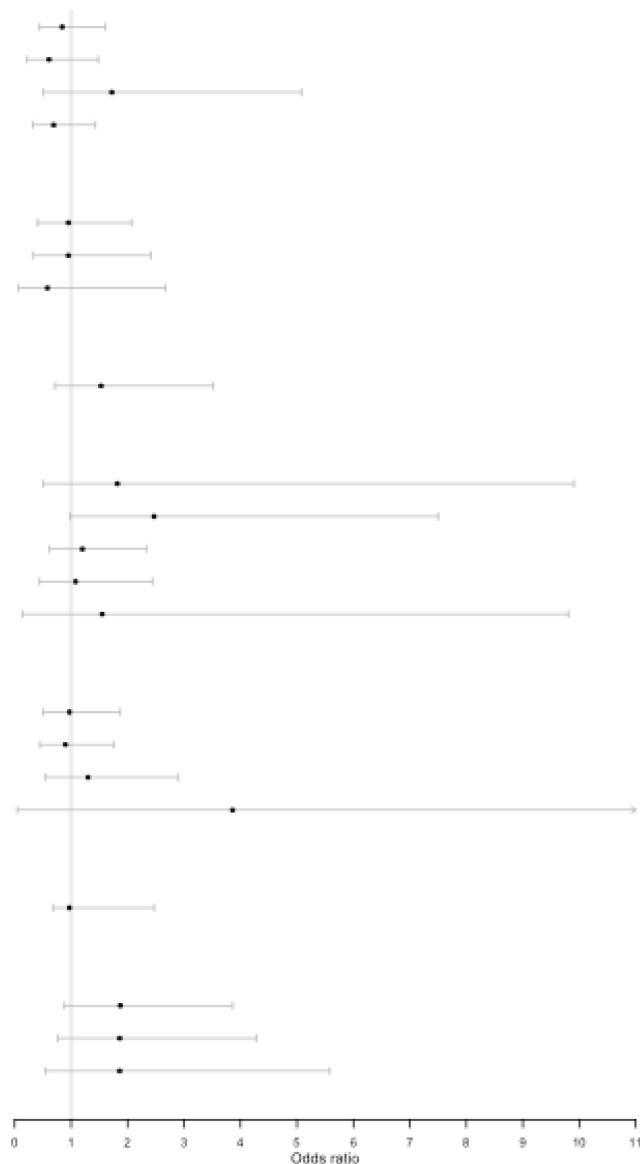


Figure 17. Forestplot of risk factors for Phlebovirus seropositivity in the Kosovo. Dots represent odds ratios and horizontal lines represent 95% CIs.

3.5.3.3 Risk factors in soldiers returning from Syria

No significant association between Phlebovirus antibodies and risk factors was observed, however a strong trend towards a correlation between anti-Phlebovirus antibodies and fever was detected (OR=4.03, 95% CI=0.71-21.28, p-value=0.06) (Figure 18, Table 22). Moreover, a strong trend towards an association between a titer of 1:320 and fever was observed (OR=8.12, 95% CI=0.59-84.42, p-value=0.06) (Table 24).

Factors

Missions

Prior mission
Kosovo
Syria
BIH

Symptoms

Symptoms
Fever
Limb pain

Vacation

Vacation during mission

Outdoor activities

Sports
Running
Mountaineering
Cycling

Animal contact mission

Animal contact
Dog
Cat

Pets

Pet

Leishmania ELISA

Overall antibodies
Positive antibodies
Borderline antibodies

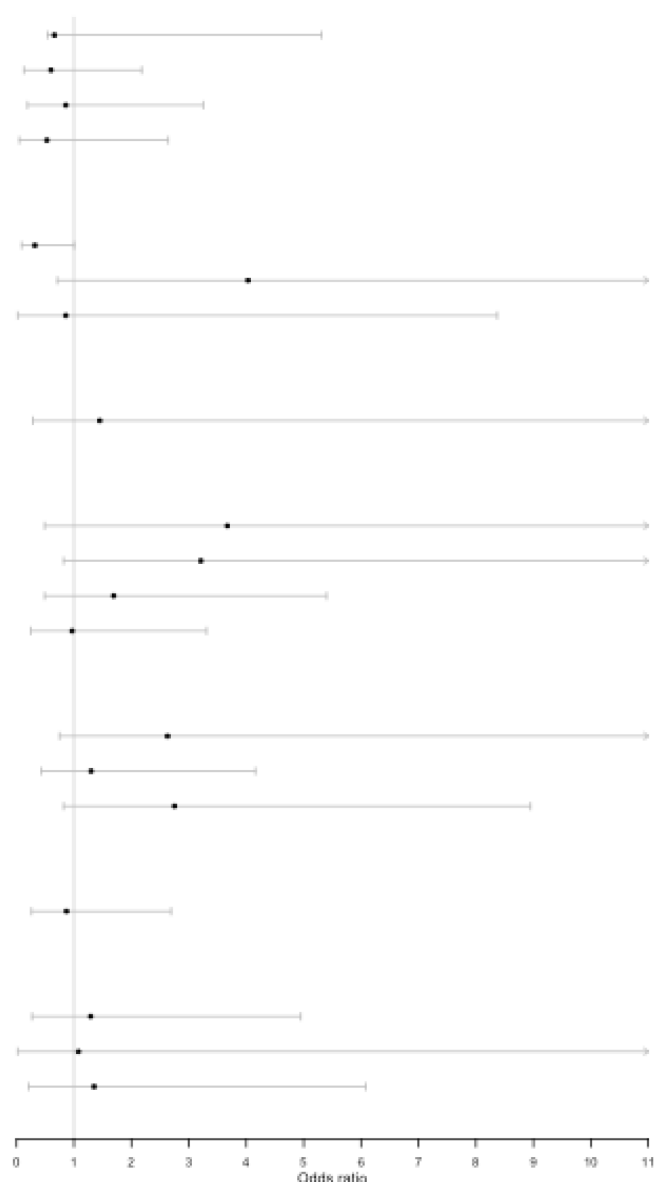


Figure 18. Forestplot of risk factors for Phlebovirus seropositivity in Syria. Dots represent odds ratios and horizontal lines represent 95% CIs.

3.5.3.4 Risk factors in the Lebanon

No significant associations were observed in soldiers returning from the Lebanon, however a trend towards a correlation between anti-Phlebovirus antibodies and anti-*Leishmania* spp. antibodies was detected (OR=7.04, 95%CI=0.45-112.1, p-value=0.09) (Figure 19, Table 22).

Factors

Missions

Prior mission
Kosovo
Syria
BIH

Symptoms

Symptoms
Fever
Limb pain

Outdoor activities

Sports
Running
Mountaineering
Cycling

Animal contact mission

Animal contact
Dog
Cat

Pets

Pet

Leishmania ELISA

Overall antibodies
Positive antibodies

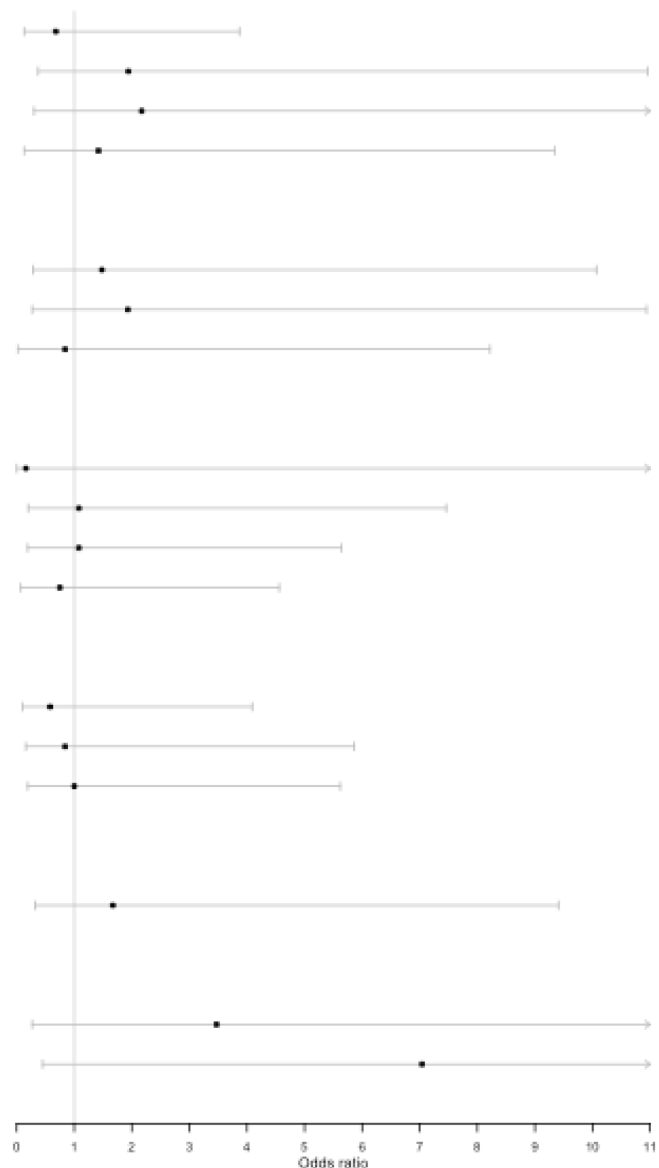


Figure 19. Forestplot of risk factors for Phlebovirus seropositivity in the Lebanon. Dots represent odds ratios and horizontal lines represent 95% CIs.

Table 22. Risk factors for anti-Phlebovirus antibodies in the respective operational areas.

Risk factors	BIH OR/ 95% CI (p-value)	Kosovo OR/ 95% CI (p-value)	Syria OR/ 95% CI (p-value)	Lebanon OR/ 95% CI (p-value)
Missions				
Prior mission	0.27/ 0.04-1.81 (0.27)	0.84/ 0.44-1.6 (0.65)	1.66/ 0.54-5.31 (0.45)	0.68/ 0.13-3.87 (0.71)
Kosovo	0.38/ 0.05-2.52 (0.24)	0.61/ 0.22-1.48 (0.33)	0.6/ 0.13-2.18 (0.57)	1.94/ 0.37-10.96 (0.47)
Syria	-	1.72/ 0.51-5.08 (0.27)	0.86/ 0.19-3.25 (1)	2.17/ 0.3-9.34 (0.65)
Bosnia	-	0.69/ 0.32-1.4 (0.32)	0.53/ 0.05-2.64 (0.5)	1.42/ 0.13-9.34 (0.65)
Symptoms				
Symptoms	1.04/ 0.09-7.24 (1)	0.95/ 0.41-2.07 (1)	0.32/ 0.09-1.003 (0.03)	1.48/ 0.28-10.07 (0.73)
Fever	-	0.95/ 0.33-2.41 (1)	4.03/ 0.71-21.3 (0.06)	1.93/ 0.27-10.94 (0.4)
Limb pain	8.25/ 0.1-701.03 (0.22)	0.58/ 0.06-2.67 (0.74)	0.86/ 0.02-8.37 (1)	0.84/ 0.02-8.22 (1)
Vacation				
Vacation during mission	1.25/ 0.18-14.3 (1)	1.53/ 0.71-3.52 (0.3)	1.45/ 0.28-14.57 (1)	-
Outdoor activities				
Sports	1.36/ 0.14-68.9 (1)	1.82/ 0.51-9.91 (0.43)	3.67/ 0.49-165.2 (0.29)	0.16/ 0.002-13.32 (0.27)
Running	2.28/ 0.24-113.6 (0.66)	2.47/ 0.98-7.5 (0.06)	3.21/ 0.82-18.56 (0.1)	1.08/ 0.2-7.46 (1)
Mountaineering	-	1.2/ 0.61-2.33 (0.63)	1.69/ 0.49-5.4 (0.4)	1.08/ 0.19-5.64 (1)
Cycling	1.33/ 0.25-14.5 (1)	1.08/ 0.44-2.44 (0.84)	0.97/ 0.25-3.3 (1)	0.75/ 0.07-4.56 (1)
Football	4.17/ 0.06-92.31 (0.31)	1.55/ 0.14-9.81 (0.64)	-	-
Animal contact				
Animal contact	0.87/ 0.08-6.02 (1)	0.97/ 0.5-1.87 (1)	2.63/ 0.75-11.86 (0.11)	0.58/ 0.1-4.09 (0.43)
Dog	1.12/ 0.02-11.74 (1)	0.90/ 0.45-1.76 (0.87)	1.3/ 0.43-4.16 (0.62)	0.84/ 0.16-5.86 (1)
Cat	0.95/ 0.08-6.59 (1)	1.3/ 0.55-2.9 (0.54)	2.75/ 0.82-8.95 (0.07)	1/ 0.19-5.62 (1)
Rodent	-	3.86/ 0.05-305.8 (0.37)	-	-
Pets				
Pet	0.87/ 0.12-5.70 (1)	1.29/ 0.68-2.47 (0.45)	0.87/ 0.26-2.69 (1)	1.67/ 0.32-9.41 (0.5)

Leishmania ELISA

Overall antibodies	-	1.87/ 0.88-3.86 (0.09)	1.29/ 0.27-4.95 (0.74)	3.47/ 0.27-30.14 (0.2)
Positive antibodies	-	1.86/ 0.76-4.28 (0.12)	1.08/ 0.02-11.84 (1)	7.04/ 0.45-112.1 (0.09)
Borderline antibodies	-	1.86/ 0.55-5.88 (0.24)	1.35/ 0.21-6.08 (0.7)	-

Table 23. Correlations between Phlebovirus species and risk factors in soldiers returning from the respective operational areas.

	TOSV	SFSV	CYPV	SFNV
	OR/ 95% CI	OR/ 95% CI	OR/ 95% CI	OR/ 95% CI
	(p-value)	(p-value)	(p-value)	(p-value)

Bosnia and Herzegovina**Prior mission**

Prior mission	0.25/ 0.02-2.41 (0.15)	-	-	-
Kosovo	0.35/ 0.03-3.32 (0.34)	-	0.56/ 0.007-45.4 (1)	-
Syria	-	-	-	-
BIH	-	-	-	-

Symptoms

Symptoms	1.8/ 0.14-17.4 (0.61)	-	-	-
Fever	-	-	-	-
Limb pain	12.4/ 0.14-1085.4 (0.16)	-	-	-

Vacation

Vacation during mission	2.03/ 0.18-106.5 (1)	-	0.48/ 0.006-39.23 (1)	-
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Outdoor activities

Sports	-	-	0.2/ 0.003-17.6 (0.33)	-
Running	-	-	0.35/ 0.004-28.53 (0.46)	-
Mountaineering	-	-	-	-
Cycling	2.05/ 0.04-26.1 (0.49)	-	-	-
Football	6.4/ 0.13-15.9 (0.63)	-	-	-

Animal contact

Animal contact	1.52/ 0.12-14.55 (0.64)	-	-	-
Dog	1.7/ 0.03-21.3 (0.52)	-	-	-
Cat	1.65/ 0.13-15.9 (0.63)	-	-	-
Rodent	-	-	-	-

Pet

Pet	1.84/ 0.2-23.7 (0.65)	-	-	-
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Leishmania ELISA

Overall antibodies	-	-	-	-
Positive antibodies	-	-	-	-
Borderline antibodies	-	-	-	-

Kosovo**Prior mission**

Prior mission	0.76/ 0.29-1.9 (0.67)	0.95/ 0.4-2.2 (1)	-	-
Kosovo	0.38/ 0.04-1.6 (0.27)	0.88/ 0.25-2.50 (1)	-	-
Syria	1.11/ 0.12-5.14 (0.7)	2.06/ 0.50-7.10 (0.26)	-	-
BIH	1.19/ 0.42-3.12 (0.82)	0.43/ 0.13-1.22 (0.14)	-	-

Symptoms

Symptoms	2.48/ 0.9-6.5 (0.06)	0.24/ 0.03-1.01 (0.05)	-	-
Fever	2.38/ 0.71-6.94 (0.11)	0.2/ 0.005-1.3 (0.09)	-	-
Limb pain	1.56/ 0.16-7.63 (0.64)	-	-	-

Vacation

Vacation during mission	1.9/ 0.60-7.92 (0.34)	1.21/ 0.47-3.52 (0.83)	-	-
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Outdoor activities

Sports	1.07/ 0.23-10.02 (1)	3.04/ 0.46-130.2 (0.49)	-	-
Running	1.37/ 0.43-5.76 (0.79)	4.15/ 0.99-37.10 (0.05)	-	-

Results

Mountaineering	0.98/ 0.35-2.56 (1)	1.36/ 0.57-3.16 (0.54)	-	-
Cycling	0.65/ 0.12-2.3 (0.78)	1.5/ 0.51-3.9 (0.44)	-	-
Football	1.67/ 0.04-14.7 (0.5)	1.29/ 0.03-11.25 (0.51)	-	-
Animal contact				
Animal contact	2.09/ 0.83-5.4 (0.12)	0.47/ 0.16-1.18 (0.11)	-	-
Dog	1.63/ 0.63-4.14 (0.27)	0.52/ 0.18-1.33 (0.16)	-	-
Cat	1.69/ 0.52-4.8 (0.27)	0.96/ 0.27-2.76 (1)	-	-
Rodent	10.07/ 0.13-802.8 (0.18)	-	-	-
Pet				
Pet	1.67/ 0.66-4.38 (1)	0.99/ 0.43-2.29 (1)	-	-
Leishmania ELISA				
Overall antibodies	1.66/ 0.55-4.52 (0.29)	1.77/ 0.67-4.36 (0.23)	-	-
Positive antibodies	1.7/ 0.48-5.31 (0.35)	1.67/ 0.51-4.67 (0.27)	-	-
Borderline antibodies	1.97/ 0.34-7.73 (0.4)	1.5/ 0.26-5.72 (0.47)	-	-

Syria

Prior mission

Prior mission	0.52/ 0.13-1.99 (0.38)	-	-	-
Kosovo	0.72/ 0.12-3.1 (0.75)	-	-	-
Syria	1.02/ 0.17-4.5 (1)	-	-	-
BIH	0.38/ 0.01-2.92 (0.69)	-	-	-

Symptoms

Symptoms	0.3/ 0.07-1.31 (0.06)	-	-	-
Fever	4.02/ 0.56-22.75 (0.09)	-	-	-
Limb pain	1.38/ 0.03-14.0 (0.57)	-	-	-

Vacation

Vacation during mission	0.87/ 0.16-9.04 (1)	-	-	-
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Outdoor activities

Sports	2.26/ 0.29-103.8 (0.67)	-	-	-
Running	3.11/ 0.6-30.66 (0.21)	-	-	-
Mountaineering	1.76/ 0.41-6.84 (0.34)	-	-	-
Cycling	0.8/ 0.13-3.48 (1)	-	-	-
Football	-	-	-	-

Animal contact

Animal contact	2.19/ 0.51-13.2 (0.36)	-	-	-
Dog	3.45/ 0.81-20.81 (0.08)	-	-	-
Cat	0.9/ 0.15-3.94 (1)	-	-	-
Rodent	-	-	-	-

Pet

Pet	0.42/ 0.07-1.77 (0.24)	-	-	-
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Leishmania ELISA

Overall antibodies	0.82/ 0.08-4.34 (1)	-	-	-
Positive antibodies	1.74/ 0.03-19.65 (0.51)	-	-	-
Borderline antibodies	0.53/ 0.01-4.26 (1)	-	-	-

Lebanon**Prior mission**

Prior mission	0.85/ 0.09-10.1 (1)	1.16/ 0.06-71.35 (1)	-	-
Kosovo	2.25/ 0.24-28.92 (0.64)	0.95/ 0.15-181.6 (0.56)	-	-
Syria	0.96/ 0.02-10.95 (1)	8.48/ 0.41-533.8 (0.11)	-	-
BIH	3.53/ 0.26-35.7 (0.21)	-	-	-

Symptoms

Symptoms	1.06/ 0.11-13.57 (1)	1.42/ 0.07-87.6 (1)	-	-
Fever	-	7.65/ 0.37-479.7 (0.12)	-	-
Limb pain	2.43/ 0.04-35.6 (0.43)	-	-	-

Vacation

Vacation during mission	-	-	-	-
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Outdoor activities

Sports	0.06/ 0.001-5.41 (0.12)	-	-	-
Running	0.52/ 0.04-7.65 (0.61)	1.08/ 0.05-66.5 (1)	-	-
Mountaineering	1.08/ 0.19-5.64 (1)	0.66/ 0.01-13.29 (1)	-	-
Cycling	-	5.8/ 0.28-360.7 (0.18)	-	-
Football	-	-	-	-

Animal contact

Animal contact	0.93/ 0.07-52.5 (1)	0.14/ 0.002-3.0 (0.14)	-	-
Dog	1.31/ 0.1-72.9 (1)	0.20/ 0.003-4.15 (0.21)	-	-
Cat	0.8/ 0.05-11.6 (1)	0.39/ 0.006-7.84 (0.58)	-	-
Rodent	-	-	-	-

Pet

Pet	4.99/ 0.3-220.07 (0.31)	0.62/ 0.01-12.43 (0.62)	-	-
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Leishmania ELISA

Overall antibodies	3.49/ 0.06-54.55 (0.34)	-	-	-
Positive antibodies	5.88/ 0.09-109.28 (0.24)	-	-	-
Borderline antibodies	-	-	-	-

Table 24. Correlations between titers and risk factors in soldiers returning from the operational areas.

	1:10 OR/ 95% CI (p-value)	1:100 OR/ 95% CI (p-value)	1:320 OR/ 95% CI (p-value)	1:1000 OR/ 95% CI (p-value)
Bosnia and Herzegovina				
Prior mission				
Prior mission	0.4/ 0.03-5.9 (0.57)	0.2/ 0.003-4.02 (0.21)	-	-
Kosovo	0.55/ 0.04-8.07 (0.6)	0.27/ 0.004-5.47 (0.29)	-	-
Syria	-	-	-	-
BIH	-	-	-	-
Symptoms				
Symptoms	0.86/ 0.02-11.6 (1)	1.3/ 0.02-26.76 (1)	-	-
Fever	-	-	-	-
Limb pain	16.3/ 0.2-1465.9 (0.13)	-	-	-
Vacation				
Vacation during mission	1.5/ 0.11-82.8 (1)	0.98/ 0.05-60.5 (1)	-	-
Outdoor activities				
Sports	0.64/ 0.05-36.8 (0.56)	-	-	-
Running	1.07/ 0.08-60.0 (1)	-	-	-
Mountain	-	-	-	-
Cycling	-	-	-	-
Football	-	12.5/ 0.16-367.3 (0.14)	-	-
Animal contact				
Animal contact	0.73/ 0.01-9.78 (1)	1.1/ 0.02-22.6 (1)	-	-
Dog	-	3.5/ 0.05-76.5 (0.35)	-	-
Cat	0.79/ 0.01-10.6 (1)	1.2/ 0.02-24.5 (1)	-	-
Rodent	-	-	-	-

Pet

Pet	1.2/ 0.08-17.46 (1)	0.58/ 0.01-11.7 (1)	-	-
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Leishmania ELISA

Overall antibodies	-	-	-	-
Positive antibodies	-	-	-	-
Borderline antibodies	-	-	-	-

Kosovo**Prior mission**

Prior mission	0.7/ 0.32-1.4 (0.31)	2.2/ 0.3-24.8 (0.43)	1.5/ 0.24-10.2 (0.7)	-
Kosovo	-	-	0.74/ 0.02-6.3 (1)	-
Syria	2.0/ 0.5-6.13 (0.2)	2.5/ 0.05-23.8 (0.38)	-	-
BIH	0.5/ 0.16-1.1 (0.09)	2.4/ 0.31-18.3 (0.4)	1.8/ 0.26-10.8 (0.4)	-

Symptoms

Symptoms	0.5/ 0.14-1.35 (0.21)	1.9/ 0.17-13.7 (0.6)	2.9/ 0.4-17.7 (0.16)	-
Fever	0.5/ 0.09-1.66 (0.32)	1.3/ 0.03-12.2 (0.6)	2.7/ 0.24-17.1 (0.24)	-
Limb pain	0.38/ 0.01-2.6 (0.5)	-	2.8/ 0.06-26.1 (0.34)	-

Vacation

Vacation during mission	1.53/ 0.65-4.07 (0.34)	1.82/ 0.2-87.7 (1)	0.9/ 0.14-9.7 (1)	-
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Outdoor activities

Sports	2.0/ 0.46-18.2 (0.55)	-	0.6/ 0.07-27.35 (0.5)	-
Running	2.7/ 0.9-10.9 (0.09)	-	0.7/ 0.11-7.12 (0.64)	-
Mountain	0.86/ 0.49-2.27 (0.86)	0.98/ 0.09-7.01 (1)	1.5/ 0.21-9.02 (0.7)	-
Cycling	1.71/ 0.68-4.0 (0.18)	-	-	-
Football	2.27/ 0.21-14.5 (0.3)	-	-	-

Animal contact

Animal contact	0.77/ 0.34-1.64 (0.49)	1.7/ 0.22-12.73 (0.68)	2.25/ 0.37-15.68 (0.43)	-
Dog	0.87/ 0.39-1.85 (0.72)	0.92/ 0.08-6.54 (1)	1.4/ 0.2-8.41 (0.7)	-
Cat	1.24/ 0.5-3.04 (0.65)	0.96/ 0.02-8.9 (1)	1.96/ 0.2-12.44 (0.35)	-
Rodent	-	-	39.99/ 0.46-3209 (0.05)	-

Pet

Pet	0.92/ 0.44-1.91 (0.86)	0.56/ 0.05-4.01 (0.69)	-	-
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Leishmania ELISA

Overall antibodies	1.6/ 0.7-3.6 (0.29)	0.76/ 0.02-7.03 (1)	3.0/ 0.4-18.15 (0.16)	-
Positive antibodies	1.4/ 0.48-3.63 (0.46)	1.3/ 0.03-11.71 (0.6)	5.0/ 0.7-30.85 (0.06)	-
Borderline antibodies	2.1/ 0.58-6.7 (0.18)	-	-	-

Syria**Prior mission**

Prior mission	1.1/ 0.26-5.0 (1)	-	0.6/ 0.05-5.4 (0.67)	-
Kosovo	0.92/ 0.15-4.25 (1)	-	0.61/ 0.01-6.53 (1)	-
Syria	1.3/ 0.2-6.14 (0.71)	-	0.84/ 0.02-9.11 (1)	-
BIH	0.47/ 0.01-3.72 (0.7)	-	1.25/ 0.02-13.74 (1)	-

Symptoms

Symptoms	0.42/ 0.09-1.8 (0.19)	0.27/ 0.004-5.35 (0.3)	0.35/ 0.03-3.24 (0.35)	-
Fever	2.6/ 0.23-16.87 (0.25)	-	8.12/ 0.59-84.42 (0.06)	-
Limb pain	1.69/ 0.03-17.53 (0.51)	-	-	-

Vacation

Vacation during mission	1.7/ 0.21-78.8 (1)	0.31/ 0.02-19.42 (0.36)	-	-
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Outdoor activities

Sports	1.8/ 0.23-85.55 (1)	-	-	-
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Results

Running	5.72/ 0.75-258.4 (0.09)	1.02/ 0.05-61.68 (1)	2.1/ 0.2-106.2 (0.6)	-
Mountain	3.7/ 0.84-16.74 (0.07)	-	4.42/ 0.5-55.8 (0.12)	-
Cycling	0.6/ 0.06-3.1 (0.72)	-	-	-
Football	-	-	-	-

Animal contact

Animal contact	3.0/ 0.6-29.85 (0.2)	-	0.9/ 0.1-11.26 (1)	-
Dog	1.67/ 0.39-8.31 (0.53)	1.8/ 0.09-110.94 (1)	0.9/ 0.1-11.26 (1)	-
Cat	1.16/ 0.2-5.4 (1)	6.4/ 0.32-388.7 (0.15)	5.0/ 0.53-62.74 (0.1)	-
Rodent	-	-	-	-

Pet

Pet	0.54/ 0.09-2.45 (0.52)	0.76/ 0.02-15.03 (1)	2.4/ 0.26-26.63 (0.38)	-
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Leishmania ELISA

Overall antibodies	3.1/ 0.58-14.1 (0.11)	-	-	-
Positive antibodies	2.13/ 0.04-24.55 (0.45)	-	-	-
Borderline antibodies	3.0/ 0.44-15.24 (0.15)	-	-	-

Lebanon

Prior mission

Prior mission	0.28/ 0.005-5.58 (0.55)	0.28/ 0.005-5.58 (0.55)	-	-
Kosovo	0.7/ 0.01-14.22 (1)	2.95/ 0.15-181.6 (0.56)	-	2.95/ 0.15-181.6 (0.56)
Syria	-	-	-	8.48/ 0.41-533.8 (0.11)
BIH	-	-	-	10.7/ 0.5-677.54 (0.08)

Symptoms

Symptoms	-	1.42/ 0.07-87.56 (1)	-	0.34/ 0.01-6.86 (0.56)
Fever	7.65/ 0.37-479.7 (0.12)	1.8/ 0.03-39.96 (0.54)	-	-
Limb pain	-	3.7/ 0.06-79.36 (0.34)	-	-

Vacation

Vacation during mission	-	-	-	-
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Outdoor activities

Sports	-	-	-	0.04/ 0-3.98 (0.09)
Running	1.08/ 0.05-66.5 (1)	-	-	0.26/ 0.004-5.2 (0.28)
Mountain	2.76/ 0.14-169.6 (0.57)	0.66/ 0.01-13.3 (1)	-	0.66/ 0.01-13.3 (1)
Cycling	-	1.37/ 0.02-28.0 (1)	-	1.37/ 0.02-28.0 (1)
Football	-	-	-	-

Animal contact

Animal contact	0.61/ 0.03-38.45 (0.57)	-	-	0.14/0.002-2.97 (0.14)
Dog	0.86/ 0.04-53.35 (1)	-	-	0.2/ 0.003-4.15 (0.21)
Cat	1.6/ 0.08-100.0 (1)	1.6/ 0.08-100.0 (1)	-	0.39/ 0.006-7.84 (0.58)
Rodent	-	-	-	-

Pet

Pet	2.6/ 0.13-158.6 (0.26)	2.6/ 0.13-158.6 (0.26)	-	0.62/ 0.01-12.43 (1)
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Leishmania ELISA

Overall antibodies	5.24/ 0.08-118.7 (0.26)	5.24/ 0.08-118.7 (0.26)	-	-
Positive antibodies	8.8/ 0.12-220.8 (0.18)	8.8/ 0.12-220.8 (0.18)	-	-
Borderline antibodies	-	-	-	-

4 Discussion

In the current study, unexpectedly high Phlebovirus seroprevalences were detected in soldiers returning from missions in BIH, the Kosovo, Syria and the Lebanon, as well as in the control group, whereby rates of infection varied between the operational areas. Moreover, high Phlebovirus diversity was observed, varying between the operational areas and the control group. Interestingly, there was an association between Phlebovirus seropositivity and the potential risk factors “running” and “*Leishmania* spp. infection”.

4.1 Seroprevalence

Although, sandfly fever was in fact discovered during military operations of the Austrian Army 100 years ago, the impact of this disease on recent operations by the Austrian Armed Forces has never been investigated (Flamm 2008). The results of this thesis prove, that military personnel of the Austrian Army is exposed to Phlebovirus infection in different operational areas. Army troops operating in sandfly endemic countries are at an increased risk of infection with sandfly-borne pathogens, such as phleboviruses and *Leishmania* spp., because these follow the distribution of their respective vectors (Newman et al. 2014). Non-combat injuries and infectious diseases have a significant impact on military operations (Sanders et al. 2005). Observed seroprevalences of soldiers returning from operational areas are comparable to other studies on military troops operating in sandfly endemic areas. Phlebovirus infections have been reported for Swedish and Greek soldiers operating in Cyprus, for Soviet and British troops in Afghanistan and in Pakistani military personnel and soldiers from the US Army returning from Iraq (Eitrem et al. 1990, Papa et al. 2006, Gaïdamovich et al. 1989, Newman et al. 2014, Bryan et al. 1996, Ellis et al. 2008, Riddle et al. 2008).

Interestingly, the rate of Phlebovirus-infected soldiers showing symptoms varied among the operational areas. While only 20.4% and 28.6% of soldiers returning from BIH and the Kosovo, respectively, reported symptoms, 42.1% and 66.7% of the soldiers returning from Syria and the Lebanon,

respectively, reported symptoms, including febrile illnesses, fever, nausea, malaise, limb and joint pain. Of nine Phlebovirus seropositive US Army soldiers returning from Iraq, only one reported a febrile illness (Riddle et al. 2008). Military troops stationed in the Iran were positive for anti-Phlebovirus antibodies (18.4%), however the majority did not exhibit any symptoms of febrile illnesses (Shiraly et al. 2017). Also, 93.5% of UK military personnel were reported asymptomatic after a Phlebovirus infection (Newman et al. 2014). Cases of febrile illness associated with sandfly fever virus (SFV) seropositivity have been reported from Swedish soldiers stationed in Cyprus, where 9 of 11 infected soldiers were symptomatic (Eitrem et al 1990). Up to 70% of Pakistani military personnel and 92.9% of US Army soldiers operating in Iraq with febrile illness were positive for anti-Phlebovirus antibodies (Gaïdamovich et al. 1989, Ellis et al. 2008). Affected soldiers were incapacitated for at least three days, showing various symptoms, such as high fever, headache, myalgia, malaise and decreased functional capacity of up to one week. All these reports highlight the diversity of etiopathologies of a Phlebovirus infection among healthy soldiers.

Despite reports of high Phlebovirus seroprevalences, leishmaniasis remains the primary sandfly-borne disease of interest (Coleman et al. 2006, Faulde & Parola 2010). However, with the known wide distribution of sandflies in operational areas and these being potential vectors for phleboviruses, the impact of sandfly fever on the missions should not be underestimated. Seroprevalences of anti-*Leishmania* spp. antibodies were observed in returning soldiers of this study from operational areas of BIH, Syria and the Lebanon, prior to this study (Obwaller, p.o.). These seroprevalences were exceeded by those for phleboviruses detected in the current study, which directly highlight the topic of possible co-infections and the circulation of phleboviruses in the operational areas.

Interestingly, no significant age-dependent differences in seroprevalence were observed, however differences depending on the operational areas were detected. In this study, the peaks of seroprevalences were observed in different age groups of soldiers depending on the operational areas. While soldiers returning from BIH and the Kosovo showed the highest seroprevalences in the age group 20-29, soldiers returning from Syria and

the Lebanon showed the highest seroprevalences in the age group 40-49. These results perfectly reflect the age distribution among the soldiers returning from the different areas, as soldiers returning from BIH and the Kosovo were significantly younger than those returning from Syria and the Lebanon. In Phlebovirus endemic areas, seroprevalences typically increase with age. In Central Italy, a significant age-dependent TOSV immunity was observed, as well as in Croatian residents (Terrosi et al. 2009, Punda-Polić et al. 2012). A correlation between seropositivity against SFSV and SFNV and age was observed in a Phlebovirus-endemic area in Jordan (Batieha et al. 2000). However, a change towards increased and age-independent seroprevalences can be observed among risk populations, such as forestry workers, abattoir workers or military personnel. A significantly increased seroprevalence of anti-TOSV antibodies compared to the control group was observed in forestry workers in Tuscany (Valassina et al. 2003). In Egypt, employees of slaughterhouses showed a significantly increased seroprevalence of anti-RVSV antibodies compared to the control group (Abu-Elyazeed et al. 1996). Age-independent Phlebovirus seroprevalences were also observed among military personnel operating in the Iran and age-independent dengue-neutralizing antibodies among soldiers of the US Army (Shiraly et al. 2017, Hesse et al. 2017).

4.2 Risk factors

Of the outdoor activities listed in the questionnaire, running was observed to be associated with Phlebovirus infections. Usually, sports are practiced in the evening, when temperatures are moderate and appropriate for outdoor activities. This coincides with the nocturnal activity of most sandfly species, where activity rises shortly before sunset, peaks during nighttime and declines rapidly with sunrise (Roberts 1994, Roberts 1996). However, the nocturnal activity varies with the season. While during cooler months, activity peaks in the evening, increased activity during the night is observed in hotter months (Coleman et al. 2007). Running is typically associated with increased sweating and CO₂ exhalation and facultative stretching afterwards. Sandflies are attracted to CO₂ and human odor, and thus running could increase the

risk of being bitten (Pinto et al. 2001). During hot summer months, military personnel typically wear shorts and short-sleeved shirts when they are off-duty, especially during sports. Even when insect repellents are applied properly, heavy sweating could result in an improper protection during outdoor activities. US soldiers stationed in Iraq, reported insufficient protection by insect repellents because of sweating during nighttime, while they were sleeping (Coleman et al. 2006).

Another risk factor is the co-infection with *Leishmania* spp., which is possible if suitable sandfly species and both pathogens are present in an operational area. In this study, co-infection rates varied between 0.00% and 18.5% among soldiers returning from the different operational areas. Co-infections with *Leishmania infantum* and phleboviruses were observed in dogs in Turkey and Portugal (Dincer et al. 2015, Maia et al. 2017). An increased risk of co-infection is posed by the presence of sandfly species, that can carry both pathogens. *P. tobbi* is a known vector for both TOSV and *Leishmania infantum*, and moreover co-infections with both pathogens have been observed (Svoboda et al. 2009, Ergunay et al. 2014). The distribution area of *P. tobbi* includes the investigated operational areas (Miscevic et al. 1998, Hadad et al. 2003, Haddad et al. 2015). *Leishmania* spp. are endemic in the Kosovo, Syria and the Lebanon (Lazri et al. 2008, Maroli et al. 2009, Nuwayri-Salti et al. 2000). Of course, symptoms are often unspecific and not directly associated to a certain disease. Of eight army veterans returning from the operation Desert Storm and suffering from visceral leishmaniasis, seven showed unspecific symptoms and one was asymptomatic (Magill et al. 1993). A study investigating healthy Austrian military and civilians, revealed around 5% asymptomatic *Leishmania* infections (Poepl et al. 2013). This highlights the importance of considering a co-infection, when an infection with one of the pathogens is detected.

4.3 Differences between operational areas

Differences in seroprevalences were observed between the operational areas, seroprevalence being highest in the Kosovo and lowest in BIH. Additionally, Phlebovirus diversity was higher in soldiers returning from Syria

and the Lebanon, compared to soldiers returning from BIH and the Kosovo. Interestingly, Phlebovirus diversity also varied between Syria and the Lebanon, although the distance between these two operational areas is only about 70 km at a maximum. While a significant amount of TOSV seropositivity was observed in soldiers returning from Syria, similar rates of TOSV and SFSV seropositivity were observed in soldiers returning from the Lebanon. The Golan Heights could function as a natural barrier for certain sandfly species. To date, no studies on Phlebovirus seroprevalences in Syria and the Lebanon have been published. However, leishmaniasis is a major health problem in both countries, confirming the presence of sand-fly species in Syria and the Lebanon (Alawieh et al. 2014, Saroufim et al. 2014).

Austrian soldiers operating in Syria were stationed in different camps on the Central and North Golan Heights, at an elevation of around 1000 to 2000 m above sea level (asl.). Soldiers operating in the Lebanon were stationed in Naqoura, a town at the coast of the Mediterranean Sea, in the south of the Lebanon. From there, the troops operate all along the border to Israel and also Syria to a small part. The circulation of Phlebovirus species is known from countries bordering Syria and the Lebanon. Various Phlebovirus species, including TOSV, have been isolated from sandflies in Southern Anatolia, in Turkey close to the border to Syria (Alkan et al. 2016). SFSV and SFNV antibodies were observed among study volunteers in South Iraq and in Israeli soldiers (Barakat et al. 2016, Cohen et al. 1999). Also in Jordan, seroprevalences of anti-SFSV and anti-SFNV antibodies were observed in hospitalized patients (Batieha et al. 2000). High numbers of individuals of the TOSV and SFSV-associated sandfly species *P. tobbi* and *P. papatasi* respectively, have been reported from Syria, at an altitude of ~500 m (Haddad et al. 2015). The low rate of SFSV seropositivity could be explained by the increased altitude, at which Austrian soldiers are operating in Syria. It was shown that the density of *P. papatasi* decreases with altitude and this species is absent at altitudes >1000 m asl., as it is bound to arid areas. Moreover, *P. papatasi* is anthropophilic and favors indoor resting places, such as cattle barns (Maroli et al. 2009). However, other sandfly species show increased densities at higher altitudes, such as *P. perniciosus* (Gueranoui et al. 2006). The occurrence of various sandfly species is known

from the Lebanon, Israel and Jordan. The large distribution of *Leishmania major* and *Leishmania infantum* transmitted by *P. papatasi* and possibly *P. tobbi*, corroborate the presence of both sandfly species (Haddad et al. 2003, Jaffe et al. 2004, Kamhawi et al. 1995). Austrian soldiers operate at lower elevations in the Lebanon than in Syria and thus are more likely to be exposed to diverse sandfly species, which is also reflected by the higher SFSV seropositivity compared to soldiers returning from Syria. Additionally, *P. papatasi* is functioning as a vector for SFNV, which was also detected in soldiers returning from the Lebanon. This supports the hypothesis that sandfly species diversity decreases with higher altitude. Still, the risk of infection with phleboviruses remains also at rather high altitudes, as indicated by the high Phlebovirus seropositivity observed among Austrian soldiers returning from the Golan Heights.

Also, a considerable difference was observed between the seroprevalences of soldiers returning from BIH and the Kosovo, the operational areas being around 300 km away from each other. The presence of sandflies and phleboviruses in BIH has been known for more than a century (Strobl 1900, 1904, Flamm 2008). However, data on sandfly distribution and associated phleboviruses is scarce for BIH. Studies from former Yugoslavia demonstrated the presence of several important Phlebovirus-associated species, such as *P. perfiliewi*, *P. perniciosus* and *P. tobbi* (Miscevic et al. 1998). A recent study revealed anti-TOSV seroprevalences of 10.71%, 9.38% and 12.50% in the years 2006, 2007 and 2008, respectively, among hospitalized patients suffering from febrile illnesses in Sarajevo (Hukić et al. 2009). Several studies confirmed the occurrence of sandflies and phleboviruses in the neighboring countries Croatia, Serbia and Montenegro. SFNV and SFSV seropositivity was observed in the Dalmatian region in Croatia (Tesh et al. 1976). The TOSV-associated sandfly species *P. perfiliewi* and *P. tobbi* were collected during a survey in three counties of the Dalmatian region (Bosnić et al. 2006). TOSV seroprevalence of up to 9% were observed in continental areas and up to 49% in coastal areas of Croatia (Punda-Polić et al. 2012a). Additionally, the occurrence of an autochthonous TOSV strain in Croatia was suggested by Punda-Polić et al. (2012b). In Serbia, the occurrence of sandflies suitable for the transmission of

phleboviruses was proven and SFNV was isolated from *P. perfiliewi* (Zivkovic 1980, Gligic et al. 1982). However, the medical relevance of TOSV remains uncertain in this area. Also in Montenegro, suitable vectors for SFV have been observed, but no data on the circulation of TOSV are available (Ivović et al. 2003). These studies on the circulation of TOSV and on suitable vectors, and the detected TOSV seropositivity of 8.20% of the current study, support the assumption that TOSV is circulating in BIH. Interestingly, two soldiers returning from BIH were CYPV seropositive. To date, CYPV seropositivity has only been observed in Cyprus, Greece and Turkey (Papa et al. 2006, Eitrem et al. 2009, Anagnostou 2011, Çarhan et al. 2010). A suggested but not proven vector for CYPV, *P. papatasi*, is present in BIH. However, the acquisition of the infection with CYPV during a vacation in the known area of distribution seems more likely. This is supported by the fact that both soldiers showed a very low titer (1:10). In the 1970s, neutralizing antibodies against SFSV and SFNV (9.6 % and 27.9%) were observed in a human population in the Kosovo (Tesh et al. 1976). A more recent study showed a seroprevalence of 5.5% for TOSV among blood donors in Peja, Kosovo. A Plaque reduction neutralization test confirmed two cases of SFNV infection and one of TOSV infection (Venturi et al. 2011). A recent study showed anti-SFSV antibodies in 2.7-78.2% and anti-TOSV antibodies in 0.0-10.4% of livestock of several counties in the Kosovo (Ayhan et al. 2017b). These results prove the circulation of SFSV and TOSV and highlight the variance of seroprevalences between different areas in the Kosovo. In Macedonia, bordering the Kosovo in the South, anti-*Leishmania* antibodies were detected in dogs. This confirms the presence of suitable sandfly species in the sampling area (Stefanovska et al. 2012). Also in Albania, anti-*Leishmania* antibodies were observed in dogs (Lazri et al. 2008). Several Phlebovirus-associated sandfly species were found in North and Central Albania and two novel Phlebovirus species, Adria virus and Balkan virus, were found in a coastal region of Albania (Velo et al. 2005, Papa et al. 2011, Ayhan et al. 2016). These studies demonstrate that sandflies are present and Phlebovirus species are circulating in Bosnia and Herzegovina, the Kosovo and bordering countries. However, the abundance and diversity of sandflies vary and Phlebovirus seroprevalences are different between the

countries. The mostly higher seroprevalences detected in humans and livestock in the Kosovo compared to BIH, are in accordance with the results of this study and indicate an elevated risk of infection in the Kosovo compared to BIH.

4.4 Relevance for the Austrian Armed Forces

This thesis provides the first proof, that Austrian Army personnel is exposed to phleboviruses in certain operational areas. However, seroprevalences and Phlebovirus species varied between the different areas. The presence of sandflies and the circulation of phleboviruses poses a risk of infection and the risk for a febrile illness with diverse symptoms. Additionally, the infections with TOSV, detected in soldiers returning from all four operational areas, constitute a potential risk for the involvement of the central nervous system. Since no vaccine and chemoprophylaxis against phleboviruses are available, expositional prophylaxis is the only recommended prevention measure. Not all countermeasures against hematophagous insects are appropriate for the prevention of sandfly bites. The in-door spraying with insecticides in military camps has been proven to be ineffective against sandflies, as this only shows an effect against anthroponotic sandfly species (Coleman et al. 2011). Other measures, such as insect repellents, protective clothing and insecticide-treated bednets were found to be effective against sandfly bites. However, proper application is essential for optimal protection. Insect repellents should cover all the exposed skin. Moreover, the application has to be repeated several times during day and night. US Army soldiers reported many sandfly bites, although insect repellents were applied, with heavy sweating and improper use assumed to be responsible (Coleman et al. 2006). Also, DEET and IR3535® are known to be effective against various sandfly species, but only function for several hours (Naucke et al 2006). An advantage of repellents such as DEET is that they are also protective against other blood-sucking arthropods, such as mosquitoes, black flies and ticks (Katz et al. 2008).

Additionally, permethrin-treated uniforms are effective against tick bites, mites, mosquitoes and sandflies (Evans et al. 1990, Breiden et al. 1982,

Schreck et al. 1984, Fryauff et al. 1996). A combination of insect repellents and permethrin-impregnated uniforms is advisable. Also, military personnel has to be aware, that sandfly activity is highest during night time in the summer months (Killick-Kendrick 1999). Thus, prevention measures should be applied during outdoor activities and while sleeping. If used properly, insecticide-treated bed nets (ITN) prevent from the bites of sandflies. However, the appropriate usage is mandatory. ITNs are effective against biting insects, such as mosquitoes (Nahlen et al. 2003) but large mesh sized bed nets are not protective against sandflies, because of their small body size. Also, large gaps between mattress and net or skin contact with the net support sandfly bites (Coleman et al. 2006). The proper use of appropriate ITNs is highly effective against sandflies (Jalouk et al. 2007). Sandfly bites do not necessarily result in a Phlebovirus infection, however many bites can be acquired during a single night enhancing the risk of infection and allergic reactions and secondary infections can also be observed (Coleman et al. 2006).

If soldiers stationed in a sandfly-endemic area suffer from an uncertain febrile illness, a Phlebovirus infection should be taken into account.

5 Abbreviations

ADRV	-	Adria virus
asl	-	above sea level
BC	-	before Christ
BIH	-	Bosnia and Herzegovina
CEE	-	Central European Encephalitis
CF	-	Complement fixation
CG	-	control group
CI	-	confidence intervall
CNS	-	Central nervous system
CYPV	-	Cyprus virus
DNA	-	Desoxyribonucleic acid
dsDNA	-	double strand Desoxyribonucleic acid
EIA	-	Enzyme immunoessay
EU	-	European Union
F1	-	first filial generation
HIGV	-	Hunter Island Group virus
ICOV	-	Icoaraci virus
ICTV	-	International Committee of Taxonomy of Viruses
IgG	-	Immunoglobulin G
kb	-	kilobases
kbp	-	kilobasepairs
LUCA	-	Last universal common ancestor
ml	-	milliliter
mm	-	millimeter
NATO	-	North Atlantic Treaty Organization
NCBI	-	National Center for Biotechnology Information
nm	-	nanometre
OeBH	-	Österreichisches Bundesheer
OR	-	Odds ratio
OSCE	-	Organization for Security an Co-operation in Europe

PBS	-	Phosphate Buffered Saline
RNA	-	Ribonucleic acid
RNAi	-	Ribonucleic acid interference
rpm	-	rounds per minute
RVFV	-	Rift Valley fever virus
SALV	-	Salehabad virus
SFNV	-	Sandfly fever Naples virus
SFSV	-	Sandfly fever Sicilian virus
SFTSV	-	Sandfly fever with thrombocytopenia virus
ssDNA	-	single strand Desoxyribonucleic acid
TBE	-	Tick-borne encephalitis
TOSV	-	Toscana virus
WHO	-	World Health Organization

6 Glossary

Abdomen: Posterior body part.

Adult: Sexually mature animal.

Antigen: Molecules that stimulate the production of antibodies by the immune system.

Antibody: (Immunoglobulin). Proteins produced mainly by the B lymphocytes. They recognize external molecules (e.g. pathogens) and neutralize them.

Arthropod: Invertebrate animal with exoskeleton and jointed appendages: Hexapoda, Crustacea, Myriapoda, Chelicerata and Trilobitomorpha (extinct).

Asymptomatic: Without symptoms.

Base pair (Bp): In the DNA, complementary bases of a strand bind to form a base pair. Adenine binds with thymine and cytosine binds with guanine.

Classification: Hierarchical arrangement of species.

Cytoplasm: Material within the cell membrane, excluding the nucleus.

Dead-end host: An intermediate or definitive host, where the parasite cannot complete its life cycle.

Diapause: A pause in the life cycle of an insect or other invertebrates.

Diversity: Variety or variability of species.

DNA: Desoxyribonucleic acid, carrying the genetic information. A polynucleotide, consisting of sugar (Desoxyribose), a phosphate group and one of the four nucleobases, adenine, cytosine, guanine and thymine.

DNA-polymerase: An enzyme, that synthesizes DNA molecules and builds DNA strands.

Endemic: Native to a certain area.

Enzyme: Proteins, which catalyze chemical reactions in living organisms.

Epidemiology: Study of the distribution and causes of contagious and non-contagious diseases in a population.

Evolution: The progressing development of life forms through selection and mutation.

Exposition prophylaxis: A protective measure to prevent contact to pathogens and/or respective vectors.

Family: A group of related genera, above the taxonomic level of genus.

Fauna/faunal: The animal kingdom in total or a certain area.

Fluorescence: The luminescence of substances or organisms, as a result of an exposure to light of a certain wavelength.

GenBank: A database for genetic sequences.

Genome: The genetic material of an organism, either consisting of DNA or RNA.

Genus: A group of related species, above the taxonomic level of a species.

Hard tick: Ixodidae, one of the three families of ticks.

Hematophagous arthropod: Bloodfeeding arthropod.

Hemorrhagic fever: Fever with concurrent bleeding tendency.

Hepatitis: Inflammation of the liver.

Immunity: Resistance of an organism against an infection with a pathogen.

Indirect immunofluorescence test: A serological test to detect antigen-bound antibodies with fluorescent anti-antibodies.

Intracellular: Inside of the cell.

Infection: The attack of an organism by a pathogen, characterized by three features: invasion, reproduction and immune response.

Influenza: Specialist term for the flu.

Incubation time: Timespan between infection and onset of an illness.

Insecticide: Substances used for killing insects.

Instar: The growth period between ecdysis.

Kilobase (Kb): 1000 bases or basepairs.

Larva(e): Immature stage of certain animals (e.g. insects) with special features lacking in adults.

Leishmaniasis: An infectious disease caused by protozoa (*Leishmania* spp.), which is transmitted by the bite of sandflies (Phlebotominae).

Lethal: Deadly.

Leukocytopenia: The decrease of white blood cells in the blood.

Life cycle: The development of organisms from the egg stage to maturity.

Lipid: Fats and fat-like substances, which are hydrophobic.

Meningitis: Inflammation of the protective connective tissue of the brain and spinal cord.

Meningoencephalitis: A meningitis combined with an inflammation of the brain.

Mutation: Alterations in the nucleic acid sequence.

Nausea: Urge to vomit.

Nervous system: System of specialized cells, for the perception and processing of stimuli from the surroundings to certain body regions.

Nucleic acid: DNA or RNA (see DNA).

Order: A taxonomical group of related families.

Parasite: An organism that either lives in or on a host by the exploitation of energy (mostly nourishments), without killing its host.

Pathogen: A biological unit that causes an illness.

Polyphyletic: A taxon of living organisms, without a common ancestor.

Prevalence: Amount of infected or ill individuals in a population at a certain time point.

Prophylaxis: Preventive measures against an illness.

Proteine: Large molecules consisting of amino acids.

Repellent: Substances to avoid insects and other arthropods.

Seroepidemiological study: Assessment of the antibody status in a study collective.

Seropositivity: Existence of specific antibodies.

Seroprevalence: Amount of individuals with specific antibodies of a disease agent in a certain population.

Soft tick: Argasidae, one of the three families of ticks.

Species: Lowest hierarchical taxon, a group of related individuals.

Symptom: Sign of a disease.

Taxon: Taxonomic unit, e.g. species, genus, family, order.

Taxonomy: Systematic hierarchical classification of organisms, appropriate to their assumed formation and relatedness.

Temperate zone: Geographical areas between polar and subtropical regions, mostly with four seasons and moderate climate.

Thorax: Bodypart between neck and abdomen.

Transmission: The transfer of a disease or a disease agent.

Vaccine: A substance which induces an immunity against a certain pathogen.

Vector: Carrier of pathogens, which can be transmitted.

Vector competence: The ability to host a certain pathogen and maintain its life cycle.

Vertebrate: A group of animal species with common features, such as a spinal cord.

Virulence: The level of pathogenicity of a pathogen.

Virus: An infectious particle, that needs a living cell to replicate.

Zoonosis: A disease that can be transmitted from animals to humans.

Definitions largely following:

Kaushansky, K. (1996). Glossary of molecular biology terminology. *Biologicals: journal of the International Association of Biological Standardization*, 24(3), 157-175.

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8 Appendix

8.1 Abstract

8.1.1 English

Seroprevalence of sandfly-borne viruses in Austrian soldiers returning from missions abroad

Phleboviruses are transmitted by either sandflies, mosquitoes or ticks and can infect diverse vertebrates including humans. They comprise nine species with several serotypes and are widely distributed, following the distribution of their respective vectors. Infections are often asymptomatic, but can result in a self-limiting febrile illness or also more serious diseases, such as meningitis and meningoencephalitis. The aim of this study was to assess the seroprevalence of phleboviruses in Austrian soldiers returning from different operational areas abroad and to detect risk factors associated with Phlebovirus infections.

Sera of 486 soldiers returning from operational areas in Bosnia and Herzegovina, the Kosovo, Syria and the Lebanon and 500 sera from soldiers prior to their missions, functioning as a control group, were provided by the Austrian Armed forces and investigated by indirect-immunofluorescence assays and subsequent fluorescence microscopy to detect anti-Phlebovirus antibodies. Additionally, serial dilutions were performed to assess the respective antibody titers.

Altogether, 146 sera (14.8%) revealed anti-Phlebovirus antibodies including sera from the control group. Seropositivity ranged from 11.48% to 20.69% depending on the respective operational area. Also the control group showed an unexpected high seropositivity of 11.40%. The Phlebovirus seroprevalence in soldiers returning from the Kosovo almost doubled the one in soldiers returning from BIH, although the two countries are closely located. Phlebovirus diversity varied between the operational areas with highest diversity in Syria and the Lebanon. Toscana virus was the most prevalent

virus and the only virus detected in all groups. Phlebovirus positivity was found to be associated with the potential risk factors “running” and “*Leishmania* spp. infection”. Interestingly, 20.4%-66.7% of the seropositive soldiers showed symptoms during their respective missions. This thesis provides the first data on Phlebovirus seroprevalences in Austrian soldiers and in Austria altogether and shows that Austrian Army personnel is at risk of infection with phleboviruses in the different operational areas and thus should apply appropriate prophylaxis during their missions.

8.1.2 German

Seroprävalenz von Sandmücken-assoziierten Viren in österreichischen Soldaten nach dem Auslandseinsatz

Phleboviren werden von Sandmücken, Stechmücken und Zecken übertragen und können zahlreiche Wirbeltiere, unter anderem den Menschen, infizieren. Die Gattung Phlebovirus besteht aus neun Arten und über 30 Serotypen und hat eine große Verbreitung, gebunden an das Vorkommen der jeweiligen Vektoren. Die Mehrzahl der Infektionen verläuft asymptomatisch, häufig kommt es aber auch zur Ausbildung grippeähnlicher Symptome die in der Regel jedoch selbstlimitierend sind. Bei schweren Fällen kann es zu einer Infektion des zentralen Nervensystems und einer Meningitis oder Meningoenzephalitis kommen. Die vorliegende Arbeit beschäftigt sich mit der Seroprävalenz von Phleboviren bei österreichischen Soldaten nach ihrem Auslandseinsatz. Verschiedene Einsatzgebiete wurden miteinander verglichen und mögliche Risikofaktoren evaluiert.

Insgesamt wurden 486 Seren von österreichischen Soldaten die aus den Einsatzländern Bosnien und Herzegowina, Kosovo, Syrien und Libanon heimkehrten und 500 Seren von Soldaten vor ihrer jeweiligen Mission als Kontrollgruppe untersucht. Ein indirekter Immunfluoreszenztest mit anschließender Fluoreszenzmikroskopie wurde angewendet, um anti-Phlebovirus Antikörper in den Seren festzustellen. Verdünnungsreihen wurden durchgeführt um Antikörpertiter zu erheben.

Insgesamt wurden 146 Seren (14.8%) positiv auf anti-Phlebovirus Antikörper getestet, einschließlich Seren der Kontrollgruppe. Die erhobenen Seroprävalenzen variierten zwischen 11,48% bis 20,69% in den getesteten Einsatzgebieten. Eine unerwartet hohe Seropositivität von 11,40% wurde unter den Probanden der Kontrollgruppe nachgewiesen. Trotz der geringen geographischen Entfernung, wurde eine annähernd doppelt so hohe Seropositivität bei Soldaten mit Einsatzgebiet Kosovo, verglichen mit Bosnien und Herzegowina festgestellt. Auch die Phlebovirus-Diversität variierte zwischen den Einsatzgebieten, wobei Syrien und der Libanon die höchste Diversität aufwiesen. Insgesamt war das Toskana-Virus am weitesten verbreitet und wurde als einziges in allen Gruppen nachgewiesen. Als potentielle Risikofaktoren konnten „Laufen“ und eine „*Leishmania* spp.-Infektion“ ermittelt werden. Interessanterweise waren zwischen 20,4% und 66,7% der seropositiven Soldaten auch symptomatisch. Diese Studie liefert die ersten Ergebnisse zu Seroprävalenzen von Phleboviren bei österreichischen Soldaten und in Österreich generell. Es wurde gezeigt, dass österreichische Soldaten einem erhöhten Risiko ausgesetzt sind, sich während eines Auslandseinsatzes mit Phleboviren zu infizieren, eine entsprechende Prophylaxe während des Einsatzes wird empfohlen.

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