Research article

PHYLOGENETIC ANALYSIS OF HA AND NA GENES OF SWINE INFLUENZA VIRUSES IN SERBIA IN 2016-2018

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Pigs are very important for the epidemiology of influenza A viruses, being commonly infected with the lineages of most adapted H1N1, H3N2, H1N2 swine subtypes. Epidemiological complexity of swine influenza is increasing by a periodic spillover of human or avian viruses in the pig population when genetic shifts can occur. The objectives of this research were to determine the presence of the influenza A virus in nasal and tracheobronchial swabs and lung tissue samples of ill and dead pigs on commercial farms, to determine circulating subtypes and characterize them through the phylogenetic analysis of hemagglutinin (HA) and neuraminidase (NA) genes. A total of 255 samples collected from 13 farms were analyzed by means of real-time RT-PCR. The genome of influenza A virus was detected in 24 samples, which represented a 61.5% prevalence at the farms level (influenza A virus was confirmed in 8 out of 13 farms included in this study). Based on HA and NA gene sequences of 8 viruses, the circulation of H1N1 and H3N2 subtypes of influenza A viruses were determined. In addition, one farm exhibited a time separated circulation of H1N1 and H3N2 virus subtypes. Using Influenza Research Database, our viruses of the H1 subtype were classified into 1C.2.1 and 1A.3.3.2. clade. Based on the nucleotide sequences of HA genes, three viruses of the H1N1 subtype belong to the H1N1pdm09 lineage, and the other four to Eurasian "avian-like" H1avN1 lineage; while based on NA genes sequences, these seven viruses belong to Eurasian "avian-like" H1avN1 lineage. Both HA and NA genes of the virus of the H3N2 subtype belonged to the A/swine/Gent/1/1984-like H3N2 lineage.

Keywords: swine, influenza A viruses, subtypes, lineages

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INTRODUCTION

Influenza is one of the most important zoonotic diseases nowadays. The causative agents belong to the family *Orthomyxoviridae*, and are assigned to Influenza A, B, and C genus according to their genetic and antigenic characteristics. Besides, in studies from 2011 onward, a genetically distinguishable novel virus [1, 2] has been described, officially named Influenza D virus. Influenza A viruses (IAVs) through history have earned the biggest attention because they caused multiple epidemics in human and animal populations worldwide, as well as pandemics [3]. Influenza A viruses have a broad host range (humans, mammals, birds). The majority of susceptible animals become ill after infection with a certain subtype of influenza A virus (IAV) previously adapted to the specific family or class of animals. Pigs are very important for the epidemiology of influenza, being commonly infected with the lineages of most adapted H1N1, H3N2, H1N2 subtypes [4]. Besides this, pigs can be susceptible to the viruses previously adapted to humans or birds, representing in that way the potential intermediate host where genetic shifts occur. Antigenically distinct viruses can be further transmitted to both humans and bird species [5, 6].

Swine influenza is an enzootic disease in most areas of the world with dense pig populations [7, 8]. Within Europe, a wide study based on the molecular characterization of swine IAVs [6], provided details of the genome of circulating viruses, information about their diversity, pathogenicity, pandemic potential and correlation to the viruses circulating worldwide. The presence of influenza A virus in the swine population in Serbia was confirmed in an investigation conducted on commercial farms in 2011-2012 [9]. The virus was not detected among analyzed wild boars in Serbia [10]. Although the circulation of the virus in domestic pig populations has been confirmed, the molecular characterization of circulating subtypes has not been conducted yet.

The objectives of this research were to examine samples from sick and dead pigs from commercial farms from the north, central and east parts of Serbia for the presence of IAVs, to determine the circulating subtypes, and to characterize them through the sequence analysis of HA and NA genes.

MATERIAL AND METHODS

Samples

Samples for IAV detection were collected during passive surveillance conducted in the period from August 2016 until January 2018 on 13 farms (marked A, B, C, D, E, F, G, H, I, J, K, L, M) located in the north, central and east parts of Serbia (Fig 1). All farms were farrow-to-finish farms with 800 to 1500 sows each. At the time of collection the sampled farms did not vaccinate the pigs against influenza.

Samples included nasal swabs from two to four months old pigs with influenza-like symptoms and lung tissue samples and tracheobronchial swabs from dead pigs of

different ages with lung lesions resembling interstitial pneumonia. A total of 255 samples were analyzed for the presence of the virus including: 207 nasal swabs, 32 lung tissue samples, and 16 tracheobronchial swabs. The reference virus strains used as positive controls in this study were: H1N1: A/sw/It/311368/2013, H3N2: A/sw/It/311349/2013, H1N2: A/sw/It/284922/2009, H1N1pdm: A/sw/It/282866/2013 (OIE reference laboratory for swine influenza - Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" (IZSLER), Brescia, Italy).
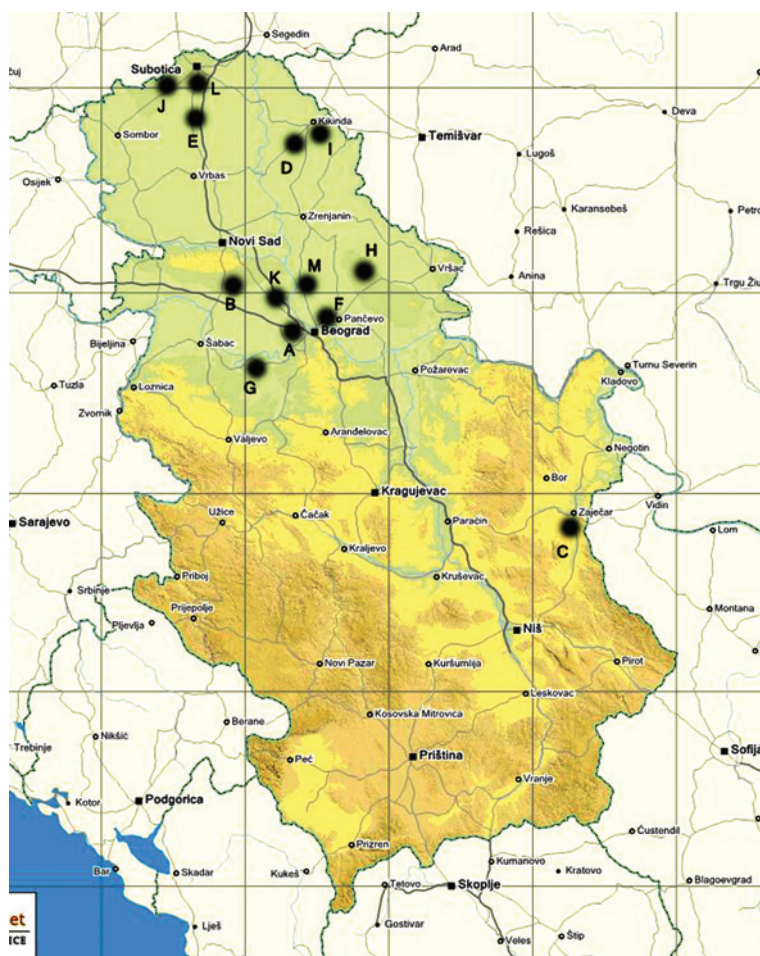

Figure 1. Geographical distribution of the farms where analyzed samples were collected
The farms are marked with black dots and Latin letters

Collected swab samples were soaked in one milliliter of cell culture medium (DMEM, Gibco, ThermoFisher Scientific, USA), with the addition of 100 µg/ml of Gentamicin (Gentamicin, Hemofarm A.D., Serbia) and 2% of Amphotericin B (250 µg/ml) (Gibco™ Amphotericin B, Gibco, ThermoFisher Scientific, USA) in accordance to the procedure described in the OIE Terrestrial Manual [11]. The lung tissue samples were

homogenized in a 1:10 ratio with the same medium. After the one-hour incubation at 4-8 °C and intense shaking of both swabs and lung tissue homogenates, they were centrifuged at 1700 g for 15 minutes [11]. The supernatant was removed and used for further analysis.

Extraction of nucleic acid, PCR, and sequencing

The viral RNA was extracted using Cador Pathogen Mini Kit (Qiagen, Germany) according to the protocol for purification of pathogen nucleic acids from fluid samples. Real-time reverse transcriptase-polymerase chain reaction (RRT-PCR) was performed using VERSO-1STEP qRT-PCR ROX Kit (Thermo Scientific, USA), and previously published primers and probes that amplify the part of the M gene of the IAVs [12, 13]. The samples producing the sigmoid curve in the RRT-PCR were considered as positive. Eight samples with the Ct values below 30 were sent for whole genome sequencing to the OIE reference laboratory for swine influenza (IZSLER). The next-generation sequencing (NGS) was performed using Illumina Miseq platform. The sequence assembly and editing were done with CLC Genomic Workbench v.11 software.

Phylogenetic analyses

The obtained sequences of complete HA and NA genes were compared with available IAV sequences using Basic Local Alignment Search Tool (BLAST). Phylogenetic and evolutionary analyses HA and NA genes of Serbian swine IAVs were performed using software MEGA 7.0. The phylogenetic trees were constructed using the Maximum Likelihood method with 1000 bootstrap replicates [14]. The most similar and reference strain sequences of HA and NA gene from NCBI ([CY083902.1], [CY067651.1], [CY070392.1], [CY057058.1], [KC781586.1], [HM189391.1], [KC780689.1], [CY054670.1], [HM189424.1], [KF667908.1], [CY122655.1], [KR701431.1], [CY115835.1], [CY115842.1], [CY115983.1], [GQ161139.1], [KC345622.1], [KR700271.1], [KC881265.1], [KR700248.1], [FJ791277.1], [KR701320.1], [KR699663.1], [FN429078.1], [FJ798777.1], [KR701344.1], [CY116466.1], [CY116479.1], [KU322687.1], [HM996939.1], [FJ770256.1], [KJ880934.1], [KU322711.1], [KU322502.1], [KU322759.1], [KU322598.1], [CY115856.1], [KR701423.1], [KR701249.1], [KR701257.1], [KR701415.1], [KR701471.1], [KR700597.1], [CY079537.1], [CY128155.1], [CY128251.1], [KT715449.1], [CY037898.1], [FJ966082.1] and [CY079539.1], [CY079546.2], [CY116468.1], [GQ161121.1], [KR700115.1], [CY116513.1], [KR700131.1], [GQ161158.1], [KR066586.1], [CY010574.2], [CY116497.1], [CY010582.1], [EU045393.2], [EU045389.2], [EU045388.2], [KR701425.1], [KR701473.1], [KR699998.1], [KR701251.1], [KR701385.1], [KR701509.1], [KR701433.1], [KR701259.1], [KR701330.1], [KR701417.1], [GQ161140.1], [KR066581.1], [KR066583.1], [FN429081.1], [KR700487.1], [FJ791288.1], [KU322713.1], [KJ880936.1],

[HM996952.1], [KU322689.1], [HM996957.1] respectively) were included in the phylogenetic analysis, as well.

Influenza Research Database (IRD) [15] was used for H3 amino acid numbering and for determination of genetic clade Swine H1 Clade Classification Tool [16] was used. The complete sequence of HA and NA gene of 8 IAVs originating from Serbian pigs are deposited in the GeneBank under accession numbers [MK453364] to [MK453371] and [MK454929] to [MK454936] respectively.

Ethical approval

The conducted research is not related to the use of animals. No ethical approval was obtained because this study did not involve laboratory animals and involved only non-invasive procedures.

RESULTS

RRT-PCR results

Out of 255 tested, the IAV genome was detected in 24 samples. At the farm level, IAV was confirmed in 8 out of the 13 farms included in this study (61.5%). Regarding the sample type, out of 207 nasal swab samples, 17 were positive, and out of the 32 lung tissue samples, the IAV genome was detected in 7. However, IAVs were not detected in any of the 16 tracheobronchial swabs (Table 1).

Table 1. Results of RRT-PCR detection of IAVs in the analyzed samples

Farms	Nasal swab			Lung tissue			Tracheobronchial swab		
	analyzed	positive	%	analyzed	positive	%	analyzed	positive	%
A	43	3	6.98	-	-	-	-	-	-
I	20	3	15.00	-	-	-	-	-	-
J	3	1	33.33	-	-	-	-	-	-
B	63	4	6.35	12	2	16.67	-	-	-
C	9	1	11.11	1	0	0.00	-	-	-
F	41	0	0.00	2	2	100.00	-	-	-
G	10	3	30.00	6	3	50.00	-	-	-
M	3	0	0.00	1	0	0.00	-	-	-
E	-	-	-	6	0	0.00	-	-	-
D	-	-	-	-	-	-	5	0	0
H	-	-	-	-	-	-	4	0	0
K	15	2	13.33	-	-	-	3	0	0
L	-	-	-	4	0	0.00	4	0	0
SUM	207	17	8.21	32	7	21.88	16	0	0

Phylogenetic analysis

Using NGS, the whole genome of 8 IAVs was successfully sequenced. The 6 sequenced viral genomes originated from viruses detected on farms A, F, G, I, J, K, whereas from farm B the complete genomes of 2 viruses were sequenced (Table 1).

The read sequences of HA and NA genes were composed of 1694-1701, and 1388-1410 nucleotides, respectively. Based on the BLAST research, detected IAVs of swine origin circulating on commercial farms in Serbia, belong to the H1N1 and H3N2 subtypes. Subtype H1N1 was detected in all farms, while the H3N2 virus was detected only in farm B. HA gene sequences of viruses from farms A, B and K showed the highest similarity, up to 98%, with the HA gene of H1N1pdm09 lineage IAVs isolated from humans in 2009 and 2010. The other group of the HA gene sequences (farms F, G, I, J) showed the highest similarity with the HA gene sequences of influenza A H1N1 viruses originating from Italian, Belgian and Spanish swine (Fig. 2).

After aligning with the reference sequences of global swine H1 clade deposited in the IRD, Serbian origin of swine IAVs were classified into the 1C.2.1 clade (Avian-like swine H1avN1 lineages) together with Italian and Belgian isolates, as well as into the 1A.3.3.2. clade (H1N1pdm09 lineages) along with USA and Mexico isolates [16, 15] (Table 2). Hemagglutinin gene of the H3N2 virus originating from farm B was most similar to the A/swine/Gent/1/1984-like H3N2 European isolates (Fig. 2).

Regarding the NA gene, 7 swine IAV strains showed the highest similarity with the sequences of NA gene of European "avian-like" H1N1 swine strains [6]. The NA gene of the H3N2 virus showed the highest similarity with the A/swine/Gent/1/1984-like H3N2 European isolates [6] (Fig. 3).

Table 2. Name, subtype, origin and IRD H1 clade assignment of the sequenced Serbian swine H1 IAVs

Name of the virus (subtype)	Originating farm	Global Swine H1 Clade Classification Clade Assignment
A/swine/Serbia/1/2017(H1N1)	F	1C.2.1
A/swine/Serbia/2/2017(H1N1)	A	1A.3.3.2
A/swine/Serbia/3/2016(H1N1)	B	1A.3.3.2
A/swine/Serbia/4/2018(H3N2)	B	-
A/swine/Serbia/5/2016(H1N1)	G	1C.2.1
A/swine/Serbia/6/2017(H1N1)	K	1A.3.3.2
A/swine/Serbia/7/2017(H1N1)	I	1C.2.1
A/swine/Serbia/8/2017(H1N1)	J	1C.2.1

Analysis of the amino acid sequence of the HA gene of H1N1 viruses from farms A, B and K using H3 amino acid numbering scheme (IRD) revealed the presence of glutamic acid (E) at position 77, aspartic acid (D) at position 190 and 225, and NTT (asparagine-threonine- threonine) glycosylation site at the position 278-280 (Table 3).
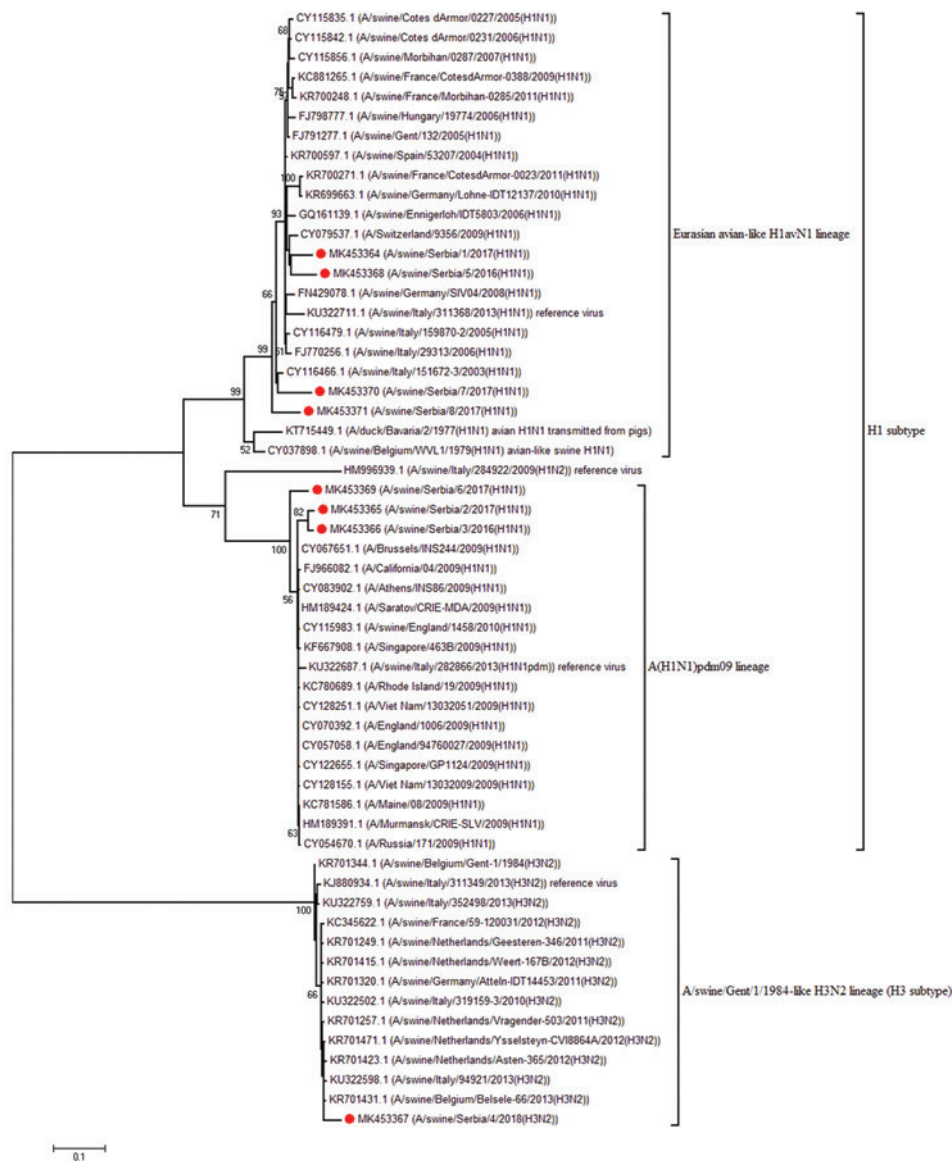
Figure 2. Phylogenetic tree constructed using Maximum Likelihood method based on the HA gene sequences

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The analysis involved 57 nucleotide sequences. All positions containing gaps and missing data were eliminated. Serbian sequences are marked with red dots, identified by accession number followed by Genus/Host/Geographic Origin/Identification/Year (Subtype).
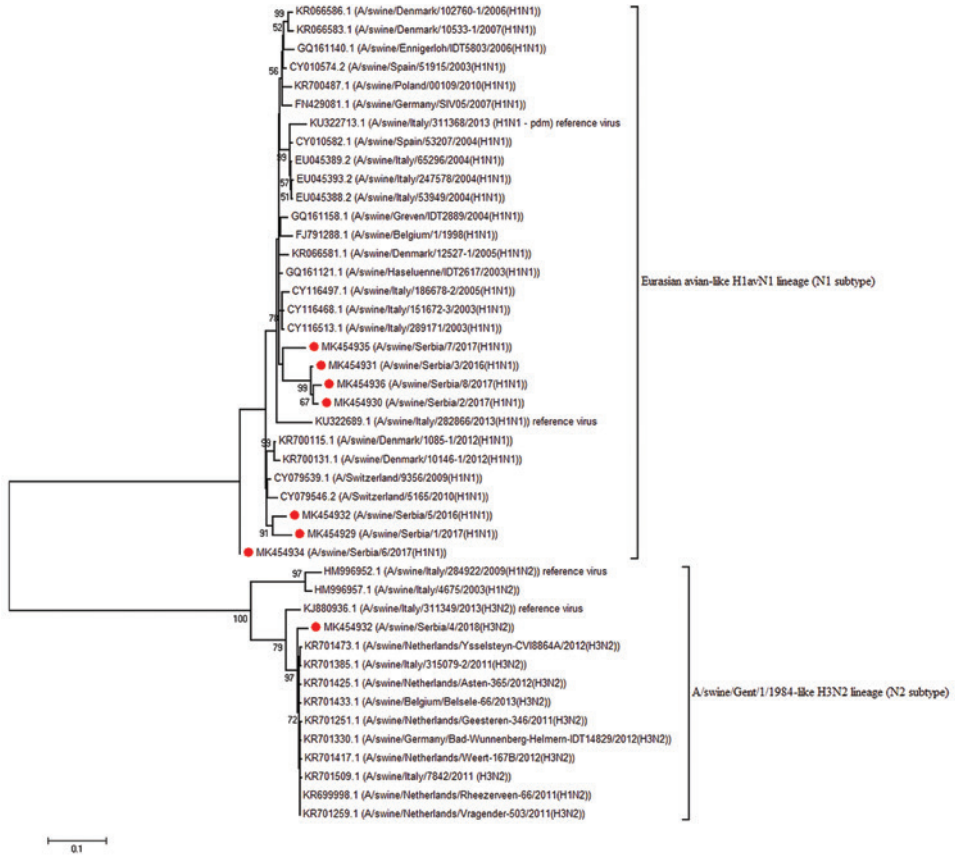
Figure 3. Phylogenetic tree constructed using Maximum Likelihood method based on the NA gene sequences

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The analysis involved 44 nucleotide sequences. All positions containing gaps and missing data were eliminated. Serbian sequences are marked with red dots, identified by accession number followed by Genus/Host/ Geographic Origin/Identification/Year (Subtype).

Viruses from farms F, G, I and J in the amino acid sequence at the cleavage site of the HA gene possessed the PSIQSR motif, and D190/E225 combination within receptor-binding site (Table 3).

Virus of the H3 subtype from farm B displayed the 226 leucine (L)/228 serine (S) combination in its amino acid sequence within the receptor-binding site (Table 3).

Table 3. Amino acid position in HA protein sequence of Serbian swine IAVs according to H3 numbering system

H3 NUMBERING	76	77	78	189	190	191	225	226	228	277	278	279	280	281	282	324	325	326	327	328	329	330	331	332
H3 (A/ACHI/2/68)	C	D	V	Q	E	Q	G	L	L	C	I	S	E	C	I	P	E	K	Q	T	R	G	L	F
A/swine/Serbia/2/2017(H1N1)	C	E	S	A	D	Q	D			C	N	T	T	C	Q									
A/swine/Serbia/3/2016(H1N1)	C	E	S	A	D	Q	D			C	N	T	T	C	Q									
A/swine/Serbia/6/2017(H1N1)	C	E	S	G	D	Q	D			C	N	T	T	C	Q									
A/swine/Serbia/1/2017(H1N1)					D		E									P	S	I	Q	S	R	G	L	F
A/swine/Serbia/5/2016(H1N1)					D		E									P	S	I	Q	S	R	G	L	F
A/swine/Serbia/7/2017(H1N1)					D		E									P	S	I	Q	S	R	G	L	F
A/swine/Serbia/8/2017(H1N1)					D		E									P	S	V	Q	S	R	G	L	F
A/swine/Serbia/4/2018(H3N2)								L	S															

DISCUSSION

This study, conducted from 2016 to 2018, reveals the presence of the IAVs in Serbian swine on 8 of 13 examined farms, representing a prevalence of 61.5% of IAVs at the farm-level. However, considering that point mutations in the virus genome may affect the result of RRT-PCR [17, 18], as well as the short time of virus excretion [19, 20], the possibility of virus circulation in the remaining five farms cannot be excluded. Although the nasal swab is considered the most preferable sample for IAV detection in swine [21], the IAV genome was most often detected in the lung tissue samples (21.88%) in this study. This is in line with the results of De Vleeschauwer et al. (2009) and Demeter et al. (2011) [22, 23], who reported the highest affinity of swine influenza viruses for epithelial cells in the lungs. Furthermore, the results of our study showed that this type of sample is also most suitable for NGS of the IAV because of its high viral concentration in the lungs. The IAV genome was detected in only 8.21% of nasal swab samples that represented the majority of the analyzed samples.

Phylogenetic analysis of HA and NA gene sequences, revealed the circulation of H1N1 and H3N2 that represent two most prevalent swine subtypes of IAV [24, 6, 25, 21]. During the survey in 2016-2018, six farms experienced the infection with H1N1 subtype of IAV, whereas in one farm two subtypes, H1N1 and H3N2, were detected in time-separate sampling. This is in line with the reports from neighboring countries, where these two subtypes were revealed in Croatia, Romania in 2009, Albania in the period 2007 to 2009, and in Hungary from 2010 to 2013 [26, 6, 27, 28]. Viruses of the H1N2 subtype are enzootic in pig populations of several European countries such as Belgium, Denmark, Germany, France, and Italy [6]. However, this subtype has not been confirmed in our study. Nevertheless, considering the small number of farms included in the study, the circulation of this subtype cannot be excluded.

Using Maximum Likelihood method Watson et al. [29] categorized each gene of recent European swine viruses into one of nine lineages circulating worldwide: classical H1N1, Eurasian "avian-like" H1$_{av}$N1, A(H1N1)pdm09, A/swine/Scotland/410440/1994-like H1$_{hu}$N2, A/swine/Italy/4675/2003-like rH1N2, North American triple reassortant, A/swine/Gent/1/1984-like H3N2, human seasonal H3N2 and avian. According to this classification, results of phylogenetic analysis of the HA gene sequences of Serbian viruses indicate that they belong to Eurasian "avian-like" H1avN1, A(H1N1)pdm09 and A/swine/Gent/1/1984-like H3N2 lineages.

Phylogenetic analyses of the HA gene sequence of three viruses of H1N1 subtype including A/swine/Serbia/2/2017(H1N1), A/swine/Serbia/3/2016(H1N1) and A/swine/Serbia/6/2017(H1N1) originating from farms A, B, and K respectively, showed the highest nucleotide similarity with the sequences of HA genes of human origin viruses isolated during 2009/2010 influenza pandemic, indicating that the HA gene of these viruses may have been derived from H1N1pdm09 human strain [30].

Further, HA genes of H1N1 viruses from farms A and B showed high nucleotide similarity (98.25%). Considering the proximity of the farms (Fig. 1) and years of sampling, the possibility of virus transmission between farms cannot be excluded. On the other side, the HA gene sequence from farm K diverges from sequences obtained from farms A and B (93.86% and 94.27% of nucleotide identity respectively) and is located in a separate branch within H1pdm09 clade indicating a different origin (Figure 2).

Analysis of amino acid sequences of the HA gene of these strains showed that they have identical amino acids at the certain position which are specific for pandemic H1N1 strains. Thus, using H3 numbering scheme, the presence of glutamic acid (E) at the position 77, corresponding to the conserved spot in the HA amino acid sequence of H1N1pdm09 strains, was revealed in all three sequences [31, 32]. Furthermore, the presence of aspartic acid (D) at positions 190 and 225 (instead of glycine) which are essential for receptor preference (high affinity to α2-6 sialylated glycan receptors dominant in human respiratory epithelium), confirms the pandemic origin of these strains [33, 34]. In addition, the viruses possessed glycosylation site (NTT) at the position 278-280 in HA amino acid sequence, which is also characteristic of pandemic H1pdm09 strains [31] (Table 3).

Hemagglutinin gene of H1N1 viruses originating from farms F (A/swine/Serbia/1/2017(H1N1)), G (A/swine/Serbia/5/2016(H1N1)), I (A/swine/Serbia/7/2017(H1N1)) and J (A/swine/Serbia/8/2017(H1N1)), showed the highest similarity with the HA gene of Eurasian "avian-like" swine H1avN1 viruses [6, 35]. Viruses from these farms showed the highest nucleotide identity, 93-95%, with European swine influenza viruses isolated in the period 2003-2011 (Figure 2). The avian origin of the HA gene of these viruses was additionally confirmed by the presence of PSIQSR motif in the cleavage site that is a trait of all low pathogenic avian influenza strains [36, 37]. Moreover, the presence of aspartic acid at position 190 and glutamic acid at position 225 suggested the preference for human-type receptors (α2-6 sialylated glycan receptors) [38] (Table 3). The phylogenetic tree of HA gene indicated the same origin of the viruses from farms F and G. However, the nucleotide identity of 93% resulted from the viral evolution during the period 2016-2017. Interestingly, the other two viruses, sharing in-between and to the viruses from farms F and G between 90.66% and 89.66% of nucleotides, had a different origin (Figure 2).

Hemagglutinin gene of the second sequenced virus from farm B (A/swine/Serbia/4/2018(H3N2)) showed the highest nucleotide similarity with the HA gene sequences of H3N2 swine viruses isolated in Europe during the period 2010-2013. This isolate is genetically closest to the IAV isolate from the Netherlands (A/swine/Netherlands/Ysselsteyn-CVI8864A/2012(H3N2), [KR701471.1]) with which it shares 97.06% of nucleotide identity.

The presence of two subtypes of IAVs on farm B (H1N1 and H3N2) in time-separate samplings is most likely a result of viral replacement by each other rather than co-

circulation considering the time interval between two samplings. The presence of L226/S228 combination within the receptor-binding site in the amino acid sequence of HA gene of this virus is typical for swine H3N2 subtypes (Table 3). Also, the L226/S228 combination determinates the virus preference for human-type receptors [38].

Analyses of the NA gene sequences revealed the circulation of two NA subtypes - N1 and N2. The N1 genes showed the highest similarity with the sequences of European swine strains isolated after 1998, which were characterized as the Eurasian avian-like H1avN1 lineage [35, 6]. By the phylogenetic analysis of the N1 NA gene sequences, Serbian isolates were grouped into two clades. One clade was composed of Serbian isolates only (A/swine/Serbia/2/2017, A/swine/Serbia/3/2016, A/swine/Serbia/7/2017, A/swine/Serbia/8/2017), and the other clade, along with Serbian isolates includes the IAV strains from Switzerland (human origin) and Denmark (Figure 3).

In addition, the A/swine/Serbia/6/2017 isolate formed a separate group (Figure 3). The phylogenetic tree of NA genes derived from IAV isolates originating from farms A, B and J and F and G shows that they most likely have a common origin, indicating the introduction of animals from the same source to these farms. Based on the analysis of HA and NA genes of A/swine/Serbia/2/2017, A/swine/Serbia/3/2016 and A/swine/Serbia/6/2017 isolates, who showed the highest similarity in the HA gene with the human A(H1N1)pdm09 lineages, and NA gene with the Eurasian avian-like H1avN1 swine lineages, we concluded that reassortment of human and swine IAVs occurred [39].

The sequence of NA genes of N2 virus originating from farm B belonged to the A/swine/Gent/1/1984-like H3N2 lineage together with European swine viruses isolated between 2011 and 2013(Figure 3) [31].

In conclusion, research confirmed the circulation of H1N1 and H3N2 IAV subtypes in the Serbian swine population. Although H1N2 subtype has not been detected, its presence cannot be excluded. Based on the phylogenetic analysis of HA genes, Serbian viruses belong to the Eurasian avian-like H1avN1, A(H1N1)pdm09 and A/swine/Gent/1/1984-like H3N2 lineages, and by sequence analysis of NA genes, they were grouped in the Eurasian avian-like H1avN1 and A/swine/Gent/1/1984-like H3N2 lineages. Furthermore, the reassortment between human and swine viruses was confirmed. Additional analyses of the internal gene cassette are needed to provide detailed insight into the molecular evolution, origin, pandemic potential and additional reassortment events. The detection of the reassorted genotype underlines the need of active surveillance implementation, aiming the public health protection.

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Authors' contributions

JMZ carried out molecular tests and sequence analysis, designed phylogenetic trees and draft the manuscript. VM, BS, OS and BK designed and coordinated the study and drafted the manuscript. CC performed Next Generation Sequencing and sequence assembly and editing and drafted the manuscript. AP, NS, LJV and VR participated in the collection and preparation of samples, molecular tests and preparation of phylogenetic trees.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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FILOGENETSKA ANALIZA HA I NA GENA VIRUSA INFLUENCE SVINJA U SRBIJI OD 2016 DO 2018 GODINE

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Svinje su veoma važne u epidemiologiji influenca A virusa, jer je većina zapata širom sveta zaražena nekim od tri podtipa (H1N1, H1N2 ili H3N2). Pored toga, one su prijemčive i za ljudske i ptičije infleunca A viruse, usled čega u njihovom organizmu može doći do genetskog reasortiranja i stvaranja genotipski i fenotipski novih virusa. Cilj ovog istraživanja je bio da se analizom nosnih i traeo-bronhijalnih briseva i pluća poreklom od bolesnih i uginulih svinja ispita prisustvo influenca A virusa, da se odrede cirkulišući podtipovi i filogenetski okarakterišu kroz analizu sekvenci HA i NA gena. Tokom ovog istraživanja sa 13 farmi je sakupljeno i metodom real-time RT-PCR pregledano je 255 uzoraka. Genom influenca A virusa je utvrđen u 24 uzorka. Prevalencija virusa na nivou farmi iznosila je 61.5%. Kompletno je sekvenciran genom 8 virusa koji su poticali sa sedam farmi. Na osnovu sekvenci HA i NA gena potvrđena je cirkulacija H1N1 i H3N2 podtipova. Na jednoj farmi ustanovlejna je cirkulacija oba podtipa, ali u različitim periodima ispitivanja. Na osnovu sekvence HA gena, sedam virusa H1 podtipa su grupisani u 1C.2.1 i 1A.3.3.2 genske grupe i H1N1pdm09 i evroazijsku "avian-like" H1avN1 linije. Na osnovu sekvence neuraminidaza gena ovih sedam H1N1 virusa su grupisani u evroazijsku "avian-like" H1avN1 liniju. Virus H3N2 podtipa na osnovu sekvenci HA i NA gena pripada liniji A/Swine/Gent/1/1984 - "like" liniji.