O. Ivashchenko

THE SPREAD OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN UKRAINE

В статье приведены результаты исследований по выявлению антител к вирусу репродуктивного и респираторного синдрома свиней (ВРРСС) из 23 областей Украины и АР Крым. Результаты исследования свидетельствуют о циркуляции ВРРСС в 28 хозяйствах 19-ти областей Украины. Обнаружено тенденцию распространения ВРРСС на территории Украины по сравнению с исследованиями эпизоотической ситуации 2006-2007 годов.

The results of this study concern the detection of antibodies against PRRSV in serum from 23 regions of Ukraine and the Crimea. The results indicate porcine reproductive and respiratory virus (PRRSSV) circulation in 28 farms from 19 regions of Ukraine. The end results show trends of spreading PRRSV in Ukraine compared with studies of epizootic situation in 2006-2007.

Porcine reproductive and respiratory syndrome virus are currently prevalent in most pork producing countries. It's one of the main causes of severe reproductive disorders in swine and respiratory disease complex [1,2]. Currently, due to losses associated with these reproductive and respiratory problem in pigs PRRSV is considered to bean economically importantviral pathogen in the pork industry [2]. This contagious disease of pigs of different ages may manifest acute or subclinical [3].

PRRSV is a member of the family *Artereviridae* in the order *Nidovirales.* Sequencing and antigenic properties showed existent of two distinct genotypes Europinian (type 1) and North-American (type 2) [2]. The two genotypes shared only about 63% nucleotide identity on the genome level. The evolution rate of PRRSV fixed as the highest among RNA viruses [5] and it is one of the causes of complexity in diagnostics, vaccination and controlling PRRSV.

From the first time PRRSV was detected in Canada in 1979, it rapidly spread in North America in the late 80s. Genetically different PRRSV, with similar clinical occurrence, appeared in Europe and spread through swine-growing regions during 1990-1992 [1, 6].

For more than10 years, PRRSV spread in most pig populations. Currently the virus is recorded in the majority of pork-producer countries. PRRSV was not found only in Australia, New Zealand, Norway, Finland, Sweden and Switzerland [1].

Clinical manifestations of PRRSV vary depending on the virulence of the virus, the immunestatus of the herd, and the age of infected animals. The disease, which clinically manifests, is a result of viremia. Ability of PRRSV to overcome transplacental barrier and infect the fetus causes abortions in sows and births of weak, nonviable piglets [1, 4].

Most of the clinical manifestations associated with PRRSV infection were investigated by experimental infection. Field observations of PRRSV infection in combination with other viral and bacterial agents can modify and complicate the clinical manifestations of disease [3, 7, 8]. The similarity of clinical signs of disease and diversity of reproductive diseases of pigs complicates setting even priordiagnosis. It is necessary to use laboratory diagnostic methods for the diagnosis of infection of PRRSV in infected animals. ELISA is simple in works, fast, reliable enough, with a high specificity method. All these advantages make ELISA one of the methods of choice for the diagnosis of PRRSV.

The epizootic situation in Ukraine of PRRSV was studied not enough and that was why our main aim was to study the spread of the porcine reproductive and respiratory syndrome virus in Ukraine.

Materials and Methods. For detection of antibodies to PRRSV, 108 herds were selected on the basis of clinical signs. This selection was based on information of the herd veterinarian. In all herds, the sampling of serum samples from animals of different technological group was carried out. Samples of serum were taken from the main herd animals with the clinical science of PRRSV and from boars in these farms. Biological material was delivered in thermal containers with ice. The volume of each sample was 3ml. It also recorded the necessary information: clinical science, animal age, name of the farm, sector, region. For this study 7,937 serum samples were collected from animals from 23 regions of Ukraine.

Sera were tested for the presence of PRRSV antibodies by ELISA (IDEXX HerdCheck PRRS 2XR ELISA, USA). Overall procedure was performed according to manufacturer's manual.

Samples of serum were collected 10-14 days after the appearance of first clinical signs [4, 9]. The presence of maternal antibodies to PRRSV and the fact of their decreasing to 9 weeks of animal life were also taken into account [4, 10].To obtain reliable results, serum samples were collected from animals of different technological groups from one farm [9] in an amount not less than 12 [4].

Results and their Discussion. Currently PRRSV was found in most pork producing countries. The genetic material of the PRRSV was detected in France, Germany, Belgium, Denmark, Spain, Italy, Czech Republic, Latvia, Lithuania, Russia, Belarus, Kazakhstan, Ukraine, China, Japan, Vietnam, Thailand, and the United States [11, 12, 13, 14].

In 2006-2007, Havrasyeva et.al and others studied the distribution PRRSV in 16 regions of Ukraine, the circulation of the virus was detected in 12 of them [15]. It should be noted that in 2007 VRRSS not diagnosed in animals of Ternopil and Chernihiv regions, while in 2011 the PRRSV positive animals in these regions were found. The results of Havrasyeva et.al. in the Dnipropetrovsk region from 2006 showed 1.25% of PRRSV positive animals by ELISA. The increase of the number of PRRSV infected animals were observed (7, 5%) in 2007 [15]. In 2011, we recorded PRRSV infection in 39% of the animals. It indicated the spread of the PRRSV through Ukraine.

In 2006, PRRSV was diagnosed in 41.3% of animals from Kyiv region, whereas in 2007 no serologically positive PRRSV animals were found in this region [15]. These results can be explained by selection oft he blood serum samples from animal of different farms in the Kyiv region, in sufficient sample size in 2007, or different age groups of animals that were studied. In 2011, we studied nine farms from the region around Kyiv and detected 30,5% of serologically positive for PRRSV animals. However, this data may no treflect that the reduction of circulating virus in the region was due to significan tdifferences in sample size. This is different from the way the serum was analyzed from two farms of this region in 2006, compared to the 9s in 2011.

It is also necessary to emphasize the epizootic situation stability in the Kherson region and in the Crimea. The antibodies against PRRSV were not detected in serum samples from this region in 2007, 2011 years [15].In general, we have analyzed 7937 serum samples from 108 farms in 23 regions of Ukraine and Crimea. 1265 serum samples were serologicaly PRRSSV positive (Table1).The circulation of the virus detected in 21 regions of Ukraine (Table 1., Fig. 1).Our results significantly complete the existing information about spreading PRRSSV and indicate the trend of spreading the pathogen in the farms of Ukraine. PRRSSV

circulation was found in 28 herds, while the situation of the disease in 4 farms remain uncertain. It requires additional studies and further monitoring of blood serum for the presence of increasing or decreasing the level of antibodies to PRRSV and determine the number of PRRSSV positive animals in these farms (Table.1).

***Table* 1. The results of detection PRRSSV antibodies in serum samples**

№	Region	No. of farms	No. of seropositive farms	No. of samples	No. of seropositiv samples	No. of Seropositiv samples %
1	Vinnitsa	6	2	46	16	34, 8
2	Volyn	2	1	110	6	5, 5
3	Dnypropetrovsk	15	3	410	160	39
4	Donetsk	10	3	754	314	41, 6
5	Zhytomyr	3	1	52	41	78, 8
6	Zaporyzha	8	4	4798	263	5, 5
7	Zakarpatska	4	2	88	25	28, 4
8	Kyiv	9	1	315	45	30, 6
9	Kirovohrad	3	1	147	24	16, 3
10	Lugansk	1	0	6	0	0*
11	Lvyv	4	2	99	61	61, 6
12	Mucolayv	1	0	10	0	0*
13	Odessa	3	1	57	7	12, 3
14	Poltava*	3	1	146	1	0,7*
15	Rivne	1	0	12	0	0
16	Sumy	4	2	176	78	44, 3
17	Ternopil*	2	1	29	1	3, 4*
18	Kharkiv	4	1	193	129	66, 8
19	Kherson	3	0	39	0	0
20	Khmelnytsky	3	2	58	46	79, 3
21	Cherkasy	8	2	99	10	10, 1
22	Chernivtsi	4	1	25	4	16
23	Chernihiv	6	1	92	33	30,3
24	Crimea	2	0	103	0	0
Total	23 regions and Crimea	109	32	7937	1265	

* regions requires further research to establish the epizootic situation based on a small number of serologically positive animals

Fig.1. The spread of the porcine reproductive and respiratory syndrome virus in Ukraine

– PRRSV circulation were detected

– No PRRSV circulation were detected

– areas that require further research to establish the epizootic situation

– areas that weren't studied

– areas that were studied in 2006-2007 [15]

With this data we can come to a conclusion about the circulation of the virus in 19 regions of Ukraine. In the herds of Poltava and Ternopol regions, there was detected one PRRSV positive serum. We believe more research is needed to monitor dynamic changes of PRRSV antibodies in serum for the establishment of the epizootic situation in the farms in these areas. PRRSV was not detected only in herds from Lugansk, Rivne, Kherson regions, and AR Crimea (Table1). However, it should be noted that the number of samples that we examined in Lugansk, Rivne and Kherson regions were insufficient for analysis of epizootic situation and therefore, more research is needed.

Within a herd, PRRSV can rapidly spread through nose-to-nose contact and inutero infections [3, 12]. There are a number of factors to the contribution of PRRSV such as animal welfare, increasing herd size, carriers from purchased animals, and sperm [3, 12]. Infection can also be spread through PRRSV-contaminated clothing via injection [3]. Active animal trade, the purchase of pigs, and semen from different farms in Ukraine and the world, non-technology holding of animals, and a high variability of the pathogen can cause the spread PRRSV in Ukraine.

Conclusions and Prospects for Further Research. 1. The results suggest the circulation of the porcine reproductive and respiratory syndrome virus in 28 farms in 19 regions of Ukraine. 2. The results significantly complete the existing information about spreading PRRSV and indicating the spreading trend of the pathogen through Ukrainein comparison with studies epizootic situation 2006-2007. 3. It is important to create a bank of PRRSV isolates to make their sequencing for further analysis of the situation with PRRSV in Ukraine.

1. Kim H.K., Yang J.S., Moon H.J. et al. Genetic analysis of ORF5 of recent Korean porcine reproductive and respiratory syndrome virus (PRRSVs) in viremic sera collected from MLV-vaccinating or non-vaccinating farms // Journal of Veterinary Science. – 2009. – Vol.10. – P.121-133. 2. Van Breedam W., Costers S., Vanhee M. et. al. Porcine reproductive and respiratory syndrome virus (PRRSV) – specific mAbs: supporting diagnostics and providing new insights into the antigenic properties of the virus // Veterinary Immunology and Immunopathology. – 2011. – P.1-12. 3. Rownald R.R.R. The interaction between PRRSV and the late gestation pig fetus// Virus Research – 2010. – Vol. 154 – P.114-122. 4. Duinhof T.F., Schaik G. van, Esch E.J.B. van, Wellenberg G.J. Detection of PRRSV circulation in herds without clinical signs of PRRSV: Comparison of five age groups to assess the preferred age group and sample siza // Veterinary Microbiology. – 2001. – Vol.150 – P. 180-184. 5. Forsberg R. Divergense time of porcine reproductive and respiratory syndrome virus subtypes// Mol. Boil. Evol. – 2005. – Vol 2. – P. 2131-2134. 6. Amonsing A., Kedkovid R., Puranaveja S. et al. Comparative analysis of complete nucleotide sequence of porcine reproductive and respiratory syndrome Virus (PRRSV) isolates in Thailand (US and EU genotypes) // Virology Journal. – 2009. – Vol.6. – P.280-289. 7. Инфекционные болезни животных / Б.Ф. Бессарабов, Е.С. Воронин и др.; Под ред. А.А. Сидорчука. – М.: КолосС, 2007. – 671 с. 8. World organization for modern health. PRRS: the disease, its diagnosis, prevention and control // Report of the OIE AD HOC group on reproductiveandrespiratorysyndrome. – Paris.-2004. 9. Ladinig A., Balka G., Weissenboeck H., Saalmueller A., Gerner W., Kaeser T., Rusvai M., Ritzmann M. PRRSV (Type I) challenge in finishing pigs // International symposium on emerging and re-emerging pig diseases, 2011. 10. Kim S.H., Roh I.S., Choi E.J., Lee C., Lee C.H., Lee K.H., Lee K.K., Song Y.K., Lee O.S., Park C.K. A molecular analysis of European porcine reproductive and respiratory syndrome virus isolated in south Korea// Vet Microbiol. – 2010. – Vol. 143. – P. 394-400. 11. Stankevicius A., Cepulis R., Zilinskas H., Stadejek T., Pejsak Z. Persistence of European and American type PRRSV strains within Lithuanian pig herd // Bull. Vet. Inst. Pulavy. – 2008. – Vol.52. – P.319-323. 12. Murtaugh M.P., Stadejek T., Abrahante J.E., Lam T.T.Y., Leung F. C-C. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus// Vir. Res. – 2010. – Vol.154. – P.18-30. 13. Karniychuk U.U., Geldhof M., Vanhee M., Van Doorsselaere J., Saveleva T.A., Nauwynck H.J. Pathogenesis and antigenic characterization of a new East European subtype 3 porcine reproductive and respiratory syndrome virus isolate//BMC Vet. Res. – 2010. – Vol.6. – P.30. 14. Shi M., Lam T.T.Y., Hon C-C., Hui R.K.H., Faaberg K.S., Wennblom T., Murtaugh M.P., Stadejek T., Leung F.C.C. Molecular epidemiology of PRRSV: A phylogenetic perspective//Vir. Res. – 2010. – Vol.154. – P.7-17. 15. Гаврасьєва Н.В., Головко А.М., Троценко З.Р., Абрамов А.В. Ретроспективна діагностика репродуктивного та респіраторного синдрому свиней методом імуноферментного аналізу// Ветеринана біотехнологія. – 2008. – №13.

Надійшла до редколегії 17.04.12

A. Kharina, PhD, D. Chaika, stud, S. Zaika, PGS, V. Polischuk, PhD

SELECTION OF BACTERIOPHAGES, SPECIFIC TO *SERRATIA MARCESCENS*

Представлены результаты скрининга бактериофагов специфичных к возбудителю гнили лука. Высокостабильный литический фаг, специфичный к S. marcescens выделенный из окружающей среды и обозначен как бактериофаг Smd. У выделенного фага изучена морфология вирионов и состав белков. Показана способность фага Smd1 подавлять развитие бактериальной инфекции на чешуйках лука.

The results of screening bacteriophages specific to causative agent of onion decay are presented. Highlystable lytic phage specific for ***S.marcescens was isolated from the environment and denoted as phage Smd1. The virion morphology and protein content of isolated bacteriophage was studied. There was shown the ability of phage isolate Smd1 to reduce the development of bacterial infection on an onion scales.***

Introduction. Modern society focuses on ecologically pure future. Guarantees of that are "naturalization" of production, management of natural resources and energy.The preservation of crop losses caused by bacterial pathogens is significant problem for Ukraine and international community. Nowadays pesticides and herbicides, that in chemical nature are antibiotic substances, are widely used for prophylactic and therapeutic purposes. Only the last 20-30-years scientists began to pay special attention to the processes of resistance formation of pathogenic bacteria to the antibiotics. Therefore people faced the problem of search and development of new protective forms that would be characterized by high efficiency, safety and would not cause rapid adaptation of bacteria. The hypothesis of the possibility of using bacteriophages (viruses of bacteria) for therapeutic purposes was nominated in the beginning of the 20^{th}century. Nowadays, this direction develops rapidly and expands the range of phage usage [2,3]. Significant development as acquired agricultural phage therapy that is engaged in elaboration of phage preparations to deal with pathogenic bacteria (for example, *Erwinia carotovora*, *Pseudomonas sp.*, *Serratia marcescens*).

Due to the importance and attractiveness of the area of research, the aim of our study was the selection and characterization of lytic phages, specific to the pathogen *Serratia marcescens*, which causes an onion decay and is considered to be one of the most harmful phytopathogens for agriculture in Ukraine.

Materials and methods.

Bacterial strains. Studies were carried out on bacterial culture *Serratia marcescens* IMBG291 [5], generously provided by colleagues from the laboratory of microbial ecology, Institute of Molecular Biology and Genetics (National Academy of Sciences of Ukraine). Working with phages we used an overnight culture of bacteria, in which bacteria was in exponential phase of growth. The concentration of bacteria cell culture was 10^8-10^9 c.f.u./ml. Bacteria was cultivated on plate count agar or in PC-broth. Incubation temperature was 25 ^{O}C.

Sample collection. During laboratory studies a wide range of samples was analyzed. Samples were selected from vegetable washouts (including onion, potatoes, cab-