The monitoring and molecular epizootiology of porcine epidemic diarrhea in Ukraine during 2014-2018

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Abstract

The rapid spread of porcine epidemic diarrhea (PED) in different countries in a short time while and the significant economic damage caused by it were important reasons for conducting long-term monitoring studies in Ukraine. PED monitoring researches conduct carry out during 2014-2018 using RT-PCR and ELISA showed the presence of infection in 14 (66.67%) of 21 examined regions of Ukraine. For the period 2014-2018, the proportion of PED cases rate was the lowest in 2017 (1.76%) and the highest in 2016 (48.03%). Over the entire period, the percentage seropositive animals progressively decreased to a seronegative status indicator defined in sows in 2018. The results of determination of the virulence of 40 strains of PED virus from different regions of Ukraine using the RT-PCR method proved the circulation of highly virulent strains. The phylogenetic analysis demonstrated that the endemic strain of PED virus is included in the cluster of North American strains and the Chinese strains. Important is the fact that it is not included in the group of European low-virulent S-INDEL strains. Thus, the obtained data indicate a high probability that the PED virus was introduced into Ukraine from the territory of the Asian continent or the United States of America – (a high probability that the PED virus was translocated from the territory of the Asian continent or the United States of America into Ukraine).

Key words: *PCR; ELISA; highly virulent strain; regions of Ukraine; spike (S1) gene*

Introduction

Porcine epidemic diarrhea (PED; gender – *Alphacoronavirus*, family - *Coronaviridae*) is an emergent viral infection of pigs, which is accompanied with a high morbidity and mortality rate, especially in piglets of up to 10 days old (Sun et

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al., 2012; Lin et al., 2014; Lee, 2015). Anorexia, watery diarrhea, vomiting and dehydration are usually observed in infected piglets for several days (Li et al., 2012; Stevenson et al., 2013; Wang et al., 2019).

The rapid spread of PED among the pig livestock in Ukraine coincided with the expansion of this emergent infection throughout the world. The first epizootic outbreaks of PED were detected in the territory of Asian countries: South Korea (Park et al., 2014), China (Li et al., 2012), Taiwan (Sung et al., 2015). Almost simultaneously, PED infection had been registered since 2013 in the United States (Stevenson et al., 2013). It is believed that the spread of porcine epidemic diarrhea virus (PEDV) from the USA was directed to Canada (Pasick et al., 2014; Ojkic et al., 2015), Mexico (Lara-Romero et al., 2018), and Ecuador (Barrera et al., 2017). At the same time, the spread of PEDV in the United States was extremely progressive, resulting in the deaths of more than a million animals in 32 states. Animal losses caused significant economic damage in the USA pig farming (Pogranichniy et al., 2016; Mole, 2019). The molecular research methods that allowed us to establish the genetic relationship of PEDV strains, which were first isolated in the USA in 2013 and circulated in China throughout 2011-2012 (Chen et al., 2014).

The first officially documented data the incidents of PED infection in European countries were made public in 2014-2016, in particular in the pig farms of Italy (Boniotti et al., 2016), Austria (Steinrigl et al., 2015), Portugal (Mesquita et al., 2015), Belgium (Theuns et al., 2015), Serbia (Prodanov-Radulović et al., 2017), France (Grasland et al., 2015), Germany (Stadler et al., 2015), Hungary (Valkó et al., 2017), Ukraine (Dastjerdi et al., 2015; Masiuk et al., 2017a).

It is believed that the main ways of cross-border spread of PED are contaminated feed (Pasick et al., 2014) and the movement of infected animals (Barrera et al., 2017). However, the possible role of poorly disinfected animal transport vehicles in the emergence of new outbreaks of PED is also not excluded (Lowe et al., 2014).

Airborne (Alonso et al., 2014; Beam et al., 2015; Dastjerdi et al., 2015) and fecal-oral (Hill et al., 2014) ways are primarily considered as potential mechanisms of PEDV transmission. Infection of animals can also occur through contaminated sow milk (Sun et al., 2012) and sperm (Gallien et al., 2018). According to the results of recent experimental studies, the role of the housefly (*Musca domestica vicina*) in the mechanical spread of PEDV in a confined environment is not excluded (Masiuk et al., 2019).

The comparative analysis of whole genomes showed that the PEDV strains isolated in Germany and the USA demonstrated a high nucleotide sequence similarity. This confirms the hypothesis of a one-time or simultaneous introduction of the causative agent to Germany and Central Europe in 2014 (Hanke et al., 2015, 2017).

The significant spread of PED in Ukraine and the economic damage caused in recent years is an important reason to carry out long-term monitoring studies and genotyping of PEDV.

Materials and methods

The monitoring studies were carried out based on the Biosafety Center of the Dnipro State Agrarian and Economic University.

Observed geographic areas

The monitoring studies on the emergence and PED invasion in Ukraine during 2014-2018 covered 21 (84%) from 25 regions of Ukraine: Vinnytsia, Volyn, Dnipropetrovsk, Donetsk, Zakarpattia, Zaporizhia, Zhytomyr, Ivano-Frankivsk, Kyiv, Kirovohrad, Lviv, Poltava, Sumy,

Ternopil, Odesa, Kharkiv, Kherson, Khmelnytskyi, Cherkasy, Chernivtsi and Chernihiv.

Samples collection

During 2014-2018, 1061 samples of blood serum and 1093 samples of faeces or intestinal fragments of pigs from 291 farms were examined. At the time of the studies, no specific PED immunoprophylaxis was carried out in the farms. The population of sows in experimental farms was ranged from 200 to 10,000 heads.

From the animal of each farm, blood serum samples were taken from 3-6 sows, and in the detection on the farm of diarrhea piglets, an additional were taken 3-10 sample feces. Fecal samples or rectal swabs were collected from animals with the signs of diarrhea for examination. Besides, intestinal fragments with marked pathological changes were taken from some dead piglets. From each animal, one sample was taken. Additionally, intestinal fragments with pronounced pathological changes were taken from dead pigs.

Blood samples were collected from the cranial hollow vein or orbital venous sinus of the animals to detect PED by serological methods. After settling, serum was stored at -20 °C until the study.

Enzyme immunoassay

The detection of anti-PEDV antibodies in serum samples was performed by ELISA using the commercial Swinecheck®PED (Biovet, Canada) and ID Screen®PEDV Indirect (IDVet, France) test systems on a BioTek ELx800 enzyme immunoassay analyzer (USA) according to the manufacturers' instructions for use.

Polymerase chain reaction (PCR)

The detection of PED virus in fecal samples or intestinal fragments of pigs was performed by PCR using the commercial BIO-T KIT® PEDV / TGEV / PDCOV (BIOSELLAL, France) and EXOone PEDV OneMIX qPCR test systems (EXOPOL, Spain) according to the manufacturers' instructions for use. The detection of amplification results was performed on a CFX 96 Real-Time Systemfirms instrument (BioRad, USA) with the BioRad CFX Manager software. The detection of highly virulent PEDV strains was performed by PCR using the commercial BIO-T KIT®PEDV-all / PEDV-HV (BIOSELLAL, France) test system according to the manufacturers' instructions for use.

The spike (S1) gene sequencing

A PEDV-positive sample using RT-PCR was collected from an animal in Zaporizhia region in 2018. Sequencing was performed in the EXOPOL laboratory (Zaragoza, Spain).

First, TA cloning into the pGEM-T vector (manufactured by Promega, USA) was performed. Plasmid DNA was isolated by alkaline lysis (Lee and Rasheed, 1990). Next, Sanger bidirectional sequencing for the isolated plasmids with primers (M13-forward: 5'-GTAAAACGACGGCCAGT-3' and M13-reverse: 5'-AACAGCTATGACCATG-3') flanking the insert (MCS-multiple cloning site) was performed. For this purpose, an automatic sequencer 3500 GeneticAnalyzer (Applied Biosystems, FosterCity, CA) with the BigDye® Terminator v1.1 Cycle Sequencing-Kit (Applied Biosystems) was used.

Phylogenetic analysis

Alignment was performed by using the MAFFT bioinformatic software (V.7.2). Phylogenetic analysis was performed using the nucleotide sequences of the strain under study with those available in GenBank.

Results

Analysis of the results of monitoring studies showed that PED outbreaks were

detected in 14 (66.67%) from 21 regions of Ukraine, in particular in Vinnytsia, Dnipropetrovsk, Zakarpattia, Zaporizhia, Zhytomyr, Kyiv, Lviv, Poltava, Odesa, Kharkiv, Kherson, Khmelnytskyi, Cherkasy and Chernihiv regions. The pig farms in Volyn, Ivano-Frankivsk, Ternopil, Chernivtsi, Kirovohrad, Donetsk and Sumy regions were free from PEDV.

In Ukraine, the proportion of PED cases over the study period was 28.62% in 2014, 33.88% in 2015, 48.03% in 2016, 1.76% in 2017, and 13.15% in 2018. At the same time, percentage of seropositivity dynamically decreased and was at the level of 10.65% in 2014, 9.09% in 2015, 13.04% in 2016, 1.23% in 2017, and 0% in 2018. The presence of PEDV was confirmed by PCR in 10.65% of samples of biological material in the first year of the study, in 43.18% in the second, in 61.08% in the third, in 2.71% in the fourth and in 33.70% in the fifth (Table 1).

PEDV had been revealed in Dnipropetrovsk, Zaporizhia, Kherson, Cherkasy and Chernihiv regions, starting from 2014. The subsequent spread of PEDV had been demonstrated in Kyiv, Poltava and Kharkiv regions since 2015. Starting from 2016, PEDV had been detected in Odesa, Vinnytsya, Zakarpattia and Lviv regions, and from 2018 in Zhytomyr and Khmelnytskyi (Fig. 1).

The high proportion of PED cases was determined in Zaporizhia, Cherkasy and Vinnytsya regions of Ukraine, while the average proportion of PED cases was shown in Dnipropetrovsk and Kharkiv regions and low proportion of PED cases in Kherson, Poltava, Zakarpattia, Lviv, Khmelnytskyi, Odesa, Kyiv and Chernihiv regions (Fig. 2).

The results of PCR studies of 40 samples of biological material from different regions (Dnipropetrovsk, 2014, 2016; Zaporizhia, 2014, 2015; Cherkasy,

Table 1. Dynamics confirmed of PED cases in Ukraine (Biosafety Center Results during 2014-2018)

Time of collection	Methods of diagnostic	Number of samples tested	Positive samples	Percent positivity
2014	ELISA	169	18	10.65
	PCR	142	71	50.00
	Total	311	89	28.62
2015	ELISA	66	6	9.09
	PCR	176	76	43.18
	Total	242	82	33.88
2016	ELISA	138	18	13.04
	PCR	370	226	61.08
	Total	508	244	48.03
2017	ELISA	405	5	1.23
	PCR	221	6	2.71
	Total	626	11	1.76
2018	ELISA	283	0	0
	PCR	181	61	33.70
	Total	464	61	13.15

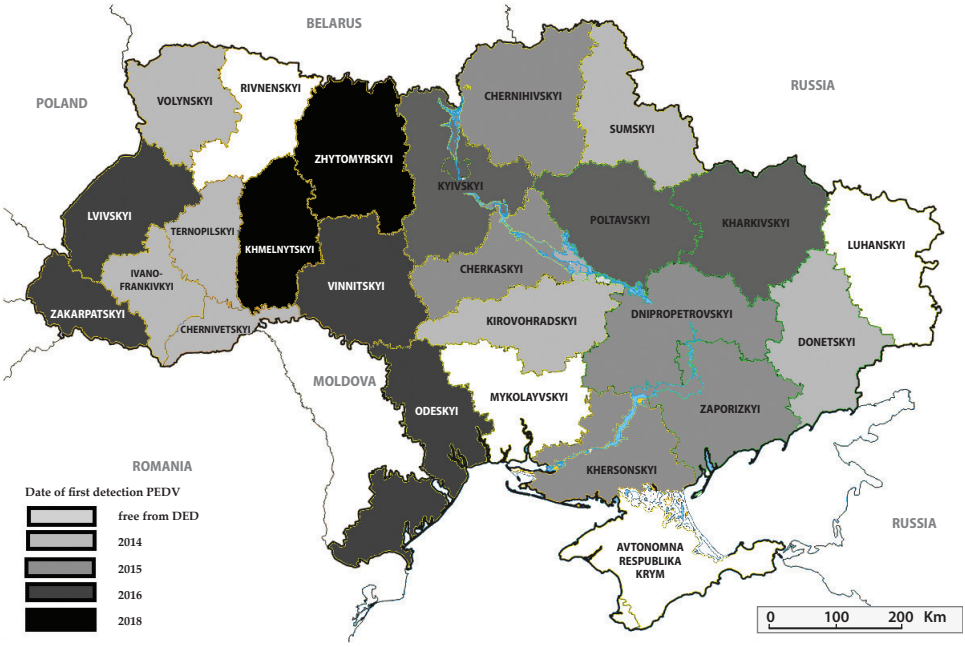

Fig. 1 PED Outbreaks Map by Regions of Ukraine (Biosafety Center Results during 2014–2018)

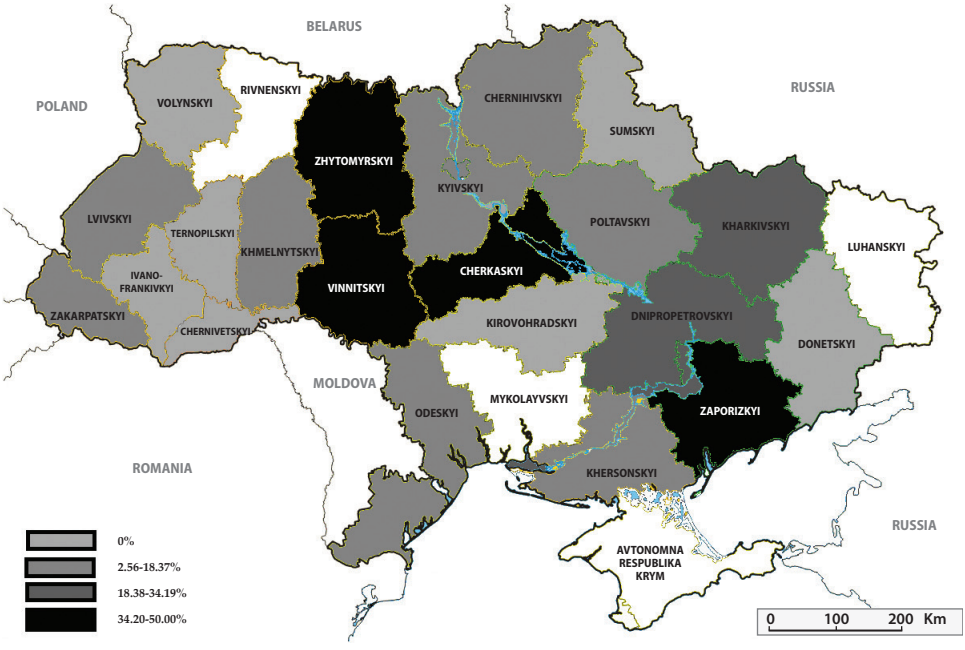

Fig. 2 Map by Regions of Ukraine of the proportion of PED cases (Biosafety Center Results during 2014-2018)

2016; Chernihiv, 2014; Kyiv, 2015, 2016; Cherkasky 2018) indicated the circulation of highly-virulent PEDV strains in Ukraine.

It should be noted that Dnipropetrovsk, Zaporizhia, Kherson, Cherkasy and Chernihiv regions, in which there had been an unfavorable situation in terms of PED since 2014, have different levels the proportion of PED cases. This indicates the absence of a causal relationship between the time of the first outbreaks and the level the proportion of PED cases in the regions of Ukraine.

The phylogenetic analysis based on spike (S1 gene) sequencing showed that the strain under study had 89% of homology to PEDV deposited in GenBank from Slovenia, 2015 (No. KU297956), France, 2014 (No. KR011756), Italy, 2016 (No. KT0274413), Germany, 2014 (No. LM645057), Belgium, 2015 (No. KR003452), as well as 89.5% of homology to the PEDV S-INDEL strain described in the USA in 2014 (Fig. 3).

The largest similarity (from 97.6% to 98.5% of homology) of the spike (S1) gene nucleotide sequence of the strain under study was found with several highly virulent PEDV strains described in the USA and China in 2011-2013, which clusters them into the North American group of strains (No. KF468752, No. KF468753, No. KF452322), and the Chinese strains (No. JX489155, No. JX088695, No. KC210145). In addition, 98.4% of homology was found between the highly virulent strain studied in present work and the recently described PEDV strain, isolated in Poltava region of Ukraine (No. KR403954).

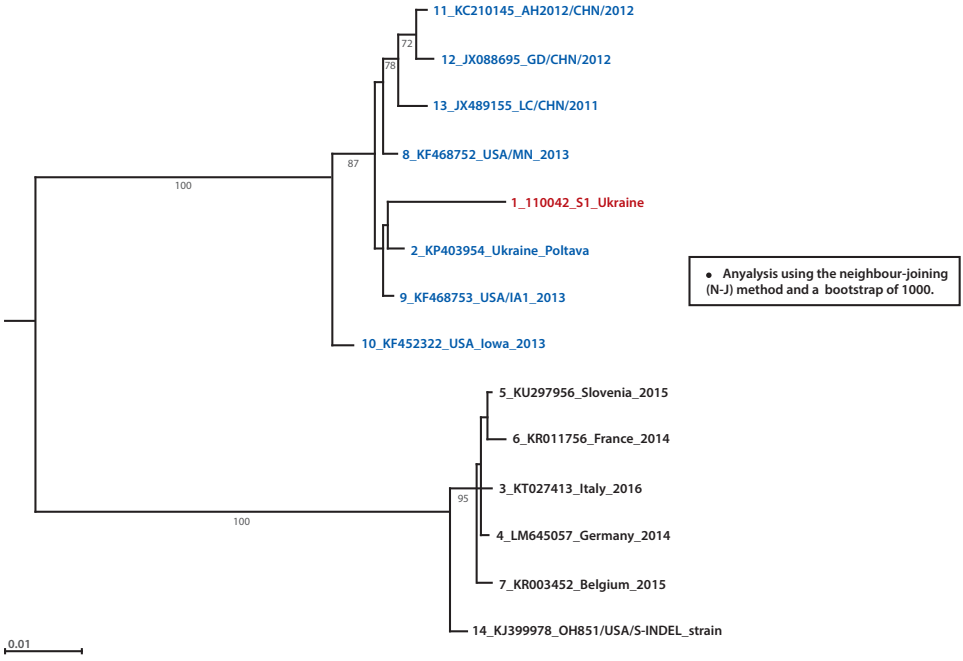

Fig. 3 Phylogenetic tree: the partial sequence for the Spike gene (S1) of investigated case (in red) was compared with sequences of PEDV described from some European countries (deposited at the GenBank), and also with the highly virulent PEDV strains reported in USA, China and recently in Ukraine (in blue)

Discussion

The sudden invasion and extremely rapid spread of PEDV in the United States led to significant economic losses (Mole, 2019). In a short period of time, PED spread across the countries of the American and European continents. Cases of PED infections had been identified in Ukraine, as in most European countries, since 2014 (Dastjerdi et al., 2015). The results of monitoring studies attested PEDV circulation in farms of most regions that covered the western (Zakarpattia, Lviv and Khmelnytskyi regions), eastern (Kharkiv region), southern (Zaporizhia, Odesa and Kherson regions), northern (Zhytomyr, Kyiv and Chernihiv regions) and central (Vinnytsya, Dnipropetrovsk, Poltava and Cherkasy regions) parts of Ukraine. Using PCR and ELISA, it was found that during 2014-2018, the proportion of PED cases in Ukraine was the lowest (1.76%) in 2017 and the highest (48.03%) in 2016. The negative serological status of animals was determined in 2018.

The wide spread of PED at the farms of different regions of Ukraine can be explained by the insufficient level of biosafety and biosecurity of disadvantaged farms. On the other hand, this was also facilitated by the lack of timely comprehensive information about the emergence of the infection in the country and the characteristics of its course, and by the fact that the ways of its ingress to the farms and preventive measures were not discussed. Thus, it was shown in our previous report that the development of PED infection in a significant number of farms was caused by the contact of pigs with contaminated transport of meat-processing plants (Masiuk et al., 2017b). Only some time later, the farms demanded that the pork purchasers wash the vehicles before each shipment of animals, and also began to disinfect contact surfaces of the vehicles and shipping platforms themselves. In a number of farms, no farm bioprotection during the shipment of animals for their further transportation to the meat-processing plant was envisaged at all. Contacts with small purchasers of sanitary spoilage that are in constant contact with a large number of small farms with an unsatisfactory level of bioprotection, and also constantly mix livestock of pigs and piglets from different sources (Masiuk et al., 2017b) were especially dangerous.

The results of our studies to determine the virulence of 40 PEDV isolates from different regions of Ukraine by PCR proved the circulation of highly virulent strains. Accordingly, the detected PED outbreaks were characterized by significant morbidity and mortality rate of young stock (Dastjerdi et al., 2015; Masiuk et al., 2017a). The Ukrainian strain, first identified in 2014 in Poltava region, was determined as highly virulent (Dastjerdi et al., 2015), which is fully consistent with the scale of the identified PED outbreaks.

The European PEDV strains, isolated since 2014, belong to the S-INDEL group. These strains are low virulent, as they cause a benign course of PED and lower morbidity and mortality rate (Stadler et al., 2018). The analysis of whole-genome sequencing showed that S-INDEL PEDV strains circulating in European countries have low similarity with highly virulent strains from the USA and China. At the same time, they differ from the European strains isolated before 2014 (Hanke et al., 2015).

The phylogenetic analysis carried out by us showed a high similarity of the PEDV strain under study with the highly virulent Ukrainian strain (No. KP403954) and the group of North American strains (No. KF468752, No. KF468753, No. KF452322), and the Chinese strains (No. JX489155, No. JX088695, No. KC210145). In addition, the Ukrainian

strains differed from the S-INDEL group of low-virulent strains isolated in the territory of Europe (No. KU297956, No. KR011756, No. KT0274413, No. LM645057, No. KR003452) and America (No. KJ399978). The data obtained in our study indicate that the Ukrainian strains belong to the non-S-INDEL group of highly virulent PEDV strains. Moreover, the conducted phylogenetic analysis showed a high probability of the spread of highly virulent PEDV strain/strains from the Asian continent or the United States of America in the territory of Ukraine.

Conclusion

The results of monitoring studies conducted in 2014-2018 showed a complex time-geography dependence of PED spread in the regions of Ukraine due to the factors independent of each other, such as the time of the first outbreak of infection and the proportion of PED cases in certain regions of Ukraine. PEDV was found in 14 (66.67%) of 21 regions of Ukraine. The phylogenetic analysis showed that the endemic strain belongs to the non-S-INDEL group of highly virulent PEDV strains with a high probability of PEDV strain/strains entering the territory of Ukraine from countries of the Asian continent or the United States of America.

Declaration of conflicting interests

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Praćenje i molekularna epizootiologija epidemijskog proljeva svinja u Ukrajini tijekom 2014.-2018. godine

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Brzo širenje epidemijskog proljeva svinja (PED) u različitim zemljama tijekom kratkog razdoblja, koje je rezultiralo znatnom ekonomskom štetom, potaknulo je potrebu za dugoročnim studijama praćenja u Ukrajini. Praćenje PED uporabom RT-PCR i ELISA u razdoblju 2014.-2018. godine pokazalo je prisutnost infekcije u 14 (66,67 %) od 21 ispitane regije Ukrajine. Tijekom ovog razdoblja pojavnost slučajeva PED bila je najniža 2017. godine (1,76 %), a najviša 2016. godine (48,03 %), s progresivnim padom postotka seropozitivnih životinja i seronegativnog indikatora statusa definiranog u krmača 2018. godine. Rezultati određivanja virulencije 40 sojeva PED virusa iz različitih regija Ukrajine uporabom RT-PCR metode dokazali su kruženje vrlo virulentnih sojeva. Filogenetska analiza pokazala je da se endemski soj PED virusa grupirao sa sjevernoameričkim i kineskim sojevima. Potrebno je napomenuti da se nije grupirao s europskim S-INDEL sojevima niske virulentnosti. To navodi na zaključak da je PED virus vrlo vjerojatno unesen u Ukrajinu iz Azije ili SAD-a.

Ključne riječi: *PCR, ELISA, vrlo virulentni soj, regije Ukrajine, Spike (S1) gen*