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Original article

AFRICAN SWINE FEVER IN THE KRASNODAR REGION

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Summary

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Monitoring of the epidemic situation with African swine fever was carried out in the Krasnodar region (federal subject of Russia) in 2008–2013. This made it possible to determine sources, routes and factors promoting African swine fever spread. The major sources of the epidemic were presented by sick and dead wild and domestic pigs, non-decontaminated condemned meat products. During this time forty three outbreaks of this disease were reported in this region; more than 280 ths of pigs died and were destroyed, 36 pig enterprises were closed and it caused a great economic damage to pig farming industry of the region. Transmission factors were as followed: transport facilities that visited affected focal points and were not decontaminated to an accomplished standard, contaminated by African swine fever virus feeds, equipment, etc. Predictions on a possibility of occurrence of new disease cases in time and space are made using available information on ASF spread in the territory of the RF. As a result, a possibility to coordinate future efforts aimed at the disease surveillance in corresponding territories independently of reports from the given region emerges.

Key words: African swine fever, spread, economic damage, Krasnodar region, outbreak eradication

INTRODUCTION

Pig production is an important sector of agriculture and the economies of many countries and the major supplier of meat in the world. In economically developed countries intensive pig production predominates. More than half of the pig stock is reared in Asia, where China holds a leading position with more than 1/3 of the world population. Another third of the pig stock is grown in Europe, one-tenth in the United States. South America and Africa account for a small portion of the world's pig stock because pig production here is poorly developed. Exporters of pork products are Belgium, Denmark, The Netherlands, Germany, China and importers: Great Britain, Russia and other countries of Europe.

The effective development of intensive pig farming is hindered to a large extent by different infectious diseases which cause a great economic damage characterised with significant mortality and animal productive capacity losses

(Sobko *et al.,* 1988; Balyshev *et al.,* 2010). At present, African swine fever (*Pestis Africana suum*, ASF) constitutes a threat of highest priority for global pig industry (Makarov *et al.,* 2013). It is a highly contagious disease characterised with fever, haemorrhagic diathesis, inflammatory, dystrophic and necrotic changes in different organs and a high mortality level. ASF is caused by an icosahedral cytoplasmic DNA-containing virus of Iridoviridae family. A virion has two capsid layers and an outer envelope formed by budding through cell membrane (Dixon *et al.,* 2005). It is a complex virus containing 28 structural polypeptides.

By now 8 variants of ASF virus antigenic types are known. The virus isolates found in different regions of the world or within chronologically different epizootics, differ significantly in virulence. Animals surviving after an illness become resistant to infection with the homologous isolate, but are not protected from death after infection with heterologous isolates (Norley & Wardley, 1982; Garsia-Barreno *et al.,* 1986; Mebus, 1988).

During the period from 2007 to July, 2014 a total of 688 outbreaks of disease, including domestic pigs – 353, wild boars – 303 and infected objects (32) have been registered in the Russian Federation.

In the Krasnodar region ASF was registered for the first time in November 2008 in the Novokubansky Rayon. From that time ASF is registered annually in different backyards and enclosed type enterprises causing great economic damage to pig farming industry (Kurinnov, 2009).

The Krasnodar region is a federal subject of Russia, located in the Southern Federal District. Its administrative centre is the city of Krasnodar (Fig. 1). The Krasnodar region ranks No. 2 in the Russian Federation after the Belgorod region in

Fig. 1. Map showing the geographical location of the Krasnodar region.

terms of pig population (547 ths at the end of 2011 and 270.3 ths in October 2013). Many enterprises of different business patterns appeared: agricultural organisations producing 52.4% of meat products, farm households with different sanitary levels engaged simultaneously in swine breeding, raising and slaughter as well as in processing of swine products.

In the region there are 194 enterprises dealing with swine slaughter and processing of meat products of different categories. They include such large enterprises as Armavirsky, Kanevsky and other meat-processing plants and small slaughter units in households, on farms. All of them have different levels of equipment status, different levels of zoosanitary situation (compartment). Thus, 34 enterprises out of 194 have compartment level I and 42 enterprises – level II, i.e. 39.1% of enterprises are subject to shut-down. There are such large enterprises as Vasyurinsky meat-processing complex, Dinsky cold storage facility, Korenovsky "Progress" breeding enterprise "Industrialny", meat-processing plant "Tikhoretsky" etc. There are 23 enterprises with compartment level III, 95 enterprises with compartment level IV, in total 60.9%.

The activities were aimed at carrying out monitoring of ASF epidemic situation in the Krasnodar region, studying of sources and spreading routes for prediction of ASF outbreak occurrence in the region Therefore, an investigation of epidemic situation in the region during 2008–2013 was conducted and major sources, routes and factors contributing to ASF spread in the Krasnodar region were determined. Also, seasonal patterns in ASF occurrence and spread among domestic pigs were studied. An analysis of taken diagnostic, preventive and final measures aimed at eradication of disease focal points and prognosis of ASF spread in the Krasnodar region were performed.

MATERIALS AND METHODS

The epidemic situation was studied using commonly known research methods: epidemic and economic analysis, microbiological and serological tests, analysis of data of official veterinary statistics provided by the State Veterinary Authority of the region, Ministry of Agriculture and Processing Industry of the region, Administration of the Federal Service for Veterinary and Phytosanitary Surveillance for the Krasnodar region, Krasnodar Interoblast Veterinary Laboratory during 2008–2013.

The immunofluorescence assay, polymerase chain reaction and solid-phase immunosorbent assay were used for detection of an agent in tested 70,748 samples during 2008–2013.

The direct immunofluorescence test (DIFT) was conducted for the direct detection of viral antigen in cells of organs from pigs suspected of being infected with ASF virus. Intracellular antigen was detected by the fluorescent microscopy of preparations (impression smears), stained with specific antibodies to the ASF virus, marked with fluorescein isothiocyanate. The best organs are the spleen and lymph nodes. Set of preparations for differential immunofluorescence diagnosis of African swine fever (ASF), classical swine fever (CSF) and Aujeszky's disease (AD), produced by the State Science Institution National Research Institute of Veterinary Virology and Microbiology (VNIIVViM) in Pokrov (Vladimir region) were used. The evaluation of the results of microscopy carried out on the conventional scale with four crosses, with use of ocular

lens 10×20 and objective lens 10. In positive cases sparkling, yellow-green fluorescence of cells with inclusions or focal fluorescent complexes diffusion and granular antigens were seen in one of 10–50 observation fields.

For detection of antigen and antibodies against ASF virus the solid phase sandwich enzyme-linked immunosorbent assay (ELISA) was used, based on the interaction between antibodies specific to the ASF virus and with specific ASF antigens immobilised on the surface of wells and subsequent identification of antigen-antibody complex by the conjugate antibodies specific to the ASF virus labelled with horseradish peroxidase. Peroxidase causes decomposition of hydrogen peroxide in the chromogen-substrate solution and oxidation of the chromogen, resulting in development of colour whose intensity is directly proportional to the amount of antigen in the test sample.

For detection of the genome of the ASF virus, PCR was also performed using test-system for detection of DNA of the ASF virus by PCR (production VNIIVViM). The principle of the reaction is repetition of cycles of denaturation of tested DNA at a temperature of 94°C, hybridisation of DNA with specific primers at a temperature of 53 °C and synthesis of complementary strands of DNA using the thermostable DNA-polymerase at a temperature of 72 °C. As a result of amplification, the concentration of the synthesised fragment in the test sample is increased a million times, which allows assessing the results of the analysis using agarose gel electrophoresis.

The results of electrophoresis were detected under ultraviolet light at a wavelength of 254 nm. The reaction is considered positive if the band in the corresponding track is located in the gel exactly as the band of positive control of amplification. The reaction is considered negative if bands are not detected or do not correspond to the size of a fragment in the control sample.

The epidemic monitoring was carried out in conformity with a method of epidemic investigation (Tarshis, 1979).

RESULTS

According to monitoring results, clinical signs in pigs in ASF focal points in the Krasnodar region coincided with the classical description. In some cases they were less pronounced and time stretched but resulted in high mortality. There was a sudden increase in temperature of animals, and on the 2–3rd day they died. The fever was maintained for 3–4 days. One or two days before death, diseased pigs exhibited loss of appetite, distrurbed coordination of movements and cough; animals were weak, the majority showed cough, respiratory and pulse rates were accelerated. The skin on the ears, head, legs, chest, and the base of the tail was covered with cyanotic swollen spots. In some animals mucous discharges were present from the nasal cavity and eyes. The pregnant diseased breeding pigs often aborted.

The examination and autopsy of dead pigs demonstrated typical signs of the disease. Haemorrhages occurred predominantly in lymph nodes, kidneys, and heart; haemorrhages in other organs were of variable incidence and distribution.

The epidemiological analysis identified the following major reasons and factors for the disease occurrence and spread:

- inadequate understanding of ASF threat by economic entities and citizens;

- disinterest of citizens and owners of animals in ASF eradication due to misunderstanding of the problem and unwillingless to change anything in reference to animal keeping, registration as well as sale of pigs and pork without veterinary documents;
- illegal turn-over, purchase and sale of animals and animal products;
- failure on the part of executive managers to disclose early cases of disease and animal deaths;
- low level of protection of pig farms against biological risks and specific virus properties;
- presence of pigs in backyards of pig farm specialists and employees;
- negligence of biosafety elements in case of selection and shipment of purchased pigs in the territory of a pig farm.

Wild pigs play one of major roles in ASF spread. Fig. 2 shows the population of wild boars in hunting areas of the Krasnodar region from 2007, i.e. from the time of the given population being infected by ASF virus. As could be seen, the wild boar population started to decrease drastically from 2010 after the decision of the *ad hoc* commission on ASF eradication among wild boars.

Evidence of the fact that wild boars were the source of ASF for domestic pigs were confirmed by results of multiple testings of lesions from dead and shot off wild boars in the Tuapsinsky, Severskу, Apsheronsky, Yeysky, Mostovskoy and other areas (2009–2013).

Since 2009, ASF was diagnosed among wild animals in:

- FGT "Sochi republican state wildlife reserve" mountain area "Bolshoy Bznych" mountain area "Tri duba";
- FGI "Caucasian state natural biosphere reserve" in the territory of the warren complex "Laura";
- Republic of Adygea – settlement Guzeripl, mountain range Skazhenny, mountain area "NovyVolok";
- Apsheronsky region – settlement Tuby, distant rural settlement of mountain Shessi;
- Tuapsinsky region – territory of wooded mountain area "3 rota"; territory of wooded area of mountain Paporotnaya, mountain area "Redkina

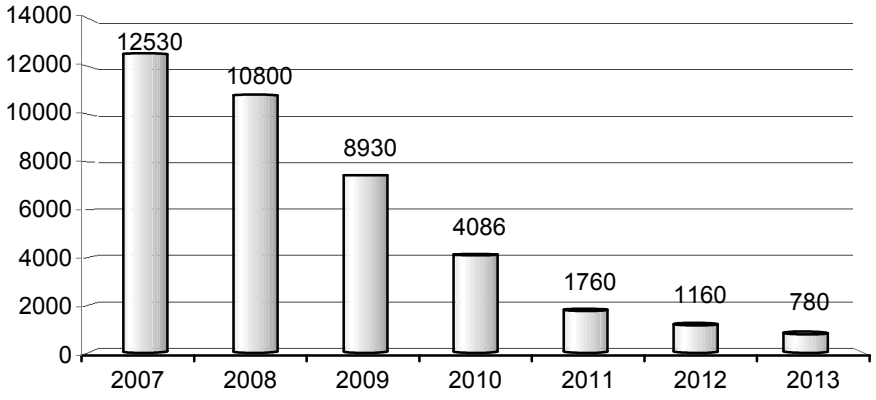

Fig. 2. Population of wild boars in hunting areas of the Krasnodar region in 2007–2013.

Table 1. Results of laboratory examinations of materials from domestic pigs suspected for ASF from 2008 to 2013 (70748 samples) in the Krasnodar Interoblast Regional Veterinary Laboratory by various methods

	Pathological material	Blood serum
Polymerase chain reaction		
Total number of samples	3781	50855
Number of positive samples	150	116
Immunofluorescence assay		
Total number of samples	13068	0
Number of positive samples	97	0
Solid-phase immunosorbent assay		
Total number of samples	2016	665
Number of positive samples	0	0

polyana" lot No. 31; mountain area "Ivankova polyana" lot No. 38; settlement Goytkh; mountain area "Mashinkhoz" lot No. 28;

- GoryachiyKluch – state wildlife reserve, mountain area "Kurgannoye";
- Novorossiysk – a pig carcass at the stage of decomposition was found by the sea, between oil harbor "Sheskharis" and naval base under construction;
- Mostovskoy region – mountain area "Borisovka" 10 km from station Barakaevskaya, Gubsk rural settlement; mountain area "Sukhoy Ruchey" hunting farm "Solyonovskoye";
- Seversky region – mountain area "BalkaSolyonaya Schel" hunting farm "Planchenskaya Schel"; quarter "53" forest area Mirny of Verkhne-Afipsk hunting farm, area of Armyanskiye Polyany;
- Yeysky region – Kopansk flooded areas between costal lakes Gusiny and Krugly.

The Krasnodar interoblast laboratory analysed 76 samples from wild boars from different rayons, 14 samples gave a positive result in direct immunofluorescence assay.

Results of laboratory examinations of materials from domestic pigs suspected for ASF from 2008 to 5/10/2013 are given in Table 1. The materials of the farms in all districts of the region were investigated by three methods: PCR, the immunofluorescence assay and solid-phase immunosorbent assay. According to the table, the PCR method was the most widely used and effective.

The analysis of seasons of ASF occurrence among domestic pigs for 2008–2013 showed that 26 out of 43 registered outbreaks were observed in the summer (June-August), and 17 outbreaks – in the autumn. This could be explained by the fact that the intensity of transportation, movements of agricultural workers and specialists, movements of animals, namely a human factor, was increased with the incoming of a warm season.

During the period 2008–2013, 43 ASF focal points were registered, more than 90 ths pigs died and were destroyed. The billionth damage was caused to pig farming industry in the region.

ASF has become the main reason for sharp decrease of swine population in the region. At the end of 2011 in the region there were 547,529 pigs and by 1 September 2012 – only 303,333 pigs remained. Moreover, if in 2011 there were 12 areas with more than 20,000 pigs then in 2012 there were only 5 such rayons, and only 12 areas had more than 10,000 pigs.

DISCUSSION

In Europe, African swine fever has appeared in Portugal in 1957, then re-emerged in 1960 and has extended over Spain. In Spain and Portugal, the disease has remained endemic up to 1995. From the Iberian Peninsula ASF has extended over France (1964, 1967, 1977), Italy (1967, 1978, 1980), Belgium (1985) and the Netherlands (1986), on the Islands of Madeira (1965, 1974, 1976) and Malta (1978). The last outbreak in the Iberian Peninsula was registered in Portugal in 1999. Currently, ASF is still endemic in Sardinia (Plowright *et al.,* 1994). Outside Africa and Europe, the ASF virus was revealed in the Dominican Republic (1978), Brazil (1978), Haiti (1980) (Wilkinson, 1989) and Cuba (1971, 1980) (Seifert, 1996). The disease has never been recorded in Asia.

In 2007, further transcontinental spread of ASF occurred with the introduction of ASF to Georgia in the Caucasus region. Delays in recognising ASF resulted in its widespread distribution to neighbouring countries, including Armenia, Azerbaijan and several territories in Russia. The Russian epidemic has since been reported from the territories of Chechnya, North Ossetia-Alania, Ingushetia, Orenburg, the Stavropol region, the Krasnodar region and now further westwards into the Rostovskaya Oblast, which has common borders with the Ukraine (Dudnikov *et al.,* 2013). The reports of infection in wild boars on several occasions will complicate eradication (Beltran-Alcrudo *et al.* 2008; OIE, 2009).

The analysis of the situation showed that ASF virus was introduced into the Russian Federation from Transcaucasia and, more precisely, from Georgia where it had been introduced with non-decontaminated meat products from sea vessels coming from European and South African countries where ASF is registered on a regular basis.

A significant number of ASF focal points are located on the border with the Rostov region (Yeysky, Scherbinovsky, Starominskoy, Krylovskoy and Novopokrovsky districts) and the Stavropol region (Gulkevichsky and Novokubansky districts) which territories have become ASF affected before the Krasnodar region. It highlights the high probability of ASF virus introduction on farms of the Krasnodar region from the given territories.

The disease occurred among domestic pigs and was transmitted to wild pigs living in Caucasian mountains and submountain regions. In 2007–2008 about 12000 wild boars inhabited hunting farms of the Krasnodar region only and they had permanent contacts with domestic pigs in the Chechen Republic, North Ossetia, Kabardino-Balkaria, Stavropol and Krasnodar regions. Moreover, wild boars constantly migrated and reproduced in multiple flooded areas, rosh grounds of the Caucasian plain part. Therefore, first ASF outbreaks were reported from North Caucasis from aforementioned republics and regions.

At a later stage a human factor, non-decontaminated condemned goods, trans-

port facilities, feeds, clothes, etc. were "switched on". The analysis of cases of ASF occurrence shows that ASF virus contaminated mixed feeds, meat and meat products, transport and other objects of environment, unauthorised movements and reallocations of animals, slaughter and sale of pigs suspected as being ASF virus-infected, contacts of domestic pigs and humans with wild boars-carriers of ASF virus are sources and routes of the disease spread in the region (Bolotsky *et al.,* 2007).

ASF started to be registered in the Rostov, Astrakhan, Volgograd and other districts. New ASF focal points can occur in other regions with intensive pig farming industry and spread along main transport routes, in Povolzhye, along transport route Moscow-Rostov as well as on farms of other regions which have contacts with people and animals from affected farms of southern regions. The main causes of such ASF wide spread in Russia were associated with the omission to observe veterinary and sanitary regulations and sometimes with their crude violation. The causes were also associated with the fact that the main focal point was among wild pigs and their destruction is a great problem. ASF virus is resistant in the environment and therefore its rapid and complete destruction is limited.

It is evident that farms of our neighbours engaged intensively in pig farming in Ukraine and Belarus will not escape the same fate.

In the Krasnodar region it is difficult to eradicate ASF rapidly and completely as a significant quantity of swine population is in backyards and on private farms (28.5% by the end of 2011) where conditions for breeding, keeping and slaughter do not meet the most frequently elementary veterinary and sanitary regulations. It is difficult to put things right in the given sphere, therefore it is necessary to switch pig farming industry over to large-scale production, to build and startup large specialised pig complexes meeting all aforementioned requirements. It is necessary to bring under strict control movements of pigs and meat products in the region; to develop and encourage alternative animal farming aimed at provision of citizens with meat; to carry out the complete depopulation of wild boars especially in the regions plain area, in flooded areas, etc. The way of thinking should also be changed; pig farmers should know that there is no other way in the pig farming industry. Such problems cannot be solved rapidly; minimum 1.5–2 years will be required. Therefore at present ASF epidemics are liable to occur in the region.

In its ASF prediction in the Russian Federation for 2012 the Information and Analytical Centre of the Federal Service for Veterinary and Phytosanitary Surveillance (FGBI "ARRIAH") states in point 4 that "There is a tendency of ASF spread in cross-border regions of the enzootic zone (SFO and NCFO of the RF) where the occurrence of 55 new focal points is possible. These are the Belgorod, Voronezh, Saratov Oblasts.

There is a probability of new focal point occurrence within the endemic zone including the Krasnodar region. Judging from the current situation the disease eradication in the Russian Federation is a problematic task which is complicated by serious economic expenditures that are necessary for implementation of corresponding programmes, qualified workforce potential and consistent efforts not only of specialists but

also citizens engaged in the pig breeding sector of national economy.

The economic damage caused by ASF to pig farms is a combination of many components: death of affected animals, slaughter and disposal of pigs in the first zone under threat; restrictions in activities due to quarantine; expenses for interned farmers in an outbreak, disinfection agents and other materials. The damage can be different on different farms and it can depend on swine population in an ASF outbreak.

In the absence of preparations for specific prevention the only self-sustainable strategy for ASF eradication or elimination is the depopulation in the given area. The damage can be reduced by taking strict restrictive, sanitary-veterinary and economic measures.

The analysis of aforementioned facts shows that the higher concentration of swine population and the heavier and more developed traffic network, the more frequent occurrence of ASF epidemics. Therefore, it is more reasonable to build large meat-processing plants (and it is justified in the region) in areas with a high level of swine population so that to spread infectious diseases to a lesser degree.

In view of the experience of foreign countries (Spain, Portugal, Italy, etc.) – (Sanchez-Vizcaino, 2012) it is necessary to carry out in the Krasnodar region recording of pig population for the purpose of the complete control over their movements and slaughter. All establishments for keeping and raising pigs, meat-processing plants, slaughter points should pass commission compartmentalization for the protection level and should arrive at a valuation. Farm enterprises and peasant households that do not meet corresponding veterinary and sanitary requirements and have a low compartment level (I and II) should be closed up. In the region it is necessary to build more large specialized pig breeding complexes meeting all rules for protection against infectious diseases, to build them in conventionally corn-growing areas of central and north-western zones of the region where a network of well-appointed autoroads is the most developed and where a significant number of meat-processing plants and slaughter points is located.

Natural and climatic conditions and grain production potential of the region makes it possible to keep simultaneously at least 3–4 mln of pigs and to have in the turnover 7–8 mln of pigs. Thus, the region is able to provide pork for all native citizens as well as visitors and export it in significant volumes. Until a network of specialised pig breeding complexes is build and put into mass production it is necessary to develop in the region alternative production of meat: poultry, cattle, rabbit etc.

CONCLUSIONS

The conducted epidemic situation monitoring made it possible to determine sources, routes and factors promoting ASF spread in the Krasnodar region. In the former Soviet Union territory the disease occurred in 2007 in Georgia. Wild pigs in Caucasian mountains got infected and then the disease affected domestic pigs in neighbouring Caucasian Republics, Stavropol and Krasnodar regions and widely spread.

It was established that in the period from 2008 to 2013, 43 epizootic foci of ASF were registered in Krasnodar region. Most outbreaks were observed in summer (June-August) – 26 outbreaks, and in autumn – 17 outbreaks.

The main sources of ASF epidemics were presented by sick and dead wild and domestic pigs, non-decontaminated seized meat products. The transmission factors were transportation vehicles from outbreaks and insufficiently decontaminated ASF virus – infected feeds, implements, etc. In the analysis of ASF outbreaks in pig farms of Krasnodar region found that the leading role in the spread of ASF is the human factor. Under such situation the tendency to ASF spread can not only continue to persist but also to expand.

The success in ASF eradication can be attained under the condition that veterinary sanitary and economic measures will be implemented.

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