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Short communication

SEROLOGICAL SURVEY OF AVIAN INFLUENZA VIRUS INFECTION OF BACKYARD CHICKENS IN BOSNIA AND HERZEGOVINA

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Summary

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The aim of this research is to examine seroprevalence of avian influenza (AI) in backyard chickens from several localities in the entity of the Republic of Srpska, Bosnia and Herzegovina. Up to date there was no previous research in this topic. Our study, using ELISA test, confirmed the presence of AIV antibodies in 2.87% of examined chicken sera samples. These serological findings show lower prevalence compared to other studies from countries with previously recorded AI outbrakes. Nevertheless, AI remains a significant risk for public health and poultry industry output in the Republic of Srpska. This is first serological survey of AI in domestic birds from Bosnia and Herzegovina.

Key words: avian infuenza, Bosnia and Herzegovina chickens, Republic of Srpska

Avian influenza (AI) is highly contagious disease of domestic and wild avian species with high zoonotic potential. Causative pathogen is a virus that belongs to the family Orthomyxoviridae, genus *Influenzavirus*, type A. Avian influenza virus (AIV) can be divided in two groups: highly pathogenic avian influenza virus (HPAI) with 100% mortality rate and low pathogenic avian influenza virus (LPAI) that is often detected in wild birds (Rebel *et al*., 2011).

According to the literature data, domestic bird species that are predisposed to the AIV infection are chickens, turkeys and guinea fowls, while many species of wild birds are carriers of virus, especialy waterfowls and wild ducks (Rebel *et al*., 2011). Epidemics of AV were reported in Asia (China), North America (Mexico), Middle-Eastern countries, and recently in Europe – Netherlands, Belgium and Italy (De Jong *et al*., 2005; Perdue & Swayne, 2005; Bonfanti *et al*., 2014). Netherlands, Belgium and Italy are known for modern

industrial poultry breeding, organiszed veterinary service and strict biosafety levels. In particular, these epidemics in European countries were good examples which show the extent of unpredictability and various difficulties with controlling the spread of AI.

Molecular detection of highly pathogenic avian influenza virus subtype H5N1 in mute swans (*Cygnus olor*) from central Bosnia and Herzegovina was reported with prevalence of 0.51% (Goletic *et al*., 2004). Following this record in Bosnia, Sekler *et al*. (2009) observed high seroprevalence (3.47%) of avian influenza virus infection among wild bird species in Serbia. Furthermore, spreading of avian influenza in the population of wild birds from neighbouring Croatia with seroprevalence (ELISA) of 28.2% has been reported (Jurinovic *et al*. ,2014).

However, a field study to determine seroprevalence of avian influenza virus infection in poultry from Bosnia and Herzegovina has not been done, although such a survey would have its importance due to many observable reasons. First of all, there are no similar investigations in Republic of Srpska and Bosnia and Herzegovina, which means that risk factors for disease transmission in domestic birds are also unknown. However, possible epidemics even in these conditions would create catastrophic losses in poultry farming and would also increase the risks for public health.

Earlier mentioned reports from Bosnia and Herzegovina, Serbia and Croatia confirm dynamic virus circulation in wild bird species in the Balkans. Poultry production in Republic of Srpska is the most developed branch of animal husbandry which creates the need for epidemiological surveys.

The aim of this study, first of its kind, is to examine seroprevalence of avian influenza in backyard chickens from several localities in Republic of Srpska and Bosnia and Herzegovina.

A total of 1500 poultry sera from municipalities of Srbac, Gradiska, Derventa, Prnjavor and Donji Zabar (Fig. 1) were tested for the presence of antibodies against avian influenza virus. This region was chosen because these locations were known for intensive poultry farming in this part of Republic of Srpska, but in this study blood was randomly collected only from backyard chickens. It was hypothesized that backyard chicken can be possible AI virus infection source and risk factor for virus transmission to farm poultry in this region. Chicken blood was taken from the wing vein and transported to Laboratory for Immuno-serological investigation of the Public Veterinary Institute of the Republic of Srpska "Dr. Vaso Butozan" in Banja Luka within 12–24 hours. For the examination, standard and commercially available indirect enzyme-linked immunosorbent assay (ELISA) was used (IDEXX, Flockcheck ELISA test kit, Westbrook, Maine, USA). This ELISA test is designed to detect influenza A group specific virus nucleoprotein (NP) antibodies in chicken sera.

After examining obtained poultry blood serum samples for the presence of antibodies against AIV by the ELISA test, 43 of them (2.87%) turned positive (Table 1). Specific antibodies in chicken sera from households in Srbac, Gradiska, Derventa and Prnjavor municipalities were detected. Only in Donji Zabar antibodies in chicken sera to AIV were not observed. Predominantly, the antibodies were found mainly in samples that originate from backyard chickens in Srbac, Gradiska and Derventa.

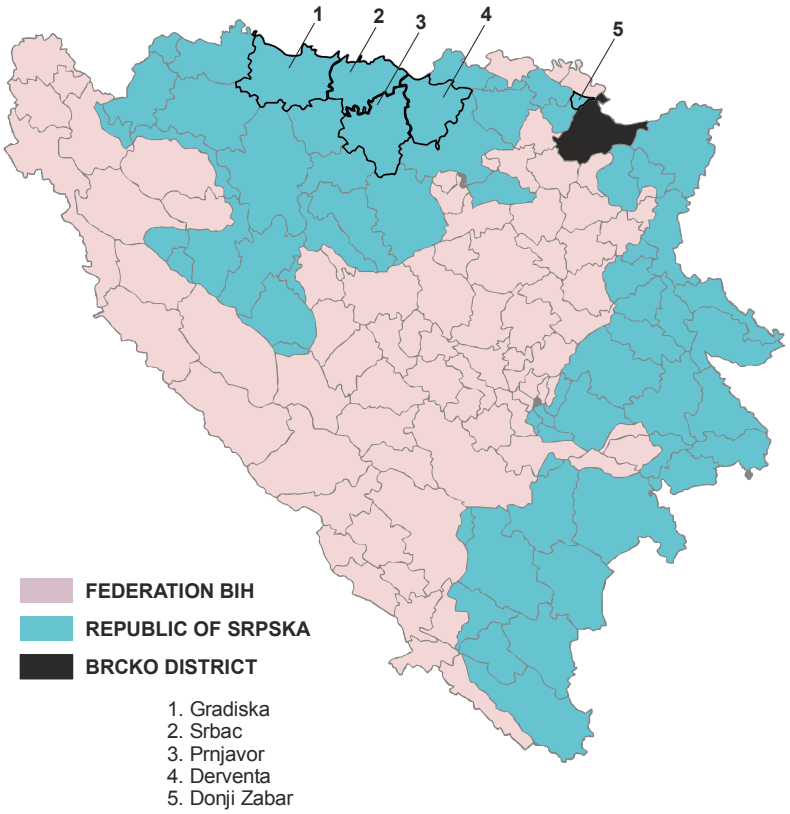

Fig. 1. Geographical map of Bosnia and Herzegovina with examined municipalities in the Republic of Srpska.

In our study, lower AI sero-prevalence was noted, which means lower risk for possible outbreak of AI in Republic Srpska and Bosnia and Herzegovina. From the scenarios of EU countries, we can learn that a single AI outbreak is always possible and in most of the cases unpredictable. Investigated poultry flocks in this study were kept in free range rearing conditions and backyards, so it was possible a direct contact with other bird species (ducks, turkeys) can be an initial source for virus infection. All investigated households are located in region of river Sava and some ponds and lakes throughout the region are inhabited by many species of water wild birds. Additionally, the region is on a route for seasonal avian migration. In this context it might be of interest to examine some bird sera samples from the special ornithological reserve of Bardaca, located in wider Srbac region, a locality with highest seroprevalence in this survey. It is possible that wild birds in Bardaca can be reservoir of AI virus in this part of Bosnia and Herzegovina.

The first major outbreak of avian influenza in poultry occurred in 1997 in Hong Kong, with cases of fatal infection in humans recorded as well. Due to this

outbreak, about 1.5 million of poultry had to be destroyed in order to reduce the risk of further spread of the AIV in the human population of Hong Kong (Sims *et al*., 2003; Alexander, 2007). Also, in 2001 and 2002, new outbreaks happened in Hong Kong, but this time, human cases were not observed. The next major outbreaks of avian influenza has been described in 2003 in South Korea, Vietnam, Japan, Thailand, Laos, Cambodia, Indonesia and Malaysia and thereafter, there was a spread of the disease in Europe, such that the disease was diagnosed in birds in Turkey, Russia, Romania, Italy and the Netherlands (Alexander, 2007).

AIV distribution to Europe from Asia has further increased the risk for public health. This new epidemiologic situation in the world has created the need for active disease monitoring in flocks of domestic poultry and wild birds which were

Table 1. Serological results of AIV antibodies from five municipalities in northern Republic of Srpska, Bosnia and Herzegovina

Municipalities	Location (villages)	Number of households	Examined chicken sera	Number (%) positive
Srbac	Srbac	2	60	16 (5.33%)
	Inadjol	4	10	
	Razboj	3	55	
	Povelic	3	35	
	Brezovljani	1	40	
	Kaoci	3	100	
Gradiska	Kozinci	4	175	10 (3.33%)
	Bok Jankovci	–	65	
	Donja Dolina	1	60	
Derventa	Trstenci	30	150	7 (2.23%)
	Gornji Cerani	1	10	
	Velika Socanica	-	15	
	Polje	1	25	
	Gornji Detlak	1	40	
		1		
	Donji Detlak	1	25	
	Drijen	–	10	
	Miskovci	2	20	
		1		
	Kelenderovci	–	10	
Prnjavor	Gornji Smrtici	25	300	10 (3.33%)
		3		
Donji Zabar	Donji Zabar	5	150	0 (0%)
		5		
	Loncari	1	50	
		4		
		2		
	Covic Polje	1	100	
		4		
		2		
	Total:	111	1500	43 (2.87%)

reservoirs of the virus and potential risk for further disease expansion. So, one of the most common ways of disease monitoring is systematic serological surveys, which were already performed in the early seventies in poultry and wild birds (mostly in the species which are virus reservoirs). Therefore, one of the first seroepidemiological analyses, which were conducted in New Guinea, has confirmed the presence of anti-influenza immunoglobulins in domestic poultry (Van Kammen, 1982). However, more extensive epidemiological studies were performed in Middle-East after above mentioned human epidemics. For example, authors from Pakistan reported that high prevalence in chickens (in some flocks 100%) correlated with high mortality and decreased egg production (Naeem *et al*., 2003).

From the epidemiological data on prevalence of AI in Eastern and Middle-Eastern countries it can be concluded that prevalence of AI in domestic poultry in Bosnia is lower, but sensitivity of tests must be taken in consideration. Main reasons are that Middle-Eastern countries are known for high infection rates and permanent dynamic virus distribution in poultry flocks (Naeem *et al*., 2003). Also, poultry production in these lands is the main branch of animal husbandry – an additional risk factor for easier virus spread. In the case of AI however, risk factors are not always most relevant and best examples can be seen in Europe. Recently, outbreaks in Italy (Bonfanti *et al*., 2014) and the Netherlands (Stegeman *et al*., 2004) confirmed the fact that it is almost impossible to predict emergence of the disease. In Italy, LPAI H5 N2 virus, that later mutated to HPAI, was detected in domestic poultry industry and other cases from Netherlands AI outbreak of HPA subtype H7N7 occurred in commercial poultry farms (Stegeman *et al*., 2004).

Confirmed HPAI virus presence in wild birds in central part of land by Goletic *et al*. (2004) and serological findings in non-commercial backyard chickens in this study may be the important information for AIV epidemiology in the Republic of Srpska and Bosnia and Herzegovina. However, more comprehensive epidemiological, molecular and virological (virus sub-typing) analyses are needed in case of avian influenza and a more detailed surveillance strategy.

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