

# ANALYSIS OF LABORATORY TESTS FOR SEVERAL VIRAL SWINE DISEASES IN RUSSIA IN 2011–2017

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## ABSTRACT

Retrospective analysis of laboratory diagnostics carried out in Russia for dangerous viral swine diseases demonstrated that during the last seven years (the period when the Rosselkhoz nadzor orders to implement National Monitoring Plans were executed) the amount of performed tests had increased. But it is not always possible to read the test results unambiguously and that requires the improvement of diagnostic practices supported by established rules of sampling, laboratory tests and obtained result submission. It means random tests shall be representative for tested populations, correlate with the prevalence of concerned infection and meet the required certainty. It is also needed to establish the techniques of primary and confirmation tests, regulate the use of test kits (validated) preferring the use of the most effective and inexpensive. Appropriately planned and implemented surveillance activities (including national monitoring) will contribute to minimization of financial expenses and improve their effectiveness at the same time.

**Key words:** laboratory diagnosis, African swine fever, classical swine fever, Aujeszky's disease, porcine reproductive and respiratory syndrome, surveillance, monitoring.

## INTRODUCTION

The Russian Federation (RF) remains infected by several dangerous viral swine diseases [6, 12]. Infection spread prevention and disease eradication in the country depends on the efficiency of the surveillance system in place. Due to the absence of official surveillance programs, the current RF system of epidemic data collection and analysis does not comply with a number of international recommendations. Therefore, taking into account complicated epizootic situation, it is necessary to improve the surveillance system, as well as to regulate the procedure of sampling and analysis of the obtained results.

Up-to-date laboratory tests for African and classical swine fever (ASF, CSF), Aujeszky's disease (AD), porcine reproductive and respiratory syndrome (PRRS) allow rapid and reliable confirmation of the diagnosis [2, 12]. Taking into consideration a wide variety of diagnostic tests, it is necessary to choose tests that fully comply with the aim of study.

The main objectives of the surveillance system are:

- confirmation of disease/infection absence or presence;

- determination of disease development tendencies in susceptible populations;

- early detection of exotic or emerging diseases [10, 12].

CSF and AD prevention and control measures, which are implemented currently in the RF, do not allow to apply for the international disease-free status recognition of the whole country or its individual subjects. For this purpose, the following conditions shall be fulfilled:

- absence of vaccination of domestic pigs and captive wild boars within the last two years (against Aujeszky's disease) or within the last year (against CSF),

- or the possibility of differentiation of vaccinated from infected pigs (DIVA strategy – Differentiation of Infected from Vaccinated Animals) [12].

Precise data on animal infectious diseases spread (as well as latent carriers) is necessary to make a reasonable decision concerning introduction of changes in control strategy (for example no use of vaccination), as well as for gaining disease-free status of country's territories. This data shall be confirmed not only by the absence of the reported outbreaks but also by the results of reliable laboratory studies. If these requirements are not complied with, the diagnosis may be delayed and some territories of the country may be declared disease free prematurely.

The records of laboratory tests (for ASF, CSF, AD and PRRS) performed in the territory of the RF, as well as the national methodology on improvement of diagnostic studies [4, 5] and international recommendations [12] presented in this paper will be useful for development of regulatory acts on surveillance system organization.

## MATERIALS AND METHODS

Data on laboratory studies submitted into the electronic national database (GIS) "Vetis" [1], veterinary reports of the

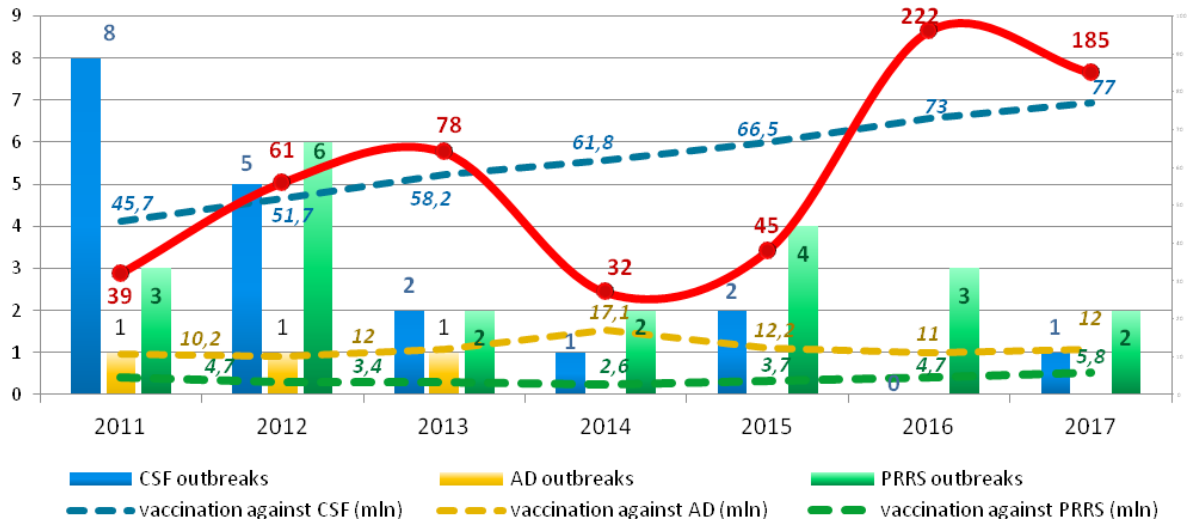


Fig. 1. Dynamics of ASF, CSF, AD, PRRS infection and vaccination of pigs in the RF in 2011–2017 (data obtained from the OIE, FGBI “Veterinary Centre”)

FGBI “Veterinary Centre” [6], OIE data were used for the analysis [12].

### RESULTS AND DISCUSSION

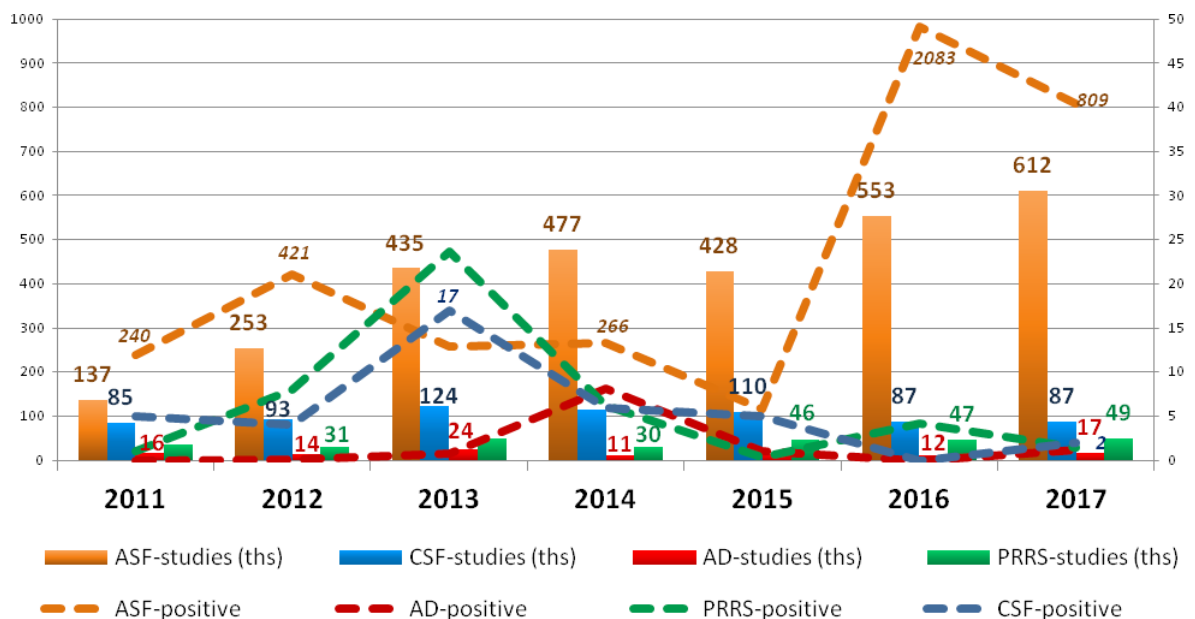
The ASF situation in Russia has become more complicated. The infection spreads further to the east of the country, to previously ASF-free regions. In 2011–2015 about 50 outbreaks in pigs were reported per year, but in the last 2 years the number of outbreaks increased 3.6 times (Fig. 1).

For other viral diseases: CSF, AD, PRRS – there is some improvement in the epizootic situation, which is partly associated with the large-scale vaccination of pigs. But the disadvantage of the prolonged mass immunization of animals (using traditional preparations) is the development of vaccine dependence in pig farms. But the refusal

of vaccination leads to the manifestation of infection, previously masked by the specific prevention. Disease control measures in such cases will often include the reinitiation of vaccination of pigs, since the notification of the disease outbreak infection means significant economic losses for the pig farm due to the introduction of correspondent restrictions.

Despite the decrease in the number of CSF cases registered in the population of domestic pigs (from 8 cases in 2011 to 1 case in 2017), the number of vaccinations against this disease in the Russian Federation is increasing annually. It increased 1.7 times over the last 7 years. CSF live vaccines are widely used in our country, because the existing DIVA vaccines are more expensive, they are characterized by lower immunogenicity and lower ability to form early and colostral protection [9, 11].

Fig. 2. Dynamics of laboratory studies for ASF, CSF, AD, PRRS in the RF in 2011–2017 (data obtained from the FGBI “Veterinary Centre”)



Non-DIVA vaccines are widely used for the AD immunization of pigs in Russia, despite of the availability of labelled AD vaccines, the immunogenicity of which is comparable to that of the traditional vaccines [8]. Current instructions on the use of such preparations prescribe immunization of pigs only in AD infected farms and farms at risk. Nevertheless, 12 million animals are immunized against AD annually in the Russian Federation, which does not correspond with the AD-free status of our country declared during the last four years.

According to veterinary reports, 22 PRRS cases have been registered during the last 7 years. 4 million animals are immunized against PRRS annually in the Russian Federation.

Laboratory diagnosis of swine viral diseases in Russia is carried out within the framework of various programs, both regional and federal. Veterinary executive authorities of the RF subjects collect and timely send information (according to the approved forms 4-vet A and 4-vet B) on diagnostic tests carried out in the Ministry of Agriculture of Russia (data are processed in the FGBl "Veterinary Centre") [6, 7]. The analysis of this data shows that the amount of ASF, CSF, AD, PRRS tests performed in the Russian Federation in recent years corresponds to the level of threat posed by these infections. For example, the number of ASF-tests is 4 times higher than that of CSF-tests, and 26 times higher than the number of AD-tests (Fig. 2).

The presented data demonstrates only total amount of studies carried out in the country. The reported data available for analysis does not contain information on laboratory diagnostic methods.

Due to this, more detailed reports of 32 Rosselkhozndzor subordinate laboratories have been subjected to detailed analysis. These laboratories have conducted studies on the implementation of the Plan of state epizootic monitoring of highly dangerous animal diseases (hereinafter – state monitoring) since 2011, according to the Orders "On laboratory research within the Rosselkhozndzor activities aimed at ensuring compliance with the WTO SPS Agree-

ment on Russia's accession to the WTO". Data on the research results is entered into the GIS "Vetis". At the end of the year, the institutions involved in the implementation of the state monitoring submit the final reports for their subsequent analysis conducted at the FGBl "ARRIAH" [1, 3].

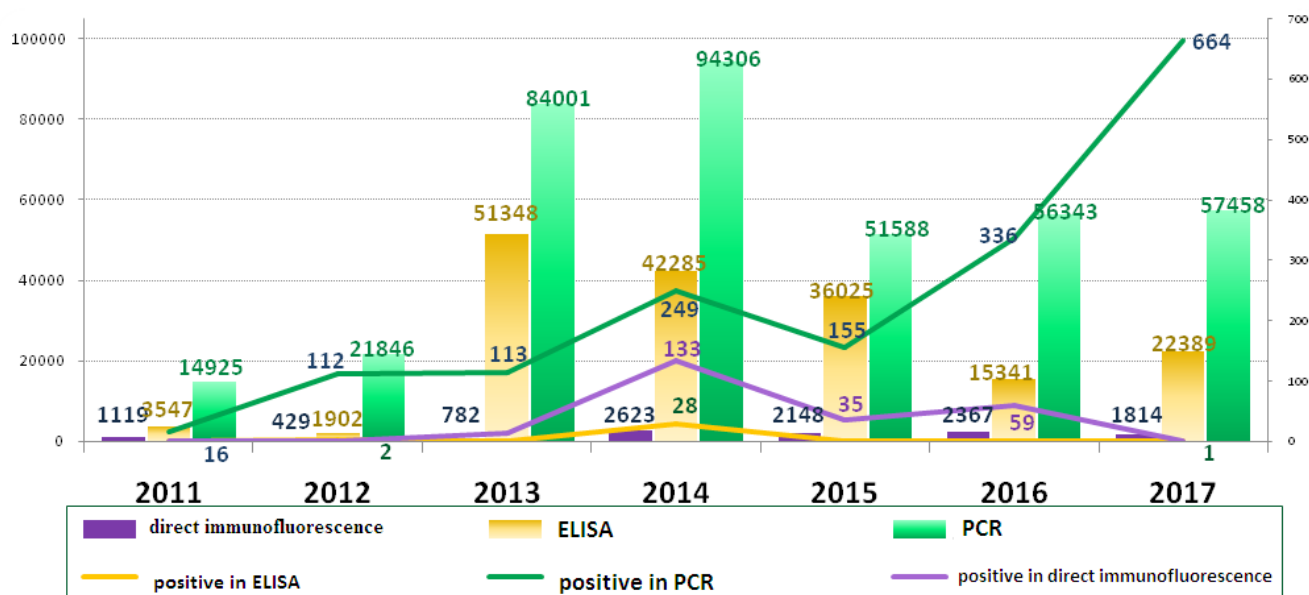
Within the framework of state monitoring the following infectious swine diseases are monitored: ASF, CSF, AD, PRRS, transmissible gastroenteritis of swine (TGS), erysipelas, pasteurellosis, salmonellosis, chlamydiosis, trichinellosis, etc. The number of studies and laboratory diagnostic methods depend on the object of study (infectious agent). The laboratory diagnostic methods are those recommended by the OIE [12]:

- detection of virus genetic material using different variants of polymerase chain reaction (PCR);
- virus identification using direct immunofluorescence method;
- virus isolation on susceptible cell cultures;
- bioassay using laboratory animals;
- detection of virus antigen or virus-specific antibodies using ELISA.

The dynamics of ASF laboratory studies within the framework of state monitoring (Fig. 3) demonstrates that during the last 7 years more than 550,000 ASF-tests have been performed, most of them using PCR (67%) and ELISA (31%). 1,645 positive samples (0.4%) were detected in 380,000 tests by PCR, and only 31 positive sample (0,018%) was detected in 173,000 tests by ELISA (detection of antibodies against ASF virus). And 28 of them were detected during the ASF outbreak in one of the large pig farms in Tula Oblast in 2014.

At the same time, according to European veterinary services, ASF virus antibodies were detected in 350 samples collected from wild boars in Estonia (I. Nurmoja, 2017), in more than 150 samples collected in Poland (G. Wozniakowski, 2017), and in more than 90 samples in Lithuania (S. Pileviciene, 2017) during the period from 2014 to 2017. Totally about 115,000 samples from wild boars were tested in these countries during the above-mentioned period,

Fig. 3. ASF laboratory studies carried out in Russia within the framework of state monitoring (data obtained from GIS "Assol")



and detected seroprevalence increased every year (mostly as a result of shooting animals): from 0.1% in 2014 to 0.4% in 2015, 0.6% in 2016 and 1.0% in 2017.

For ASF diagnosis, PCR is often used as a primary test, and positive and doubtful samples are additionally tested by direct immunofluorescence method and/or virus isolation. During these studies, a large number of positive results is obtained. Thus, 249 positive samples (2%) were detected in 11,700 samples tested by direct immunofluorescence method, and 606 positive samples (40%) were detected in 1,517 samples tested by virus isolation.

The dynamics of CSF laboratory studies (Fig. 4) demonstrates that most of diagnostic studies are performed using ELISA: more than 243,000 ELISA tests have been performed during 7 years (65.9% of the total amount). 33.7% of tests are performed using PCR and only 0.4% of tests are performed using direct immunofluorescence method.

The analysis of the available reports demonstrated that the greatest number of positive results was obtained when testing samples by ELISA (54%). However, these results are difficult to interpret. Thus, according to the reports of a number of laboratories, all ELISA-tested samples were CSF-seronegative in some regions of the country. But it cannot happen because vaccines against this disease are widely used. Either the detection of antibodies in vaccinated pig herds was not registered as positive cases during filling out the report forms (so as not to be confused with infection), or quarantined, unvaccinated pigs imported from abroad were massively examined, but the latter does not correspond to the purpose of monitoring studies.

Data from other laboratories' reports indicate that in a number of regions of the country (Moscow, Kursk, Oryol Oblasts in 2014, Kaliningrad, Kursk, Oryol Oblasts in 2015, Zabaykalsky Krai, Irkutsk Oblast, the Republic of Altai in 2016) specific antibodies against CSF virus were detected in samples from animals from the unvaccinated herd, which may indicate the infection of animals. Meanwhile, the CSF cases were not registered during the analyzed period in the listed regions. Unfortunately, according to the

reports of veterinary laboratories (including GIS "Assol"), it is not possible to trace the implementation of subsequent laboratory tests proving the absence of infection in suspicious animal subpopulations.

During 7 years, 54 positive results were obtained by PCR (CSF virus genome detection) and 14 positive samples were detected using direct immunofluorescence method. The number of CSF outbreaks notified during these years does not coincide with the data given, since not all detected cases were diagnosed within the framework of state monitoring. Besides, more than one sample was tested in the same outbreak.

Notwithstanding the foregoing, the results of PCR and direct immunofluorescence method can be easier interpreted than the results obtained in ELISA. Moreover, there is the possibility of accurate differential diagnosis of CSF virus (vaccine or field) (according to the results of restriction analysis/sequencing).

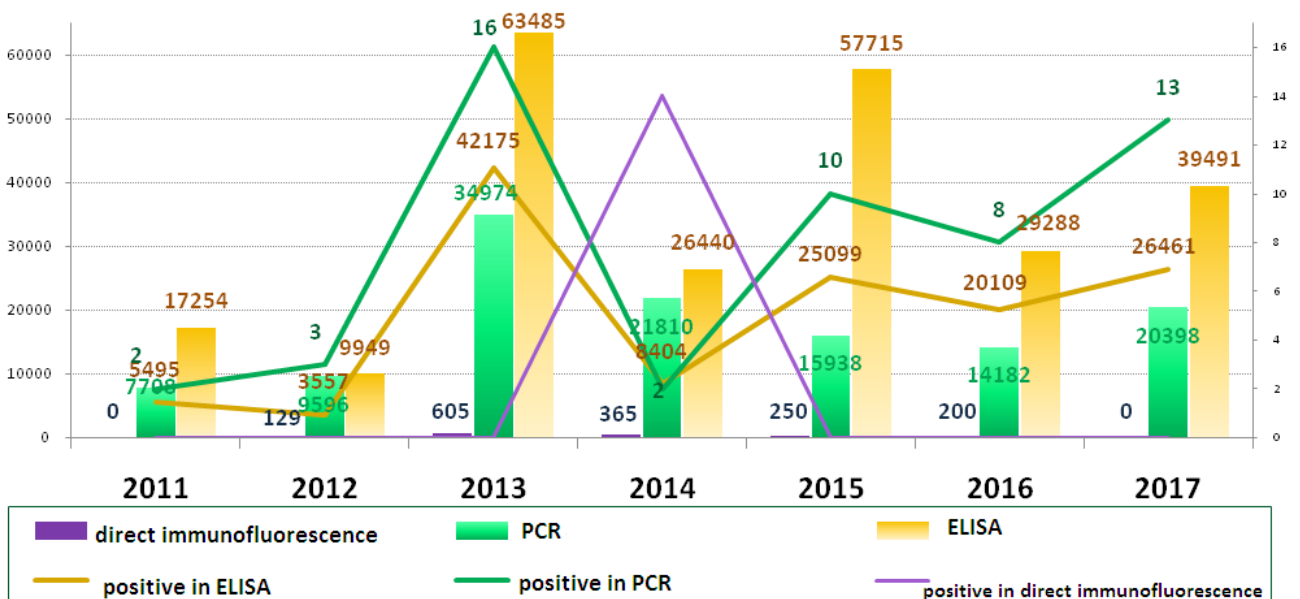
To study the biological properties of recovered isolates and to enlarge the collection of microorganisms, the FGBI "ARRIAH" carried out the studies on the isolation of CSF virus on susceptible cell cultures. 343 such studies were conducted during the analyzed period.

The dynamics of AD laboratory studies (Fig. 5) demonstrates that 251 tests were performed using direct methods (virology, bioassay), with negative results.

The majority of all AD tests (up to 99.8%) was performed using indirect methods (detection of specific antibodies in ELISA). However due to mass AD vaccination in Russia, it is difficult to interpret the ELISA results (44% positive).

According to the reports of a number of veterinary laboratories, AD virus specific antibodies were detected in samples from unvaccinated pig herds (Belgorod, Voronezh, Moscow Oblasts, Krasnoyarsky, Stavropolsky Krai in 2014, Republic of Khakassia, Krasnoyarsky, Stavropolsky Krai, Sverdlovskaya Oblast in 2015, Irkutsk Oblast, Stavropolsky Krai in 2016, the Republics of Buryatia, Khakassia, Zabaykalsky, Krasnoyarsky, Stavropolsky Krai, Irkutsk, Sverdlovsk Oblasts in 2017). This causes suspicion, because according

Fig. 4. CSF laboratory studies carried out in Russia within the framework of the state monitoring (data obtained from GIS "Assol")



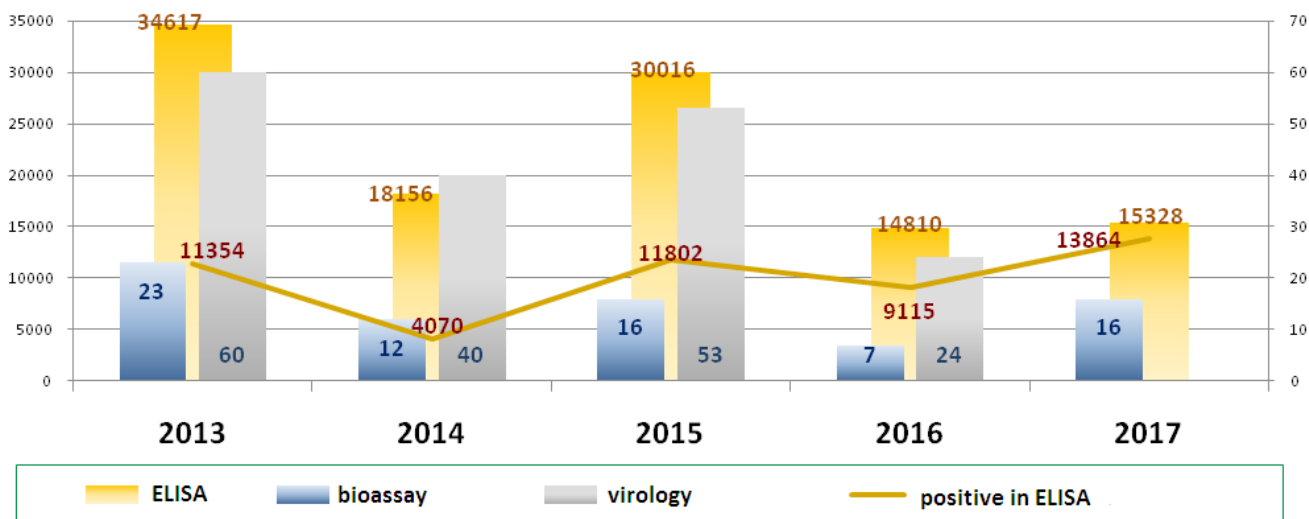


Fig. 5. AD laboratory studies carried out in Russia within the framework of the state monitoring (data obtained from GIS "Assol")

to available reports it is not possible to confirm whether the measures demonstrating AD freedom in these herds have been carried out to the full extent.

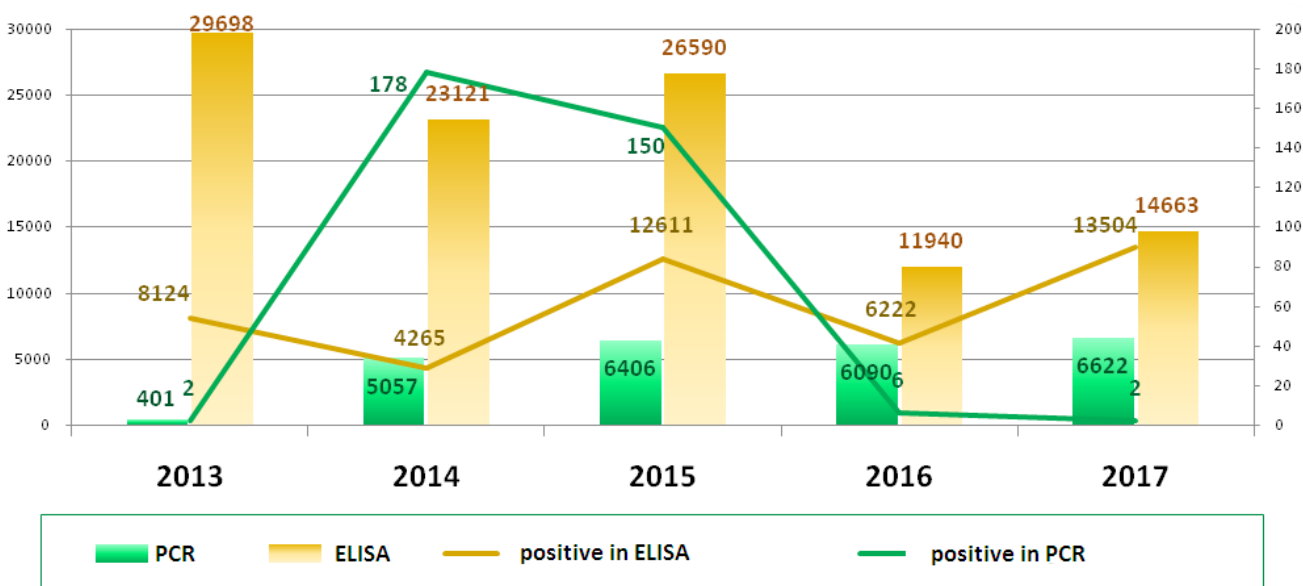
The dynamics of PRRS laboratory studies (Fig. 6) demonstrates that most of diagnostic studies (81%) were performed using ELISA, and the large amount of samples (31%) were positive. It can be explained by the absence of regulatory restrictions on the implementation of research (indirect methods) based on the profile of vaccinated livestock.

Out of 11,581 PCR tests, 328 were positive (2.8%), which demonstrates PRRS infection in a number of pig farms in Russia. PRRS infection can also be demonstrated by the detection of PRRS virus antibodies in animals from unvaccinated herds (Belgorod, Kursk Oblast, Primorsky, Khabarovsk Krai in 2015; Tyumen, Novosibirsk Oblasts, Primorsky, Khabarovsk Krai in 2016; Kursk Sverdlovsk,

Tver, Irkutsk Oblasts, Primorsky, Permsky Krai in 2017). Reports for 2017 contain data on the detection, by ELISA, of samples "positive for the presence of the pathogen" in the Moscow (599 samples), Novosibirsk (20), Pskov (3), Sverdlovskaya (20), Tomsk (6), Tyumen (4) Oblasts and in the Republic of Bashkortostan (1).

The analysis of the reported data indicates that in the absence of the RF regulatory surveillance programs, the laboratory tests performed do not always correspond to the main objectives of the surveillance. Therefore, mass use of ELISA method can hardly be considered to be effective for confirmation of conducted vaccination. Besides, it is more reasonable to evaluate vaccination efficacy using quantitative methodology. For example, the neutralization test, unlike ELISA, allows not only to detect specific antibodies, but also to determine the titer of virus neutralizing antibodies.

Fig. 6. PRRS laboratory studies carried out in Russia within the framework of the state monitoring (data obtained from GIS "Assol")



Besides it is not reasonable to stop using ELISA method, because its correct application allows to obtain reliable results (when both antigen and antibody are detected) confirming the infection in unvaccinated animals, and discriminating ELISA kits for the detection of marker antibodies provide a good possibility of identifying infected animals among those vaccinated with DIVA-vaccine against CSF, AD, etc.

However, the simplicity, low cost, speed of ELISA, the possibility of conducting mass studies should not be the key factors for choosing this method as the main one. In addition, ELISA is less sensitive (for example, in antigen detection) than PCR, direct immunofluorescence method, virus isolation. Therefore, it would be reasonable to establish a number of limitations on ELISA application within the framework of state monitoring.

Therefore, studies should not be performed to detect specific antibodies in animals after vaccination (with the exception of DIVA vaccines against CSF, AD) or for early detection of ASF in disease-free subjects of the Russian Federation (because the latter is efficiently performed by PCR and direct immunofluorescence method [2]). Taking into account the European experience of using ELISA test for antigen/antibody detection, ASF should be complemented by PCR testing of samples from the same suspicious animals [12].

At present, the RF system of veterinary reports collection comprises the information provided by subject veterinary institutions in accordance with established forms of the relevant regulations [7]. The disadvantages of this system are its labor-consuming and non-operative nature associated with the manual collection and processing of information, excessive generalization of data (for example, on the methodology of the studies), inconsistency in amount of provided information, and discrepancy of information on certain infectious diseases.

Wider use of electronic information systems, for example, the Rosselkhoz nadzor's GIS "Vesta", "Assol", "Mercury", etc. can correct these defects. Integration of these GIS into a single environment (with the merging of databases) can significantly increase their functionality and provide an opportunity to use them as automated systems of infectious animal diseases surveillance and control.

At the same time, it is necessary to improve the existing GIS (in particular, "Assol", "Vesta"), and it is necessary to correct data entry protocols for unambiguous interpretation of research results with the improvement of electronic reporting forms, not only for rapid statistical processing of accumulated information, but also for epizootic analysis, including the possibility to trace research results (both positive and negative) at the level of individual herds of animals (with the indication of examined subpopulation size for assessment of the representativeness of the studies). The functionality of existing systems shall be expanded by information from epizootic investigations.

## CONCLUSION

The RF surveillance diagnostic activities shall be documented, coordinated according to the main surveillance objectives. The costs associated with reforming the current system can be reduced through the following actions:

- ranking of the list of controlled diseases with reduction of test number for diseases that do not pose potential risk of causing significant harm;
- using test methods which provide unambiguous results in current epizootic situation;

- increasing the number of laboratory studies conducted within passive surveillance (incident diagnosis), by reducing active surveillance in cases where the research objectives are questionable and/or it is not possible to study representative samples at the level corresponding to the prevalence of the disease and the required reliability of the studies;

- regulation of the reasonable procedure for testing samples with the definition of the possibility of their pooling to perform initial examination of similar groups of animals, and to avoid duplicate tests (sending of already examined negative samples to the reference laboratories);
- drawing up of a general research plan, carried out both within the framework of the federal monitoring plan, and within the framework of the Subject's programs.

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