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ВИРУС ПЯТНИСТОГО УВЯДАНИЯ ТОМАТА НА РАСТЕНИЯХ ПЕРЦА СЛАДКОГО (*CAPSICUM ANNUM L.*) В ВЕНГРИИ

С середины 1990-х годов вспышки заболевания, вызванного вирусом пятнистого увядания (бронзовости) томата (Tomato spotted wilt virus (TSWV)) в Венгрии, часто приводит к значительным потерям урожая коммерческих посевов перца сладкого. Изначально усилия при борьбе с TSWV направлялись на контроль векторов вируса – трипсов (путем использования различных инсектицидов или пластиковых ловушек) и сорняков, которые выступают хозяевами для вируса и трипсов. Позже были созданы разнообразные трансгенные сорта перца с интродуцированным геном устойчивости к данному вирусу – Tsw. Начиная с 2010-2011 гг в регионе Сентеш в Венгрии случались единичные, а с 2012 г. – все чаще случаи появления новой формы TSWV, которая была способна к преодолению устойчивости трансгенных растений перца. Считается, что вспышки инфекции, вызванной TSWV, вызванные невыполнением рекомендаций по контролю трипса Frankliniella occidentalis и прекращением использования некоторых эффективных пестицидов (например, Unifos 50 EC). Данная работа посвящена изучению этой проблемы.

Ключевые слова: вирус пятнистого увядания томата, трипс Frankliniella occidentalis.

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EFFICIENCY OF SEROLOGICAL KITS OF DIFFERENT MANUFACTURERS IN DEFECTION OF ANTIBODIES AGAINST PRRVS CIRCULATION IN UKRAINE

Stadejek et al. have studied the efficiency of five serological test kits, which are in wide use across the world, showing that their sensitivity differs in relation to the geography of PRRSV isolation. Karniychuk et al. confirmed an essential genetic and antigenic distinctions between East European PRRSV isolates and West European and North American strains of the virus. Our results on differences in the sensitivity of serological kits to detect anti-PRRSV antibodies comply with the data published earlier. When using a BIONOTE kit false positive results were received whereas the application of a CIVTESTsuisPRRS kit might lead to the significant number of false negatives questioning the expediency to use these test kits. Our findings lead us to conclude that a serological IDEXX HerdCheck PRRS 3XR ELISA kit is the most sensitive to Ukrainian PRRSV strains while an Ingezim PRRS Universal test kit may also be used to detect antibodies against this virus in Ukraine.

Key words: PRRVS, strains, serological kits, antibodies.

Introduction. A porcine reproductive and respiratory syndrome virus (PRRSV) is a member of Arterevirus genus, Artereviridae family and Nidovirales order [1]. Virus isolates are usually attributed either to the North American and European strains that have some distinctions in genome. The fact that these genetically different types of the virus appeared practically simultaneously presents one of the today's mysteries [2]. The analysis of their nucleotide sequences and antigen properties demonstrated that the North American (type 2) and European (type 1) PRRSV strains are only 63% identical at genome level [1]. Evolutional variability of this virus is suggested to be the highest among RNA viruses [3].

Until 2010 it was thought that the ORF7 is the most conservative gene of PRRSV. That is why it was widely used in diagnostic in test kits based on RT-PCR and real time PCR techniques [4]. However, Stadejek et al. analyzing European isolates of the virus found out the significant genetic variability among the ORF7 sequences which was especially high in East Europe where four main virus subgroups were revealed [4, 5]. Such high variability makes significantly harder to diagnose the related disease correctly, to develop efficient and safe vaccines as well as to control the disease.

Clinical signs of PRRSV infection vary depending on the virus virulence, immune status of the herd and age of infected animals. Viremia leads to clinical manifestation of the disease. PRRSV capable to cross transplacentary barrier and infect fetus causing abortions, stillbirths and births of weakened piglets [6, 7].

The International Epidemiological Bureau marks out, as the most efficient, several techniques of PRRSV diagnostics: virus isolation, serological tests, PCR and real time PCR. Serological tests are a potent and sensitive approach used in schemes of PRRSV control [3, 8]. Seroconversion can be identified 7-11 days after animals been infected using proven, highly sensitive and specific serological kits [9]. An analysis is performed in serum sampled from animals of an infected herd belonging to different age groups.

Blood serum specimens are tested with time intervals (for example, at the time of clinical signs development and then in 2–3 weeks) providing the basis for serological diagnostics. Such approach is also applied to control the results of vaccination. It is important to take into account the presence of maternal anti-PRRSV antibodies in serum specimens. The level of these antibodies gradually decreases up to 9th week of animal life [10]. According to literature the number of blood serum specimens sampled from a single

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farm has to be no less than 12 in order to obtain statistically valid results [11, 12].

At the moment, an immuno-enzyme analysis (IEA) is regarded as an easily available, sufficiently reliable technique the advantages of which include fast results and high specificity. Taking into account the widespread occurrence of PRRSV in Ukraine [13, 14] as well as its antigen variability [15] the aim of our study was to evaluate the efficiency of four basic serological test kits being used in Ukraine for detection of antibodies against Ukrainian isolates of the virus.

Material and methods

Blood serum samples were collected from animals of the main herd in different farms to assess titers of anti-PRRSV antibodies. The biological material was transported in thermo containers filled with ice. The volume of a sample was equal to 3 ml. When sampling specimens the following data were recorded: clinical signs, farm's technology details, animal age, farm name, province. In total, 93 samples of blood serum from eight farms in seven provinces of Ukraine were taken for analysis.

The titer of anti-PRRSV antibodies in blood serum samples was assessed with IEA using a diagnostic HerdChek*PRRS X3 kit manufactured by IDEEX (USA). Tests were performed in accordance with the instruction of a kit manufacturer.

11 serum samples originated from five Ukrainian farms were selected. Tests were performed simultaneously in order to minimize the influence of a sample quality, test conditions and kit series. Farms providing serum samples use different breeding companies to rebuild their herds, are territorially separated and do not trade with one another. Herds in these farms were serologically PRRSV positive during six months preceding the tests. This allowed us to exclude management as a factor influencing the similarity/difference of properties for pathogens circulating in these farms.

Further, blood serum samples having different titers of anti-PRRSV antibodies were selected (low titer, high titer and titer near the cutoff). These samples were analyzed on the presence of anti-PRRSV antibodies using the following kits: Ingezim PRRS Universal 11.PRU.K1 (Spain), BIONOTE (Korea), CIVTESTsuisPRRS (HIPRA, Spain) and repeatedly IDEXX HerdCheck PRRS 3XR ELISA (USA). Tests were performed in accordance with the instruction of a kit manufacturer.

Results and Discussion

A PRRSV variability presents the greatest challenge to its efficient diagnostics. Stadejek et al. have studied the efficiency of five serological test kits which are in wide use across the world demonstrating that their sensitivity differs in relation to the geography of PRRSV isolation. In addition, Stadejek stressed that a strict separation in genetic characteristics of PRRSV exists between East and West Europe explaining it by the absence of active trade between countries of the former Soviet Union and Europe. For example, according to their genetic characteristics PRRSV samples isolated on the territory of Poland were attributed to a single PRRSV subgroup while Belorussian isolates were thought to belong to three genetic subgroups [4].

Data received in two Ukrainian farms show that all four test kits produced similar results in detection of anti-PRRSV antibodies (Table 1).

***Table* 1. Comparison of serological kits in tests of pig blood serum samples on the presence of anti-PRRSV antibodies**

Farm	№ Sample	"IDEXX HerdCheck PRRS 3XR ELISA", USA *			"CIVTESTsuisPRRS", HIPRA, Spain **		"BIONOTE", Korea ***		"Ingezim PRRS Universal 11.PRU.K1", Spain ****	
		OD	Titre	Result	OD	Result	OD	Result	OD	Result
1	1	2,984	7554	pos	150	pos	1,53	pos	2,504	pos
2	2	3,012	7612	pos	80	pos	1,53	pos	2,845	pos
	3	0,699	1551	pos	19	sub	0,91	pos	0, 53	pos
3	4	2,932	7480	pos	125	pos	1,53	pos	1,606	pos
	5	0,396	837	sub	9	neg	0,77	pos	0,412	sub
	6	0,059	105	neg	0	neg	0,46	pos	0,119	neg
	7	0,456	976	pos	2	neg	0,74	pos	0,432	sub
4	8	0,556	1208	pos	0	neg	0,18	neg		neg
	9	2,601	6494	pos	0	neg	0,27	neg		neg
	10	0,042	74	neg	0	neg	0,24	neg	0,141	neg
5	11	0,384	805	sub	24	pos	0,84	pos	0,609	pos

* "IDEXX HerdCheck PRRS 3XR ELISA", USA – pos >0,4; titer> 844.
** "CIVTESTsuisPRRS", HIPRA, Spain – pos >20.
***"BIONOTE", Korea – pos >0,4.
****"Ingezim PRRS Universal 11.PRU.K1", Spain – pos >0,450.

Only an IDEXX HerdCheck PRRS 3XR ELISA kit identified anti-PRRSV antibodies in farm #4 samples while the results of tests in these samples performed with other test kits were negative ones. It is worth to note that false negative results enable the spread of the disease, which is highly contagious, among animals of the herd causing significant economic losses due to abortions, stillbirths, births of weakened piglets and development of respiratory symptoms in adult animals. From the other hand, false positives are also impermissible drawbacks in PRRSV diagnostics. In order to exclude the possibility that test results for farm #4 samples were false positives, blood serum was sampled again from the same animals. Titers of anti-PRRSV antibodies and their dynamics pointed to the presence of PRRSV infection.

False positives were received in two specimens when using a BIONOTE kit. A CIVTESTsuisPRRS kit appeared to be less sensitive to detect anti-PRRSV antibodies in samples with levels near the cutoff. It is also necessary to add that sample #11 was shown to be PRRSV positive by tests performed with a Ingezim PRRS Universal, BIONOTE and CIVTESTsuisPRRS kits whereas IDEXX HerdCheck PRRS 3XR ELISA kit showed a negative result the value of which was, however, near the cutoff.

Thus, data received indicate the difference in the sensitivity between all commercial serological kits being currently used in Ukraine that comply with results Karniychuk et al. published earlier. These authors confirmed significant genetic and antigenic distinctions between PRRSV isolated in East Europe and the virus isolated in West Europe and USA [15].

The study of a reproductive and respiratory swine syndrome is greatly important because this disease may cause enormous economic losses for pig farms. Contagiousness,

high levels of abortions and mortality related to aforementioned disease stipulates the necessity to implement efficient diagnostic strategy, as one of the measures allowing to combat the spread of PRRSV in pig farms of Ukraine. The use of serological tests is one of approaches to solve this problem. These tests help to ascertain a farm's epizootic situation, to identify infection times and intensity that is necessary for comprehensive estimate of PRRSV involvement in the development of respiratory and reproduction-related symptoms. In order to design an optimal preventive vaccination scheme it would be the most practical to obtain serological profiles at farm level.

Taking the aforementioned into account one may conclude that the sensitivity of serological test kits is a critical factor assuring that test results comply with real data. False positives are possible when using a BIONOTE kit whereas CIVTESTsuisPRRS kit may show significant number of false negatives questioning the expediency to apply these test kits. Data obtained allow to conclude that a serological IDEXX HerdCheck PRRS 3XR ELISA kit is the most sensitive with respect to Ukrainian PRRSV isolates. However, it should be stressed that the use of an Ingezim PRRS Universal test kit is also possible in Ukraine.

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ЕФЕКТИВНІСТЬ ВИКОРИСТАННЯ СЕРОЛОГІЧНИХ ТЕСТ-СИСТЕМ РІЗНИХ ВИРОБНИЦТВ ДЛЯ ДЕТЕКЦІЇ АНТИТІЛ ВРРСС, ЩО ЦИРКУЛЮЮТЬ В УКРАЇНІ

Tomasz Stadejek та його колеги дослідили ефективність 5-ти найпоширеніших серологічних тест-систем у світі та показали їх різну чутливість в залежності від географії виділення ізолятів ВРРСС. Karniychuk та співав. підтвердили значну генетичну та антигенну відмінність ВРРСС, ізольованого зі східної Європи, у порівнянні із західноєвропейським та американським штамами ВРРСС. Наші результати різності чутливості серологічних тест-систем для детекції антитіл до ВРРСС корелюють з раніше опублікованими результатами. При використанні тест-системи виробництва "BIONOTE" детектовані хибно позитивні результати, тоді як тест-система "CIVTESTsuisPRRS" може показувати значну кількість хибно негативних результатів, що в свою чергу вказує на недоцільність використання даних тест-систем. За отриманими даними можна зробити висновок, що найчутливішою до українських штамів ВРРСС є серологічна тест-система "IDEXX HerdCheck PRRS 3XR ELISA", проте використання в Україні тест-системи "Ingezim PRRS Universal" також можливе.

Ключові слова: PRRVS, серологічні комплекти, антитіла.

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ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ СЕРОЛОГИЧЕСЧКИХ ТЕСТ-СИСТЕМ РАЗНЫХ ПРОИЗВОДИТЕЛЕЙ ДЛЯ ДЕТЕКЦИИ АНТИТЕЛ ВРРСС, КОТОРЫЕ ЦИРКУЛИРУЮТ НА ТЕРРИТОРИИ УКРАИНЫ

Tomasz Stadejek и его коллеги исследовали эффективность 5-ти распространенных серологических тест систем в мире и показали их различную чувствительность в зависимости от географии выделения изолятов ВРРСС. Karniychuk и соавт. подтвердили значительное генетическое и антигенное различие ВРРСС, изолированных из восточной Европы по сравнению с западноевропейским и американским штаммами ВРРСС. Наши результаты о разной чувствительности серологических тест- систем для детекции антител к ВРРСС коррелируют с ранее опубликованными результатами. При использовании тест-системы производства "BIONOTE" детектировались ложноположительные результаты, тогда как тест-система "CIVTESTsuisPRRS" может показывать значительное количество ложноотрицательных результатов, что в свою очередь указывает на нецелесообразность использования данных тест-систем. По полученным данным можно сделать вывод, что наиболее чувствительной к украинским штаммам ВРРСС является серологическая тест-система "IDEXX HerdCheck PRRS 3XR ELISA", однако использование в Украине тест-системы "Ingezim PRRS Universal" также возможно.

Ключевые слова: PRRVS, серологические комплекты, антитела.