

The potential of environmental and demographic factors in prediction of the presence of Aujeszky disease in wild boars (*Sus scrofa* L.)

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ABSTRACT

Aujeszky disease is a viral disease, primarily of suids, caused by Suid-alpha herpesvirus 1, also known as the Aujeszky disease virus. The potential transmission of virus from wild boars to domestic pigs underlines the necessity of studying the epidemiology of Aujeszky disease in wild boars. The aim of this study was to evaluate the potential of demographic, environmental and population management predictors in predicting that ELISA tested animals will be seropositive. We used 222 wild boar blood samples, collected from 10 different hunting grounds and Medvednica Nature Park. In total, 12 predictors were used in this study, three of which were demographic (age category, sex, population density), two represented population management (hunting method, relative hunting bag) and seven environmental characteristics (altitude, watercourses, meadows, scrublands, forests, agricultural area and infrastructure area). The model selection was based on the Akaike Information Criterion, corrected AIC and Akaike weight. The model with the lowest value ($\Delta AIC < 2$ units) was selected as representing the better model fit. Predictors were selected using logistic forward stepwise regression. The age of the wild boars proved to be positively correlated with prediction of seropositive animals, while altitude and population density proved to be negatively correlated. The selected models were shown to be able to predict ADV seropositive animals with an average accuracy between 77.12 and 78.67%, and seronegative animals between 82.46 % and 85.09. The odds ratio values ranged between 12.53 and 14.52. Our results are in accordance with previous studies, and the impact of population density in the case of Aujeszky disease in wild boars remains unclear.

Key words: wild boar; Aujeszky disease; epidemiology; modelling; prediction

Introduction

Aujeszky disease is a viral disease of wild and domestic animals caused by Suid alpha herpesvirus 1, also known as the Aujeszky disease virus (ADV). The synonym "pseudorabies" originates from the fact that the symptoms in infected

carnivores closely resemble those caused by rabies, and therefore the virus is also known as a pseudorabies virus. In its natural hosts (suids), the clinical manifestation of AD largely depends on the age of the infected animal, but also on the virulence

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of the ADV strain (CARD and ENQUIST, 1995; GORTÁZAR et al., 2002; METTENLEITER et al., 2012; FREULING et al., 2017). Depending on these factors, the disease can have a mild or severe form. In younger animals, severe symptoms related to the digestive and neural systems may be present, while in adults they are usually milder, and the disease manifests itself through retarded growth and reproductive disorders. In wild boars the disease is usually asymptomatic, even though clinical signs in the nervous system and weight loss have been observed in younger animals (GORTÁZAR et al., 2002; SCHULZE et al., 2010). However, in the case of free-ranging wild boar it is very difficult to estimate true mortality among piglets, since it is extremely difficult to find carcasses due to the dense vegetation, the large area involved, rapid carcass degradation during warmer periods, and the presence of predators and scavengers. Also, according to other studies, diseased wild boars tend to seek cover in humid and cool habitats, areas where is usually difficult to find them (MORELLE et al., 2019).

So far, several risk factors essential for the maintenance and spread of AD in domestic pigs have been addressed, including the distance between farms, the density of pigs on the farm, the number of farms in a certain area, the management system and topographical features (MARSH et al., 1991; AUSTIN and WEIGEL, 1992; TAMBA et al., 2002). The epidemiology of AD in wild boar has also been studied by several authors (LUTZ et al., 2003; RUIZ-FONS et al., 2007, 2008; FERRARA et al., 2021). In the majority of these studies, the presence of wild boar in the vicinity of domestic pig farms is characterized as a risk factor for ADV transmission to domestic pigs. On the other hand, potential transmission of ADV from domestic pigs to wild boars has been studied less frequently. In any case, this relationship between ADV infection in wild boars and domestic pigs is still unclear, since RUIZ-FONS et al. (2008) concluded that there is no evidence of a statistical association between ADV seropositive pigs and wild boars in the same region.

In this study, we developed 21 models to predict ADV infection in wild boars based on

12 environmental, population management and demographic predictors.

Material and methods

Animals and habitat characteristics. The seroprevalence of wild boars against ADV was obtained from SUČEC (2021). In total, blood samples from 222 wild boars were analysed using a commercial ELISA test (IDEXX ADV/ADV gI AB test) in accordance with the manufacturer's instructions (IDEXX B.V., Hoofddorp, The Netherlands). Blood samples were collected (from the heart or large blood vessels) during the execution of regular game management operations immediately after shooting, and analysed within 24 hours. The animals were divided into age categories as piglets (<1 yr), yearlings (1-2 yrs) and adults (>2 yrs). Age was evaluated using the criteria provided by BRIEDERMANN (2009) and MERTA et al. (2015). For this study we used the individual serostatus of animals in each hunting ground, marked as 1 (seropositive) or 0 (seronegative). Sampling was performed in 10 different hunting grounds and Medvednica Nature Park (Table 1). In accordance with the regulations (ANONYMOUS, 2006), localities were divided as follows: lowland habitats – more than 75 % of the area is below 200 meters above sea level (m.a.s.l.) (7 localities), lowland-hilly habitats that include both areas below 200 m.a.s.l., and between 200 and 800 m.a.s.l. (3), and hilly habitats where more than 75 % of the area is between 200 to 800 m.a.s.l. (1). Classification of areas according to altitude was performed using a digital relief model derived from digitalized layers of a 1:25000 topographic map. Data about land utilization were obtained from the available maps (<http://www.bioportal.hr/gis/>). The game management area was calculated on the basis of the difference between the total hunting ground area and the area under infrastructure or watercourses. Data about the average number of wild boars in spring (spring stock) and hunting bag were obtained for each analysed year from the Central Hunting Record (<http://lovistarh.mrrsvg.hr/sle>), or research data in the case of Medvednica Nature Park. To avoid potential bias caused by errors in estimation of the wild boar spring stock, we also

used the relative hunting bag (RHB, number of shot animals per 100 ha). The study was approved by the Committee for Ethics in Veterinary Medicine,

Veterinary Faculty, University of Zagreb (Class: 640-01/20-17/02; No.: 251-61-44-20-11).

Table 1. Hunting grounds used in this study and their characteristics.

Hunting ground	Total area (ha)	Hunting area (ha)	water	meadows	scrublands	forests	Agricultural land	Infrastructure area	Population density	Relative hunting bag	Type of the hunting ground
I/2 ČESMA-BOLČANSKI LUG	5 266	5063	1.9	6.7	3.1	55.1	31.2	1.9	1.60	3.08	lowland
I/3 ČRNOVŠČAK	2 156	2094	1.1	2.1	8.0	41.9	45.1	1.8	1.58	2.48; 2.20	lowland
III/124 DIVUŠA	8 894	8255	3.9	19.9	17.9	34.9	20.1	3.3	0.48	1.70	lowland-hilly
III/134 BUČICA	6 104	5895	2.0	13.5	18.1	54.9	10.0	1.4	0.68	1.27	lowland
III/28 POSAVSKE ŠUME	11 700	11260	3.1	5.0	12.6	67.8	10.9	0.7	1.24	1.36	lowland
III/39 OPEKE II	8 341	8158	1.4	3.3	6.3	66.3	21.9	0.8	2.45	2.21; 2.81	lowland
IV/11 VELIKA KAPELA	5 388	5310	0.5	5.8	0.3	92.3	0.1	1.0	0.38	0.08	hilly
V/3 KALNIK	9 469	9337	0.0	0.2	0.2	91.3	6.9	1.4	1.65	2.53	Lowland-hilly
XIV/1 BREZNICA	7 919	7763	0.0	10.3	0.0	66.4	21.3	2.0	2.45	5.40	lowland
XIV/9 PODUNAVLJE-PODRAVLJE	28 386	23030	18.2	36.8	1.8	33.8	8.8	0.7	1.93	2.64	lowland
NP Medvednica	8 440	8071	0.5	2.9	8.1	83.8	0.8	3.8	4.09	1.30	hilly

RHB – two variables in one cell are in the case of different values in different hunting season

Model selection. In total, 12 predictors were used in this study, three of which were demographic (age category, sex, population density), two represented population management (hunting method, RHB), and 7 environmental characteristics (altitude, watercourses, meadows, scrublands, forests, agricultural area and infrastructure area). The model selection was based on Akaike Information Criterion (AIC) and corrected AIC (AIC_c), using the following formula: $AIC_c = AIC + 2(k+2)(k+3)/T-k-3$, in which T is the number of observations used for estimation, and k is the number of predictors

in the model (BURNHAM and ANDERSON, 2002). Following the calculation, the model with the lowest AIC ($\Delta AIC < 2$ units) was selected as the one representing the better model fit. Additionally, Akaike weight (w_i), was calculated to help assess the most appropriate model.

To select predictors of ADV infection in wild boar, logistic forward stepwise regression was used (HOSMER and LEMESHOF, 2000). Forward stepwise regression was used instead of backward due to the large number of potential predictors. The significance of each model was determined by

the log-likelihood ratio test, while the significance of the coefficients of the dependent variables was based on χ^2 Wald statistics. The relative importance of the independent variables within each model was evaluated by multiplying the coefficients of logistic regression (β) by the standard deviation of each variable. Positive coefficients, larger than 0, indicate a positive relationship with the dependent variable. Contrary to this, negative coefficients indicate a negative relationship, or in other words, as the independent variable increases, the dependent variable tends to decrease. The impact of coefficients is made visible through the formula: $p=1/(1+e^{-(\beta_0+\beta_1 x_1+\beta_2 x_2+\dots)})$ where p is the dependent variable and $e^{-(\beta_0+\beta_1 x_1+\beta_2 x_2+\dots)}$ the logarithm value. The odds ratio was calculated to estimate the impact of independent variables.

To evaluate the amount of variation in the dependent variables explained by the model we used Nagelkerke R^2 .

Results

The ELISA test revealed an average seroprevalence of 33.78%. According to location, the seroprevalence ranged from 87.50% (Posavske

šume), to 83.33% (Bučica), 44.82% (Opeke), 39.47% (Črnovščak), 35% (Česma-Bolčanski lug), 33.33% (Divuša), 30.30% (Podunavlje-Podravlje), 26.66% (Medvednica NP), 25.80% (Breznica), 20% (Kalnik) and 4.76% (Velika Kapela).

In accordance with the $\Delta AIC < 2$ criterion, a total of 21 models were developed to predict ADV seropositive/seronegative animals (Table 2). The number of predictors ranged from 4 (model 12) to 7 (models 4, 7, 9, 10, 11, 13, 15, 17, 18, 19, 20 and 21). Model 16 had 5 predictors, while models 1, 2, 3, 5, 6, 8 and 14 had 6 predictors each (Table 3). The most frequent predictors were age category and hunting method, which were included in all models. Population density was another frequently used predictor, which appeared in 20 models. Other predictors were rarely included. Sex was included in 5 models (4, 7, 9, 10 and 15), while RHB was included in only two models (14 and 21). The positive coefficient was the wild boar's age, while negative ones were altitude and population density. The value of the estimated variance (Nagelkerke R^2) ranged between 42.7% (model 12) to 46.2 % (model 4), but was mainly around 45% in other models.

Table 2. Selection of models to predict AD seropositive/seronegative animals

Model number	K	AIC	ΔAIC	w_i	Nagelkerke R^2
1.	6	178.90	0.00	0.02	0.457
2.	6	179.19	0.29	0.02	0.455
3.	6	179.29	0.39	0.02	0.455
4.	7	179.79	0.89	0.01	0.462
5.	6	179.88	0.98	0.01	0.452
6.	6	180.05	1.15	0.01	0.451
7.	7	180.09	1.19	0.01	0.461
8.	6	180.24	1.34	0.01	0.450
9.	7	180.38	1.48	0.01	0.459
10.	7	180.71	1.81	0.01	0.458
11.	7	180.71	1.81	0.01	0.458

Model number	K	AIC	ΔAIC	w_i	Nagelkerke R^2
12.	4	180.73	1.83	0.01	0.427
13.	7	180.74	1.84	0.01	0.458
14.	6	180.75	1.85	0.01	0.447
15.	7	180.77	1.87	0.01	0.457
16.	5	180.77	1.87	0.01	0.437
17.	7	180.84	1.94	0.01	0.457
18.	7	180.85	1.95	0.01	0.457
19.	7	180.86	1.96	0.01	0.457
20.	7	180.87	1.97	0.01	0.457
21.	7	180.87	1.97	0.01	0.457

Table 3. Predictors and their values.

Model	Intercept	Age category	Sex	Hunting method	Altitude	Water	Meadows	Scrublands	Forests	Agriculture area	Infrastructure area	Population density	RHB
1	4.74	1.80		-5.44	-1.71		0.04		0.07			-2.20	
2	5.93	1.79		-4.44	-1.57				0.04	-0.03		-1.91	
3	5.21	1.78		-5.69	-1.79	0.09			0.07			-2.31	
4	5.17	1.77	-0.42	-5.54	-1.73		0.04		0.07			-2.22	
5	7.08	1.77		-3.16	-1.34		-0.05			-0.06		-1.52	
6	6.16	1.79		-2.98	-1.23	-0.08				-0.06		-1.41	
7	6.34	1.76	-0.42	-4.56	-1.59				0.04	-0.03		-1.93	
8	8.48	1.83		-6.27		-0.37	0.16				-1.48	-1.60	
9	5.55	1.75	-0.38	-5.74	-1.79	0.08			0.07			-2.31	
10	6.65	1.76	-0.46	-3.15	-1.27	-0.08				-0.06		-1.45	
11	9.99	1.81		-6.82	-2.00			-0.07	0.05	-0.03		-2.68	
12	-4.28	1.74		1.75				0.12		-0.03			
13	7.17	1.81		-6.77	-1.95		0.04	-0.04	0.07			-2.63	
14	9.92	1.78		-9.98				0.15			-1.63	-2.39	1.16
15	7.45	1.74	-0.42	-3.31	-1.36		-0.05			-0.06		-1.55	
16	4.13	1.72		-4.09	-1.25				0.04			-1.70	
17	5.27	1.80		-5.69	-1.67		0.04		0.07		-0.10	-2.23	
18	4.89	1.80		-5.56	-1.75	0.02	0.03		0.07			-2.25	
19	4.01	1.81		-5.97	-1.77		0.07		0.09	0.02		-2.36	
20	15.01	1.80		-6.70	-2.06		-0.08	-0.12		-0.08		-2.69	
21	4.52	1.80		-5.22	-1.71		0.04		0.07			-2.14	-0.04
Frequency in models	-	21	5	21	18	6	11	5	13	10	3	20	2

The selected models proved capable of predicting ADV seropositive or seronegative animals with average accuracy between 77.12 and 78.67 % (Table 4). However, there were differences between the models in their potential to predict seropositive or seronegative animals. Prediction of seropositive animals was less accurate and varied

between 70.15 % (model 14) and 74.63 % (models 5, 6 and 8). On the other hand, accurate prediction of ADV seronegative animals ranged between 82.46 % (models 5, 6 and 8) and 85.09 % (models 4, 7, 9 and 15). Odds ratio values ranged between 12.53 and 14.52.

Table 4. Prediction accuracy that wild boar will test ADV seropositive/seronegative.

Model	Accuracy to predict seropositive	Accuracy to predict seronegative	Average	Odds ratio	Log odds ratio	log likelihood (LL)	-2LL
1	73.13433	84.21053	78.67243	14.51852	2.67543	-82.45034	164.90068
2	73.13433	84.21053	78.67243	14.51852	2.67543	-82.59464	165.18927
3	73.13433	83.33333	78.23383	13.61111	2.61089	-82.64543	165.29086
4	71.64179	85.08772	78.36476	14.41486	2.66826	-81.89688	163.79376
5	74.62687	82.45614	78.54150	13.82353	2.62637	-82.93964	165.87927
6	74.62687	82.45614	78.54150	13.82353	2.62637	-83.02690	166.05380
7	71.64179	85.08772	78.36476	14.41486	2.66826	-82.04676	164.09352
8	74.62687	82.45614	78.54150	13.82353	2.62637	-83.11963	166.23926
9	71.64179	85.08772	78.36476	14.41486	2.66826	-82.18930	164.37860
10	73.13433	83.33333	78.23383	13.61111	2.61089	-82.35300	164.70600
11	73.13433	83.33333	78.23383	13.61111	2.61089	-82.35348	164.70696
12	71.64179	83.33333	77.48756	12.63158	2.53620	-85.36542	170.73084
13	73.13433	84.21053	78.67243	14.51852	2.67543	-82.37058	164.74117
14	70.14925	84.21053	77.17989	12.53333	2.52839	-83.37681	166.75361
15	71.64179	85.08772	78.36476	14.41486	2.66826	-82.38363	164.76726
16	70.14925	85.08772	77.61849	13.40882	2.59591	-84.38669	168.77339
17	73.13433	84.21053	78.67243	14.51852	2.67543	-82.41911	164.83821
18	73.13433	84.21053	78.67243	14.51852	2.67543	-82.42586	164.85173
19	73.13433	84.21053	78.67243	14.51852	2.67543	-82.42833	164.85665
20	73.13433	83.33333	78.23383	13.61111	2.61089	-82.43318	164.86636
21	73.13433	84.21053	78.67243	14.51852	2.67543	-82.43537	164.87075
min	70.14925	82.45614	77.17989	12.53333	2.52839	-85.36542	163.79376
max	74.62687	85.08772	78.67243	14.51852	2.67543	-81.89688	170.73084

Discussion

The occurrence, clinical manifestation and outcome of a disease is the result of complex interactions between the hosts, pathogens and their environment, creating the classical epidemiological triangle (ENGERING et al., 2013). Environmental characteristics can influence the population size, distribution and horizontal/vertical migration of animals. These, on the other hand, can constrain or enhance the transmission of pathogens (SMITH

et al., 2002; TRACEY et al., 2014; PODGÓRSKI et al., 2020; WILK-DA-SILVA et al., 2022). From the example of African swine fever (ASF), PODGÓRSKI et al. (2020) concluded that the probability of finding ASF cases in wild boars increases in more forested areas. However, since knowledge of the transmission and epidemiology of wild boar diseases is still rather scarce (KRAMER-SCHADT et al., 2007), we analysed the potential

of various factors that may help in the prediction of ADV seropositive or seronegative animals.

Our results are in accordance with RUIZ-FONS et al. (2008), who found that the age of wild boars was the most important predictor of the probability that an animal would test positive. The same trend was observed in our study, showing that older animals will be more likely to test positive. In our models, the age of the wild boar was always a positive coefficient, with values ranging between 1.72 and 1.83.

Regarding the impact of wild boar density on the occurrence of diseases, it seems that the only reasonable explanation is that an increase in population will lead to the establishment of easier contact between positive (infective) and susceptible animals. Indeed, for the majority of infectious diseases, population density is characterized as one of the most important factors for the maintenance and transmission of the disease. Below the threshold population density, contact between potential hosts and infected animals will be reduced, leading to the cessation of the disease. Accordingly, PODGÓRSKI et al. (2020) reported that the probability of ASF positive animals will increase from 3 to 20% if the population density increases from 0.4 to 2 wild boars/km². In contrast to ASF, in the case of AD the influence of population density is still rather unclear as many studies have not statistically confirmed that a higher abundance of wild boar will result in an increase in seroprevalence (RUIZ-FONS et al., 2008; BOADELLA et al., 2012; CHIARI et al., 2015). In our study, population density had negative coefficient values, ranging from -2.69 to -1.41, meaning that an increase in population density will lead to a lower probability that tested animals will be ADV seropositive. Here, this effect of population density can also be explained by the fact that the data obtained from the Central Hunting Record are probably not the most accurate, and the wild boar population is probably larger than reported. In accordance with this opinion is the fact that the Relative Hunting Bag had a positive value, and the RHB is in direct relationship with population size. In other words, a larger wild boar population will result in a larger RHB. There is also another potential explanation for the unclear

influence of wild boar density reported in other studies. When considering animal density in the case of wild boars, it is necessary to take their social life into consideration. Females and piglets form strongly related groups which do not mix frequently with other groups. Bachelor males will form small groups of 5 to 6 animals, while older males live solitarily (TACK, 2018). Therefore, the number of wild boars in one area cannot be transferred directly to calculate the number of individuals per surface unit. This can diminish the influence of population size. The results obtained also indicate the necessity of using other potential estimates of wild boar population size, such as aggregation index and frequency-based indirect index (ACEVEDO et al., 2007).

Altitude was another negative predictor, indicating that the probability that tested animals will be ADV seropositive decreases with higher altitudes. In a study which included high altitude areas in Switzerland, MEIER et al. (2015) found ADV seroprevalence in wild boar of 0.57 % (95 % CI: 0.32–0.96 %). However, this might also be a consequence of other factors. The negative impact of higher altitudes was found in a study on Tibetan pigs (WU et al., 2018). In this study, seroprevalence decreased from 21.43% at 2800 m.a.s.l. to 11.11% at 3700 m.a.s.l. In our case of wild boars, one potential explanation is the fact that despite being highly opportunistic animals, lowland and lowland-hilly habitats are preferred by wild boars, and normally sustain larger populations. Even if AD in wild boars is less density dependent, intragroup transmission will result in higher seroprevalence if the population size is larger.

Regarding other predictors, their influence is rather marginal, except the negative influence of urbanized areas. This is understandable, since despite increasing reports of wild boars in urban areas, wild boars are still a rare finding in villages. A higher percentage of watercourses and scrublands increases the probability that tested animals will be seropositive, but this value is also marginal. Similarly, RUIZ-FONS et al. (2008) included the type of management (open vs. fenced), the percentage of pastures and agricultural areas, and the percentage of trees and scrublands in

their model to predict ADV seropositive wild boar. All of these predictors proved to have marginal, statistically non-significant effects. Potential use of the presence of carnivores as one of predictors was not analysed since the population of badgers (*Meles meles*) and red foxes (*Vulpes vulpes*) in Croatia is relatively small. In the case of future analysis, the presence of golden jackal (*Canis aureus*) should be used as one of the predictors, as well as the presence of wolves (*Canis lupus*) and lynx (*Lynx lynx*) in mountain habitats.

Models with higher accuracy to predict ADV seropositive animals are less accurate in the prediction of seronegative ones and vice versa. The best models for prediction that animals will test seropositive are models 5, 6 and 8, providing 74.63% accuracy. The odds indicate that there is a 13.82 times higher possibility that an animal will test positive. The best models to predict seronegative animals are 4, 7, 9 and 15, with accuracy of 85.09% and an odds ratio of 14.41. Despite the fact that -2LL values are far from ideal, these models offer relatively accurate predictability of finding ADV seropositive wild boars in the analysed hunting grounds.

Conflict of interest: Authors declare no conflict of interest.

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SAŽETAK

Bolest Aujeszzkoga je virusna bolest prvenstveno divljih i domaćih svinja uzrokovana svinjskim alfaherpesvirusom 1, također zvanim i virusom bolesti Aujeszzkog. Mogućnost prijenosa virusa s divljih na domaće svinje naglašava nužnost provedbe epidemioloških istraživanja ove bolesti u divljih svinja. Cilj ovoga istraživanja bio je procijeniti ulogu demografskih, okolišnih i uzgojnih mjera u predviđanju da će životinja testirana ELISA testom biti seropozitivna. Koristili smo 222 uzorka krvi divljih svinja prikupljenih na području 10 lovišta i Parka prirode Medvednica. Ukupno je korišteno 12 pretkazivača, od čega tri demografska (dob, spol i gustoća populacije), dva uzgojna (metoda lova i relativna odstrjelna kvota) i sedam okolišnih (nadmorska visina, vodotoci, livade, šikare, šume, oranice i izgrađeno zemljište). Izbor modela proveden je na temelju Akaike Informativnog Kriterija, korigiranog kriterija i Akaike težine. Model s najnižim Akaike Informativnim Kriterijem odabran je kao najprikladniji. Pretkazivači su odabrani primjenom logističke stupnjevite regresije s izborom unaprijed. Dob divljih svinja pokazala je pozitivan odnos prema predviđanju seropozitivnih jedinki, dok su nadmorska visina i gustoća populacije pokazali negativan odnos. Odabrani modeli pokazali su mogućnost predviđanja seropozitivne jedinice s prosječnom točnošću između 77,12 i 78,67%, a seronegativne između 82,64 i 85,09%. Omjeri vjerojatnosti varirali su između 12,53 i 14,25. Naši rezultati su podudarni s prijašnjim istraživanjima, pri čemu utjecaj gustoće populacije na bolest Aujeszzkog u divljih svinja ostaje nedostatan poznat.

Ključne riječi: divlja svinja; *Sus scrofa*; bolest Aujeszzkoga; epidemiologija; modeliranje; predviđanje
