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AFRICAN SWINE FEVER IN ROMANIA: RETROSPECTIVE ANALYSIS 2017-2020

PESTA PORCINĂ AFRICANĂ ÎN ROMÂNIA: ANALIZĂ RETROSPECTIVĂ 2017-2020

Florica BĂRBUCEANU[1),2),*)], Paula TAMBA[1),2)], C. NITESCU[1)], N. LAZĂR[1)], D. DONESCU[1)], G. PREDOI[2)], S. BĂRĂITĂREANU[2)]

ABSTRACT

The evolution of African swine fever (ASF) in Europe was severe in last years, despite various control procedures applied. In the epidemiology of ASF, wild pigs acted as a reservoir and transboundary spreaders of ASFV, but in some countries, low biosecurity standards of backyard pig farms played a more significant role in the transmission of the virus. To reduce the risk of transmitting ASFV, European countries implemented passive and active surveillance programs for an early detection of the introduction of the virus in a certain area. This study aims to present a retrospective analysis of African Swine Fever evolution in Romania, between 2017 and 2020. During the analysed period, 810563 samples were investigated and 5680 outbreaks were detected. From the total number, 3942 were outbreaks in domestic and 1738 cases in wild pigs.

Keywords: ASFV, emerging infectious disease, swine diseases, diagnostic

REZUMAT

În ciuda măsurilor de control aplicate, în ultimii ani evoluția europeană a focarelor de pestă porcină Africană (PPA) a fost severă. În epidemiologia PPA, porcii sălbatici au fost considerați rezervor silvatic și vectori de transmitere transfrontalieră a ASFV, dar în unele țări, standardele scăzute de biosecuritate din unitățile de creștere tradițională a porcilor au jucat un rol mult mai mare în transmiterea acestui virus. Pentru a reduce riscul transmiterii ASFV, țările europene au implementat programe de supraveghere pasivă și activă pentru detectarea precoce a virusului. Acest studiu își propune să prezinte o analiză retrospectivă a evoluției pestei porcine africane în România, între 2017-2020. În perioada analizată, au fost investigate 810563 probe și au fost identificate 5680 focare. Din numărul total, 3942 au fost focare la porcii domestici și 1738 cazuri la porcii sălbatici.

Cuvinte cheie: ASFV, boală infecțioasă emergentă, suine, diagnostic

The emergence of African swine fever (ASF) in Eastern Europe tested sorely pig farmers, veterinary practitioners and authorities. The emergence has begun in the Caucasus region in June 2007 when ASF was confirmed in Georgia (14).Unfortunately, the control of ASF in that region failed and, in the following months, the virus spread and become endemic in Georgia, Armenia, and parts of Russia. In the next 10 years, outbreaks were reported in Azerbaijan (2008), Ukraine (2012), Belarus (2013) Lithuania (2014), Poland (2014), Estonia (2014), Latvia (2014), Moldova (2016), and Czech Republic (2017) (2). In a retrospective study, Baraitareanu et al. (2018) concluded that the origin of the first outbreak reported by each Eastern European country remains unknown or inconclusive and any means of spreading should be considered (1). However, wild boars were the mainly incriminated source of ASFV spreading and they should be given more attention (2). The epidemiological situation of ASF in Eastern and Central European countries evolved severely, despite multifaceted preventive and hygienic procedures implemented by each country. The cross-border movement of wild pigs and low biosecurity standards of backyard pig farms may have played a significant role in the rapid spread of the virus in Eastern European countries (2, 5).

In the absence of a specific measure of prevention, the only option of ASF prevention is strict biosecurity. The identification of the most feasible, practical, and affordable biosecurity measures will be the main challenge for all stakeholders because biosecurity measures are often unaffordable or impossible to implement in small-scale pig farms (13).

The aim of this study is to present a retrospective analysis of African swine fever in Romania, between 2017 and 2020.

MATERIALS AND METHODS

The sampling, procedures and methods used in this study were set out following the European Union guidelines and regulations, the OIE Manual of Diagnostic Tests and Vaccines for Terrestrials Animals (on-

1) Institute for Diagnosis and Animal Health, Bucharest, Romania
2) University of Agronomic Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Bucharest, Romania
*) Corresponding author: florica.barbuceanu@idah.ro

line edition) and carried out in biosafety level 2 and 3 facilities of the Institute for Diagnosis and Animal Health (Bucharest – Romania).

Samples

A total of 810563 samples were included in the Romanian surveillance program for ASF between 2017 and 2020 (Table 1). Sampling and testing for ASF were parts of the Romanian active surveillance program, involving all Sanitary Veterinary and Food Safety Directorates (SVFSD) and the Romanian Reference Laboratory for ASF (located at the Institute for Diagnosis and Animal Health, Bucharest, Romania).

iELISA

The iELISA recommended by EURL was used by NRL following the OIE Manual of Diagnostic and Tests (11). In the SVFSD territorial laboratories commercial kits iELISA or Blocking ELISA were used.

IPT

The indirect immunoperoxidase test (IPT) was used as a confirmatory technique for positive and doubtful ELISA results in accordance with the recommendations of Gallardo et al. (2015), OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals and the standard operating procedures of CISA-INIA (Madrid, Spain) EURL-ASF (6, 8, 11).

FAT

The FAT was used to detect ASFV-antigen in pig tissues sampled from animals with clinical signs or lesions possibly associated with ASFV infection. FAT was an additional method of diagnostic used in primary outbreaks on samples positive in PCR Test, in per acute and acute diseases. In accord with Sanchez-Vizcaino and Arias (2012) recommendation, FAT was not used in the suspicions of subacute and chronic ASF where the low sensitivity of test can provide false-negative results (15). The procedure of the test followed the general recommendations of OIE reference laboratories for African swine fever. Briefly, impression smears of test tissues were air-dried, fixed with ace-tone (10 min), and stained with FITC-conjugate anti-ASFV-Ig (60 min, 37°C, in a humid chamber). Samples were considered positive if a specific granular cytoplasmatic fluorescence was recorded in fixed macrophages.

IBT

The method is the confirmatory test recommended by OIE for samples that have been positive and doubtful with ELISA tests and also in case of sera incorrectly handled, stored, or transported (11). The method used Protein A-peroxidase conjugated according to the recommendations of CISA-INIA (Madrid,Spain) EURL-ASF (see the last version of the protocol at: https://asf-referencelab.info/asf/images/ficherosasf/SOP%202018/SOP-ASF-IB-1_REV 2018.pdf).

Real-Time RT-PCR

The real-time RT-PCR was performed using the Fernández-Pinero et al. (2013) protocol recommended in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (7, 11). The method proved suitable in the detection of infected animals in a very early or chronic stages of infection (7). The DNA extraction was performed in accord with the standard operating procedure for the extraction of African swine fever virus (ASFV) DNA of CISA-INIA (Madrid, Spain) EURL -ASF (see the last version of the protocol at: https://asf-referencelab.info/asf/images/ficherosasf/SOP%202018/SOP-ASF-DNA-EXTRACTION-1_REV 2018.pdf). The real-time RT-PCR method followed the Fernández-Pinero et al. (2013) recommendations, described in OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (7, 11). The procedure is also deailed in the standard operation procedure for the detection of African swine fever virus (ASFV) by a real-time polymerase chain reaction of CISA-INIA (Madrid, Spain) EURL-ASF (see the last version of the protocol at: https://asf-referencelab.info/asf/images/ficherosasf/SOP%202018/SOP-ASF-PCR 2_REV2018.pdf).

Table 1

Domestic and wild pigs tested in Romania for African swine fever between 2017 and 2020

Method	2017	2018	2019	2020	Total
iELISA	7551	83774	189695	273896	554916
IPT	64	2323	905	346	3638
FAT	1	836	2066	1391	4294
IBT	0	0	5	1	6
Real-Time PCR	8969	61315	94603	82812	247699
PCR-sequencing	7	0	3	0	10
Total	**16592**	**148248**	**287277**	**358446**	**810563**

iELISA = indirect enzyme-linked immunosorbent assay; IPT= indirect immunoperoxidase test; FAT= fluorescent antibody test; IBT = immunoblotting test; PCR = polymerase chain reaction

Table 2

The annual number of outbreaks of African swine fever in domestic pigs in Europe (16, 17)

Country	Outbreaks			
	2017	2018	2019	2020
Romania	**2**	**1163**	**1724**	**1053**
Bulgaria	0	1	44	19
Estonia	3	0	0	0
Greece	0	0	0	1
Italy	17	10	1	0
Latvia	8	10	1	3
Lithuania	30	51	19	3
Moldavia	0	0	0	2
Poland	81	109	48	103
Serbia	0	0	18	16
Slovakia	0	0	11	17
Ukraine	124	105	42	23
Total	**265**	**1449**	**1908**	**1240**

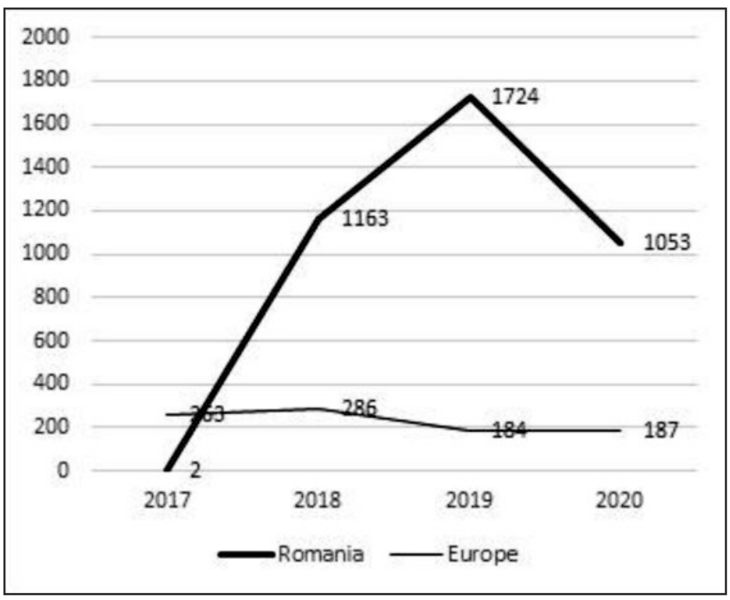

Fig. 1. Comparative dynamics of the number of outbreaks notified by Romania and other European states (excluding Romania) between 2017 and 2020 (16, 17) - Domestic pigs

PCR-sequencing

The procedure for the genotyping of African swine fever virus (ASFV) isolates followed the standard operating procedure of CISA-INIA (Madrid, Spain) EURL-ASF (see the last version of the protocol at: https://asf-referencelab.info/asf/images/ficherosasf/SOP%202018/SOP-ASF-GENOTYPING-1_REV 2018.pdf).

RESULTS AND DISCUSSIONS

The first Romanian outbreaks of ASF were reported in 2017 when two cross-border outbreaks were reported in the Northern part of the country.In 2018 new outbreaks were reported in the South-eastern Romanian territory. ASFV spread quickly and the number of outbreaks increased progressively to 1163 in 2018, 1724 in 2019, and 1053 in 2020 only in domestic pigs. The Romanian evolution of ASF outbreaks in domestic and wild pigs did not follow the epidemiological pattern of the ASF in the European countries (Table 2) where the majority of outbreaks were linked with wildlife. Moreover, between 2017 and 2020, Romania reported 81.07% (3942/4862) of all European outbreaks. Romania reported the highest number of outbreaks in 2019, and this accounted for 90.35% of all European outbreaks (1724/1908).

In wild pigs, Romania reported the first cases in 2018 (Table 3).

Table 3

The annual number of reported cases of African swine fever in wild pigs in Europe (16, 17)

Country	Cases			
	2017	2018	2019	2020
Romania	**0**	**170**	**683**	**885**
Belgium	0	161	482	3
Bulgaria	0	5	165	533
Czech Republic	202	28	0	0
Estonia	637	230	80	68
Germany	0	0	0	403
Hungary	0	138	1594	4001
Italy	93	64	60	41
Latvia	947	685	369	320
Lithuania	1328	1443	464	230
Moldavia	0	0	0	30
Poland	741	2438	2464	4070
Serbia	0	0	0	63
Slovakia	0	0	27	375
Ukraine	37	41	11	5
Total	**3985**	**5403**	**6399**	**11027**

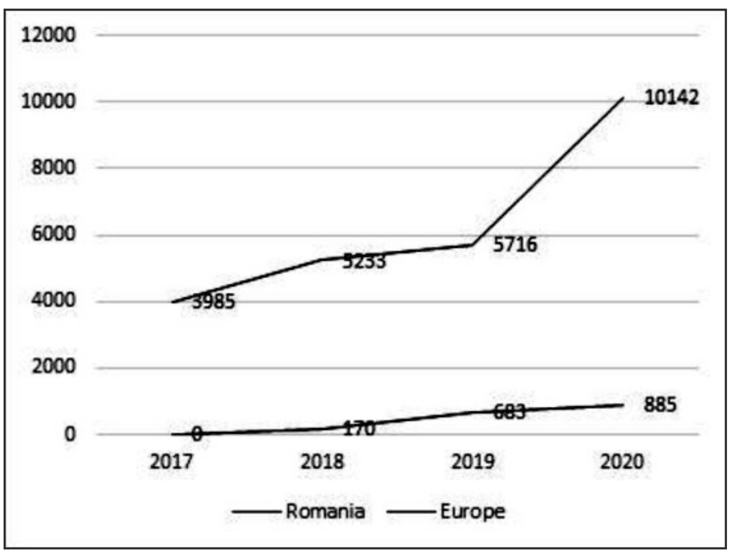

Fig. 2. Comparative dynamics of the number of cases notified by Romania and other European states (excluding Romania) between 2017 and 2020 (16, 17) - Wild boars

The number of reported cases increased progressively from 170 in 2018 to 683 in 2019, and 885 in 2020, covering 6.48% (1738/26814) of European ASF outbreaks in wild pigs, between 2017 - 2020. Compared to the situation in other European countries, the reported cases of ASF in Romania represented 3.14% (170/5403) of European wild pigs' reported cases in 2018, 10.67% (683/6399) in 2019, and 8.02% (885/ 11027) in 2020.

In Romania, the outbreaks of ASF were predominantly in the backyard and small-scale pig farms, following an epidemiological pattern of spreading similar to that of Georgia (3), the North Caucasus region, and the Russian Federation (9).

The highest involvement of backyard farms in Romanian outbreaks can be associated with several risk factors of infection and spreading. In some areas, owners release backyard pigs to scavenge in the field, and in other areas, free-ranging pigs were recorded. These breeding practices have increased the risk of contact with wild pigs or feeding on contaminated feed. Boklund et al. (2020) associated outbreaks of ASF in backyard pig farms in Romania with visits of professionals, proximity to outbreaks in wildlife, and feeding with contaminated forage (4).

Other risks were associated with the long-term survival of ASFV in refrigerated or frozen pork products and mechanical transmission by stable flies (4, 10, 12). It remains to be established to what extent the various fodder and insects are involved in the transmission of ASFV on the Romanian territory. The studies carried out so far cannot ignore this risk, but in order to obtain certain information, more detailed epidemiological studies are needed.

CONCLUSIONS

Between 2017 and 2020, the Romanian Veterinary Authorities tested 810563 samples, that allowed the detection of 3942 outbreaks in domestic and 1738 cases in wild pigs. The most used diagnostic tests were iELISA (68.46%,554916/810563) and Real-Time PCR (30.55%, 247699/810563). During the investigation period, most outbreaks have been identified in domestic pigs (69.40%, 3942/5680), with a maximum of outbreaks reported in 2019 (30.35%, 1724/5680). This study revealed the high incidence of ASF in Romanian backyard pig farms and the high risk of spreading ASF from them to commercial farms and wild animals.

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