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Results of serological and molecular analysis of African and classical swine fever in the population of wild boars in Ukraine

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Abstract

The results of the study of lymphoid organs and sera of wild boars for the presence of DNA of African swine fever (ASF) virus and RNA of classical swine fever (CSF) virus are presented, as well as the results of a serological examination for the presence of ASF and CSF virus antibodies. The study was conducted in Ukraine between 2008 and 2013. Biological samples were obtained from wild boars shot during the hunting season, and were examined by real-time PCR and ELISA. In total, 5759 sera were tested for CSF virus antibodies and 4856 for ASF virus antibodies by ELISA. Samples of lymphoid organs totalling 1129 were examined by PCR for the detection of CSF virus RNA and 8102 such samples were examined for the detection of ASF virus DNA. CSF virus antibodies were detected in 6.56% of wild boar sera. RNA of CSF virus was also identified in 1 out of 1129 samples tested. ASF virus antibodies or DNA in lymphoid organ samples were not detected.

Keywords: wild boars, African swine fever, classical swine fever, antibodies, molecular analysis.

Introduction

African swine fever (ASF) has caused very substantial losses in the swine population in Africa, Europe, and North and South America, and remains a dangerous infectious disease impacting the economy worldwide, mainly in countries with developed pork industries. The entry and spread of ASF in the Caucasus and Russia is characterised by high epizootic intensity and a tendency for the appropriate epizootic forecast to be adverse and long-term (5-7, 13).

Classical swine fever (CSF) remains a significant problem worldwide. Despite the constant struggle with this disease, as well as the development of a number of specific prevention measures and eradication programmes, World Organisation for Animal Health (OIE) reports indicate that CSF is periodically registered on almost all continents. Furthermore, ASF has heterogeneous clinical manifestations, due to the peculiarities of the pathogen and its antigenic variability. The gradual transfer of the virus to a state of low virulence leads to the asymptomatic chronic form of the disease among wild and domestic pigs that conceals constant virus carriers in their populations. This fact was registered in the People's Republic of the Congo (2), Portugal, Spain, and among wild boars in Sardinia (13). The virus can change its virulence properties when in contact with susceptible animal populations.

Considering these specifics of the CSF and ASF risk, their monitoring is particularly important for Ukraine, because its territory borders Russia, the Caucasus, and Belarus, which report the occurrence of these diseases.

As ASF and CSF are related diseases, have similar disease manifestations and cause similar pathological changes (3), the aim of the study was to monitor the presence of both viruses and specific antibodies in the population of wild boars in Ukraine.

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Material and Methods

Biological material was obtained from wild boars shot during hunting seasons in different regions of Ukraine. The sampling of the material (serum samples and fragments of lymphoid organs) was conducted according to the approved procedures for the selection, packing, and transportation of biological material for CSF and ASF virus research.

Between 2008 and 2013, 5759 serum samples (135 in 2008, 1284 in 2009, 1282 in 2010, 199 in 2011, 1851 in 2012, and 1008 in 2013 were tested for CSF antibodies. Lymphoid organ samples comprising 1129 (22 in 2011, 385 in 2012, and 722 in 2013) were also analysed for CSF virus. Between 2009 and 2013, 4856 serum samples (1165 in 2009, 623 in 2010, 531 in 2011, 761 in 2012, and 1776 in 2013) were tested for ASF antibodies and between 2011 and 2013, 8102 samples of lymphoid organs (2116 in 2011, 3174 in 2012, and 2812 in 2013) were analysed for ASF virus.

Serological analysis for ASF was conducted by ELISA using the commercial "Ingezim PPA Compac" kit (Ingenasa, Spain). The detection of ASF virus DNA was performed by PCR using the "Test system for the detection of African swine fever virus by polymerase chain reaction with hybridisation fluorescent detection in real-time" (FBIS CRIE Rospotrebnadzor, Russia) and diagnostic kit ASF-PCR GNP (JSC PSE "Ukrzoovetprompostach", Ukraine).

The detection of CSF virus antibodies was conducted by ELISA using a classical swine fever virus antibody test kit (IDEXX, the Netherlands). Molecular detection of CSF virus RNA was performed by PCR using a kit with hybridisation fluorescent detection in real-time (FBIS CRIE Rospotrebnadzor, Russia).

All analyses were performed at the Laboratory of Diagnostics and Veterinary and Sanitary Expertise of the State Research Institute and at the Institute of Veterinary Medicine of the Ukrainian National Academy of Agrarian Sciences, Kiev.

Results

The results of serological and molecular analyses of biological samples from wild boars for CSF and ASF antibodies and genetic material are presented in Tables 1–4.

The data in Table 1 indicate that the overall rate of CSF virus seroprevalence in the wild boar population in Ukraine is 6.56%, and the rates by region are 2.75% in the western, 3.13% in the southern, 10.62% in the northern, 5.95% in the eastern, and 9.17% in the central regions of the country.

In the western regions of Ukraine, the highest percentage of positive samples was found in Zakarpattia (7.69%). In the Volyn, Ivano-Frankivsk, Lviv, Rivne, Ternopil, Khmelnytskyi, and Chernivtsi regions, the seroprevalence was 2.38%, 2.36%, 0.71%, 2.65%, 1.52%, 6.78%, and 0.70% respectively.

In the southern regions, the highest percentage of positive samples was found in the Odessa region (5.74%). In the AR Crimea, Zaporizhia, and Mykolaiv regions, the seroprevalence was 2.15%, 2.86%, and 3.08% respectively. It should be noted that no positive sera were detected in the Kherson region during the period tested.

In the northern part of Ukraine, the highest percentage of positive samples was reported in the Kyiv (17.73 %) and Chernihiv (13.71 %) regions. Significantly lower numbers were recorded in Zhytomyr (8.44 %) and Sumy (6.25 %).

In the eastern regions, the percentages of positive sera were: 3.19% in Donetsk, 8.51% in Luhansk, and 4.81% in Kharkiv.

In the central regions, the highest percentages of serum positive samples were found in Cherkasy (16.72%), Vinnytsia (10.14%), and Dnipropetrovsk (9.78%) oblasts. In the Poltava oblast, this parameter was lower (4.27%). No positive serum samples containing CSF virus antibodies were found in Kirovohrad.

The majority of serum samples (1851) were examined in 2012. In 2008, the number of investigated sera was much lower (135). In 2009, 1284 serum samples were investigated and a year later almost exactly as many were evaluated (1282). In 2011, 199 serum samples were analysed, whereas in 2013, the number of analysed serum samples was 1008.

During the hunting seasons, the percentages of positive sera containing CSF virus antibodies were as follows: no positive sera were recorded in the 2008 season, whereas in subsequent years the percentage of positive sera tended to increase and was equal to 8.02% in 2009; 6.47% in 2010; 3.52% in 2011; 5.94% in 2012; and 7.44% in 2013.

Between 2008 and 2013, 1129 samples of lymphoid organs were analysed by PCR (Table 2). The samples were received from different oblasts excluding Zakarpattia, Khmelnytskyi, Zhytomyr, Vinnytsia, and Kirovohrad.

The results of CSF virus RNA detection in fragments of the lymphoid organs by PCR are presented in Table 2.

During the analysed period, only 1 out of 1129 samples contained RNA of CSF virus. This sample was received from a wild boar shot in the Kyiv region in 2012.

Due to the outbreaks of ASF in the Caucasus region and Russian Federation in 2007, serological monitoring of this disease in wild boars in Ukraine was initiated. The results of ASF virus antibody detection by ELISA in wild boar sera are presented in Table 3.

Between 2009 and 2013, 4856 serum samples from all regions of Ukraine were tested by ELISA, however antibodies specific to CSF virus were not detected.

Table 1. CSF virus antibodies in wild boar sera from different regions of Ukraine, detected by ELISA

Region	Oblast	Year												Total		Percentage of positive results
		2008		2009		2010		2011		2012		2013				
		tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	
West	Volyn	29	0	49	2	25	3	0	0	107	0	0	0	**210**	**5**	2.38
	Zakarpattia	0	0	12	3	16	0	9	3	26	0	15	0	**78**	**6**	7.69
	Ivano-Frankivsk	0	0	40	0	23	3	6	0	42	0	16	0	**127**	**3**	2.36
	Lviv	0	0	45	0	80	2	2	0	90	0	65	0	**282**	**2**	0.71
	Rivne	0	0	50	5	30	0	0	0	112	1	34	0	**226**	**6**	2.65
	Ternopil	0	0	17	1	15	0	1	0	16	0	17	0	**66**	**1**	1.52
	Khmelnytskyi	22	0	48	1	44	11	0	0	35	0	28	0	**177**	**12**	6.78
	Chernivtsi	0	0	53	0	26	0	10	1	46	0	8	0	**143**	**1**	0.70
Total		**51**	**0**	**314**	**12**	**259**	**19**	**28**	**4**	**474**	**1**	**183**	**0**	**1309**	**36**	2.75
Percentage of positive results		0.00		3.82		7.34		14.29		0.21		0.00		**2.75**		
South	AR Crimea	0	0	44	4	34	0	8	0	159	0	34	2	**279**	**6**	2.15
	Zaporizhia	0	0	33	3	34	0	8	0	48	1	17	0	**140**	**4**	2.86
	Mykolaiv	0	0	22	0	15	0	17	2	11	0	0	0	**65**	**2**	3.08
	Odessa	0	0	79	7	49	3	21	0	44	2	16	0	**209**	**12**	5.74
	Kherson	0	0	31	0	16	0	0	0	22	0	5	0	**74**	**0**	0.00
Total		**0**	**0**	**209**	**14**	**148**	**3**	**54**	**2**	**284**	**3**	**72**	**2**	**767**	**24**	3.13
Percentage of positive results		-		6.70		2.03		3.70		1.06		2.78		**3.13**		
North	Zhytomyr	0	0	91	6	108	0	0	0	103	10	77	16	**379**	**32**	8.44
	Kiev	0	0	47	15	78	14	0	0	38	1	57	9	**220**	**39**	17.73
	Sumy	0	0	50	3	105	6	31	0	188	9	74	10	**448**	**28**	6.25
	Chernihiv	48	0	105	4	63	0	13	0	117	37	48	13	**394**	**54**	13.71
Total		**48**	**0**	**293**	**28**	**354**	**20**	**44**	**0**	**446**	**57**	**256**	**48**	**1441**	**153**	10.62
Percentage of positive results		0.00		9.56		5.65		0.00		12.78		18.75		**10.62**		
East	Donetsk	0	0	45	0	32	4	18	0	51	2	42	0	**188**	**6**	3.19
	Luhansk	0	0	73	11	129	1	0	0	144	16	124	12	**470**	**40**	8.51
	Kharkiv	8	0	119	3	109	11	17	0	143	2	207	13	**603**	**29**	4.81
Total		**8**	**0**	**237**	**14**	**270**	**16**	**35**	**0**	**338**	**20**	**373**	**25**	**1261**	**75**	5.95
Percentage of positive results		0.00		5.91		5.93		0.00		5.92		6.70		**5.95**		
Central	Vinnytsia	0	0	12	0	42	4	26	1	44	9	14	0	**138**	**14**	10.14
	Dnipropetrovsk	0	0	30	9	13	0	9	0	20	0	20	0	**92**	**9**	9.78
	Kirovohrad	0	0	23	0	27	0	2	0	37	0	30	0	**89**	**0**	0.00
	Poltava	0	0	87	7	88	7	0	0	96	1	50	0	**351**	**15**	4.27
	Cherkasy	28	0	79	19	81	14	1	0	112	19	10	0	**311**	**52**	16.72
Total		**28**	**0**	**231**	**35**	**251**	**25**	**38**	**1**	**309**	**29**	**124**	**0**	**981**	**90**	9.17
Percentage of positive results		0.00		15.15		9.96		2.63		9.39		0.00		**9.17**		
Total in Ukraine		**135**	**0**	**1284**	**103**	**1282**	**83**	**199**	**7**	**1851**	**110**	**1008**	**75**	**5759**	**378**	6.56
Percentage of positive results		0.00		8.02		6.47		3.52		5.94		7.44		**6.56**		

Table 2. CSF virus RNA in fragments of wild boar lymphoid organs from different regions of Ukraine, detected by PCR

Region	Oblast	Years												Total		Percentage of positive reults
		2008		2009		2010		2011		2012		2013				
		tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	tested	positive	
West	Volyn	0	0	0	0	0	0	0	0	11	0	0	0	**11**	**0**	0.00
	Zakarpattia	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	-
	Ivano-Frankivsk	0	0	0	0	0	0	0	0	0	0	17	0	**17**	**0**	0.00
	Lviv	0	0	0	0	0	0	0	0	15	0	61	0	**76**	**0**	0.00
	Rivne	0	0	0	0	0	0	0	0	0	0	34	0	**34**	**0**	0.00
	Ternopil	0	0	0	0	0	0	0	0	5	0	5	0	**10**	**0**	0.00
	Khmelnytskyi	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	-
	Chernivtsi	0	0	0	0	0	0	0	0	24	0	17	0	**41**	**0**	0.00
Total		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**55**	**0**	**134**	**0**	**189**	**0**	0.00
Percentage of positive results		-		-		-		-		0.00		0.00		**0.00**		
South	AR Crimea	0	0	0	0	0	0	0	0	28	0	34	0	**62**	**0**	0.00
	Zaporizhia	0	0	0	0	0	0	0	0	24	0	24	0	**48**	**0**	0.00
	Mykolaiv	0	0	0	0	0	0	0	0	5	0	0	0	**5**	**0**	0.00
	Odessa	0	0	0	0	0	0	0	0	20	0	16	0	**36**	**0**	0.00
	Kherson	0	0	0	0	0	0	5	0	0	0	4	0	**9**	**0**	0.00
Total		**0**	**0**	**0**	**0**	**0**	**0**	**5**	**0**	**77**	**0**	**78**	**0**	**160**	**0**	0.00
Percentage of positive results		-		-		-		0.00		0.00		0.00		**0.00**		
North	Zhytomyr	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	-
	Kiev	0	0	0	0	0	0	7	0	1	1	17	0	**25**	**1**	4.40
	Sumy	0	0	0	0	0	0	0	0	92	0	79	0	**171**	**0**	0.00
	Chernihiv	0	0	0	0	0	0	0	0	2	0	0	0	**2**	**0**	0.00
Total		**0**	**0**	**0**	**0**	**0**	**0**	**7**	**0**	**95**	**1**	**96**	**0**	**198**	**1**	0.51
Percentage of positive results		-		-		-		0.00		1.05		0.00		**0.51**		
east	Donetsk	0	0	0	0	0	0	10	0	0	0	52	0	**62**	**0**	0.00
	Luhansk	0	0	0	0	0	0	0	0	0	0	114	0	**114**	**0**	0.00
	Kharkiv	0	0	0	0	0	0	0	0	51	0	222	0	**273**	**0**	0.00
Total		**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**51**	**0**	**388**	**0**	**449**	**0**	0.00
Percentage of positive results		-		-		-		0.00		0.00		0.00		**0.00**		
Central	Vinnytsia	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	-
	Dnipropetrovsk	0	0	0	0	0	0	0	0	0	0	20	0	**20**	**0**	0.00
	Kirovohrad	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0**	-
	Poltava	0	0	0	0	0	0	0	0	44	0	0	0	**44**	**0**	0.00
	Cherkasy	0	0	0	0	0	0	0	0	63	0	6	0	**69**	**0**	0.00
Total		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**107**	**0**	**26**	**0**	**133**	**0**	0.00
Percentage of positive results		-		-		-		-		0.00		0.00		**0.00**		
Total in Ukraine		**0**	**0**	**0**	**0**	**0**	**0**	**22**	**0**	**385**	**1**	**722**	**0**	**1129**	**1**	0.09
Percentage of positive results		-		-		-		**0.00**		**0.26**		**0.00**		**0.09**		

Table 3. ASF virus antibodies in wild boar sera from different regions of Ukraine, detected by ELISA

Region	Oblast	Years										Total	
		2009		2010		2011		2012		2013			
		tested	tested	tested	positive	tested	positive	tested	positive	tested	positive	tested	positive
West	Volyn	49	0	0	0	0	0	59	0	81	0	**189**	**0**
	Zakarpattia	12	0	16	0	16	0	0	0	35	0	**79**	**0**
	Ivano-Frankivsk	40	0	5	0	6	0	16	0	26	0	**93**	**0**
	Lviv	45	0	12	0	2	0	15	0	94	0	**168**	**0**
	Rivne	50	0	30	0	0	0	80	0	80	0	**240**	**0**
	Ternopil	17	0	9	0	1	0	0	0	33	0	**60**	**0**
	Khmelnytskyi	48	0	0	0	0	0	0	0	62	0	**110**	**0**
	Chernivtsi	53	0	0	0	10	0	23	0	22	0	**108**	**0**
Total		**314**	**0**	**72**	**0**	**35**	**0**	**193**	**0**	**433**	**0**	**1047**	**0**
South	AR Crimea	39	0	3	0	42	0	62	0	69	0	**215**	**0**
	Zaporizhia	33	0	16	0	8	0	14	0	17	0	**88**	**0**
	Mykolaiv	22	0	0	0	17	0	0	0	16	0	**55**	**0**
	Odessa	79	0	20	0	15	0	22	0	52	0	**188**	**0**
	Kherson	31	0	0	0	30	0	2	0	5	0	**68**	**0**
Total		**204**	**0**	**39**	**0**	**112**	**0**	**100**	**0**	**159**	**0**	**614**	**0**
North	Zhytomyr	52	0	77	0	0	0	68	0	183	0	**380**	**0**
	Kiev	47	0	0	0	0	0	0	0	57	0	**104**	**0**
	Sumy	35	0	45	0	73	0	85	0	158	0	**396**	**0**
	Chernihiv	94	0	48	0	86	0	82	0	127	0	**437**	**0**
Total		**228**	**0**	**170**	**0**	**159**	**0**	**235**	**0**	**525**	**0**	**1317**	**0**
East	Donetsk	33	0	20	0	16	0	0	0	87	0	**156**	**0**
	Luhansk	51	0	97	0	85	0	0	0	124	0	**357**	**0**
	Kharkiv	131	0	72	0	42	0	96	0	325	0	**666**	**0**
Total		**215**	**0**	**189**	**0**	**143**	**0**	**96**	**0**	**536**	**0**	**1179**	**0**
Central	Vinnytsia	12	0	0	0	0	0	16	0	24	0	**52**	**0**
	Dnipropetrovsk	30	0	13	0	5	0	0	0	20	0	**68**	**0**
	Kirovohrad	23	0	20	0	2	0	15	0	15	0	**75**	**0**
	Poltava	60	0	54	0	50	0	45	0	0	0	**209**	**0**
	Cherkasy	79	0	66	0	25	0	61	0	64	0	**295**	**0**
Total		**204**	**0**	**153**	**0**	**82**	**0**	**137**	**0**	**123**	**0**	**699**	**0**
Total in Ukraine		**1165**	**0**	**623**	**0**	**531**	**0**	**761**	**0**	**1776**	**0**	**4856**	**0**

Table 4. ASF virus DNA in fragments of wild boar lymphoid organs from different regions of Ukraine, detected by PCR

Region	Oblast	Years						Total	
		2011		2012		2013			
		tested	tested	tested	positive	tested	positive	tested	positive
West	Volyn	78	0	125	0	29	0	**232**	**0**
	Zakarpattia	0	0	0	0	24	0	**24**	**0**
	Ivano-Frankivsk	25	0	9	0	30	0	**64**	**0**
	Lviv	2	0	35	0	61	0	**98**	**0**
	Rivne	116	0	244	0	347	0	**707**	**0**
	Ternopil	1	0	10	0	5	0	**16**	**0**
	Khmelnytskyi	0	0	0	0	42	0	**42**	**0**
	Chernivtsi	25	0	47	0	36	0	**108**	**0**
Total		**247**	**0**	**470**	**0**	**574**	**0**	**1291**	**0**
South	AR Crimea	114	0	168	0	117	0	**399**	**0**
	Zaporizhia	52	0	70	0	30	0	**152**	**0**
	Mykolaiv	32	0	5	0	18	0	**55**	**0**
	Odessa	43	0	23	0	16	0	**82**	**0**
	Kherson	0	0	2	0	7	0	**9**	**0**
Total		**241**	**0**	**268**	**0**	**188**	**0**	**697**	**0**
North	Zhytomyr	143	0	184	0	174	0	**501**	**0**
	Kiev	255	0	528	0	380	0	**1163**	**0**
	Sumy	210	0	314	0	140	0	**664**	**0**
	Chernihiv	340	0	607	0	467	0	**1414**	**0**
Total		**948**	**0**	**1633**	**0**	**1161**	**0**	**3742**	**0**
East	Donetsk	30	0	107	0	129	0	**266**	**0**
	Luhansk	235	0	158	0	198	0	**591**	**0**
	Kharkiv	216	0	178	0	487	0	**881**	**0**
Total		**481**	**0**	**443**	**0**	**814**	**0**	**1738**	**0**
Central	Vinnytsia	2	0	50	0	19	0	**71**	**0**
	Dnipropetrovsk	26	0	23	0	30	0	**79**	**0**
	Kirovohrad	2	0	37	0	15	0	**54**	**0**
	Poltava	98	0	111	0	0	0	**209**	**0**
	Cherkasy	71	0	139	0	11	0	**221**	**0**
Total		**199**	**0**	**360**	**0**	**75**	**0**	**634**	**0**
Total in Ukraine		**2116**	**0**	**3174**	**0**	**2812**	**0**	**8102**	**0**

Due to the proliferation of ASF in Russia and the constant threat of the pathogen's entry into the territory of Ukraine, the State Veterinary and Phytosanitary Service of Ukraine performed PCR analyses of the lymphoid organs of shot wild boars for the presence of ASF virus DNA. The results of the studies performed between 2011 and 2013 are presented in Table 4.

Between 2011 and 2013, 8102 samples of biological material were analysed: 2116 in 2011, 3174 in 2012, and 2812 in 2013. No sample containing ASF virus DNA was detected.

Discussion

Since 1995, the intensity of CSF outbreaks has been decreasing, and in 2001 only one outbreak of the disease in wild boar was detected, on the territory of

the "Trakhtemyriv" reserve (Myronivka and Kaniv districts). Since then CSF has not been recorded. The application of two live virus vaccines against CSF (ADV and "LC-M" strains) seems to have greatly contributed to the absence of the disease in domestic pigs. It should be noted that the absence of CSF disease in Ukraine does not indicate the absence of the pathogen among the population of domestic and wild pigs. Due to mass vaccination of domestic pigs against CSF, the virus has reduced its virulence and it may exist among vaccinated populations without clinical symptoms (11, 15–7).

Monitoring studies of CSF in wild boars in Ukraine were initiated in 2000 in the Research Centre for the Study of CSF at the Institute of Veterinary Medicine of the Ukrainian National Academy of Agrarian Sciences. Due to the emergence of ASF in the Russian Federation in 2007, since 2008 monitoring studies of wild boar in Ukraine have involved studies on ASF outbreaks.

The studies of wild boar infectious diseases conducted by the Institute of Veterinary Medicine of the Ukrainian National Academy of Agrarian Sciences have been motivated by the continuous determination of the number of wild boars and the number of them shot on the territory of Ukraine (Fig. 1).

The data in Fig. 1 show an increase in the population of wild boar in Ukraine. During the 1999–2000 hunting season, the number of wild boar was 37 000, and by 2010 their number had increased 1.7 times. The trend towards excessive decrease or increase in any species population leads to the loss of biological equilibrium among the environment's species, which is arrived at as the aggregate of biocenotic factors of a certain area. Migration, expansion of the habitat, threat to farmland and, most importantly, the increase in various infectious and parasitic diseases occurrence in domestic herds, as well as the entry of pathogens into new territories may have an impact on the increase of diseases in wild boar. Thus much emphasis should be put on the annual trend observed in monitoring studies on the wild boar population in the Russian Federation, which had reached 404 400 animals in 2010. Furthermore, the threat of dangerous transboundary diseases, including CSF and ASF entering other territories of Russia and neighboring countries (Ukraine) should be emphasised. The border traverse of such diseases into Ukraine is actually a current phenomenon.

The density and number of wild boar population in the regions of Ukraine is shown Fig. 2.

Between May 2012 and May 2013, the Laboratory of Diagnostics and Veterinary and Sanitary Expertise of the State Research Institute, in cooperation with the Institute of Veterinary Medicine of the Ukrainian National Academy of Agrarian Sciences, carried out the international project "Analysis of the classical and African swine fever threats in wild boar populations in Ukraine" within the framework of the Ukraine–USA Biological Threat Reduction Program.

On 1 May 2012, 697 sera and 653 lymph node samples were collected from 8 oblasts: Crimea, Donetsk, Zaporizhya, Luhansk, Odessa, Sumy, Kharkiv, and Kherson. No samples were collected from Mykolaiv oblast. These oblasts were selected in agreement with the Veterinary and Phytosanitary Service of Ukraine as the most vulnerable to the incursion of ASF due to their close proximity to the Russian Federation or to the sea.

The results of the study showed the seroprevalence of CSF to be 5.31% in the wild boar population. ASF virus DNA and specific antibodies were not detected.

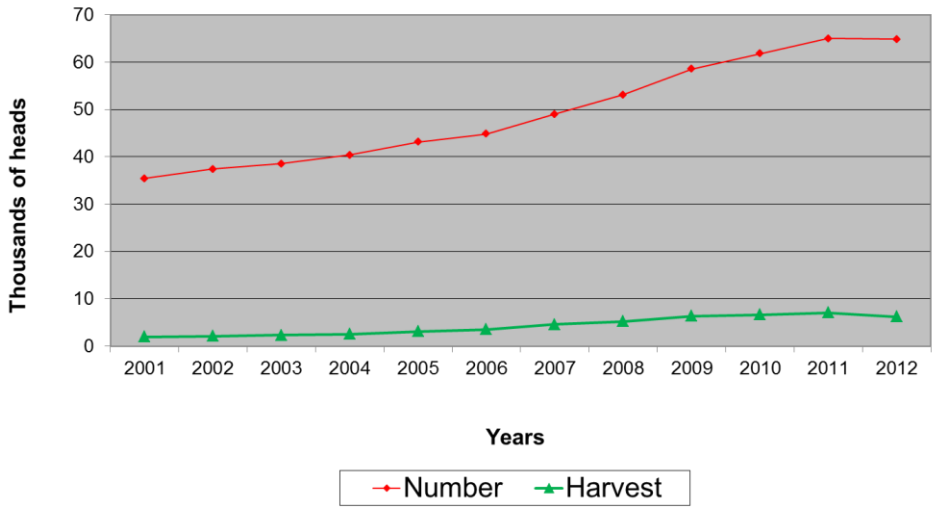


Fig. 1. Indicators of wild boar number and total kill in Ukraine in 2001-2012

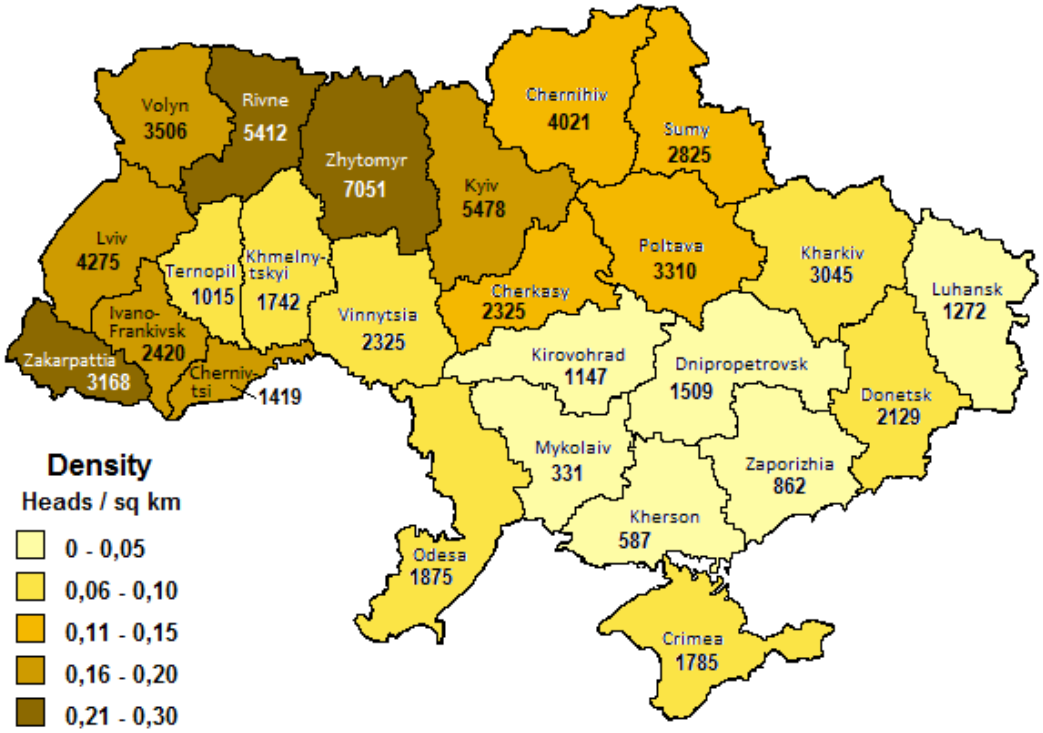


Fig. 2. The density and number of wild boars in particular oblasts of Ukraine in 2012

The monitoring studies described in our paper were necessary for epidemiological surveillance. Their results indicate the presence of humoral CSF virus antibodies in sera of wild boars in almost every area of the territory of Ukraine under study. The annual registration of seropositive animals indicates the circulation of the virus in the population of wild boar. During the monitoring studies of ASF, it was impossible to detect the presence of humoral antibodies in wild boar sera for several years. This can be explained by the fact that in the threat-posing countries of the Caucasus and Russia there circulates the genotype II of ASF virus, which is characterised by high virulence and lethality. The duration of this disease is, on average, between 2 and 7 d in both domestic and wild pigs. On the one hand, in this short period humoral antibodies cannot be formed and therefore they will not be detected through serological examination. This was confirmed by the data obtained during the epizootological survey of the ASF outbreak in domestic pigs in Ukraine in 2012, when antibodies against genotype II of ASF virus were not detected in pigs with the acute form of the disease (12). On the other hand, it is known that serological tests are preliminary ones, particularly in the case of ASF, and their results cannot be reliable as antibody synthesis does not occur in practice or is expressed weakly. For example, there are cases when ASF virus antibodies were detected in countries where this disease is endemic, such as Kenya, Uganda (8, 9, 14), and Sardinia (10), as well as Spain and Portugal (1, 4) where circulation of low virulence ASF virus was confirmed.

The results of serological and molecular monitoring studies of wild boars in the territory of Ukraine demonstrate the circulation of classical swine fever virus in this population. No specific antibodies in serum or ASF virus DNA in lymphoid organs were detected. Our studies revealed the absence of ASF virus in the population of wild boars in the territory of Ukraine between 2009 and 2013. Due to the unfavourable epizootic situation regarding classical and African swine fever in Russia, there is a permanent threat of these pathogens being introduced into Ukraine. Therefore, it is necessary to enhance monitoring studies covering a larger number of wild boars especially in the eastern regions bordering with Russia.

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