Open Access

Berl Münch Tierärztl Wochenschr 126, 468–475 (2013)
DOI 10.2376/0005-9366-126-468

ISSN 0005-9366

Korrespondenzadresse:
paul.becher@tiho-hannover.de

Eingegangen: 13.07.2013
Angenommen: 14.08.2013

Online first: 14.10.2013
http://vetline.de/open-access/158/3216/

EU and OIE Reference Laboratory for Classical Swine Fever, Institute of Virology, University of Veterinary Medicine Hannover, Germany

Classical Swine Fever in Europe – the current Situation

Klassische Schweinepest in Europa – die aktuelle Situation

Alexander Postel, Volker Moennig, Paul Becher

Summary

Classical swine fever (CSF) is considered to be one of the most important viral diseases in pigs worldwide. In many parts of the world great efforts are being undertaken to reduce economic losses caused by CSF or to eradicate the disease. Among the member states of the European Union (EU) a harmonized strategy for diagnosis, control and eradication of CSF is applied. Success of the common strategy is documented by the decreasing number of outbreaks during the last decade. The present article summarizes the recent situation concerning CSF in Europe with special focus on the situation in the EU member states. In particular, outbreaks in domestic pigs and wild boar, the identified virus isolates, and eradication and monitoring programs actually performed in the EU are described. Despite achieved progress towards eradication, CSF remains a continuous threat to the European pig and wild boar population. After introduction of CSF virus (CSFV) into the domestic pig population rapid spread as a consequence of high frequency of animal movements and intensive trade within Europe can be suspected. Platforms like the CSF sequence database and the CSF in wild boar surveillance database have been implemented as tools to easily exchange information concerning CSF. The improved availability of data about circulating CSFV isolates will help to elucidate possible sources of virus introduction and to better understand routes of virus transmission.

Keywords: CSF, Europe, eradication programs, monitoring, databases

Zusammenfassung

Die Klassische Schweinepest (KSP) wird als eine der weltweit bedeutendsten viralen Erkrankungen des Schweines betrachtet. In vielen Teilen der Welt werden enorme Anstrengungen unternommen, die wirtschaftlichen Verluste durch KSP zu minimieren oder die Krankheit zu tilgen. Innerhalb der Mitgliedsstaaten der Europäischen Union (EU) wird eine einheitliche Strategie zur Diagnose, Kontrolle und Tilgung der KSP verfolgt. Der Erfolg dieser gemeinschaftlichen Strategie wird dokumentiert durch die rückläufige Anzahl an Krankheitsausbrüchen. Der vorliegende Artikel fasst die aktuelle KSP-Situation in Europa und insbesondere die Lage in den EU-Mitgliedsstaaten zusammen. Insbesondere werden die registrierten Ausbrüche bei Haus- wie auch bei Wildschweinen, die ursächlichen Virusisolate sowie die Eradikations- und Monitoring-Programme beschrieben, die derzeit in der EU durchgeführt werden. Trotz aller Fortschritte hin zur Tilgung bleibt die KSP eine fortwährende Bedrohung für die europäische Haus- und Wildschweinpopulation. Im Falle eines Eintrags von KSP-Virus (KSPV) in die Hausschweinpopulation ist aufgrund hoher Zahlen an Tiertransporten und intensiven Handels innerhalb Europas eine rasante Ausbreitung zu befürchten. Plattformen wie die KSP-Sequenz-Datenbank und die Surveillance-Datenbank zur Schweinepest bei Wildschweinen wurden eingerichtet, um Informationen zur KSP einfacher austauschen zu können. Die verbesserte Verfügbarkeit von Daten zu zirkulierenden KSP-Virusisolaten wird künftig dazu beitragen, mögliche Quellen einer Viruseinschleppung ausfindig zu machen und die Wege der Virusübertragung besser zu verstehen.

Schlüsselwörter: KSP, Europa, Bekämpfungsprogramme, Monitoring, Datenbanken

U.S. Copyright Clearance Center Code Statement:
0005-9366/2013/126011-468 $ 15.00/0

Introduction

Classical swine fever (CSF) is a viral pig disease of great economic impact worldwide (Edwards et al., 2000; Moennig et al., 2003). Causing agent is the classical swine fever virus (CSFV) a small enveloped RNA virus belonging to the genus Pestivirus within the family *Flaviviridae* (Simmonds et al., 2012). CSFV can be classified into the genotypes 1, 2 and 3 and several subgenotypes (currently 1.1–1.4, 2.1–2.3, 3.1–3.4) by nucleotide sequencing and phylogenetic analyses of short genome fragments of the 5´non-translated region (5´NTR: 150 nucleotides) and the E2 encoding region (E2 fragment: 190 nucleotides) and of the entire E2 encoding region (1119 nucleotides) (Paton et al., 2000; Postel et al., 2012).

Only very few regions like North America and Australia are free of the disease for a longer period of time. In many countries the status concerning CSF is unknown, because no systematic laboratory diagnosis is performed or disease information is not reported (OIE, 2012b). Referring to the disease reports to the World Organization for Animal Health (Office International des Epizooties, OIE), for many countries of the African continent CSF status remains uncertain. Especially in some countries of South and Central America, the Caribbean and also in many countries of the Asian continent, CSF has been endemic since decades. Reasons for problems in controlling and eradicating CSF are various. In many cases no legal CSF control programs exist, lack of resources hamper effective control, veterinary services have limited capacities or control measures are difficult to implement. Besides the virological aspects, control and eradication of CSF strongly depend on socio-economic factors, on matters of international trade, and also on basic political conditions. For example, the CSF situation in Europe was highly influenced by foundation and development of the European Union (EU) in the past.

The establishment of the Common Market and the increasing number of Member States led to the need of harmonized diagnostic procedures and a concerted CSF control strategy. This common strategy was implemented by EU legislation in the 1980ies and is currently regulated by Council Directive 2001/89/EC and Commission Decision 2002/106/EC. The EU Reference Laboratory for CSF (EURL) was founded in Hannover, Germany, according to Council Decision 80/217/EEC in a time of many severe CSF outbreaks that occurred in the 1970ies/1980ies. In 1990, a non-vaccination policy was implemented in the EU. Thereafter many devastating CSF outbreaks occurred in the 1990ies (Fritzemeier et al., 2000; Greiser-Wilke et al., 2000a). Between 2000 and 2006, the majority of the EU member states was free from CSF in domestic pigs, with exception of outbreaks seen in Germany, Italy, Luxembourg, Spain and the United Kingdom. During that time CSF cases in wild boar were seen in several EU member states and in some countries (e. g. in Germany and Luxembourg) were causative for outbreaks in domestic pigs. Oral vaccination campaigns of wild boar were implemented in Bulgaria, France, Germany, Luxembourg, Romania, and Slovakia. Among the new member states joining the EU in 2004 (Cyprus, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Malta, Poland, Slovakia and Slovenia) only Slovakia was not free from CSF in domestic pigs. CSFV was circulating in the wild boar population of the Slovak border regions to Hungary for a longer period of time causing repeated outbreaks also in domestic pigs (Bartak and Greiser-Wilke, 2000; Stadejek et al., 1997; Vilcek and Paton, 1998). In 2006, just before Romania and Bulgaria joined the EU in 2007, still many CSF outbreaks were observed in these two countries and control measures were implemented (Commission Decisions 2006/805/EC and 2006/779/EC). Bulgaria was faced with seven outbreaks in domestic pigs and Romania reported more than 800 outbreaks in domestic pig holdings and 17 cases in wild boar.

The present article gives an overview on the current CSF situation in Europe since 2007 and provides full-length E2 sequence data of recent CSFV isolates which will be the basis for an improved molecular characterization of isolates obtained in future outbreaks.

The recent epidemiological situation in Europe (2007–2013)

France, Germany

In the French-German border region CSF was present in wild boar over a longer time period. In the French region Vosges du Nord the last case in wild boars was detected in 2007. In Germany, wild boar on the territories of the Federal states of Rhineland-Palatinate and North Rhine-Westphalia were affected and last virus positive wild boars were recognized in 2009 (OIE, 2012b). Sequence analysis revealed that isolates of both affected regions grouped into distinct phylogenetic clades within subgenotype 2.3. Full-genome sequencing exhibited a very high genetic similarity of the isolates originating from the same region (Leifer et al., 2010b). Molecular epidemiology based on analysis of complete genome sequences suggests that CSFV was persistent over a period of more than one year (mid 2007 until end 2008) in a vaccinated wild boar population at very low level. Nevertheless, CSFV was not detected despite very intensive surveillance was carried out (Leifer et al., 2010b). This example demonstrates that CSFV persistence at very low level obviously is a limiting factor hampering successful eradication.

Hungary, Slovakia

In Slovakia and Hungary CSFV was endemic in the wild boar population over years affecting also domestic pig holdings. The last outbreaks of CSF in domestic pigs were recognized in Slovakia in association with cases in wild boar in 2008. In Hungary, the last cases in wild boar were recorded in 2009. Based on very intensive full-length E2 sequencing it was possible to identify at least three (slightly) different variants of the 2.3 subgenotype in locally distinguishable wild boar populations of the border region. Outbreaks in the Slovakian domestic pig holdings in 2008 could be clearly linked to one of the respective wild boar populations (Mojzis, State Veterinary Institute Zvolen, personal communication). In Slovakia, a vaccination campaign was performed in wild boar from 2007–2009 using the C-strain vaccine. On the Hungarian side of the border no wild boar vaccination was performed and CSF was eradicated as well.

Lithuania

The most recent CSF outbreaks occurred in the Baltic States Lithuania (2009 and 2011) and Latvia (2012/13). The CSF outbreak in Lithuania 2009 affected exclusively

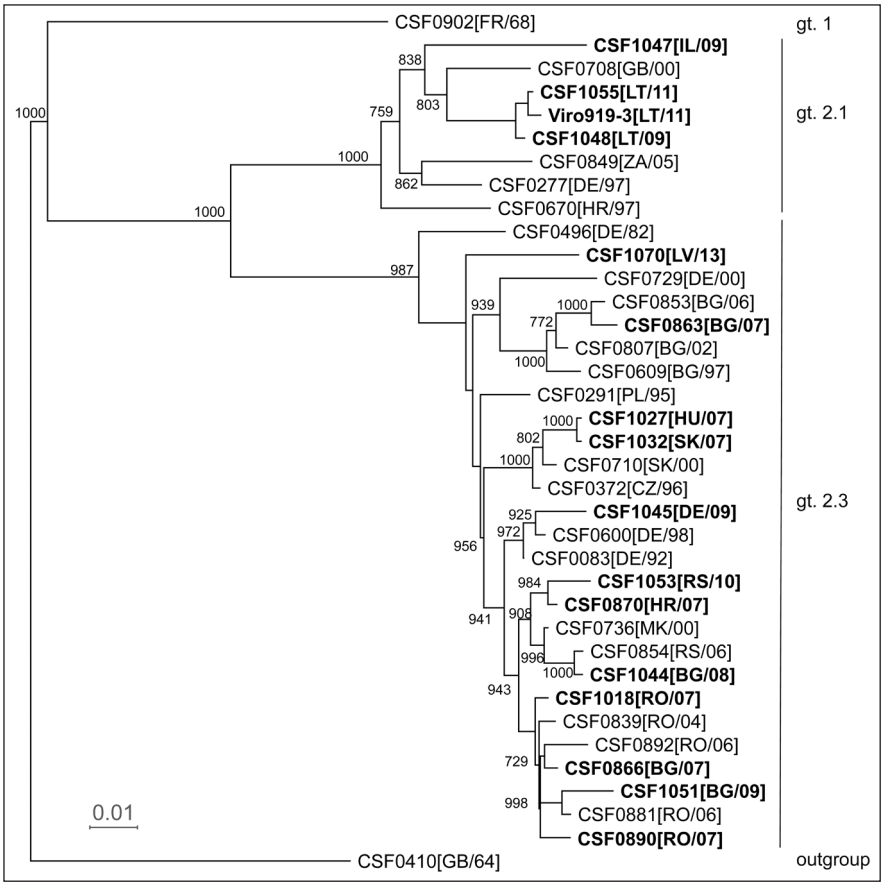

FIGURE 1: *Neighbor Joining phylogenetic tree of full-length E2 encoding sequences (1119 nt) from recent European CSFV isolates and reference sequences. Sequences from recent outbreaks (since 2007) are indicated in bold. For statistical analysis bootstrap values for 1000 iterations were calculated. Only significant bootstrap values of ≥ 70% are shown. Phylogenetic tree was generated with Dendroscope 3 software (Huson and Scornavacca, 2012).*

a commercial pig holding of a Danish owner. Interestingly, the causative virus isolate belonged to subgenotype 2.1, whereas the other recent outbreaks in Europe were caused by subgenotype 2.3 (Tab. 1, Fig. 1: CSF1048). Contact holdings in Denmark were tested to be free of infection and epidemiological data point towards a new introduction on Lithuanian territory. Monitoring of shot and dead wild boar as well as domestic pigs kept in local Lithuanian backyard holdings did not reveal association with CSF in wild boar or with low level biosecurity backyard holdings. An introduction via human activities was supposed to be probable, but the source of infection remained unclear. A very similar virus strain of subgenotype 2.1 was causative for outbreaks in five domestic pig holdings in 2011 (Tab. 1, Fig. 1: CSF1055, Viro919-3) and again no association with CSF in wild boar could be proven. The genetic similarity between 2009 and 2011 isolates was so high that determination of the full-length E2 sequences was necessary for discrimination (Postel et al., 2012). Interestingly, phylogenetic analysis revealed that there might have been two independent introductions in 2011 (Postel et al., 2012). It can be speculated that introduction might have occurred from outside EU as 2.1 subgenotype has not been found in the EU for many years. In Europe 2.1 subgenotype isolates were last detected in the United Kingdom (2000), in Germany and the Netherlands (1997/1998). Last recognized isolates of the 2.1 subgenotype were identified in Israel in 2009 and in South Africa in 2005 (David et al., 2011; Sandvik et al., 2005), but sequence analysis revealed only a low degree of genetic relatedness (Fig.1: CSF1047, CSF0849). The highest genetic similarities of E2 full-length sequences of the Lithuanian subgenotype 2.1 isolates are shared with isolates from Asia. Against this background introduction from East-European countries or from Asia appeared to be likely. In many regions of Asia isolates of the 2.1 subgenotype are circulating in pigs and extensive E2 full-length data is available for isolates from China. In contrast, no sequence information of recent CSF isolates is available from Russia or Belarus. Lack of such data hampers elucidation of the infection routes and possible source of infection. The last OIE report of CSF in domestic pigs from Belarus was dated from 1995 and only very poor information is available on the current situation concerning CSF in this country (OIE, 2012b). According to recent OIE reports, CSF is circulating in wild boar populations of Russian districts neighboring to the Belarus border (Smolenskaya Oblast and Bryanskaya Oblast). Information about subgenotype affiliation and sequences from isolates of these cases is not publicly available.

Latvia

The latest CSF outbreak in Europe was recorded in Latvia in November 2012 and CSF cases in wild boar are still seen (April 2013). First CSFV seropositive samples were found in context of a surveillance program to monitor the disease situation in wild boar on October 15th 2012. Intensified CSF monitoring of wild boar was performed and the first two cases of CSF in wild boar from counties Dagdar and Zilupe were notified on November 20th. Two weeks later outbreaks in domestic pigs were reported from three small backyard holdings (2–9 pigs/holding). The infected area was located in regions near the border to Russia and Belarus. By mid of March 2013, 53 cases with 79 mostly subclinically infected wild boar have been reported by the Latvian authorities (OIE, 2013). The respective isolates (represented by CSF1070; Tab. 1, Fig. 1) could be assigned to subgenotype 2.3. Partial E2 encoding sequences (190 nt) displayed greatest homology to the sequence of a Russian virus isolate from 2005 and full-length E2 sequences (1119 nt) revealed closest relationship to an ancient (1995) isolate from Poland (CSF0291; Fig. 1). E2 full-length encoding sequences of five isolates obtained from Latvian wild boar shot in different areas of the infected region revealed to be (almost) identical and displayed only 0–3 nucleotide differences. CSF in domestic pigs was reported from the Russian authorities in Pskovskaya Oblast, a district neighboring to the Latvian border, in June 2012 (OIE, 2012a). A more detailed analysis is hampered by the lack of epidemiological data and sequence information on currently circulating isolates from Russia and Belarus.

Balkan region

During the last years the CSF situation in the Balkan region was of particular interest to the EU member states as Romania and Bulgaria joined the EU in 2007 and some Western Balkan countries got the status of a candidate country (Montenegro, Former Yugoslav Republic of Macedonia, Serbia) or started accession negotiations with the EU (Croatia joined the EU in 2013). CSF outbreaks in domestic pigs as well as in wild boar were diagnosed in the new EU Member States Bulgaria and Romania in 2007 and 2008. In several other countries of the Balkan area CSF outbreaks were recognized at this time as well. Between 2007 and 2008 outbreaks in domestic pigs were reported for example from Albania, Bosnia Herzegovina, Croatia, Former Yugoslav Republic of Macedonia (FYROM), Montenegro, and Serbia. Due to vaccination of domestic pigs the true distribution of the disease hardly could be determined and virtually the whole Balkan region was affected during that time. Sequence and phylogenetic analysis revealed that in all cases the causative CSF subgenotype was 2.3 (Fig. 1, Tab. 1). In 2009, the last case of CSF in Bulgaria was recognized in a wild boar next to the border to Romania in the region Silistra. The respective virus isolate (CSF1051) showed highest genetic similarity to isolate CSF0881 (Romania/2006) and forms a clade with other older isolates from Romania and Bulgaria indicating that there was an on-going circulation of CSFV in the wild boar population of this region (Fig. 1). In Serbia, two outbreaks were seen in domestic pigs in 2010 resulting in almost 9000 domestic pigs to be culled (Anonymous, 2012). Analysis of the full-length E2 encoding sequence of the isolate CSF1053 (Serbia/2010) revealed close relationship to older isolates from the Balkan region like e. g. isolate CSF0870 (Croatia/2007). The last CSF case in wild boar was detected in Croatia in 2009. As Croatia joined the EU in July 2013, great efforts were made in the past years to eradicate CSF in the country (Sučec and Kiš, 2012). Being the first of the Western Balkan countries, Croatia has stopped the C-strain based vaccination of domestic pigs (January 1st, 2005) and implemented a non-vaccination policy for eradication of the disease. In consequence, a very intensive monitoring is performed now. 25 303 blood samples were tested serologically (0.1% positive) and 16 670 samples were tested virologically (all negative) in the Croatian surveillance program for domestic pigs (pig population: approx. 1.5 million) in 2011. In 2012, (Jan to Sept 30) 18 123 blood samples were tested serologically and 8265 samples virologically negative. Furthermore, 7635 wild boars (estimated population: approx. 53 000) were tested virologically negative in the hunting season 2011/2012. Serological testing of 12 465 wild boars revealed 15 antibody positive animals originating from five different hunting grounds (Sučec and Kiš, 2012).

TABLE 1: *Isolates used for phylogenetic analysis of full-length E2 encoding sequences*

Cat. No.	Isolates Name	Genotype	Country/ Year	Acc. No.
CSF0083	Rostock I	2.3	DE/1992	JQ411563
CSF0277	Paderborn	2.1	DE/1997	JQ411566/ GQ902941/ AY072924
CSF0291		2.3	PL/1995	JQ411569
CSF0372		2.3	CZ/1996	JQ411572
CSF0410	Congenital Tremor	outgroup	GB/1964	JQ411575
CSF0496		2.3	DE/1982	JQ411578
CSF0600		2.3	DE/1998	JQ411580
CSF0609		**2.3**	**BG/1997**	**KF233944**
CSF0670		**2.1**	**HR/1997**	**KF233945**
CSF0708		2.1	GB/2000	JQ411582
CSF0710		2.3	SK/2000	JQ411583
CSF0729		2.3	DE/2000	JQ411584
CSF0736		**2.3**	**MK/2000**	**KF233946**
CSF0807		**2.3**	**BG/2002**	**KF233947**
CSF0839		**2.3**	**RO/2004**	**KF233948**
CSF0849		2.1	ZA/2005	HM190299
CSF0853		**2.3**	**BG/2006**	**KF233949**
CSF0854		**2.3**	**RS/2006**	**KF233950**
CSF0863		**2.3**	**BG/2007**	**KF233951**[1]
CSF0866		**2.3**	**BG/2007**	**KF233952**
CSF0870		**2.3**	**HR/2007**	**KF233953**
CSF0881		**2.3**	**RO/2006**	**KF233954**
CSF0890		**2.3**	**RO/2007**	**KF297337**
CSF0892		**2.3**	**RO/2006**	**KF233955**
CSF0902	Alfort187	1.1	FR/1968	X87939
CSF1018		**2.3**	**RO/2007**	**KF233956**
CSF1027		2.3	HU/2007	JQ411589
CSF1032		2.3	SK/2007	JQ411590
CSF1044		**2.3**	**BG/2008**	**KF233957**
CSF1045	Roesrath	2.3	DE/2009	GU233734
CSF1047		**2.1**	**IL/2009**	**KF233958**
CSF1048	Panevezys	2.1	LT/2009	JQ411591/ HQ148063
CSF1051		**2.3**	**BG/2009**	**KF233959**
CSF1053		**2.3**	**RS/2010**	**KF233960**
CSF1055		2.1	LT/2011	JQ411592
Viro919-3		2.1	LT/2011	JQ411598
CSF1070		**2.3**	**LV/2013**	**KF233961**

CSFV isolates sequenced for this study are indicated in bold.

[1] Sequence is identical to previously published sequence of isolate from same outbreak (HQ148062).

EU regulations, eradication and monitoring programs

France, Germany

In the French region Vosges du Nord annual vaccination campaigns for wild boar (2004–2010), intensive surveillance in wild boar as well as in domestic pig holdings of the infected zone led to successful eradication of the disease. In consequence, restrictions could be lifted in November 2011. In the formerly infected zone virological and serological surveillance of dead and hunted wild boar continues and provides information on the CSF situation.

In Germany, in the bordering region to France in the Federal State of Rhineland-Palatinate and in the region west of the river Rhine in the Federal States of North

Rhine-Westphalia and Rhineland-Palatinate, CSF circulated in wild boar until 2009. To eradicate the disease, vaccination of wild boar was performed until March 2012 (three double campaigns per year). For discrimination of infected from vaccinated animals (DIVA) a multiplex real-time PCR was implemented (Blome et al., 2011; Leifer et al., 2009; Leifer et al., 2010a) allowing differentiation of the C-strain vaccine virus (subgenotype 1.1) from the field virus belonging to subgenotype 2.3 (Fig. 1, e. g. CSF1045). Intensive surveillance was performed and restricted zones were lifted in March 2012. After lifting the restrictions in 2012, the monitoring program has been continued with virological and serological testing considering still all wild boars shot or found dead in the risk areas. Furthermore, in these areas non-specific symptoms in domestic pigs have to be clarified by clinical examinations as well as virological and serological testing.

Bulgaria, Romania

Currently Bulgaria and Romania are the only member states in the EU that are regulated by trading restrictions due to CSF as stipulated by Commission decision 2008/855/EC (Tab. 2). According to this decision dispatch of live pigs and consignments of porcine semen, ova and embryos to other EU member states is prohibited for the listed areas (Bulgaria and Romania). Dispatch of fresh pig meat, certain meat preparations, and meat products from areas listed in Part II of the Annex of decision 2008/855/EC (only listed country is Bulgaria) to other EU member states is possible under certain conditions. Romania is the only country listed in Part III of the Annex that includes areas from which dispatch of live pigs, fresh meat, and fresh meat products is prohibited. Pig meat and pig meat products may be dispatched to other Member States if they are treated in such a way that any classical swine fever virus present is destroyed. In addition, a so-called "channeling system" was implemented, meaning that dispatch of fresh meat/fresh meat preparations originating from selected pig farms, slaughterhouses and establishments with high biosecurity, hygiene management and surveillance for CSF can be permitted.

In Bulgaria, pig holdings (approx. 613 000 pigs) were classified into industrial pig holdings, family farm type A (with biosecurity measures), family farm type B (without biosecurity measures), backyard holdings and free ranging East Balkan pig herds (European Commission, 2013a). The latter three categories pose a higher risk of being infected by CSFV and therefore are in the focus of the monitoring program. A similar concept based on categorization of pig holdings with respect to biosecurity level was also successfully applied in Serbia after the last outbreaks in 2010 and also in Latvia in 2012/2013 (Anonymous, 2012). In Bulgaria, surveillance is performed by clinical examination using specific checklists and by serological investigations of collected blood samples. Blood samples are taken in industrial farms, in Type A and Type B farms and in East Balkan pig herds every six months to detect a prevalence of 10% with 95% confidence. In backyard holdings pigs are examined for clinical signs of CSF by a registered veterinarian every six months and blood samples are taken from sick pigs. In 2011, 14 288 samples were tested serologically by ELISA with negative results. Additionally, a small number of samples (n = 66) was tested virologically (Ag-ELISA, FAT, RT-PCR) also giving negative results (European Commission, 2013a). Oral vaccination of wild boar in 2013 will be carried out in districts near to the borders to Serbia, Former Yugoslav Republic of Macedonia (FYROM), and Romania. Vaccination strategy in 2013 will be the same as in 2012 comprising three campaigns with two distributions each (European Commission, 2013a).

In Romania, emergency vaccination of domestic pigs was performed over a three-year period between 2007 and 2009 using the E2 subunit marker vaccine (industrial holdings) and the live attenuated C-strain vaccine (backyard holdings) according to decision 2006/802/EC. The vaccination program was focusing especially on backyard pigs in order to eradicate CSFV from low biosecurity holdings. Vaccination of domestic pigs was stopped in December 2009. Since 2007, no CSF outbreaks in domestic pigs or in wild boar have been detected and in consequence also vaccination of wild boar was stopped in 2011 (Tab. 2). Today, an intensive CSF monitoring is performed in context of two different programs – a surveillance program with the aim to demonstrate the status to be free from the disease and a program to allow trade within the EU Member States by implementing a "channeling system". Surveillance of domestic pigs (clinical, virological and serological monitoring) depends on the type of the holding and its location (European Commission, 2013b). The surveillance program for domestic pigs (population: approx. 1.7 million) in non-professional holdings was designed to detect a prevalence of 5% with 95% confidence according to decision EC/106/2002. In 2012, a prevalence of 5% was applied at two levels: herd level (infected herds in a locality) and animal level (infected animals in an infected holding). In 2013, this prevalence will also be applied at locality level (infected localities in the country). In consequence, 208 475 serum samples originating from 1445 localities and

TABLE 2: *Selected CSF outbreaks in Europe since 2007*

Country	Last confirmed case of CSF since 2007		Control measures		
			Vaccination		
	Wild boar	Domestic pig	Wild boar	Domestic pig	Restricted zones[1]
Bulgaria	2009	2008	on-going	–	Part II
Croatia	2009	2008	–	–	–
France	2007	–	until 2010	–	lifted 2012
Germany	2009	–	2009–2012	–	lifted 2012
Hungary	2009	–	–	–	county Heves/Borsod: lifted 2011 county Nogard: ifted 2012 county Pest: lifted 2013
Latvia	2013	2013	(2013)[2]	–	–
Lithuania	–	2011	–	–	–
Romania	2007	2007	until 2011	–	Part III
Russia	2012	2012	–	yes	–
Serbia	2007	2010	–	yes	–
Slovakia	2008	2008	until 2010	–	lifted 2011

[1] applicable only for EU Member States, according to Annex of decision 2008/855/EC (Part I–III);

[2] Vaccination is planned in infected/risk zones near borders to Russia and Belarus in 2013.

49 723 holdings were analyzed in 2012 (Romanian National Sanitary Veterinary and Food Safety Authority, unpublished). Surveillance in wild boar (estimated population: 77 000) was performed serologically (by ELISA) for all animals born after the last vaccination campaign and virologically (RT-PCR, fluorescent antibody test) for all shot wild boar and all animals found dead. In the hunting season spring 2013, serological testing revealed 4 ELISA-positive out of 4090 tested serum samples, but all tested samples (FAT: n = 7112; RT-PCR: n = 7020) were virologically negative.

In addition to the surveillance program, the above mentioned "channeling system" was implemented in Romania to allow trade of fresh pig meat, meat preparations and meat products with other EU member states. In the framework of this program industrial pig farms that are part of the channeling system perform a strict CSF monitoring. Included in this program are also farms that are not part of this "channeling system", like non-professional pig holdings surrounding the participating farms (European Commission, 2013b). For example in 2012, backyard farms situated in a 10 km radius around a farm of the "channeling system" were serologically tested four times a year.

Latvia

In Latvia, restriction measures were implemented in all three affected holdings according to Council Directive 2002/8/EC. Protection and surveillance zones could be lifted in January 2013. Monitoring in the wild boar population has been intensified. It is planned to test approx. 2400 wild boar samples from the infected zone (approx. 9000 km^2) and 1400 samples from the risk zone (approx. 5000 km^2). Oral vaccination of wild boar (three double campaigns) with live attenuated C-strain vaccine will be conducted in parts of the infected zone, a belt neighboring the borders to Russia and Belarus. Vaccination area (approx. 5000 km^2) is located in the south of the infected zone and has an estimated population of 6000 wild boar. The DIVA PCR that was previously used in Germany to discriminate between 2.3 field virus and 1.1 vaccine strain will be implemented and used for monitoring the CSF status of wild boar (Blome et al., 2011; Leifer et al., 2009; Leifer et al., 2010a). Latvian CSF eradication and emergency vaccination plans comprise oral immunization of wild boar, evaluation of vaccination efficiency, surveillance of wild boar and domestic pigs and categorization of pig holdings. The eradication plan was approved by decision 2013/90/EU.

CSF in wild boar surveillance database and CSF sequence database

Wild boars play a crucial role as reservoir for CSFV in Europe. The above mentioned CSFV infected or risk areas are mainly located in regions next to borders. This demonstrates that implementation of effective control measures is highly dependent on rapid exchange of wild boar surveillance data between neighboring countries. In 2002, a working group on CSF in wild boar of the Directorate General Health and Consumer Protection (DG SANCO) of the European Commission (EC) initiated the establishment of a common database on the epidemiology of CSF in wild boar based on Council Directive 2001/89/EC, which initially involved the countries Belgium, France, Germany, Luxembourg and the Netherlands. This CSF database was implemented by the Friedrich-Loeffler-Institut (Institute of Epidemiology, Wusterhausen) and the EU and OIE Reference Laboratory for CSF (Institute of Virology, University of Veterinary Medicine, Hannover). The first version of the CSF in wild boar surveillance database was released end of 2003. An updated version that is now accessible for all EU Member States was officially released in February 2011 (http://public.csf-wild boar.eu/Default.aspx). Aim of the CSF in wild boar surveillance database is to provide and exchange data of wild boar surveillance between the EU Member States and other interested European countries (Staubach, 2010).

In parallel to collecting CSFV isolates as reference material, for many years now sequence data as well as epidemiological information are collected by the EU and OIE Reference Laboratory for CSF in Hannover. Information is stored in a CSF database (CSF-DB) that can be accessed and used by the National Reference Laboratories and other interested institutions worldwide (Dreier et al., 2007; Greiser-Wilke et al., 1998; Greiser-Wilke et al., 2000b; Postel et al., 2013). Obtained sequence data of partial 5´NTR (150 nucleotides) and partial E2 encoding region (190 nucleotides) are used for molecular typing of CSFV, which proofed to be a powerful tool to identify and genetically characterize CSFV isolates, and to distinguish primary and secondary CSF outbreaks (Greiser-Wilke et al., 2006; Paton et al., 2000). Moreover, genetic similarities of isolates together with epidemiological information can help to identify possible sources of infection and to trace routes of virus transmission. Simple tools for sequence and phylogenetic analysis are provided along with the database to allow a quick identification of a new isolate sequence. For graphical display of genetic similarities to other known CSFV sequences, phylogenetic trees can be generated by the typing module of the CSF-DB and are routinely provided by the EU and OIE Reference Laboratory in case of an CSF outbreak. The relatively high degree of genetic similarities between isolates found in the EU defines the need to determine and analyze longer sequence stretches of the CSFV genome (Postel et al., 2012). Therefore, the EU and OIE Reference Laboratory recently started to generate and analyze the nucleotide sequences encoding for the entire glycoprotein E2 (1119 nt) to achieve higher resolution of molecular analysis (Postel et al., 2012). Along with implementation of space for E2 full-length sequences in the CSF-DB, tools of the database have been updated and server capabilities were extended to be able to handle the larger data sets. The full-length E2 sequences provide valuable data for a more detailed characterization of CSFV isolates and will support effective control measures.

Conclusion

Taken together, in several East-European countries CSF is still present in domestic pigs or the CSF status is at least uncertain, like for example in Belarus, the Russian Federation, in the Ukraine, in the Balkan and Caucasus regions. In the EU Member States, only sporadic CSF outbreaks in domestic pigs were observed since 2009, two years after the extension of the EU in 2007. Nevertheless, the example of reoccurrence of CSF in wild boar

in Germany in 2009 might be a hint that CSFV can persist at very low levels in local wild boar populations and even intensive surveillance cannot exclude the presence of CSF. During the last five years in different European countries, including several EU Member States, CSF was still present in wild boar and therefore posed a continuous threat to domestic pigs – especially when direct or indirect contact to the wild boar population cannot be excluded. Contact between wild and domestic animals is also a threat to the wild boar population and is of great epidemiological relevance as wild boar serves as reservoir host. Continuous and intensive surveillance in wild boar as well as emergency vaccination of wild boar are the main elements of the eradication programs performed in the EU and have proven to be powerful tools. Pig rearing facilities without any biosecurity measures or with low biosecurity level, like typically seen in backyard holdings or in small family scale farms, are of particular relevance in eradication programs and surveillance activities as they pose a higher risk to become infected than farms with implemented hygiene management. Sporadic CSF outbreaks in domestic pigs caused by spill over from wild boar, by introduction from neighboring countries or via other infection routes from unidentified sources were seen in the last years and will be relevant in the future as well. A high local wild boar density, regions with high density of domestic pigs and high transport frequencies of live pigs, pig meat, pig products, animal feed, and slurry can favor the spread of this highly contagious disease. Rapid identification of an outbreak, immediate containment of the diseased area, effective control measures and in particular rapid trans-boundary transfer of information and international cooperation will be key factors to avoid supra-regional spread and devastating outbreaks of CSF in times of on-going globalization.

Acknowledgement

We want to thank Dr. Sandra Blome (FLI, Riems), Dr. Meindl-Boehmer (LAVES, Hannover), Dr. Staubach (FLI, Wusterhausen) and DG SANCO of the European Commission for critically reading the manuscript and for giving helpful comments.

Conflict of interest: The authors declare that they have no competing interests that might have influenced the content of this manuscript.

References

Anonymous (2012): Support for the control/eradication of animal diseases IPA Multi-beneficiary Project – Western Balkans: Serbia. In: Second Regional Workshop, Belgrade, Serbia, 8./9.10.2012, http://www.ipawb-csf-rabies.eu/joomla/attachments/article/147/7a.%20RS%20%20-%20Second%20Regional%20Workshop%20-%20Update%20Oct%2012%20part%201.pdf

Bartak P, Greiser-Wilke I (2000): Genetic typing of classical swine fever virus isolates from the territory of the Czech Republic. Vet Microbiol 77: 59–70.

Blome S, Gabriel C, Staubach C, Leifer I, Strebelow G, Beer M (2011): Genetic differentiation of infected from vaccinated animals after implementation of an emergency vaccination strategy against classical swine fever in wild boar. Vet Microbiol 153: 373–376.

David D, Edri N, Yakobson BA, Bombarov V, King R, Davidson I, Pozzi P, Hadani Y, Bellaiche M, Schmeiser S, Perl S (2011): Emergence of classical swine fever virus in Israel in 2009. Vet J 190: e146–149.

Dreier S, Zimmermann B, Moennig V, Greiser-Wilke I (2007): A sequence database allowing automated genotyping of Classical swine fever virus isolates. J Virol Methods 140: 95–99.

Edwards S, Fukusho A, Lefevre PC, Lipowski A, Pejsak Z, Roehe P, Westergaard J (2000): Classical swine fever: the global situation. Vet Microbiol 73: 103–119.

European Commission (2013a): Control and monitoring programme for Classical Swine Fever – Bulgaria. In: Programmes for the eradication, control and monitoring of certain animal diseases and zoonoses, http://ec.europa.eu/food/animal/diseases/eradication/programme2013/csf_bg_en.pdf

European Commission (2013b): Control and monitoring programme for Classical Swine Fever – Romania. In: Programmes for the eradication, control and monitoring of certain animal diseases and zoonoses, http://ec.europa.eu/food/animal/diseases/eradication/programme2013/csf_ro_en.pdf

Fritzemeier J, Teuffert J, Greiser-Wilke I, Staubach C, Schluter H, Moennig V (2000): Epidemiology of classical swine fever in Germany in the 1990s. Vet Microbiol 77: 29–41.

Greiser-Wilke I, Depner K, Fritzemeier J, Haas L, Moennig V (1998): Application of a computer program for genetic typing of classical swine fever virus isolates from Germany. J Virol Methods 75: 141–150.

Greiser-Wilke I, Dreier S, Haas L, Zimmermann B (2006): [Genetic typing of classical swine fever viruses – a review]. Dtsch Tierarztl Wochenschr 113: 134–138.

Greiser-Wilke I, Fritzemeier J, Koenen F, Vanderhallen H, Rutili D, De Mia GM, Romero L, Rosell R, Sanchez-Vizcaino JM, San Gabriel A (2000a): Molecular epidemiology of a large classical swine fever epidemic in the European Union in 1997–1998. Vet Microbiol 77: 17–27.

Greiser-Wilke I, Zimmermann B, Fritzemeier J, Floegel G, Moennig V (2000b): Structure and presentation of a World Wide Web database of CSF virus isolates held at the EU reference laboratory. Vet Microbiol 73: 131–136.

Huson DH, Scornavacca C (2012): Dendroscope 3: An interactive tool for rooted phylogenetic trees and networks. Syst Biol.

Leifer I, Depner K, Blome S, Le Potier MF, Le Dimna M, Beer M, Hoffmann B (2009): Differentiation of C-strain "Riems" or CP7_E2alf vaccinated animals from animals infected by classical swine fever virus field strains using real-time RT-PCR. J Virol Methods 158: 114–122.

Leifer I, Everett H, Hoffmann B, Sosan O, Crooke H, Beer M, Blome S (2010a): Escape of classical swine fever C-strain vaccine virus from detection by C-strain specific real-time RT-PCR caused by a point mutation in the primer-binding site. J Virol Methods 166: 98–100.

Leifer I, Hoffmann B, Hoper D, Bruun Rasmussen T, Blome S, Strebelow G, Horeth-Bontgen D, Staubach C, Beer M

(2010b): Molecular epidemiology of current classical swine fever virus isolates of wild boar in Germany. J Gen Virol 91: 2687–2697.

Moennig V, Floegel-Niesmann G, Greiser-Wilke I (2003): Clinical signs and epidemiology of classical swine fever: a review of new knowledge. Vet J 165: 11–20.

Oie (2012a): Follow-up report No. 18 (15/08/2012). In: WAHID Disease Information, http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=12200

Oie (2012b): WAHID home page. In: WAHID Disease Information. Disease timelines, http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasetimelines

Oie (2013): Follow-up report No. 5 (11/03/2013). In: WAHID Disease Information, http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?reportid=13118

Paton DJ, Mcgoldrick A, Greiser-Wilke I, Parchariyanon S, Song JY, Liou PP, Stadejek T, Lowings JP, Bjorklund H, Belak S (2000): Genetic typing of classical swine fever virus. Vet Microbiol 73: 137–157.

Postel A, Schmeiser S, Bernau J, Meindl-Boehmer A, Pridotkas G, Dirbakova Z, Mojzis M, Becher P (2012): Improved strategy for phylogenetic analysis of classical swine fever virus based on full-length E2 encoding sequences. Vet Res 43: 50.

Postel A, Zimmermann B, Schmeiser S, Becher P (2013): The Classical Swine Fever Database – Version 3, EU and OIE Reference Laboratory for Classical Swine Fever, http://viro08.tiho-hannover.de/eg/csf/

Sandvik T, Crooke H, Drew TW, Blome S, Greiser-Wilke I, Moennig V, Gous TA, Gers S, Kitching JA, Buhrmann G, Bruckner GK (2005): Classical swine fever in South Africa after 87 years' absence. Vet Rec 157: 267.

Simmonds P, Becher P, Collett MS, Gould EA, Heinz FX, Meyers G, Monath T, Pletnev A, Rice CM, Stiasny K, Thiel H-J, Weiner A, Bukh J (2012): Family *Flaviviridae*, In: Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses. San Diego, USA.

Stadejek T, Vilcek S, Lowings JP, Ballagi-Pordany A, Paton DJ, Belak S (1997): Genetic heterogeneity of classical swine fever virus in Central Europe. Virus Res 52: 195–204.

Staubach C (2010): Database for Monitoring Classical Swine Fever in Wild Boar in the EU, http://public.csf-wildboar.eu/Docs/EU_CSF-DB_EC_13_12_10_v2.pdf

Sučec I, Kiš T (2012): Support for the control/eradication of animal diseases IPA Multi-beneficiary Project – Western Balkans: Croatia. In: Second Regional Workshop, Belgrade, Serbia, 8./9.10.2012, http://www.ipawb-csf-rabies.eu/joomla/attachments/article/147/3.%20HR%20-%20Second%20Regional%20Workshop%20-%20Update%20Oct%2012_rid.pdf

Vilcek S, Paton DJ (1998): Application of genetic methods to study the relationship between classical swine fever outbreaks. Res Vet Sci 65: 89–90.

Address for correspondence:
Prof. Dr. Paul Becher
Institute for Virology
EU und OIE Reference Laboratory for Classical Swine Fever
University of Veterinary Medicine Hannover
Buenteweg 17
30559 Hannover
Germany
paul.becher@tiho-hannover.de