At present, one of the global problems of poultry is Marek's disease, not only because of its unpredictability, but also because effective vaccines have been used in the past may be ineffective in modern times and it can lead to that future losses from this disease may be catastrophic.

Economic losses thus can vary from 10% to 60%, depending on the forms of the disease. You also need to take into account the costs of veterinary-sanitary measures on elimination of disease and losses due to reduced productivity.

Vaccination of poultry is one of the most important methods to control Marek's disease. At the present state, the commercial market, there are plenty of vaccines against the disease, made of different strains of Marek's disease virus, which is prepared in the form of mono-, bi- and polyvalent vaccines of cultural.

On modern, the most common vaccines against Marek's disease are three living made from attenuated strains first Marek's disease virus serotype, with natural strains of attenuated neonkonennyh second serotype and heterologous strains of herpes virus of turkeys third serotype.

In Ukraine you can find a large amount of vaccines against the disease (Nobillis Rismavac, TAD Marec vac forte, Cryomarex Rispens+HVT, "Marek Avivak-3", "Marek Avivak 1+3", "Marek Avivak 1+2+3"), but they are all overseas. Therefore, the development of domestic vaccine on current strains in Ukraine is an important issue.

Keywords: Marek`s disease, serotype, etiology, pathogenesis, prevention.

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COMPARATIVE MOLECULAR EPIDEMIOLOGY OF BOVINE VIRAL DIARRHEA IN UKRAINE AND CHINA

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Today and for Ukraine and for Republic of China, agriculture and the livestock component are the most important social and economic points. Sustainable development of strategic industries such as dairy and beef cattle have the greatest significance. The economic feasibility of livestock industries closely related to animal performance and compliance technologies of livestock products. Obstacle to obtaining products cattle often become economically significant viral infection.

Keywords: bovine viral diarrhea, PCR, genotyping, phylogenetic analysis.

Bovine viral diarrhea - pestivirus disease of ruminants, accompanied by various lesions of epithelial tissue, abortion, pneumonia, vaginitis, enteritis and diarrhea, and acute syndrome of exhaustion and sometimes high loss [1]. BVDV causes infection of the fetus, leading to persistent infection of calves born, whose rates of growth and development is unsatisfactory [2]. In populations of cattle there are several genetically heterogeneous subpopulations of the pathogen. Their thorough study of the position of molecular epizootiology will effectively create a specific means of prevention and diagnosis of infection.

Because of the exclusive economic importance bovine viral diarrhea, the main purpose of the presented project is to study the epizootic situation of bovine viral diarrhea, isolation and genetic characterization of pathogens circulating in China and Ukraine, their phylogenetic comparison.

The main aim of this study is investigate of the epidemiological situation for BVD, isolation and genetic characterization of viral strains, circulating in both countries (China and Ukraine) and their phylogenetic comparison.

Material and method. *Cattle and sample collection.* 224 clinical samples of cattle from 11 region (Dnepropetrovsk, Kirovohrad, Kyiv, Lviv, Mykolayiv, Poltava, Sumy, Kharkiv, Kherson, Cherkassy, Chernihiv) of Ukraine and Simferopol region of the Crimea were used for this study.

Animals were selected of different ages from newborns. Materials for detection of BVDV RNA by PCR were stabilized blood by sodium citrate (5 %), blood serum, semen, vaginal swabs and nasal washings. After sampling the sample were stored at 4 °C for starting investigation, after there - at minus 70 °C.

PCR. Extraction of RNA from clinical samples was performed using silica-based extraction method [3].

Second strand cDNA synthesis and amplification was applied using «GenePak RT-PCR Core» and «GenePak PCR Core» (Ltd. Lab. Isogene, Russian Federation) according.

The primers 324/326 were used for PCR amplification of 5’ UTR gene, designed by S. Vilcek *et al.* [4]. The PCR-amplification was performed with temperature program consisting of initial denaturation (95 °C, 2 min)

and 35 cycles of denaturation (95 °C, 1 min), primer annealing (56 °C, 1 min) and primer extension (72 °C, 1 min) in an automatic thermalcycler Biometra T3000. A final step of extension at 72 °C for 10 min. (Table 1).

Table 1 – PCR amplification cycle

Cycle	Temperature	Duration	Repeats
1	95 °C	2 min	1
2	95 °C	1 min	35
	56 °C	1 min	
	72 °C	1 min	
3	72 °C	10 min	1

As positive controls were used samples of BVDV strains Oregon, Osloss and Kosice. 25 μL of the PCR products were evaluated by gel electrophoresis in 1,5 %

Phylogenetic study. Samples, determined as positive in PCR were studied with sequencing. Sequencing of obtained by PCR fragment of BVDV gene was done in Lanzhou Veterinary Research Institute (China). Phylogenetic analysis in 5'-UTR (245 bp fragment) was used for the genetic typing of BVDV isolates into subgenotypes. Phylogenetic trees were constructed by Neighbor Joiningand Maximum parsimony algorithms. Pair distance was determined by Murakami algorithm. All phylogeny trees buildings and analyses were done with modules of MEGA 5 software.

Results and discussion. Ukraine currently has 4.5 million cattle [5]. These animals are kept in 4350 herds, of which 10 to 20 % have 900 and more animals, with the biggest ones approaching 7000 animals. In addition to the very large herds, there are an unknown number of small privately owned herds in Ukraine. About 60 % of the herds produce milk and about 30 % are in mixed milk and beef production. The rest are beef herds. The extent of animal traffic between the herds is not known in detail but most likely a large fraction of male calves end up in beef herds because only a relatively small number of bulls are required for natural breeding and artificial insemination. The intensity of import and export is not known at this time. There is no systematic testing for BVD, nor is there a systematic approach to controlling BVD. Inactivated and attenuated BVDV vaccines are in use, but the extent of use and the efficacy of these vaccines are unknown.

With a view of the investigating of the epidemiological situation for BVD was conducted molecular-genetic study of animals clinical samples from different Ukrainian farms.

Monitoring studies during 2013 have covered 34 farms from 11 regions of Ukraine (Dnepropetrovsk, Kirovohrad, Kyiv, Lviv, Mykolayiv, Poltava, Sumy, Kharkiv, Kherson, Cherkassy, Chernihiv) and Simferopol region of the Crimea (Table 2).

Table 2 – Research results of clinical material from cattle for presens BVDV

Region	Number of samples tested	Number of positive samples
Dnepropetrovsk	8	6
Kirovohrad	5	-
Kyiv	10	-
Lviv	4	3
Mykolayiv	9	-
Poltava	8	1
Sumy	11	1
Kharkiv	134	11
Kherson	15	5
Cherkassy	3	-
Chernihiv	5	2
The Crimea	12	-
Total	224	29 (12,9 %)

At this stage of study the 224 samples of clinical material from cattle was investigated, including samples of blood, serum, semen, vaginal scrapings and nasal washings. BVDV was detected in 29 samples (12,9 %) of the investigated.

18 Ukrainian isolates of BVDV were studied by sequencing. The genetic typing of viral isolates revealed that only BVDV type 1 viruses were presented. The phylogenetic analysis confirmed two BVDV-1 subtypes, namely b and f (Fig. 1).

16 viruses were typed as BVDV-1b and all of them were absolutely identical in 5'-UTR, but 2 samples of BVDV were typed as BVDV-1f. The genetic diversity, demonstrated in the study, releases the belonging of characterizedviruses to BVDV-1b strains with the distance not more 2–4 %. This is typical in the current genetic studies of worldwide characterized viruses. Allocated viruses of this subtype are truly same inside this clade of Ukrainian viruses.

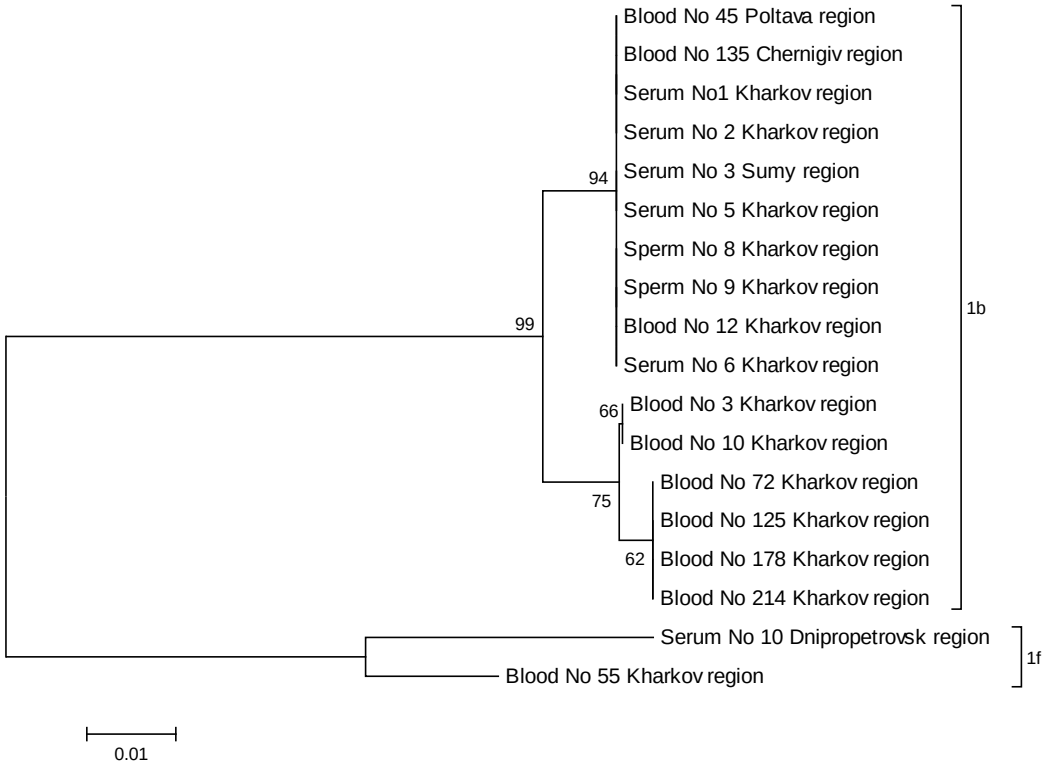

Fig. 1. Genetic typing of BVDV isolates in the 5'-UTR region

Another detected subtype was 1 f. This group of BVDV-1 was also detected in several countries of the Central and Western Europe, so they are not unique. Characterized isolate had 4.5 % differences among subtype-belonged related viruses of BVDV-1 f genotype.

Viral genetic divergence studies allows to study the molecular diversity of virus for the creation of effective prevention means, and gives the opportunity to determine viral origin and source for recognition of the epidemiology of bovine viral diarrhea and its eradication strategy development.

BVDV represented by two genotypes: BVDV-1 and BVDV-2. Virus of genotype 1 is widely distributed in the world and often registered in the territory of Ukraine. BVDV genotype 2 circulates mainly in North America. While BVDV-1 strains usually cause a mild form of diarrhea in immune cattle, some strains of BVDV-2 is highly virulent and leads to severe thrombocytopenia, multiple hemorrhages and inflammation of the mucous membranes.

It was established that the occurrence of nucleotide mutations in the genome of BVDV is constrained by the need to maintain the enzymatic and other biological features essential to the survival of organisms [6]. However, it is shown that BVDV-1 contains, in addition to the long-known genetic groups 1 a and 1 b [7], an additional group. For example, in the study [8] demonstrated the possible existence of 11 genetic groups. Scientists of the Institute of Veterinary Research (Harbin, China) proved the existence of 15 genetic groups of BVDV-1 [9]. Based on the results of phylogenetic studies conducted on the basis of genomic RNA sequences Ukrainian and Chinese isolates of BVDV, we possibility of the existence at least five genetic groups of BVDV-1 (Fig. 2).

In the phylogenetic tree, shown in Fig. 2 BVDV isolates of different genotypes form a clearly separated clusters. Each genetic group within the cluster isolates of BVDV-1 forms individual cluster. Analysis of the phylogenetic trees topology suggests in relation to the proximity of sequences of gene 5 'UTR genetic groups 1 b and 1 c; 1 p and 1 m. Draws attention to the genetic group 1 f, which has been classified in Ukraine, and often recorded in Europe.

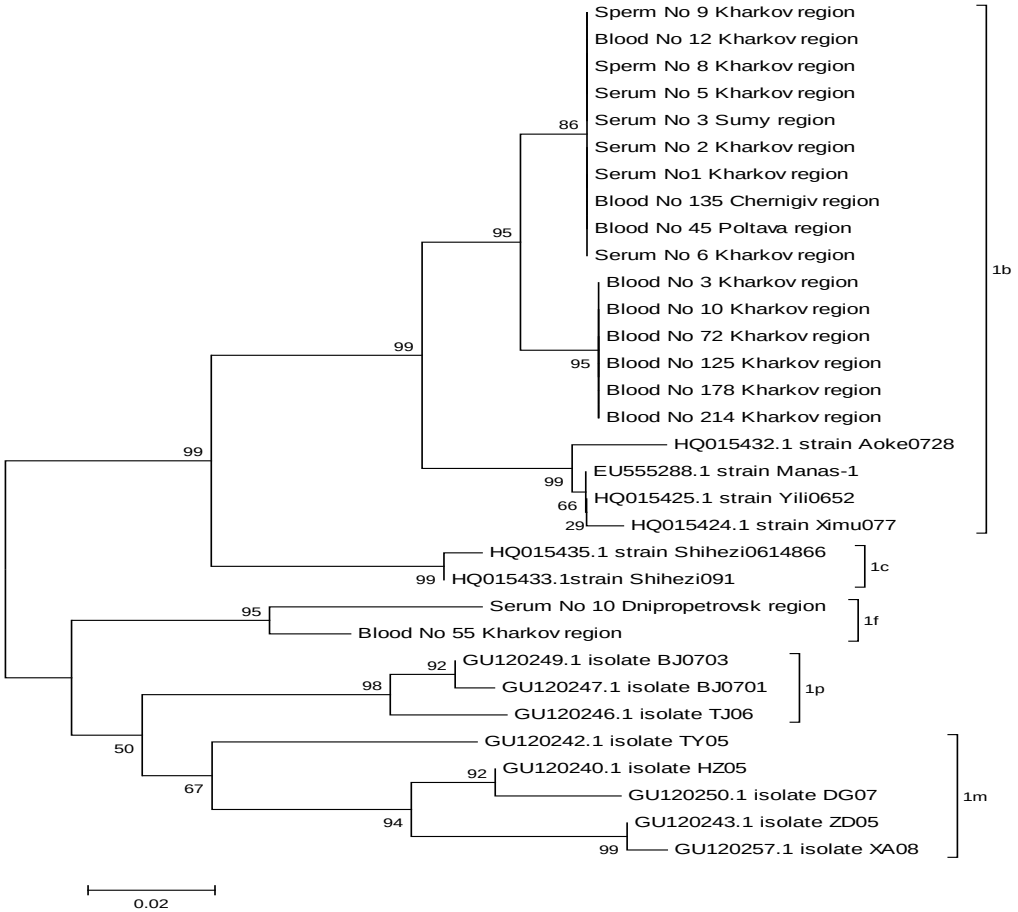

Fig. 2. Genetic typing of Chinese and Ukrainian BVDV isolates in the 5'-UTR region

Philogenetic studies of 5' UTR BVDV gene allowing the identification of viral genotypes and produce differentiated genome groups within the same cluster.

Conclusion. Based on the results of PCR screening of the BVDV spread in the Ukraine found that 29 out of 224 (12.9 %)studied samples of the animal clinical material from 34 farms were positive for presence of the BVDV RNA.

Conducted sequencing and phylogenetic analysis of Ukrainian BVDV isolates, which was established by the virus belonging for two subtypes of genotype 1: 1 b and 1 f.

Conducted a phylogenetic comparison of BVDV circulating in China and Ukraine, which found that virus subtype 1 b is widespread in both countries.

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ПОРІВНЯННЯ МОЛЕКУЛЯРНОЇ ЕПІДЕМІОЛОГІЇ ВІРУСНОЇ ДІАРЕЇ ВРХ В УКРАЇНІ ТА КИТАЇ

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Мета. Вивчення епізоотичної ситуації щодо вірусної діареї ВРХ, ізоляція і генетична характеристика штамів вірусу, що циркулюють в обох країнах (Китай і України) та їх філогенетичне порівняння.

Методи. Молекулярно-генетичні, філогенетичні. Були використані праймерні системи 324/326 для ПЛР ампліфікації 5' UTR гену збудника ВД ВРХ.

Результати. Вірус діареї ВРХ був виявлений в 29 зразках (12,9 %) з 224 досліджених. За допомогою подальшого філогенетичного аналізу 5'-UTR (ділянка 245 п.н.) було проведено генотипування виявлених ізолятів, яке показало належність 16 вірусів до підтипу 1 b та 2 виявлених збудників до підтипу 1f.

Висновки. Отримані результати вказують на значне поширення ВД ВРХ у господарствах різних областей України. У результаті проведених філогенетичних досліджень українських ізолятів вірусу діареї ВРХ встановлено їх зв'язки з вірусами виділеними в Китаї.

Ключові слова: ВД ВРХ, ПЛР, генотипування, філогенетичний аналіз.