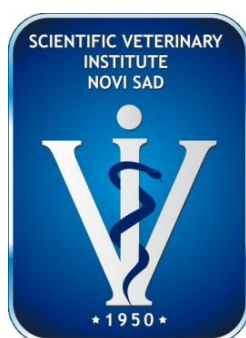


SCIENTIFIC VETERINARY INSTITUTE „NOVI SAD“  
INSTITUTE OF VETERINARY MEDICINE OF SERBIA

*„One Health – New Challenges“*

# First International Symposium of Veterinary Medicine

(ISVM2015)



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*Invited lecture*

**IMPORTANT ZONOTIC VIRAL DISEASES OF WILDLIFE AND THEIR IMPACT ON HUMAN HEALTH**

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**Abstract:**

Zoonoses are infectious diseases which can be transmitted from animals to humans and, in rare cases *vice versa*, from humans to animals. It is estimated that the majority of causative agents are viruses and approximately 75% of all known zoonoses involve wild animals. Wildlife is important for epidemiology of most zoonoses as reservoir for the maintenance and spreading of infection. Many mammalian species, such as wild boar, roe deer, wild carnivores, wild rodents, bats and also fish or reptiles are recognised as wildlife reservoirs. Wild birds are equally very important, as they have a significant role in disseminating of various pathogens over long distances.

This paper presents the overview of some literature data and results of testing conducted in Serbia of several important emerging viral zoonoses that occurred in wildlife: West Nile disease, Usutu virus, hepatitis E virus, avian and swine influenza and rabies. In addition, the results of zoonotic disease investigation in humans in Serbia and their impact on human health are also provided.

Introduction of new pathogens, especially into the industrialized countries, increased the global threat to public health and intensified the efforts to establish control for prevention emerging zoonoses.

**Keywords:** zoonoses, viral diseases, wildlife

**Introduction:**

Many well-known human viruses have been recognised for a long time ago and persist in human population in balance with their host. Most of these viruses almost never or very rarely cause large-scale health problems. But, when pathogens cross the species barriers, the consequences can be disastrous with a high morbidity and mortality rate (Haagmans et al., 2009).

Wildlife represents free-roaming animals, such as: mammals, birds, fish, reptiles and amphibians. Throughout the history wild carnivores, roe deer, wild boars, wild rodents or birds had an important role in the chain of pathogen spreading, whether they serve as a reservoir for many viral agents or are involved in viral dissemination over long distances (Daszak et al., 2001).

*Definition of zoonoses*

Zoonoses are defined as infectious diseases that can be transmitted from animals to humans, or *vice versa*, from humans to animals (Messenger et al., 2014; Venkatesan et al., 2010). The total number of zoonoses is unknown, but according to Taylor et al. (2001), 1415 infectious agents are pathogenic for humans. It is estimated that about 61% of these pathogens are zoonotic, and approximately 75% originate in wildlife (Jones et al., 2008). The WHO/FAO/OIE joint consultation on emerging zoonotic diseases held in Geneva in 2004 defined emerging and re-emerging zoonoses. Emerging zoonoses are infections that are recognised in humans for the first time, while re-emerging zoonoses are described as diseases that have already infected humans previously, but appear in new locations, show an increase in incidence or expand geographic, host or vector range

(WHO, 2004). Many emerging zoonoses represent a threat to human health and have large economic impacts.

There are rare reports on the transmission of viruses from humans to animals, named: „reverse zoonoses“. We will mention just a few: Rotavirus, Human Adenovirus A-F, Hepatitis E, Influenza A (H1N1), etc. (Messenger et al., 2014).

### *History of zoonoses*

The history of zoonotic diseases is long for centuries. The most fatal of all so far was Black Death pandemic (bubonic plague) in the 14th century, which caused 25-40 million deaths. The first known report on rabies dated from 2300 years BC in Mesopotamia, in hunting dogs (Kruse et al., 2004). Some modern hypotheses suggest that even Aleksandar the Great died with the symptoms of encephalitis in Babylon in 323 BC, probably infected with West Nile virus acquired from the dead ravens. The 1918 influenza pandemic, known as „Spanish flu“, has taken about 50 million lives. In the modern history the deadliest is the HIV/AIDS pandemic, which was reported in early 80´ and caused 35 million deaths so far (Morens and Fauci, 2013). In 2014, Ebola caused 11007 deaths in Sierra Leone, Liberia and Guinea, according to CDC (2015) report, and was the largest Ebola outbreak known so far.

### *Transmission modes*

The wildlife is considered to be the main reservoir of emerging infections and zoonotic agents can be transferred in many ways from animals to humans. Some viruses can be transmitted directly, by bite (rabies). Rodents constitute reservoir for Hantaviruses, which are transferred to humans by aerosol and excretes in direct contact (Kruse et al., 2004). In 2003, in the USA occurred epidemic of monkeypox with 37 confirmed human cases. It is considered that probably African squirrel is the natural host of the disease. Laboratory infections as a consequence of handling with infected material from animal tissue have also been recorded (Venkatesan et al., 2010).

The majority of viruses is transmitted indirectly and for their transmission is necessary the presence of different kind of vectors (vector-borne diseases). Arthropod-borne viruses or arboviruses involve blood-sucking arthropods. The virus is replicating in the insect and transferred to the animal by bite. Another insect suck the blood of a viremic animal or human and cycles of replication is repeating. In the nature are recognized different insect vectors which are responsible for spreading of many viral zoonotic diseases: mosquitoes (West Nile disease, Rift Valley fever, Equine encephalitis, Dengue fever, Yellow fever, Chikungunya virus), ticks (Tick-borne encephalitis -Eastern and Western, and Cremen-Congo hemorrhagic fever), sand flies (Vesicular stomatitis) and midges (Blue tongue disease) (Venkatesan et al., 2010).

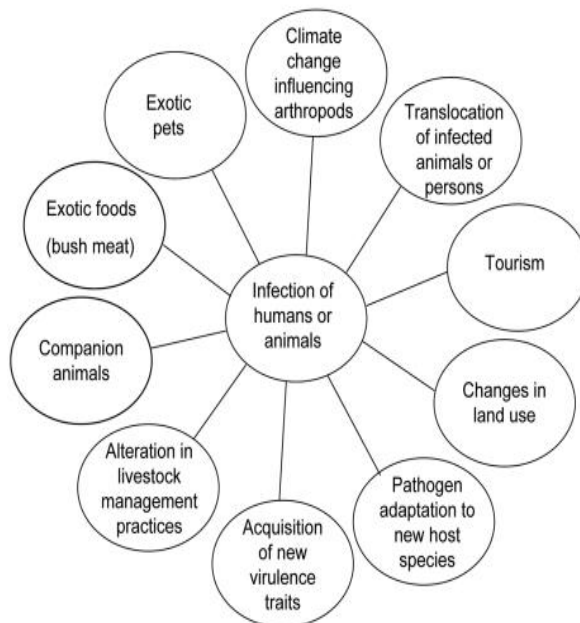
In the last few years, viral zoonoses with bat origin attract the attention of scientific society. Bats counts over 1200 species and are spread in almost every part of the world. They live in the large colonies and can disseminate viruses over the long distances. The most significant are: Hendra and Nipha virus and Coronaviruses (SARS and MERS). Recent data revealed that fruit bats are also reservoir for Ebola and Marburg viruses in Africa (Wang and Cramer, 2014).

Wild birds are important for public health and have a major role in spreading of many viruses (Haagmans et al., 2009). Wild ducks played a role in the emergency of highly pathogen H1N1 influenza A virus in 2004 in Asia, which causes 28 deaths. The virus is maintained in nature between the waterfowls and domestic poultry, which can result in reassortment of viruses with the new antigenic characteristics for which humans are immunologically naive (Kruse et al., 2004).

Some viruses showed a remarkable ability that after replication in their animal host transfer to human, and with correct circumstances can result in human-to-human transmission. This scenario is typical for Ebola fever, influenza A and SARS (Cutler et al., 2010).

*Factors influencing the emergency of zoonotic diseases*

The frequency of many zoonotic diseases outbreak is on rise (Wang and Cramer, 2014). There are recognized many factors that can influence the emergency of different viruses (Graph 1). Climate and environmental changes and global warming lead to the expansion of vector distribution. Vectors are moved from their limited geographical area, expanded the habitat and introduced the zoonotic agents into naive animal and human population. Epidemiologist suspect that appropriate weather with lots of rain and increased mosquito population are the main reasons for emergency of West Nile virus. Agricultural development, modernisation of agricultural practise and deforestation pushed together wildlife and livestock and overlapped their natural surroundings. Zoonotic diseases are lately closely linked to hunting and consuming meat from wild animals, especially non-human primates. “Bush meat” is considered delicacy and exportation and trading increased the risk of cross-species transmission. For example, Ebola and Marburg virus are among the most deadly viruses for human kind and is believed that these emergent zoonoses are transferred through “bush meat” activities (Wang and Cramer, 2014). Microbial changes are responsible for the occurrence of potentially new emerging pathogens. These changes include mutations (genetic drift) or genetic recombination (genetic shift). Typical example is influenza A virus. Natural infection with influenza A is reported in many animal species (birds, pigs, horses, sea mammals) and humans. So far, four major antigenic shifts resulted with influenza pandemics causing many human fatalities (Haagmans et al., 2009). Movement of the people, livestock and food provides rapid spread of pathogens and appearance of the viruses in new locations and continents. In this way rabies is established in red foxes and spread from Russia to the rest of Europe in early 1940` (Cutler et al., 2010). The role of companion animals and exotic pets is also important in dissemination of some virus infections; such as rabies, *Chlamydia psittaci*, etc.



Graph 1: Factors influencing new and re-emerging zoonoses (source: Cutler et al., 2010)

*Manifestations of Viral Zoonoses*

The majority of viral zoonoses can be classified according to predominant clinical signs into: 1) diseases causing no illness, 2) non-specific viral syndrome and 3) severe illness. The third group is further classified into: 1) hemorrhagic fever, 2) encephalitis and /or rash arthralgia, 3) emerging and re-emerging and 4) rare zoonotic infections.

Most of zoonotic diseases that cause encephalitis are transmitted through mosquito or tick bites (West Nile virus, Tick-borne encephalitis, Kunjin virus) or through a bite of infected host (rabies). Viruses that belong to the group of diseases with clinical signs of hemorrhagic fever causing internal bleeding from various openings of the body and internal organs (Ebola, Marburg, Dengue fever, Cremen-Congo hemorrhagic fever, Yellow fever). Rashes and arthralgia are typical clinical signs for some viruses from Togaviridae family and most of them are transmitted through mosquito bites (Chikungunya virus). Emerging and re-emerging diseases appear suddenly, with a wide range of symptoms: flu-like signs and respiratory infections (influenza A, SARS), hemorrhagic fevers (Ebola, Marburg), hepatitis (Hepatitis E), fever and abdominal pain (Hanta), fever and encephalitis (Hendra and Nipah) etc. Several viral infections cause nonspecific illness with febrile conditions and occur rarely. In these cases humans can acquire the disease mostly by contact with infected animals (FMD, Newcastle disease, Bluetongue) (Venkatesan et al., 2010).

### **Selected Viral Zoonoses with a Wildlife Origin in Serbia**

#### *Rabies*

Rabies is one of the oldest known zoonoses, but still neglected in some parts of the world. Rabies is a neurotropic disease with the clinical signs of encephalomyelitis and with almost 100% mortality rate. The disease takes every year, according to WHO (2013), more than 60 000 human lives, mostly in developing countries of Asia and Africa. The victims are usually children younger than 15 years. Many European countries obtained rabies-free status, but for these countries the main threat represents importation of rabid animals, mainly through the trading activities of pets or livestock. In infected countries, where Serbia belongs, rabies is maintained in the foxes and other wildlife (Fooks et al., 2014).

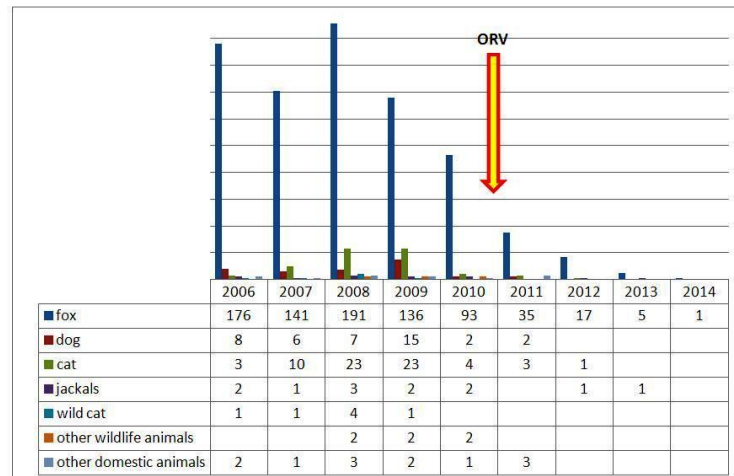
The causative agent of rabies is a negative-strained RNA virus that belongs to the genus *Lyssavirus*, family *Rhabdoviridae*. Rabies is transmitted through the bite of infected animals, but there are also reports of other ways of transmission, through the transplantation of cornea and solid organs (Maier et al., 2010).

After the World War II, rabies existed in former Yugoslavia in both urban and sylvatic form. Extensive veterinary measures and massive vaccination of cats and dogs, eradicated urban rabies and consequently, human exposure. In 1977, rabies entered Serbia from the north, during the large enzootic emergency in red foxes in Europe and established in wildlife (Petrović, 1987).

The first trial of the oral vaccination of foxes (ORV) was carried out in Switzerland in 1978. After that, many European countries followed this example and implemented ORV of foxes. In 2010, Veterinary Directorate started a multi-annual program of oral vaccination of foxes in Serbia, supported by European Commission and funded by IPA projects (*Instrument for Pre-Accession assistance*). Monitoring of the effectiveness of oral vaccination campaigns has been conducted from 2011 and is based on: a) *post mortem* laboratory examination of brain tissue of target animals (foxes, jackals and other carnivores) by fluorescent antibody test (FAT), b) detection of antibodies against rabies virus in serum samples by ELISA and c) detection of tetracycline biomarker in the mandibles for the evaluation of vaccine bait uptake. From September 2011 to May 2014, the total number of 4943 brain tissue samples, 4241 sera and 4971 mandibles were analyzed. Confirmed rabies-positive brains decreased from 10 in 2011/2012 to 6 in 2012/2013 and finally to 1 positive case in 2013/2014. The seroconversion rate increased from 10.48% in 2011/2012 to 20.11% in 2012/2013 and 42.23% in 2013/2014. Along with the seroconversion, the number of detected tetracycline positive mandibles raised from: 49.67% in 2011/2012 to 62.60% in 2012/2013 and eventually to 90.33% in 2013/2014 (Lupulović et al., 2015).



In Graph 2 is presented rabies situation in Serbia from 2006 to 2014. The highest prevalence of rabies was recorded in the population of foxes and just sporadically in other wildlife or in domestic animals. Foxes made 84.66% (795/939) of all positive samples. From 2011, when started the first campaign of vaccine distribution, the number of confirmed positive rabies cases constantly decreased confirming that ORV of foxes and other wildlife in Serbia was successful.



Graph 2: Prevalence of rabies in different animal species between 2006 and 2014 (Lupulovic et al., 2015)

### West Nile Fever

West Nile virus (WNV) is a mosquito-borne arbovirus that belongs to the genus *Flavivirus*. In nature, WNV is maintained in the cycle between mosquitoes and birds. Humans and horses can be infected, but they do not spread the disease (so called “dead-end” hosts). Approximately, 80% of infections are subclinical and less than 1% of people can develop clinical signs of the disease. The disease in horses is also unapparent, but about 10 % of animals got ill and 50% of them die with the signs of neurological disorder (Martin-Acebes and Saiz, 2012).

The first isolation of WNV was in Uganda in 1937. The disease has been occurred sporadically in Africa, Asia and Middle East, but the situation dramatically changed when WNV infection outbreak was reported in 1999 in New York, with over 1100 human fatalities. Since then, the virus spread and new human cases were reported in the USA, Canada and Mexico. In Europe, the epidemiological situation changed in 1990, when several countries of Mediterranean basin, Romania and Russia have faced with humans and horse deaths (Calistri et al., 2010).

In 2012, Greece reported re-emergence of WNV with 18 human deaths. Italy and Hungary also announced the outbreak of WNV infection. At the same time, in 2012, West Nile virus infection was reported for the first time in Serbia with 9 human victims (Popović et al., 2013) and in 2013 with 35 fatalities. Before that, WNV fever in Serbia was neglected disease with the limited data of virus dissemination. First serological testing of horses was conducted during 2009-2010 in Vojvodina province and 12% of 349 tested horses reacted seropositive (Lupulović et al., 2011). In addition, further examination was carried out and the established seroprevalence was 28.6% (Medić et al., 2014). After the human outbreak in 2012, 130 horses were examined. The detected anti-WNV antibodies were much higher comparing to previous surveys, with 49.23% seropositivity rate (Petrović et al., 2014).

According to presented data, it was assumed that WNV is circulating among wild birds too. In total, 192 blood sera and 88 pooled tissue samples from 133 different wild resident and migratory birds were analyzed for the presence of WNV during 2012. Anti-WNV antibodies were detected in 8% (7/92) of tested blood sera and the presence of WNV RNA was confirmed in 10% (8/81) of pooled

tissue samples. Phylogenetic analyzes showed that identified WNV RNA belongs to lineage 2 strain, that is circulating in this part of Europe (Petrović et al., 2013). Based on the obtained data, WNV surveillance program has been launched in Serbia since April 2014. The program encompassed serological testing of seronegative horses and poultry and RT-PCR analyzes of pooled mosquitoes samples (Petrović et al., 2014)

The recent investigation showed that WNV is present in the population of wild boars and roe deer, as well as in the population of domestic pigs in Serbia. For this purpose, 279 swine sera, 318 wild boars and 91 roe deer were tested by enzyme-linked immunosorbent assay (ELISA) and viral neutralisation test (VNT). Antibodies against WNV were detected in 43 (15.4%) pigs, 56 (17.6%) wild boars and 17 (18.7%) roe deer sera (Escribano-Romero et al., 2015)

There is evidence that another mosquito-borne flavivirus, Usutu virus (USUV), is circulating in Serbia. Anti-USUV neutralizing antibody titre is detected for the first time in one horse sera, during the examination of WNV activity in horses (Lupulovic et al., 2011). Furthermore, 4.5% (4/88) of tested patients in South Bačka has anti-USUV IgG antibodies (Hrnjaković Cvetković i sar., 2014) and four wild boar sera showed USUV specificity (Escribano-Romero et al., 2015).

### *Hepatitis E*

Wild boars are the reservoir of a various number of viruses with the potential for transmission of many diseases to domestic animals and humans. There are reports of serological examination for a couple of zoonotic agents, including Hepatitis E, swine influenza virus and Japanese encephalitis virus (Meng et al., 2009).

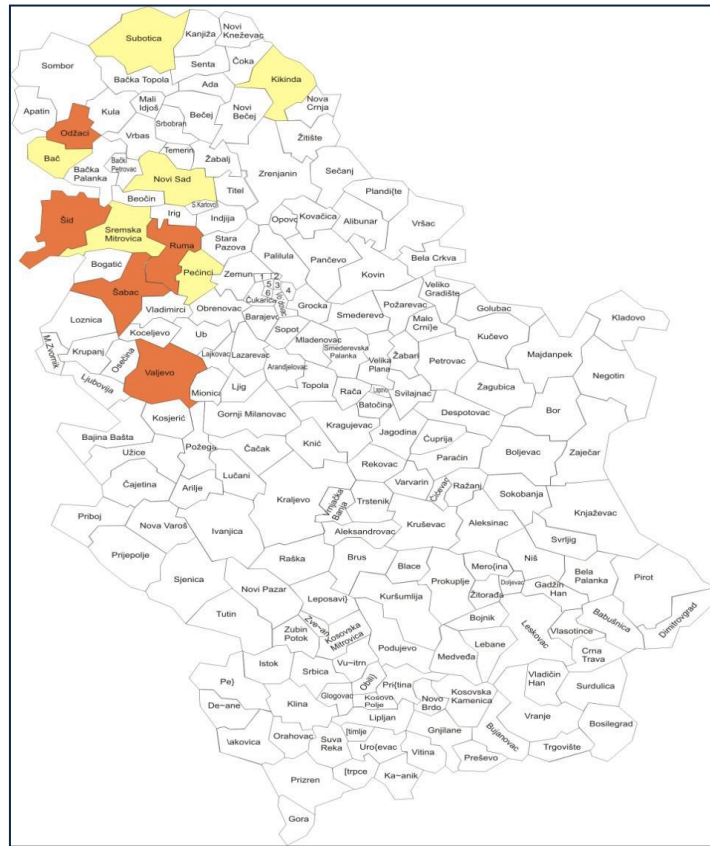
Hepatitis E (HEV) belongs to one of the five so far described types of viral hepatitis in humans (hepatitis A, B, C, D and E). The clinical signs of the disease are typical for acute hepatitis with abdominal pain, nausea, vomiting, anorexia, fever, dark urine and jaundice. The disease is spread mainly through contaminated water and caused serious epidemics in developing countries of Asia and Africa. HEV (family Hepeviridae, genus Hepevirus) is non-enveloped whose genome is a single-stranded RNA molecule (Pinto and Saiz, 2007). First isolation of HEV in pigs was reported in 1997 (Meng et al., 1997). Since then, the presence of HEV infection in pigs was confirmed in many countries, such as Spain, the Netherlands, New Zealand, France, Japan, Canada, Portugal, Germany, etc. Zoonotic potential of HEV is proved after infection of people who consumed inadequately cooked meat and liver of deer and wild boar (Tei et al., 2003; Masuda et al., 2005).

The first investigations on HEV infection in domestic and wild boars in Serbia was carried out in 2008. HEV RNA was detected in 30% of faecal samples and 45% of the tissue samples from farming pigs, while no HEV RNA was detected in pooled faecal samples from feral pigs (Petrovic et al., 2008). In another investigation, 315 swine sera from 3 to 4 months-old backyard pigs were collected during years 2006-2007 and tested for the presence of anti-HEV antibodies. Out of this number, 34.6% (109/315) of sera resulted positive by *in-house* ELISA (Lupulovic et al., 2010; Jimenez de Oya et al., 2009).

With the aim to determine the presence of HEV in wild boars in Serbia, 92 blood serum samples were analyzed by *in-house* ELISA. In 32 (34.78%) animals was detected the presence of specific antibodies against HEV genotype 3. The blood samples were collected during 2009, 2010 and 2011 from 15 hunting sites in Serbia (Lupulović i sar., 2011). The obtained results showed that hepatitis E virus is present in wild boar population in Vojvodina (Graph 3). These results are of remarkable importance because several studies suggested that HEV is food-borne pathogen with zoonotic potential.

Further examinations were undertaken and HEV RNA was detected in faeces, liver and bile samples in slaughterhouses in Serbia (Petrovic et al., 2013) as well as the presence of anti-HEV

antibodies in blood and meat juice samples in slaughtered pigs (Lupulovic et al., 2013). In order to provide more data on the potential risk for public health, 200 volunteer blood donors were tested for the presence of anti-HEV IgG antibodies by in-house ELISA. In total, 15% of blood samples were seropositive. No HEV RNA was detected in any of the analyzed blood samples (Petrović et al., 2014).



Graph 3: HEV infection in wild boars in Serbia (source: Lupulović i sar., 2011). All coloured fields - hunting areas in Vojvodina, where wild boar blood samples were collected; dark coloured fields - hunting areas with HEV IgG positive wild boars

### *Avian influenza*

Avian influenza (AI) is a viral infection of birds (especially wild water fowl such as ducks and geese), often without clinical signs of the disease. AI viruses can sometimes transfer to domestic poultry and cause large-scale outbreaks of infection. Avian influenza is zoonoses with a great potential to cross the species barrier and cause high mortality in humans.

Avian influenza viruses are divided into 2 groups based on their ability to cause disease in poultry: highly pathogenic and low pathogenic viruses. Highly pathogenic viruses can cause mortality up to 100% within 48 hours, while low pathogenic viruses are not generally associated with severe disease in poultry. Influenza A type viruses can be also subtype according to their two surface glycoproteins: hemagglutinin (HA) and neuraminidase (NA). Until now, 18 types of hemagglutinin and 11 types of neuraminidase have been identified. Human infections are usually induced with subtypes H5 and H7. Influenza A viruses are susceptible to mutations which can be minor ("drift") or major ("shift"). The special threat represents the possibility of virus to spread and replicate in the new host causing reassortment of viruses. Most cases of infection in humans were caused by direct contact with sick live or dead poultry. Until now there is no evidence that virus can be transmitted by direct contact from person to person (WHO, 2014).

A couple of pandemic outbreaks of AI have been recorded in the past. The best known is Spanish flu in 1918, when avian influenza subtype H1N1 has been transmitted to humans and killed about 50 million people. Highly pathogenic avian influenza H5N1 caused infection in poultry in Hong Kong in 1997, resulting in millions of dead chickens and several human deaths. Another emergency of AI H1N1 was reported in 2003 and 2004, and the disease spread from Asia to Europe and Africa. Influenza A subtype H7N9 infected humans in Asia in 2013, but there were no reports of human cases out of China.

Serbia announced first case of highly pathogenic AI H5N1 in March 2006 in wild birds (swans). Šekler et al. (2009) tested AI seroprevalence in 30 blood samples of 46 species of wild birds, collected from 2006 to 2008 in Serbia. Specific antibodies against AI were established in 9 (30%) samples using eELISA. In addition, the same sera were tested by hemmagglutination inhibition test (HI test) to distinguish H subtypes. Among the tested samples, antibodies against AI virus subtype H5 were detected in 4 samples, and against subtype H7 in 3 samples (Šekler et al., 2009)

Influenza pandemics are unpredictable. It is of great importance to carry out further surveys to analyze the prevalence of avian influenza, especially in wild birds in Serbia.

## Conclusions

Different viral zoonotic agents have been detected in wildlife in Serbia (rabies, WNV, hepatitis E, USUV, avian influenza). Additional investigations on important viral zoonoses should be conducted with the aim to prevent outbreak of emergency diseases with the serious consequences for human health.

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