

Seroprevalence of Aujeszky's disease in the domestic pig population in the Republic of Croatia from 2013 to 2021



Žaklin Acinger-Rogić*, Marina Pavlak i Lorena Jemeršić

Abstract

Aujeszky's disease is a viral disease of animals, primarily pigs, which are considered to be the natural hosts. It is endemic in many parts of the world, but there are areas and countries that have successfully eradicated this disease. Due to mandatory control measures and trade barriers, Aujeszky's disease causes significant economic losses. Although, it has been eradicated in most European Union Member States, there are still areas and countries where it persists. In the Republic of Croatia, the disease has not yet been eradicated from the domestic pig population, although surveillance and eradication has been

carried out since 2013. This paper presents the results of serological testing of domestic pigs for Aujeszky's disease in the period from 2013 to 2021. The prevalence of Aujeszky's disease at the farm level by year in the study period ranged from 4.66% (CI 95%, 4.39-4.95) to 0.67% (CI 95%, 0.35-1.28). Since 2016, prevalence was at an average level of 1.26% (CI 95%, 0.67-1.95%). The highest prevalence 10.28% (CI 95%, 9.15-11.53) was found in Varaždin County, and the lowest 0.14% (CI 95%, 0.07-0.31) in Krapina-Zagorje County.

Key words: *Aujeszky's disease; seroprevalence; domestic pigs; farms*

Introduction

Aujeszky's disease is a viral disease of animals, primarily pigs as the natural host. It is endemic in many parts of the world, but there are areas and countries that have successfully eradicated this disease from domestic pig population.

Due to mandatory control measures and trade barriers, Aujeszky's disease causes significant economic losses. Although this disease has been eradicated in most of the European Union (EU) Member States, there are still areas and countries

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where it persists. In the Republic of Croatia, the disease has not yet been eradicated from the domestic pig population, although monitoring and eradication has been carried out since 2013. Considering the European legislation and the fact that most EU Member States were certified as free from this disease, there are additional guarantees in relation to Aujeszky's disease, primarily that pigs originate from farms free from Aujeszky's disease (Anonymous, 2008) for the movement of pigs to free Member States.

Aujeszky's disease in Croatia gained more importance in 2013 with the accession of the Republic of Croatia to the EU. In the same year, the Veterinary and Food Safety Directorate General (VFSDG) of the Ministry of Agriculture established the first national Aujeszky's disease control and eradication programme, which enabled sampling of pigs and testing for Aujeszky's disease in accordance with the EU legislation (Maltar, 2014).

Until 2013, Aujeszky's disease was reported only sporadically, mainly due to the appearance of clinical signs of the disease or confirmation in the framework of differential diagnosis of other pig diseases, *i.e.*, classical and African swine fever. The only planned active surveillance of Aujeszky's disease was carried out in 2011 on 9407 pig blood serum samples (Ročić et al., 2013). During the same study, Aujeszky's disease was also confirmed in seropositive pigs, which etiologically proved circulation of the Aujeszky virus in the population of domestic pigs in Croatia. Since 2013, the Aujeszky's disease control and eradication programme has been continuously implemented on a larger or smaller scale, covering the smallest holdings (up to 20 pigs) where breeding pigs are kept. Within the programme, any farm where at least one serologically positive pig was confirmed positive for

Aujeszky's disease was considered positive and disease control measures were mandatory (slaughter of positive pigs, testing of the remaining pigs, biosecurity measures, movement restrictions, etc.).

Parallel to active surveillance in domestic pigs, passive surveillance of Aujeszky's disease in domestic pigs and other animals was also carried out. According to the reported cases, it is clear that Aujeszky's disease virus also circulates in the population of wild pigs (Keros et al., 2014). According to data on the occurrence of animal diseases in the Republic of Croatia (<http://veterinarstvo.hr/default.aspx?id=185>), sporadic positive findings of Aujeszky's disease virus were found in samples of dead hunting dogs that were most likely infected after close contact with wild boars, while Aujeszky's disease has been confirmed in wild boar both by molecular and serological laboratory methods.

In studies conducted in other countries, a high prevalence of Aujeszky's disease was found before and at the very beginning of its eradication. Since the prevalence of Aujeszky's disease depends on the sample size, the purpose of testing and the monitoring design, the results vary considerably. It was thought that the prevalence of Aujeszky's disease at the end of the 20th century was 30 to 40%, despite vaccination (Vannier et al., 1991). The prevalence on small farms in Vojvodina Province, Serbia was estimated at 32.72% (Pušić et al., 2009). Allepuz et al. (2009) reported a prevalence on farms with sows of 58.9% at the beginning of eradication, then after 2–3 years of 23.4%, while the prevalence after four years of the eradication programme was 9.7%. In the population of fattening animals, the prevalence at the beginning of eradication was 17.1%, as opposed to 8.5% at the end of the researched period.

Hu et al. (2016) in their cross-sectional study reported a herd (farm) prevalence of 25% and a higher prevalence on farms of 50–100 sows than on larger farms.

This study analysed the serological testing data on Aujeszky's disease of domestic pigs in the period 2013–2021. The prevalence at the animal and farm level was calculated and presented spatially and temporally.

Sampling was carried out by authorised veterinarians of the authorised veterinary organisations. Data on samples were entered into the VETIs database – (Disease surveillance – Aujeszky's disease) kept by the VFSDG, Ministry of Agriculture.

Every farm where at least one pig tested positive was considered as Aujeszky's disease positive, *i.e.*, an infected farm.

Table 1. Domestic pig census as at 31 December 2021 by farm size

Size	No. of farms	%	No. of pigs	%
< 11	53,275	73.85	211,164	16.79
11-100	18,136	25.14	438,703	34.88
> 100	733	1.02	607,886	48.33
Total	72,144	100	1,257,753	100

Material and methods

Population

The study covered the population of domestic pigs kept on farms throughout Croatia, by county. In total, there were 1,257,753 pigs (Table 1) on 72,144 farms (Table 2) in Croatia on 31 December 2021.

Samples

Blood samples were collected in the period from 2013 to 2021 within the framework of the Aujeszky's disease control and eradication programme in the Republic of Croatia. The number of samples taken at the farm was calculated based on the prevalence table according to the designed prevalence and testing dynamics, *i.e.*, in the case of a one-time test, the number of samples was calculated at a confidence interval 95% and based on 2% designed prevalence, while in double testing (interval of 4 to 12 months between testings) sample size was calculated as 5% of breeding pigs or 10% designed prevalence in fattening pigs.

Table 2. No. of farms by county

County	No. of farms
Bjelovar-Bilogora	5072
Brod-Posavina	4839
Dubrovnik-Neretva	8
City of Zagreb	854
Istria	1447
Karlovac	3644
Koprivnica-Križevci	4617
Krapina-Zagorje	7876
Lika-Senj	577
Medjmurje	2379
Osijek-Baranja	7946
Požega-Slavonija	2544
Primorje-Goranska	80
Sisak-Moslavina	5683
Split-Dalmatia	827
Šibenik-Knin	93
Varaždin	5660
Virovitica-Podravina	3849
Vukovar-Srijem	6496
Zadar	187
Zagreb	7466
Total	72,144

Laboratory testing

The samples were submitted and tested in the official laboratories of the Croatian Veterinary Institute (HVI) in Zagreb and two HVI branches, the Vinkovci Veterinary Institute and the Križevci Veterinary Institute. This paper analysed the results of serological tests for

219,974 pig blood samples taken from 36,255 farms (48,104 farms with repeated testing included) under active surveillance.

Blood samples were taken from healthy pigs under active surveillance and examined by serological examina-

Figure 1. Temporal distribution of animal prevalence

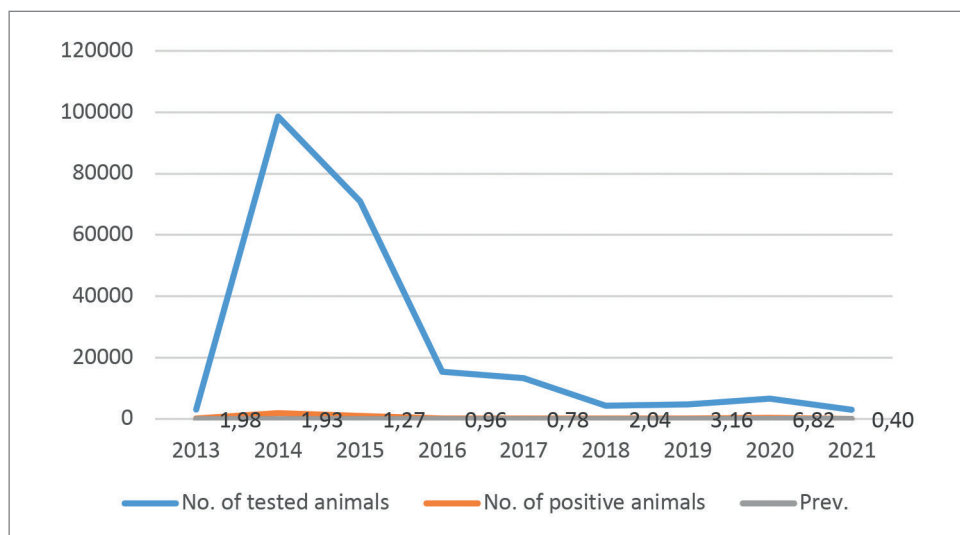


Table 3. Overview of animal testing by years

Year	No. of tested animals	No. of positive animals	Prevalence	CI 95%
2013	3074	61	1.98	1.55-2.54
2014	98,592	1901	1.93	1.84-2.02
2015	71,031	904	1.27	1.19-1.36
2016	15,359	148	0.96	0.82-1.13
2017	13,376	105	0.78	0.65-0.95
2018	4226	86	2.04	1.65-2.51
2019	4746	150	3.16	2.70-3.70
2020	6568	448	6.82	6.24-7.46
2021	3002	12	0.40	0.23-0.70
Total	219,974	3815	1.73	1.68-1.79

tion using an accredited method, *i.e.*, enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies to the gpI antigen of Aujeszky's disease virus in pigs (Pseudorabies Virus gpI Antibody Test Kit, IDEXX PRV/ADV gpI). Most commercially available ELISA's generally have a sensitivity and specific-

ity value between 96% and 99% (EFSA, 2017). In this ELISA test, pigs vaccinated with a vaccine with removed gI (gE) glycoprotein (DIVA) will be negative, while those that have been in contact with field strains of the virus, or were vaccinated with vaccines containing an attenuated or full virus, will be positive.

Figure 2. Farm prevalence by counties

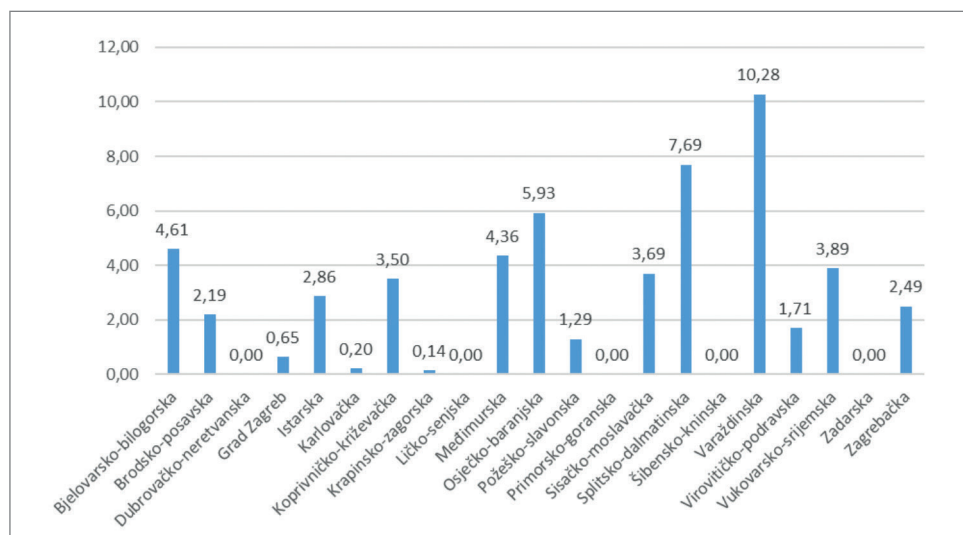


Table 4. Farm prevalence by year

Year	No. of tested farms	No. of positive farms	Prevalence	CI 95%
2013	1034	38	3.68	2.69-5.00
2014	21,915	1021	4.66	4.39-4.95
2015	13,756	428	3.11	2.83-3.41
2016	3642	71	1.95	1.55-2.45
2017	3100	40	1.29	0.95-1.75
2018	1316	18	1.37	0.87-2.15
2019	1405	18	1.28	0.81-2.02
2020	1351	9	0.67	0.35-1.28
2021	585	6	1.03	0.47-2.22
Total	48,104	1649	3.43	3.27-3.59

Data analysis

Testing results were spatially and temporally analysed and presented by county and by year in the period from 2013 to 2021. In order to visualise the spatial distribution of positive farms in Croatia, maps were created for each year of the study period using the ArcGIS system.

Prevalence data were statistically processed in the EpiTools program, and the confidence interval (CI) was calculated according to the Wilson method at CI 95%.

Results

Of the total 219,974 samples analysed, 3,815 samples were positive. An overview of the total analysed samples by testing result by year and county is presented in Table 3.

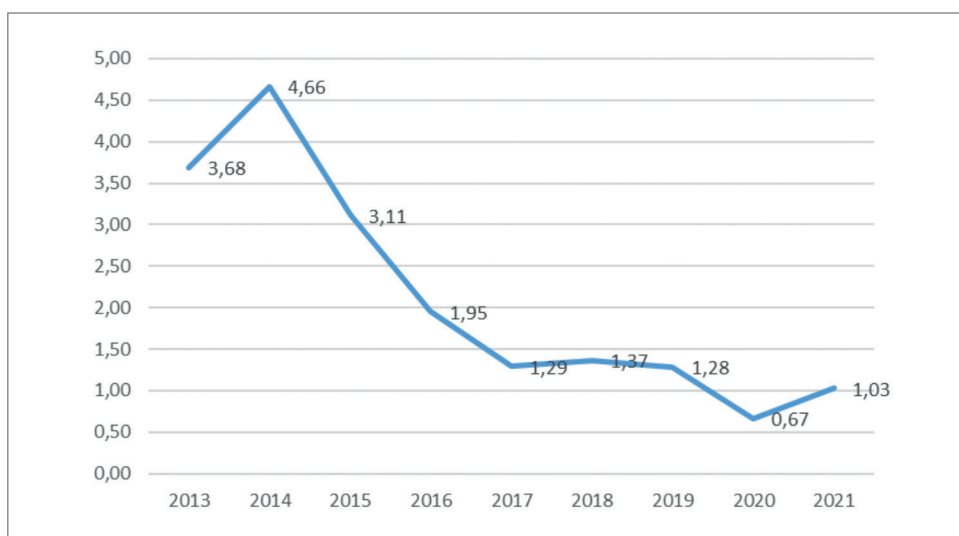
The dynamics of testing results over the years is shown in Figure 1. The largest number of samples was taken in 2014 (98,582) and 2015 (71,031), with animal

prevalence less than 2% (Table 1). The highest prevalence (6.82%) was determined in 2020, though for the interpretation, the total number of positive farms and positive samples (animals) within should be taken into account since out of the 9 positive farms, 421 positive samples come from a single farm in Osijek-Baranja County.

Farm prevalence by county is presented in Figure 2.

A farm prevalence higher than 5% was recorded in Varaždin County (10.28%, CI 95%, 9.15–11.53%), Split-Dalmatia County (7.69%, CI 95%, 1.37–3.33%) and Osijek-Baranja County (5.93%, CI 95%, 5.39–6.52%), while other positive counties showed values less than 5% (Figure 2). It was also evident that the highest number of positive farms was found in 2014, when the first comprehensive surveillance was carried out and covered the highest number of farms (21,915). The farm prevalence for each year is presented in Table 4.

Figure 3. Farm prevalence by year



The temporal distribution of farm prevalence with the decline over time is shown in Figure 3.

The spatial distribution of positive farms by year is presented on Figures 4-12.

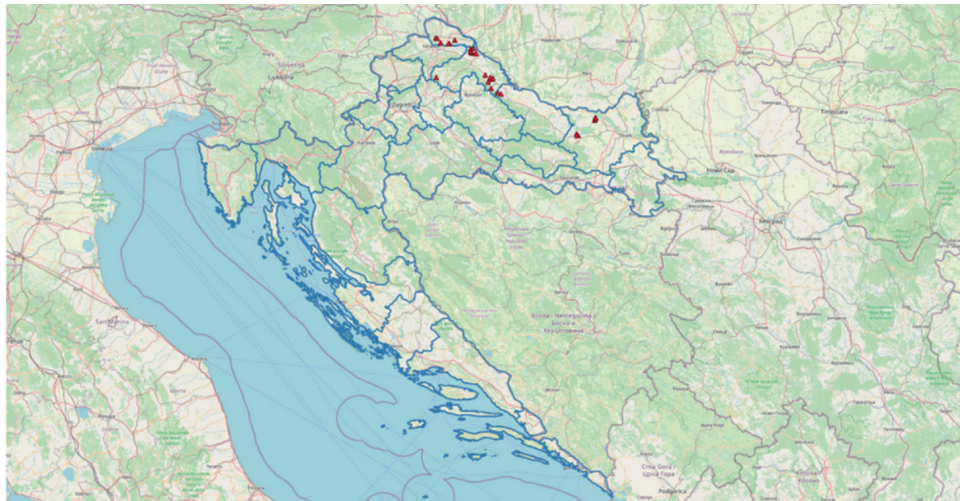


Figure 4. Spatial distribution of positive farms in 2013

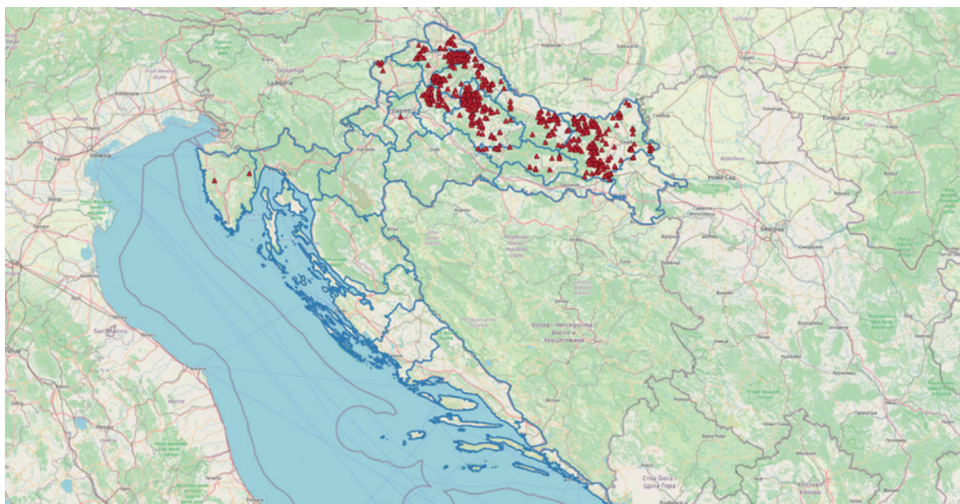


Figure 5. Spatial distribution of positive farms in 2014

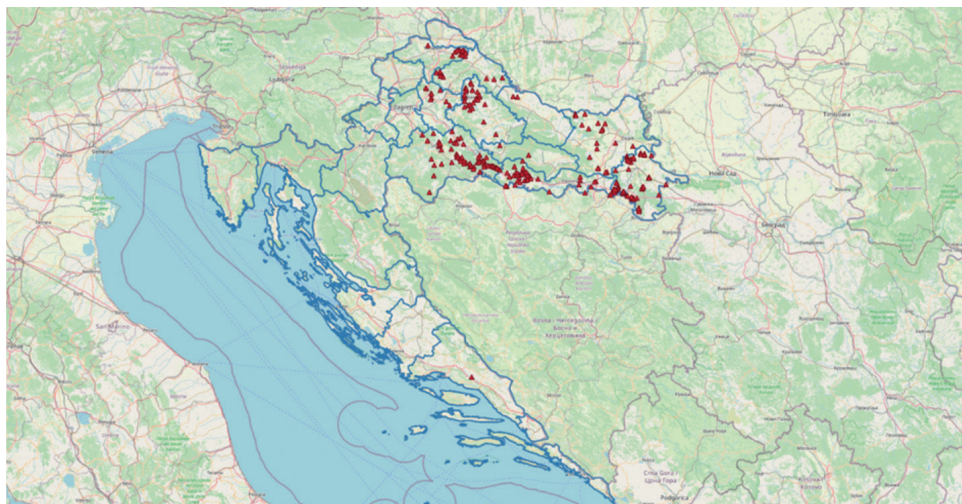


Figure 6. Spatial distribution of positive farms in 2015

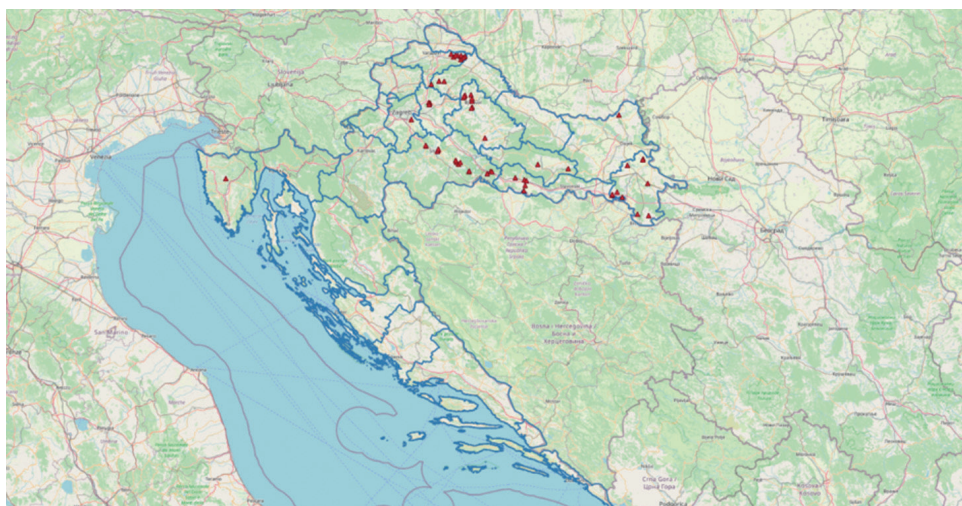


Figure 7. Spatial distribution of positive farms in 2016

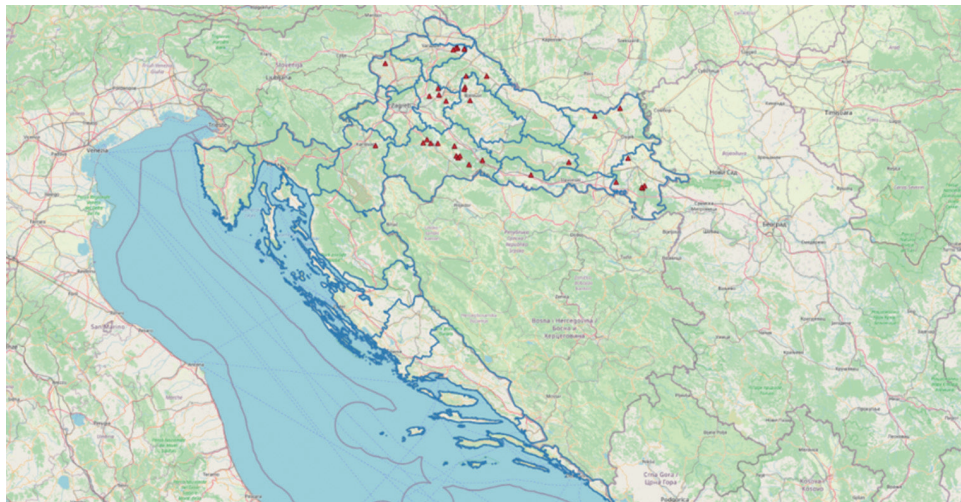


Figure 8. Spatial distribution of positive farms in 2017

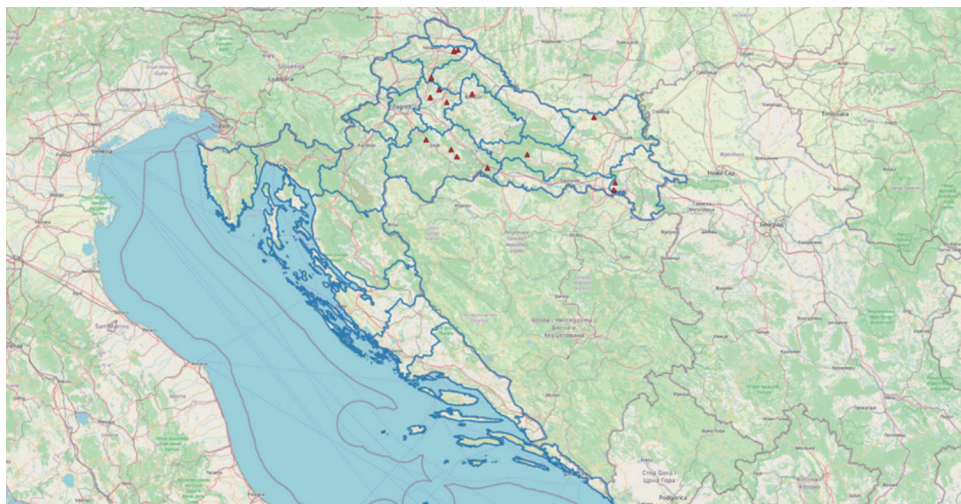


Figure 9. Spatial distribution of positive farms in 2018

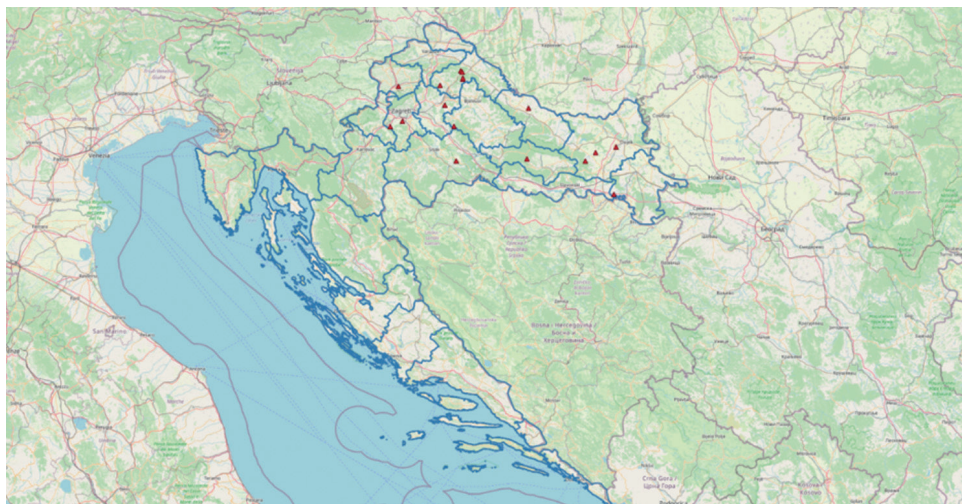


Figure 10. Spatial distribution of positive farms in 2019

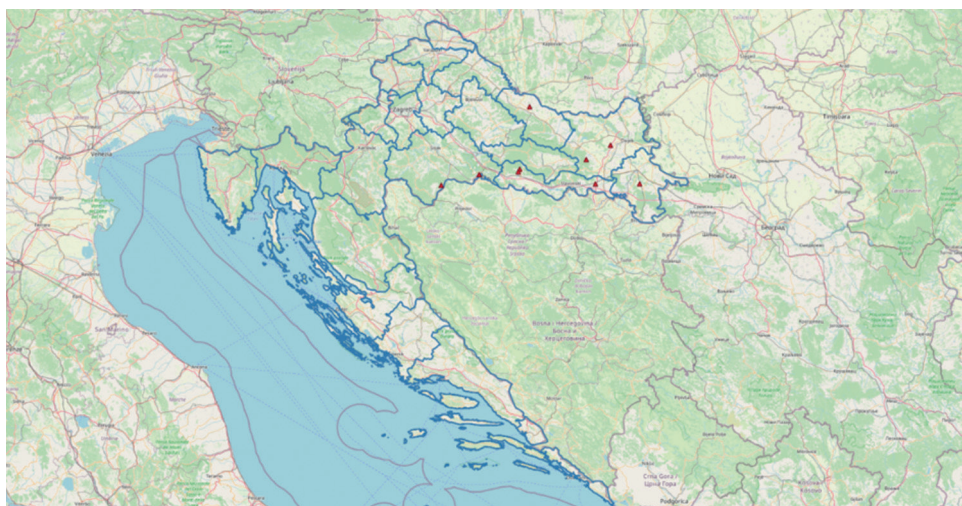


Figure 11. Spatial distribution of positive farms in 2020

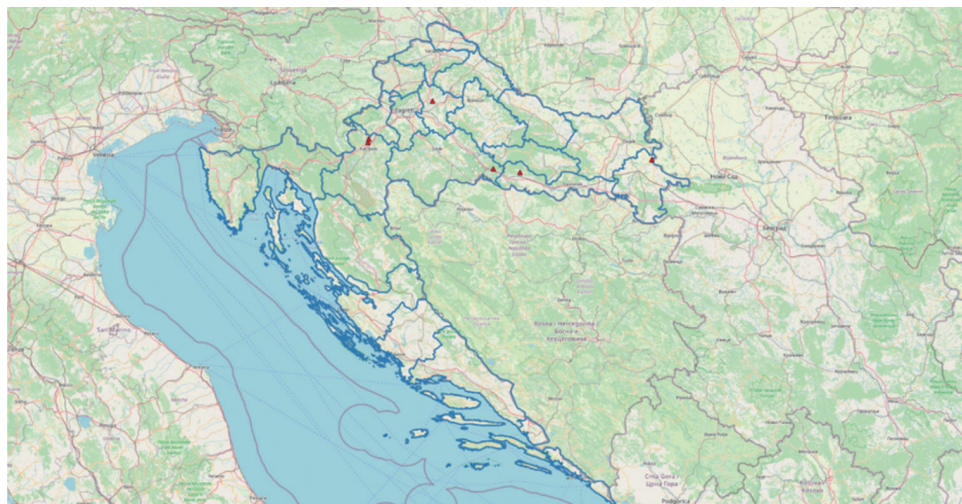


Figure 12. Spatial distribution of positive farms in 2021

Discussion

The Aujeszky's disease surveillance and eradication programme 2013–2021 was designed in such a way to enable sampling on pig farms in order to grant Aujeszky's disease-free status to farms with negative results in accordance with the European legislation, and to conduct eradication on positive farms. All farms where pigs are kept and primarily bred were tested, though the farms for sampling were not chosen randomly. Accordingly, a possible bias should also be taken into account in the assessment of prevalence and interpretation of the results with regard to the scope of the tested farms in a certain area (county), selection of the farms, coverage of certain areas by the veterinary service, and the availability of farms. On the contrary, the possibility of bias influencing the results at the animal level is insignificant knowing that the sample size was determined depending on the breeding or fattening category of pigs and the sampling dynamics in relation to the estimated prevalence of 2%, 5% and 10% at CI 95% (Christensen et al., 2000).

As the programme aimed to identify positive farms with at least one pig positive for Aujeszky's disease immediately at the first ELISA test, the high sensitivity of the diagnostic test (99%) was counted on and no additional confirmatory tests were carried out to increase specificity. There is a possibility that a farm was classified as positive, although it may have been a false positive result, which is usually considered acceptable at the beginning of the eradication implementation, while the possibility that a farm was tested as a false negative is negligible at this stage of eradication. The fact that the entire population of domestic pigs was not tested during the study period, i.e., that surveillance was not always carried out on a randomly selected farms within the representative sample, leaves the possibility of an overestimated or underestimated prevalence of Aujeszky's disease. Despite the fact that the number of tested farms varied throughout the study period, the number of positive farms decreased over the years, and it

can still be considered with high certainty that the prevalence of Aujeszky's disease in the Republic of Croatia is below 4%, and probably between 2% and 1%. However, despite such a relatively low prevalence, especially taking into account the evidence of Aujeszky's disease virus circulation in the wild boar population and the fact that only 27,672 of the total 72,144 farms in Croatia have been granted disease-free status for this disease by 31 December 2021, the question arises of possible factors that can influence the maintenance of infection with Aujeszky's disease virus in the population of domestic pigs. This is particularly the case since the prevalence since 2016 has been at an average level of 1.26%, from highest 1.95% to lowest 0.67%.

Based on the data on testing carried out in the study period, it is concluded that the prevalence of Aujeszky's disease decreased from 4.66% (CI 95%, 4.39–4.95%) in 2014 to 1.03% (CI 95%, 0.47–2.22%) in 2021 following the applying of an eradication test and slaughter strategy. However, it is important to mention that disease-free farms were not included in the regular annual active surveillance of Aujeszky's disease during 2018–2021, which may represent a gap in knowledge about the true status of disease-free farms in relation to possible reinfection with Aujeszky's disease. It is known that, parallel to the decrease in prevalence, the susceptibility of the population to infection with Aujeszky's disease virus increases, and the virus can spread again in free areas (Pensaert and Morrison, 2000). Continued sporadic confirmation of Aujeszky's disease in previously free countries supports this finding (Anonymous, 2020). The discovery of new, potentially more virulent Aujeszky's disease virus strains, such as the virus isolated in Hungary that was 100% similar to the MdBio

wild-type virus isolated in Serbia (Zsolt et al., 2019), is also an additional risk and a new source of infection for the domestic pig population.

Analysing the spatial range of Aujeszky's disease in the Republic of Croatia, with the exception of several positive farms in Istria County and one farm in Split-Dalmatia County in the period from 2014 to 2015, it is evident that Aujeszky's disease is spread primarily in the continental part of the Republic of Croatia (Figures 5 and 6). In the period from 2016 to 2021, the maps (Figures 7 to 12) show a continuous and similar pattern of expansion, although towards the end of the researched period it becomes more sporadic and remains only in certain counties of continental Croatia. This is expected as the majority of all farms with pigs are located in the continental part of Croatia, and Aujeszky's disease virus could have only a limited spread to the coastal and southern parts of the country. At the same time, farms in the coastal and southern part of Croatia traditionally raise pigs for the purpose of slaughter on site, and there are almost no farms that keep breeding pigs. These six counties contain 2.46% of the total number of farms with 1.09% of the total number of sows in the Republic of Croatia. The spatial range of Aujeszky's disease has been analysed by numerous authors, but farm location has mostly not been confirmed as a risk factor. Martini et al. (2003) analysed the density of farms and pigs within a 6 km radius from the nearest farm to determine the risk of spread of Aujeszky's disease, and found that small farms in the lower density area showed a statistically significant correlation for a favourable eradication outcome. At the same time, they found that certain factors work together and that this can lead to a higher prevalence. Allepuz et al. (2009) determined that the

presence of positive fattening pigs or positive sows up to a distance of 1500 m from a farm with sows increases the risk for infection, although this variable has no effect on increasing the risk when the farm is located at a distance of up to 1000 or 2000 m. Furthermore, they found that the spatial spread was very similar to the observed infection in sow farms in all eradication periods, indicating that spatial factors may not be the main factors related to eradication of Aujeszky's disease and that other risk factors may be more strongly associated with the risk of seropositivity in sow farms.

In this paper, the density of farms with pigs as a factor that can influence the higher prevalence of Aujeszky's disease was also considered, but was not found to be significant. Namely, the highest density of farms is in Krapina-Zagorje County (2.19 farms per km²), which also has the lowest prevalence of 0.14% (CI 95%, 0.07–0.31%) while in other high density counties, the prevalence was from 3.89% (CI 95%, 3.38–4.48%) to 5.93% (CI 95%, 5.39–6.52%), and in Međimurje County was 4.36% (CI 95%, 2.99–6.30%) though the farm density was 10-fold lower (0.47 farms per km²). Nevertheless, prevalence differed significantly between counties, from the highest in Varaždin County 10.28% (CI 95%, 9.15–11.53%) to the lowest in Krapina-Zagorje County 0.14% (CI 95%, 0.07–0.31%).

Conclusions

The highest prevalence of 4.66% (CI 95%, 4.39–4.95%) was determined in 2014 when the highest number of farms was tested. Bearing in mind that disease control measures were implemented on positive farms where all seropositive pigs were slaughtered, as well as that from 2015 to 2021 the prevalence decreased

from 3.11% (CI 95%, 2.83–3.41%) to 1.03% (CI 95%, 0.47–2.22%), the actual prevalence of Aujeszky's disease is now estimated to be below 2%. However, given the small coverage of tested farms in recent years and the fact that seropositive farms are still being found to reliably determine the current prevalence, it is recommended that future prevalence studies should cover the entire pig population on all farms, including farms that have been declared free of this disease.

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Seroprevalencija bolesti Aujeszzkoga u populaciji domaćih svinja u razdoblju od 2013. do 2021. godine u Republici Hrvatskoj

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Bolest Aujeszzkoga je virusna bolest životinja, prije svih svinja koje se smatraju i njezinim prirodnim domaćinom. Bolest je u u mnogim dijelovima svijeta endemična, ali postoje područja i države koje su ovu bolest uspješno iskorijenile. Zbog obveznih mjera kontrole i trgovinskih barijera bolest Aujeszzkoga izaziva velike ekonomske gubitke. Iako je ova bolest u većini država članica Europske unije iskorijenjena, postoje područja i države u kojima ona i dalje perzistira. Iako se nadziranje i iskorjenjivanje provodi od 2013. godine u Republici Hrvatskoj bolest još uvijek nije iskorijenjena iz populacije domaćih svinja. U ovom radu prikazani su rezultati seroloških

pretraga domaćih svinja na bolest Aujeszzkoga u razdoblju od 2013. do 2021. godine. Prevalencija bolesti Aujeszzkoga na razini gospodarstava po godinama istraživanog razdoblja kretala se u rasponu od: 4,66 % (CI 95%, 4,39-4,95) do 0,67 % (CI 95%, 0,35-1,28). Od 2016. godine prevalencija se zadržavala na prosječnoj razini od 1,26 %, (CI 95%, 0,67-1,95%). Najveća prevalencija utvrđena je u Varaždinskoj županiji (10,28 %, CI 95%, 9,15-11,53), a najmanja u Krapinsko-zagorskoj županiji 0,14% (CI 95%, 0,07-0,31).

Ključne riječi: *bolest Aujeszzkoga, seroprevalencija, domaće svinje, gospodarstva*