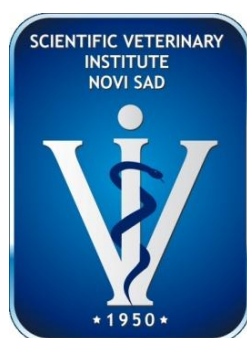


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INSTITUTE OF VETERINARY MEDICINE OF SERBIA

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A SEROLOGICAL SURVEY ON AUJESZKY'S DISEASE IN WILD BOARS IN THE REGION OF VOJVODINA IN SERBIA

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Abstract

The article presents the results on the presence of specific antibodies against Aujeszky's disease (AD) virus in blood samples of wild boars hunted in the territory of the Autonomous Province of Vojvodina in R. Serbia during hunting season 2013-2014. Blood samples were collected during the evisceration of internal organs in hunted wild boars, from the abdominal vein or heart chamber. Examination for the presence of specific antibodies against AD was carried out in two virology laboratories: Scientific Veterinary Institute of Serbia (Belgrade) and Scientific Veterinary Institute "Novi Sad" (Novi Sad), using commercial ELISA kits for the detection of antibodies against AD. The study included 434 blood samples of wild boars, originating from 6 locations, that is, epizootic units in Vojvodina. According to the evaluation of the hunting organizations for the aforementioned hunting season, the total population of wild boars in the 6 epizootic units included 7,065 animals. Thus, this examination encompassed 6.14% of the total number of wild boars in the area of Vojvodina Province.

Antibodies against AD were detected in 179 samples, making 41.24% of the total number of blood samples. The greatest number of seropositive wild boars was established in epizootic unit of Srem, where specific IgG anti-AD antibodies were detected in 68 (51.52%) of 132 tested samples. Also, high percentage of seropositive samples was determined in the epizootic unit of West Bačka. In total, 118 samples were analyzed, and 49.15% thereof reacted positive. The lowest rate of seropositive samples was detected in the unit of Severna Bačka. In this epizootic unit, 24 blood samples were tested, and antibodies against AD were found in 6 samples, which are still considered high seropositivity percentage with 25% of positive animals.

The results of this examination indicated that Aujeszky's disease virus infection is widespread in the population of wild boars in the territory of Vojvodina.

Keywords: Wild boar, Seroprevalence, Aujeszky's disease, ELISA

Introduction

Wild boar (*Sus scrofa*) populations are found in many regions worldwide. The role of wild boar as a reservoir of some viruses, and thus a possible source of infection for domestic swine and other animals is still unclear (Müller et al., 2011; Ruiz-Fons et al., 2008). Aujeszky's disease (AD, pseudorabies) is a notifiable disease caused by Suid herpesvirus 1 (SuHV), syn. Aujeszky's disease virus (ADV), which belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus* (Mettenleiter, 2000). The Aujeszky's disease virus (ADV) is an important pathogen of pigs and infects almost all mammalian species except man (Keros et al., 2014; Pannwitz et al., 2012). However, pigs are the only animal species that can survive the infection with ADV, which accounts for its ability to be subclinically (latently) infected (Martinez-López et al., 2009) while infected individuals of all other animal species succumb to the disease without shedding the virus (Komáromi and Szabó, 2005). Because of the substantial economic losses AD causes to the pig industry, it represents one of the most dangerous diseases in domestic pigs (Pannwitz et al., 2012).

In Europe, ADV has been eliminated from domestic pig populations in many countries (Müller et al., 2011). However, despite the tremendous progress made to control and eliminate the disease in domestic pigs, ADV infections appear to be widespread in populations of non-domestic swine, including feral pigs, wild boar and hybrids, across the world (Pannwitz et al., 2012). First evidence for the occurrence of ADV in wild boars was reported from the USA, Italy, and the Netherlands in the mid-1980s (Müller et al., 2000). In recent years, ADV infections in wild boar populations have also been reported from many European countries, including the Czech Republic, France, Slovenia, Croatia, Poland, Russia, Switzerland and Spain (Albina et al., 2000; Sedlak et al., 2008; Župancic et al., 2002; Vengust et al., 2006). Direct impact of AD in wild boar population dynamics is considered to be low, but AD outbreaks with associated wild boar mortality have been reported and restrictions to wild boar movements may also have an impact on wild boar production for hunting (Ruiz-Fons et al., 2008).

There are no recently published data concerning detection the ADV in wild boars population in Vojvodina province. However, Müller et al. (2011) have reported an ADV infection in border regions between Hungary and Serbia, and Keros et al. (2014) suggested the possibility of virus spread, especially among wild boars, between the borders of Croatia and Serbia. It should be stressed that domestic pig population in Vojvodina is enzootically infected with ADV (Pušić et al., 2011). One of the characteristics of outdoor swine production in some regions in Vojvodina Province is raising free-roaming domestic pigs, where they share forest habitat with wild boars (extensive grazing) (Prodanov-Radulović et al., 2011). In such conditions, the contacts between wild boars and domestic pigs kept in woods (free range) may occur occasionally (Albina et al., 2000). Since wild boars and domestic pigs have the same susceptibility to various infections, including Morbus Aujeszky virus, there is a major concern to monitor the epidemiological situation of wild boars especially when control measures in domestic pigs are implemented. The aim of the current study was to investigate the presence of ADV antibodies in hunted wild boars of Vojvodina region.

Material and methods

Blood samples and sampling procedure

Blood sampling was performed within the framework of monitoring of classical swine fever in wild boar population pursuant to the “Instruction for monitoring of classical swine fever in wild boars in 2013 and 2014” (Veterinary directorate, No. 323-02-8407/2013-05, dated 28/10/2013). During evisceration of internal organs of hunted wild boars, determination of animal’s age and blood sampling was performed. The age was determined according to the number of molar teeth in the lower jaw. All hunted animals were distributed into 4 age categories: 0-6 months, 6-18 months, 1.5-2.5 years and older than 2.5 years. Blood sampling was performed by puncture of abdominal vein and/or heart chamber using disposable sterile plastic syringes and injection needles. The samples were transferred into sterile 5 mL tubes and transported to the laboratories. Each sample was accompanied by a Form containing relevant data on the hunting ground, shooting date, identification number of hunted boar, material sample and animal’s age. Separation of blood serum was performed by the method of spontaneous coagulation and/or centrifugation. Separated blood serum was poured into the 2mL „*eppendorf*“ cuvettes and frozen at -20°C until testing. In this manner, 434 wild boar blood serum samples were collected for the purpose of this research.

Localities for blood sampling

The territory of Vojvodina is divided into 6 epizootiological units that correspond with the administrative regions, except for Banat epizootiological unit composed of two administrative regions (North Banat and Middle Banat). The number of collected samples according to

epizootiological units was as following: Srem -132; West Bačka – 118; South Bačka – 117; North Bačka – 24; Banat - 22 and South Banat – 21.

Blood sample examination

Detection of antibodies against Aujeszky's disease virus was performed in the laboratories of the Institute of Veterinary Medicine of Serbia, Belgrade and Scientific Veterinary Institute "Novi Sad" in Novi Sad using commercial ELISA kits Ingezim ADV TOTAL and IDEXX PRV/ADV gB Ab test.

Results

The obtained results were displayed in Tables. Data on the presence of ADV-specific antibodies in the examined samples in relation to the number of examine samples and number of wild boars in epizootiological units in Vojvodina, are presented in Table 1.

Table 1. Number of wild boars, number of examined blood sera and findings according to epizootiological units in Vojvodina

No.	Epizootiological unit	No. of wild boars	No. of examined blood sera	% of examined samples compared with the total number of wild boars	Finding: Number (%)	
					Positive	Negative
1	Srem	2,080	132	6.35	68 (51.52)	64 (48.48)
2	West Bačka	1,445	118	8.17	58 (49.15)	60 (50.85)
3	South Bačka	1,670	117	7.00	34 (29.06)	83 (70.94)
4	North Bačka	130	24	18.46	6 (25.00)	18 (75.00)
5	Banat	540	22	4.07	6 (27.27)	16 (72.73)
6	South Banat	1,200	21	1.75	7 (33.33)	14 (66.66)
Total		7,065	434	6.14%	179(41.24)	288(58.76)

According to the data in Table 1, the greatest number of examined wild boar blood samples originated from the epizootiological unit of Srem, which is characterized by the largest wild boar population in the territory of Vojvodina. The number of samples examined in this epizootiological unit was 132, and ADV-specific antibodies were detected in 68 samples indicating a seroprevalence rate of 51.52%. High ADV seroprevalence of 49.15% was established in wild boars from West Bačka epizootiological unit. However, in other epizootiological units, the ADV seroprevalence ranged between 25% and 33%, indicating that more than a quarter of wild boar population in Vojvodina is infected with Aujeszky's disease.

The finding of antibodies against Aujeszky's disease was presented according to the age of examined wild boars. The data on the total number of examined blood samples and antibody finding according to epizootiological units and animals' age are displayed in Table 2.

Table 2: The finding of antibodies against Aujeszky's disease according to the age of examined wild boars from epizootiological units of Vojvodina

Epizootiological unit	0-6 months			6-18 months			1.5-2.5 years			> 2.5 years		
	Exam	Pos. (%)	Neg.	Exam.	Pos.(%)	Neg.	Exam	Pos.(%)	Neg.	Exam	Pos.(%)	Neg.
Srem	14	6 (42.9)	8	40	16 (40)	24	32	17 (53.1)	15	46	29 (63)	17
West Bačka	4	0	4	40	20 (50)	20	26	10 (38.5)	16	48	28(58.3)	20
South Bačka	8	2 (25)	6	53	11(20.8)	42	22	8 (36.4)	14	34	13(38.2)	21
North Bačka	4	0	4	14	2 (14.3)	12	1	1	0	5	3 (60)	2
Banat	3	1 (33)	2	9	1 (11)	8	3	0	3	7	4 (57)	3
South Banat	0	-	-	10	2 (20)	8	7	3 (42.6)	4	4	2 (50)	2
Total	33	9 (27.3)	24	166	52(31.3)	114	91	39(42.9)	52	144	79(54.9)	65

As obvious from Table 2, antibodies against Aujeszky's disease were detected in all age categories of wild boars in the epizootiological units of Srem and South Bačka. In Srem epizootiological unit, the highest percentage of seropositive wild boars, 63%, was recorded in the age category above 2.5 years. However, the percentage of seropositive animals of all age categories was quite high in this region, ranging from 40% to 53%. In South Bačka, the highest prevalence of seropositive animals was established in the age category > 2.5 years, being 38.2%, whereas seropositivity rate among other age categories ranged between 20% and 36%. In the unit of West Bačka, high percentage of seropositive animals was detected in age categories > 2.5 years and 6-18 months, being 58.3% and 50%, respectively. As regards the age category, the majority of examined wild boars were from the age category 6-18 months (166) and > 2.5 years (144), whereas only 33 examined animals were piglets from age category below 6 months. The percentage of seropositive wild boars gradually increases with increase of animals' age. The lowest and highest seroprevalence rates were established in piglets below 6 months and boars > 2.5 years old being 27.3% and 54.9%, respectively.

Discussion

The presented results indicate the presence of ADV infection in the population of wild boars in the territory of Vojvodina, Republic of Serbia. The occurrence of the disease in wild boars was confirmed and reported by several authors from neighbouring countries such as Croatia and Hungary (Keros et al. 2014; Župancic et al., 2002; Komáromi et al., 2005) as well as from numerous countries in Europe and America (Müller et al., 2011). The infection has commonly been confirmed by serological examination; however, virus identification in hunted wild boars using molecular methods has been increasingly reported during the past few years. The seroprevalence of Aujeszky's disease in hunted wild boars in several countries and regions in Europe is variable, yet generally high. Thus, the following seroprevalence rates were reported in some countries: 51% in central Italy (Lari et al., 2006); 54.54% in Maslovačka Gora in Croatia (Župančić et al., 2002); 26% in Slovenia (Vengust et al., 2006), and 30% in Slovakia (Sedlak et al., 2008). The analysis of the results on seroprevalence of Aujeszky's disease in Germany, which was monitored throughout 24 years in 66 regions, ranges between 0.4 and 15.9%. However, in some regions, the seroprevalence rate reached even more than 30% during 2006 and 2007 (Pannwitz et al., 2012). The results on seroprevalence of Aujeszky's disease among the wild boar population in Vojvodina region, Republic of Serbia, presented in this article are similar to the results reported in the majority of European countries.

Analysis of presented data on seroprevalence of Aujeszky's disease according to age category of examined wild boars indicates close correlation of our results with those reported in the literature. The results of several authors suggested the highest seroprevalence rates in oldest age categories of wild boars (Müller et al., 2012; Lari et al., 2006; Vengust et al., 2006). According to our results, the seroprevalence of Aujeszky's disease in hunted wild boars older than 2.5 was 54.9%, whereas lowest rates were recorded in piglets below 6 months, being 27.3%

Further research of Aujeszky's disease in wild boars is necessary, especially in the aspect of the spread and persistence of the virus among the wild boar population. Wild boars are potential infection reservoir for domestic pigs but also for other animals such as hunt dogs, wild carnivores, etc. Molecular characterization of isolates of ADV in wild and domestic pigs as well as of virus isolates in other animal species could contribute to better understanding and elucidation of numerous epidemiological aspects of persistence and spread of Aujeszky's disease in the environment.

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