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Review Article

EPIDEMIOLOGY AND STATUS OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME IN THE WESTERN BALKAN REGION: CHALLENGES AND PROSPECTS

Dinko Novosel[1]*, Tamaš Petrović[2], Žaklin Acinger – Rogić[3], Marina Štukelj[4]

[1]Department of Pathology, Croatian Veterinary Institute, Savska cesta 143, 10000 Zagreb, Croatia; [2]Scientific Veterinary Institute "Novi Sad", Rumenacki put 20, 21000 Novi Sad, Serbia; [3]Veterinary and Food Safety Directorate, Ministry of Agriculture, Planinska 2a, 10000 Zagreb, Croatia; [4]Institute for Health Care of Pigs, University of Ljubljana, Veterinary Faculty, Grbiceva 60, 10000 Ljubljana, Slovenia

*Corresponding author, E-mail: dinko.novosel@gmail.com

Summary: Two decades after its emergence, porcine reproductive and respiratory syndrome (PRRS) remains a challenge to the sustainability of the porcine industry worldwide. In the Western Balkan region in particular, control of the disease is hampered by fragmentation of pig production; lack of farmer knowledge regarding health care; the fact that most farms are small, single-site pig operations with low biosecurity standards; and intensive trading and import of pigs from different countries without known health status and without quarantine. All these factors contribute to rapid disease transmission among pig operations. PRRS entered the Western Balkan region in 1995, when it appeared in Croatia and slightly later in Serbia, and again after 2004 when it entered Slovenia and potentially other countries. All PRRS cases originally described in the Western Balkans appear to have been caused by infection with type 1 subtype 1 PRRSV; more recently, infection with type 2 virus has also been reported. Veterinary services have an important role to play in monitoring and controlling spread of PRRS, but control programs in the region are either inconsistent or non-existent. Available epidemiological data suggest that new PRRSV introduction into Western Balkan countries is less likely to occur via animal transfers within the region and more likely to occur via arrivals from elsewhere in the EU. Strong efforts are needed to develop and implement guidelines for pig movement, implement biosecurity measures, establish consistent diagnostic testing for PRRS virus, and classified pig herds according to health status and farmer education.

Key words: PRRSV; Slovenia; Croatia; Serbia

Introduction

Two decades after its emergence, porcine reproductive and respiratory syndrome (PRRS) remains a challenge to the sustainability of the global porcine industry. As a result, many countries in North America and the European Union (EU) have implemented continuous monitoring programs. The picture is quite different among countries in the Western Balkans, where pig production and veterinary services are highly fragmented, and PRRS control measures are inconsistent or non-existent. This troubling state of affairs reflects a lack of harmonized diagnostic methods and sparse available data on PRRS prevalence, nature of virus and farm management practices. This poses a problem not only for control and reduction of PRRS virus (PRRSV) already present in the Western Balkans, but also for prevention of new virus introduction due to extensive trade with other EU countries.

The problem of PRRS monitoring and control in this region is even more challenging because

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Croatia, Slovenia and Serbia contain large numbers of sustainable farms where small numbers of animals are kept, mostly for household needs. For example, of approximately 168,000 breeding sows in Croatia, only 25,000 are in large pig production units; the remainder are on semi-intensive or -extensive farms, according to the Croatian Agriculture Agency (http://www.hpa.hr/, accessed 15 May 2016). Most pig farms in Slovenia are small, single-site production farms: according to a census of 4162 farms in 2013, 3909 farms had 1-20 breeding pigs; 206 farms, 21-50; 31 farms, 51-100; 12 farms, 101-200 breeding pigs; 2 farms, 501-1000 and 2 farms, 1001 or more (data from 1.2.2013 in the VOLOS database, Ministry for Agriculture, Forestry and Food, UVHVVR).

Though the first PRRS outbreak was reported in lower Saxony in 1992 (1), PRRSV appears to have emerged before that in Eastern European countries behind the "Iron Curtain" (2), when the present-day countries of the Western Balkans formed part of the single federation of Yugoslavia. This federation literally served as a bridge for PRRS: it was the only Communist country with soft borders, and it traded extensively with Eastern and Western Europe. The paths of live animal movements potentially carrying PRRSV changed often in the region as a result of complex political changes that molded and remolded trade routes. The Berlin Wall fell in 1989, Yugoslavia broke up into several republics in 1990, and war raged in Croatia, Bosnia and Herzegovina, Serbia and Montenegro in 1991-1995. Slovenia joined the Central European Free Trade Association (CEFTA) in 1996, followed by Croatia in 2003. The following year, Slovenia joined the EU and left CEFTA, while in 2007 most other former Yugoslav republics joined CEFTA. In 2013 Croatia joined the EU and left CEFTA (Figure 1). All these changes probably modified live animal movements in complex ways, making it difficult to track PRRSV epidemiology and predict prevalence.

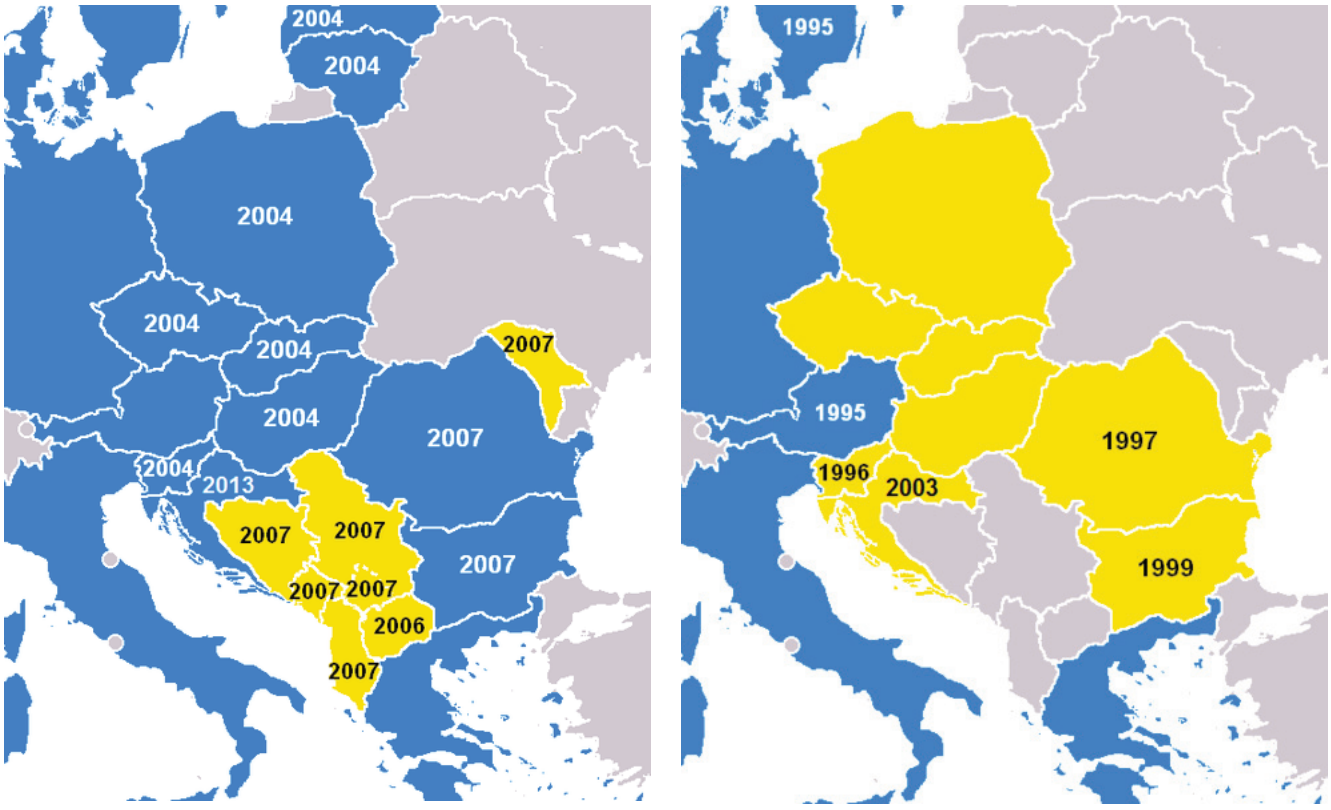

Figure 1. Evolution of political alliances affecting trade routes and therefore live animal movements in the Western Balkan region. EU member states are shown in blue; CEFTA member states, in yellow. Membership status is shown (A) before 2003 and (B) in 2015

Pig production and trade in the Western Balkans

Western Balkan countries import large amounts of pork and pigs from Western Europe. The biggest exporter to the Western Balkans in 2012 was Hungary, with the traded animals originating outside Hungary [Croatian Agriculture Agency (www.hpa.hr), accessed 15 May 2016]. This reflects the fact that large pig-exporting firms in Hungary had distribution centres for the Western Balkans but were not producing their own animals. In the same year, Croatia imported more pig and pork from the EU27 bloc than all other Western Balkan countries (Table 1). Croatia also exported large amounts to Bosnia and Herzegovina and Albania. In fact, membership in CEFTA facilitated pig and pork trading with Bosnia and Serbia (Table 2). Evidence also strongly suggests illegal trading among Western Balkan countries that bypassed veterinary inspections (3).

Before joining the EU in 2004, Slovenia was free of PRRS. The disease was introduced after entry as a result of pig import from other EU members; quarantine is not required for animals moving between EU members.

Epidemiology of PRRSV in selected Western Balkan countries and attempts at control

Epidemiological studies suggest that most pig pathogens in the Western Balkans came from outside the region, and that some local enzootic strains have emerged. Most strains of porcine circovirus type 2 (PCV2) in the Western Balkan region are highly homologous to Dutch strains (4), as are most strains of PRRSV (5, 6). One exception is porcine parvovirus genotype 3 (PPV3), which appears to have originated in Croatia (5). A different PCV2 genotype was recently discovered in wild boars (4). Some local enzootic strains appear to have spread from the Western Balkans to other regions, such as PPV3, while others have not, such as PCV2d (8).

Relatively good data exist about PRRSV seroprevalence in Slovenia and Serbia, but little is known about the situation in Croatia, and the few data available are sometimes contradictory. For example, only 11 ELISA and PCR tests were performed in 2009 (9) and only 5 in 2010 (10). Seroprevalence in Croatia is thought to be much higher than 25%, based on analyses of neighboring Slovenia and Serbia, suggesting that nearly all farms suffer economic losses due to PRRSV.

Table 1: Key data on Western Balkan countries. Source: FAO (www.fao.org, accessed 15 May 2016)

Country	Total area (10^3 Ha)	Agriculture area (10^3Ha)	Population (1000s)	GDP (mil €)	HDI	Export (%)	Import (MT)
Albania	2875	1201	3238	11781	0.19		8.6
Bosnia and Herzegovina	5121	2151	3736	16578	0.71		
Croatia	5659	1326	4379	60852	0.76		26.36
Montenegro	1381	512	633	411	0.769		7.3
Romania	23839	13982	21339	161624	0.767		65.8
Bulgaria	11100	5088	7349	47714	0.7343		37.9
Serbia	8836	5061	9835	38423	0.701		
FYROM	2571	1118	2069	9189	0.701		7.7
Slovenia	2027	458	2045	46908	0.828	18	
Western Balkans	63409	30897	54623	393480		18	153.66
EU	4381376		507890	17577000	0.876		

Abbreviation: [1] Area of country in 10^3 Ha; [2] Total agriculture area in 10^3 Ha; [3] Country population; [4] Gross domestic product; [5] Human development index; [6] Percentage of production that was exported to other countries; [7] Import in millions of tons

Table 2: Pig imports and exports to and from Western Balkan countries. Source: UN Comtrade database (http://comtrade.un.org/, accessed 15 May 2016)

Country	Pig type	Exports (MT)	Destination countries	Imports (MT)	Countries of origin
Albania	pure breed			7066	GR, H, HR, I
	< 50 kg			135	GR, NL, I
Austria	pure breed	1363	D, SLO, CZ, H	38402	D, DK, SLO, CZ
	< 50 kg	1028	SLO, H, HR, D	4814	D, SLO, CZ, H
Bosnia and Herzegovina	pure breed	16	MNE	4932	HR, SRB, H
	< 50 kg	1,6	MNE	711	H, D, SRB, HR
Croatia	pure breed	7983	BiH, SRB, AL	142	H, CZ, SLO
	< 50 kg	19	BiH	13887	NL, D, H, DK
Denmark	pure breed	46443	D, BY, I, E		
	< 50 kg	237313	D, PL, I, CZ		
Greece	pure breed				
	< 50 kg	34	AL	104	H, N, E
Montenegro	pure breed			833	H, SRB,BiH
	< 50 kg			651	SRB, BiH
Romania	pure breed			20686	H, BG, NL
	< 50 kg	11	MD	18709	NL, H ,D
Bulgaria	pure breed	89	GE, AL	18	DK, D, GR
	< 50 kg			574	NL, GR, RO
Serbia	pure breed				
	< 50 kg				
FYROM	pure breed	355	SRB		
	< 50 kg	43	SRB		
Slovenia	pure breed	2655	A, MNE, AL	306	A, H, I
	< 50 kg	19	A	1844	A, D, NL
Hungary	pure breed	55	AL	13	F, E
	< 50 kg	7948	RO, HR, NL	9042	NL, D, SK
EU27	pure breed	23443	RU, AL, SRB	1,8	CH, US
	< 50 kg	24622	HR, UA	20	CH
Western Balkans	pure breed	11098	HR, BiH	2691	
	< 50 kg	127,6		36615	

Abreviation: [1] Pure breed – reproductive gilts and boars, <50 kg – imported weaners for fattening; [2] Exports in million tons of body weight; [3] Imports in million tons of body weight

Croatia

The first outbreak of a severe reproductive disorder in breeding animals in Croatia occurred in 1995 (11), most probably resulting from sow insemination with imported Duroc semen contaminated with PRRSV. At that time, Croatia had not yet developed methods to diagnose PRRS, so serum samples were sent to the Veterinary Diagnostic Institute in Lelystad in the Netherlands; these samples tested positive for PRRSV by the enzyme-linked immunosorbent assay (ELISA). Subsequently the Croatian Veterinary Institute became a major testing centre for PRRSV, testing approximately 60,000 serum samples between 1996 and 2010 (12). During that time, the disease spread to nearly all major pig breeding herds, and seroprevalence in domestic swine was estimated to be over 90% (12).

The situation appeared to have improved in 2009, though only 709 serum samples were tested, followed by only 955 in 2010. Testing of sera from boars, sows, gilts and fatteners for the presence of anti-PRRSV antibodies using three commercial ELISA kits revealed no positives in 2009, compared to positive rates of 1.84% (5 of 272) in sows and 2.59% (5 of 193) in fatteners in 2010. These results likely underestimated the prevalence of PRRSV, since only 1.1% of sows were tested in 2009 and only 1.6% in 2010 (13). Indeed, testing of samples using nested reverse transcription (RT)-PCR, immunohistochemistry and pathology indicated the presence of PRRSV in nearly all large pig production units in Croatia.

This lack of progress in controlling or eradicating PRRS contrasts with the fact that PRRS is a notifiable disease in Croatia: every positive PRRS case must be notified to the Croatian veterinary directorate. The problem may be that the national authority has not mandated any specific control measures to combat PRRS. As a result, control measures are usually applied at the farm level by the local veterinary service assigned to that particular farm. Meanwhile, large pig production farms and units usually apply only nonspecific (mainly biosecurity) measures. Outbreaks of classical swine fever in 2006 and 2007-08 led the national authority to mandate biosecurity measures on all farms, with more stringent measures required on farms keeping more than 100 pigs.

Efforts to control and eradicate PRRS in Croatia continue to lack any coordination at the national level. As of 2015, the veterinary authority does not require PRRSV testing of national breeding stock, such as testing of boars before transfer to artificial insemination facilities or regular testing of sows and gilts. As a result, such testing is performed at farm level by the local veterinary service assigned to that particular farm based on individual health programs. No national-level data on PRRSV vaccination are available, nor are PRRSV-positive farms required to notify the veterinary authority of depopulation-repopulation programs or other control measures. In 2004, a PRRSV emergency was declared and imported inactivated vaccine was applied. The results were ambiguous and left many farmers disappointed and unconvinced of the efficacy of coordinated intervention at the national level. An attenuated vaccine against PRRSV entered the Croatian market in 2007, but it has been little used.

Epidemiological data on PRRSV-positive animals are less reliable because all animals that test positive locally are automatically recorded as PRRSV-positive, regardless of diagnostic criteria and testing method. Testing and confirmation methods have not been standardized at the national level. Available epidemiological data suggest persistence of the virus throughout Croatia: in 2009, 26 pigs on 6 farms tested positive for PRRSV; in 2010, 6 pigs on 6 farms; in 2011, 8 pigs on 3 farms; in 2012, 295 pigs on 48 farms; in 2013, 43 pigs on 7 farms; in 2014, 31 pigs on 10 farms. Positive farms were located mainly in Osijek-Baranja, Vukovar-Srijem and in Medjimurje County, consistent with the higher concentration of large pig production sites in these areas.

Slovenia

A survey of swine sera during 1999-2004 in Slovenia showed all herds to be free of PRRSV. In the beginning of 2005, soon after Slovenia joined the EU, animals positive for anti-PRRSV antibody using ELISA we first detected among breeding pigs in a few herds (14). In 2010, a survey of 267 herds revealed that 44.8% were seropositive for antibody (15). Positive herds manifested clinical signs of disease, including reproductive and/or respiratory disorders. To reduce economic losses, farms with positive animals relied on vaccination with the same

two live-attenuated vaccines currently available in the EU, as well as on herd closure, roll-over and serum inoculation. A volunteer project funded by the Slovenian Research Agency and the Ministry of Agriculture and Environment eliminated or eradicated PRRS from 7 of 19 farms (16).

The hypothesis that EU entry led to PRRSV introduction into Slovenia through import of infected animals seems more plausible given that something similar appears to have happened with Hungary. Shortly after Hungary acceded in 1996, the first seropositive pigs were recorded in 1996. In contrast to the situation in Slovenia, between 1995 and 2002, 27925 pig sera were tested and the seroprevalence remained below 3% until 2002, despite brisk pig trade between Hungary and Western Europe (17).

Although a national program to eradicate PRRS was proposed in 2011, it was never implemented. The main problem was that farmers did not want PRRS status to become publicly known.

Serbia

The first suspected cases of PRRS in Serbia occurred in 2001, when serious respiratory disorders associated with high mortality affected large numbers of pigs on two large industrial farms located in the northern region close to the borders with Croatia and Hungary. The suspected cause of the cases was boar semen illegally imported from neighbouring countries. Subsequently in 2001-02, respiratory syndrome with high morbidity and moderate mortality, which was diagnosed as PRRS, occurred on several large industrial farms in the northern Serbian province of Vojvodina, where pig production is intensive. This syndrome subsequently spread to parts of central Serbia. Severe health problems and high economic losses led the veterinary directorate to perform PRRS serology screening in 2002, 2004-2005 and 2006-2007 (Table 3). PRRSV monitoring using RT-PCR and immunofluorescence in 2002 detected the virus in 2 of 16 piglets who died on infected farms. Monitoring in 2006-2007 revealed PRRSV-positive herds in all Serbian regions at prevalences of 1.56-60.86%; the disease was most prevalent in northern, western and central parts of the country, where prevalence was 17.30-60.86%. In contrast, prevalence was only 1.56-8.98% among herds in the eastern and southern parts of the country (18).

These screening results suggest that two major PRRS introductions occurred in the Western Balkan region. The first one was in 1996 when the disease moved from Croatia into Hungary and shortly thereafter into Serbia. The second introduction occurred in Slovenia and Hungary after they joined the EU, when quarantine of imported animals from EU countries was no longer required. As a result of the first virus introduction and resulting outbreak, an emergency-vaccination campaign to control PRRSV was carried out on large industrial farms in northern Serbia in 2002-2003. Other than this limited intervention, no monitoring or control program against PRRS has ever been proposed at the national level.

Genetic diversity of PRRSV in the Western Balkan region

Extensive studies have identified only one PRRSV type (EU type 1 subtype 1) circulating in the Western Balkan region, with no evidence of other Eastern European subtypes (5, 6, 19, 20). ORF5 sequences from field samples in Croatia show 95.2-99.7% homology with the Lelystad vaccine strain used in the country (5). In Serbia, phylogenetics indicate that all 18 genetically typed isolates belong to EU subtype 1 or Lelystad-type viruses that are distributed across Europe as well as other parts of the world (19). In Slovenia, six genetically different PRRSV strains were found in circulation, all belonging to type 1 subtype 1.

Table 3: Results of PRRSV screening in Serbia

Period	Farms/animals, n	Positive animals, n / %	Positive farms, n / %
2002	32 / 880	511 / 58.07	20 / 62.50
2004-2005	43 / 1135	540 / 47.58	28 / 65.12
2006-2007	562 / 3069	No data	11 / 20.46

Median-joining (MJ) phylogenetic networks can illustrate microevolutionary process in viruses (21), particulary for viruses in which recombination frequently occurs (22). Therefore we generated an MJ phylogenetic network based on partial 281-bp ORF7 sequences from Slovenia (n = 22), Serbia (n = 17) and Croatia (n = 7) (6, 19, 20), as well as on seven additional ORF7 sequences from Croatia (5). Sequences were aligned using the ClustalW program in MEGA 5 software (23); sequence polymorphism and haplotype diversity were computed using DnaSP 5 (2). Finally, MJ networks of haplotypes were constructed using Network 4.6.0.0. (Fluxus Technology).

The MJ network clearly shows that all Serbian and Slovenian and most Croatian sequences belong to EU type 1, subtype 1. Eastern EU subtypes 2-4, previously identified in Belarus, Lithuania, Russia and Kazakhstan, were not present among the Western Balkan samples. The phylogenetic relationships suggest that the PRRSV genotype 1 was introduced six times into the Western Balkans, and these events involved the appearance of the following six strains: a strain with an ORF5 sequence similar to that of the Porcillis PRRSV strain used in vaccines in Serbia and Croatia (5), a strain that has been circulating for a long time in Slovenia and that has mutated several times, a strain recently introduced into Slovenia, a strain in Slovenia originating from an Amervac vaccine strain, a strain present in Croatia and Slovenia, and a strain circulating for a long time in Serbia that shows homology to Eastern European subtype 3. Two Croatian sequences clustered with American virus type - Genotype 2, one of which appears to be derived from a vaccine strain, while ResPRRS vaccine was never used and registered in Croatia. These sequences came from farms that imported breeding animals from the US.

These insights from MJ phylogenetic networks should be interpreted with caution when reconstructing the evolution of PRRSV strains in the Western Balkan region. The MJ analysis is based only on partial ORF7 sequences. Definitive determination of the origins of PRRSV strains in Croatia, Serbia and Slovenia requires more comprehensive analysis based on both ORF5 and ORF7 sequences. If the preliminary results in Figure 2 are verified, they may indicate that PRRSV movement among Western Balkan countries poses a much smaller threat than import of infected animals from Western Europe.

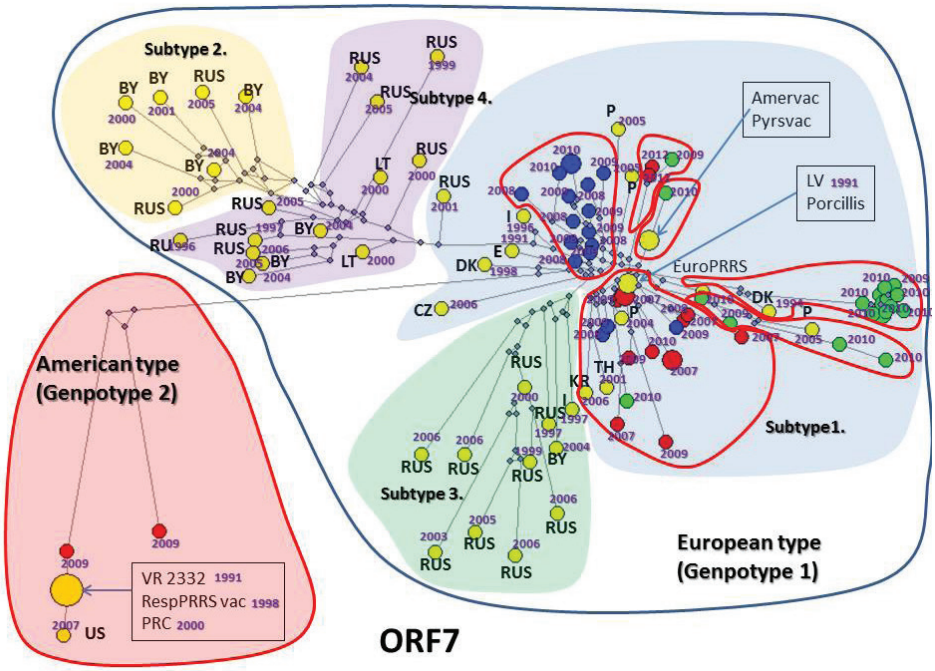

Figure 2: MJ network of PRRSV isolates from Croatia, Serbia and Slovenia based on partial ORF7 sequences, showing phylogenetic relationships among PRRSV sequences and related sequences in GenBank. Red dots indicate Croatian haplotypes; blue, Serbian; and green, Slovenian. Yellow dots indicate haplotypes isolated in other countries and deposited in GenBank. Haplotypes are labelled by geographic origin. Dot size is proportional to the frequencies of isolates belonging to the given haplotype

Conclusions

Complex trade relations and a poor understanding of the true economic costs of PRRSV have dampened political will to create control and eradication programs. Strong leadership from government agencies is needed throughout the Western Balkan region to develop guidelines for pig movements, develop diagnostic methods and screening approaches and implement requirements for clear division status at the herd and animal levels. Farmers and veterinarians need to be educated about diagnosis and biosecurity measures; uncertainties about these issues lead many to be unconcerned about PRRS as a threat. Systematic and rigorous epidemiological studies of PRRS are needed in Croatia in order to guide future monitoring, control and eradication efforts. Collaborative epidemiological studies involving the various Western Balkan countries may generate much-needed hard data on the economic impact of PRRSV, its prevalence, herd risk and effectiveness of diagnostic tools.

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NAČINI UKREPANJA IN STANJE V NEKATERIH DRŽAVAH ZAHODNEGA BALKANA GLEDE PRAŠIČJEGA REPRODUKCIJSKEGA IN RESPIRATORNEGA SINDROMA (PRRS)

D. Novosel, T. Petrović, Ž. Acinger – Rogić, M. Štukelj

Povzetek: Prašičji reprodukcijski in respiratorni sindrom (PRRS) so dokazali že pred 20 leti in še vedno povzroča velike izgube v prašičereji. V zahodnih balkanskih državah se srečujemo s specifično situacijo; v glavnem obstajajo majhne družinske reje, v katerih navadno ne izvajajo nikakršnih ukrepov zoper PRRS. Večina omenjenih držav ima nizek odstotek samooskrbe s prašičjim mesom, zato ga uvažajo iz drugih evropskih držav. Na podlagi rezultatov lahko sklepamo, da je do vnosa bolezni prišlo najprej na Hrvaškem leta 1995, malo kasneje pa v Srbiji. Drugi pomembni vnos bolezni se je zgodil pol leta po vstopu Slovenije v Evropsko unijo leta 2004. Najpogosteje v zahodnih balkanskih državah ugotavljamo genotip 1 virusa PRRS in podtip 1, medtem ko drugih podtipov, ki se pojavljajo v vzhodnoevropskih državah, nismo dokazali. Poglaviten vzrok neukrepanja zoper PRRS je v zahodnih balkanskih državah nepoznavanje dejanskih izgub, ki jih povzroča bolezen v posamezni reji, v nekaterih državah pa tudi slaba laboratorijska diagnostika. Verjetno je treba med vzroke prišteti tudi nezainteresiranost politike za izvajanje nacionalnih programov ukrepanja zoper PRRS. Izračuni so pokazali, da je PRRS na Hrvaškem leta 2011 povzročil za 17 milijonov EUR izgub v prašičereji. Poglavitni krivec za nastali položaj je veterinarska uprava, ki ni izpeljala potrebnih ukrepov. Širjene bolezni med omenjenimi zahodnimi balkanskimi državami ne predstavlja velikega tveganja, saj praktično ni trgovanja med njimi, nasprotno pa so uvozi iz drugih držav velika nevarnost za vnos novih sevov virusa PRRS. Na podlagi omenjenih dejstev bi bilo treba pripraviti načrt ukrepov zoper PRRS, ki bi med drugim vključevali tudi testiranje na prisotnost tako protiteles kot virusa PRRS in posledično vzpostavitev statusov čred, kar bi pripomoglo k zajezitvi širjenja bolezni.

Ključne besede: PRRSV; Slovenija; Hrvaška; Srbija