Received: 28 November 2018 | Revised: 21 December 2018 | Accepted: 17 January 2019

DOI: 10.1111/tbed.13132

SHORT COMMUNICATION

WILEY Transboundary and Emerging Diseases

Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017

Carmina Gallardo[1] | Alejandro Soler[1] | Ieva Rodze[2] | Raquel Nieto[1] | Cristina Cano-Gómez[1] | Jovita Fernandez-Pinero[1] | Marisa Arias[1]

[1]European Union Reference Laboratory for African Swine Fever (EURL), Centro de Investigación en Sanidad Animal, INIA-CISA, Madrid, Spain

[2]Latvia NRL: Animal Disease Diagnostic Laboratory, Institute of Food Safety, Animal Health and Environment "BIOR", Riga, Latva

Correspondence
Carmina Gallardo, Centro de Investigación en Sanidad Animal (CISA-INIA), Madrid, Spain.
E-mail: gallardo@inia.es

Funding information
European Union reference laboratory, Grant/Award Number: UE-1 LR PPA/03

Abstract

A non-haemadsorbing (non-HAD) ASF virus (ASFV) genotype II, namely Lv17/WB/Rie1, was isolated from a hunted wild boar in Latvia in 2017. Domestic pigs experimentally infected with the non-HAD ASFV developed a nonspecific or subclinical form of the disease. Two months later, these animals were fully protected when exposed to other domestic pigs infected with a related virulent HAD genotype II ASFV.

KEYWORDS
African swine fever virus, genotype II, non-haemadsorbing (non-HAD)

1 | SUMMARY

African swine fever (ASF) is a serious viral haemorrhagic disease of swine for which there is no vaccine available. Its notification to the World Organization for Animal Health (OIE) is mandatory due to the high mortality it causes, its efficient transmission rates and the great sanitary and socioeconomic impact it can have on international trade in pig (swine) products. In Eastern Europe, and starting from a single introduction of ASFV into Georgia from Southeast Africa in 2007 (Rowlands et al., 2008), ASFV spread westward reaching the Eastern European Union (EU) territory in 2014 and affecting Lithuania, Poland, Latvia and Estonia in 2014, Czech Republic and Romania in 2017 and Hungary and Bulgaria in 2018 (Gallardo et al., 2014). But seriousness of this threat was exemplified by the spread of ASFV into China in August 2018 (Ge et al., 2018) and, in Western Europe, in September 2018, when the Belgium authorities reported that ASF had been confirmed in wild boar in the province of Luxembourg (OIE, 2018). ASFV appeared to have jumped a considerable distance from previously affected countries and represent a new change in the epidemiological situation of ASF worldwide, suggesting that the disease may have reached global proportions.

The ASFV strains responsible of the European and China outbreaks have been classified as genotype II viruses and mostly induce an acute clinical form of the disease with mortality close to 100% (Gallardo et al., 2018; Ge et al., 2018; Guinat et al., 2016). However, the increased seroprevalence found in certain areas of the affected EU countries over time, in addition to the recent description of ASFVs of moderated virulence suggest virus evolution towards less virulent forms (Gallardo et al., 2018; Zani et al., 2018). Although most of the ASFV isolates cause haemadsorption (HAD) of erythrocytes to infected cells, there are several isolates that do not and these are referred to as non-haemadsorbing (non-HAD) isolates. Previous studies describe that infection of pigs with non-HAD genotype I isolates may account for some of the seropositive animals detected in the field in the absence of clinical symptoms (Boinas, Hutchings, Dixon, & Wilkinson, 2004). Therefore, in this study, the virulence pattern of a non-HAD genotype II ASFV isolated in Europe has been characterized. The ability of the non-HAD-ASFV to cross-protect has been determined as a mean of recreating potential reinfections under field conditions that could provide additional knowledge for understanding the long-term persistence of ASFV within the wild boar population.

2 | THE STUDY

Serum from a wild boar hunted in Rietumpieriga, Latvia, in February 2017 was confirmed as ASFV positive at the EU reference laboratory for ASF (INIA, CISA, Madrid, Spain) by real-time PCR (Fernandez-Pinero et al., 2013). Additionally, the animal was found to have a high antibody titre (1:327,680) to ASFV by the indirect immunoperoxidase test (IPT) (Gallardo et al., 2015). The serum sample was subjected to molecular characterization (Bastos et al., 2003), virus isolation (VI) and haemadsorption (HAD) test in primary swine blood monocyte cell cultures (PBM) (Carrascosa, Bustos, & de Leon, 2011). The virus, named Lv17/WB/Rie1, was able to replicate in PBM in the absence of HAD as it was verified in three subsequent passages by PCR (Fernandez-Pinero et al., 2013). The ASFV harvested after the first passage was titrated in PBM and used for the experiment performed in a biosafety level 3 (BSL3) animal facilities of INIA-CISA, in accordance with the EC Directive 86/609/EEC and Spanish Ethical and Animal Welfare Committee (PROEX 125/16).

Partial genome sequence analysis placed Lv17/WB/Rie1 as genotype II, and remarkably it was characterized by the presence of a single nucleotide deletion inside the *EP402R* gene (coding the CD2-like protein), which corresponds to the gene position no 395 of the HAD-ASFV Georgia 2007/1 reference genome (GenBank FR682468). This deletion results in a frame shift that produces a stop codon early in the gene, generating a truncated protein, which lacks most protein domains, making this a nonfunctional protein. In most ASFV isolates, the functional CD2-like protein is present on the surface of extracellular virions and is responsible of the specific HAD phenomenon to ASFV infected cells (Borca et al., 1994; Rodríguez, Yáñez, Almazán, Viñuela, & Rodriguez, 1993).

Animal experiment was performed as follows (Table 1): two 3-month-old European hybrid pigs (PW13 and PW17) were intramuscularly (i.m) inoculated with 10 50% tissue culture infective dose ($TCID_{50}$) of the Lv17/WB/Rie1 ASFV. At the start of the experiment, four additional pigs were co-housed with the inoculated pigs as *in-contact* animals (PW14, PW15, PW16 and PW18). Clinical signs were recorded on a daily basis and expressed with a quantitative clinical score obtained by adding values for eight clinical signs recorded on a daily basis, as previously described by Gallardo et al. (2015, 2018); fever parameters, anorexia, recumbency, skin haemorrhage or cyanosis, joint swelling, respiratory distress, ocular discharge and digestive findings were assigned points on a severity scale of 0–3 (most severe). The sum of the points was recorded as the clinical score (CS), which was also used to define humane termination points. Paired EDTA-blood and serum samples were collected twice a week starting at day 3 post inoculation (dpi)/exposure (dpe) until the end of the experiment. Twenty-one different tissues were obtained at necropsy (Table 2). ASFV detection was performed in tissues and EDTA-blood by PCR and VI (Carrascosa et al., 2011; Fernandez-Pinero et al., 2013). The ASFV antibody titres were determined by end-point dilution using IPT (Gallardo et al., 2015).

Domestic pigs infected with Lv17/WB/Rie1ASFV developed non-specific clinical signs or, in some cases, remained apparently healthy across the entire observation period. Clinical scores, viremia and humoral immune response are provided in Figure 1. Specifically, one inoculated pig (PW17) showed weak peaks of fever (40.3–40.7°C) from 8 to 12 dpi accompanied by the appearance of cyanosis in ears and swelling of joints from 14 to 32 dpi. The first PCR positive result in this pig was obtained in blood collected at 3 dpi and lasted until the termination day (45 dpi). Infectious virus was recovered from blood for 19 days (3–22 dpi) with an average titre of 4.76×10^9 TCID50/mL. The other inoculated pig (PW13) showed slight swelling of joints from 8 to 15 dpi and peaks of fever at 14, 22 and 31 dpi (40–40.2°C). A short viremia was detected from 7 to 22 dpi with virus shedding between 7 and 17 dpi which yielded similar titres as compared to PW17. Antibodies were detected in inoculated animals starting at 7 dpi and persisted until the end of the study period yielding IPT titres of 1:327,640.

TABLE 1 Design of the animal experiment

ASFV strain	Status	ID animal	Day of death (‡) or slaughtered (†)
Lv17/WB/Rie1 (non-HAD)	Inoculated	PW13	Used for challenge exposure
		PW17	† 45 dpi
	In contact	PW14	Used for challenge exposure
		PW15	† 101 dpe
		PW16	† 25 dpe
		PW18	† 101 dpe
Lv17/WB/Zieme3 (HAD)	Inoculated	P23	‡ 12 dpi
	In contact	P24	† 16 dpe
		PW13	† 126 dpi/68 dpc
		PW14	† 126 dpe/68 dpc

dpi: days post infection; dpe: days post exposure; dpc: days post challenge.

With respect to the *in-contact* pigs, 2/4 (PW15 and PW18) did not develop any detectable clinical signs other than a weak peak of fever at 10 dpe (PW18). Despite their apparent health status, intermittent weak viremia (Ct > 35) was detected in PW15 from 22 to 94 dpe, while PW18 remained aviraemic on all analysis days (up to 101 dpe). The remaining *in-contact* animals (PW14 and PW16) presented mild transitory clinical signs, specifically joint swelling, from 7 to 16 dpe, particularly in PW14. In PW14, the viremia lasted for 35 days (14–49 dpe) and the virus could be isolated from 14 to 22 dpe (average titre of 3.16×10^7 $TCID_{50}$/mL). Blood from PW16 was first detected to be PCR positive at 22 dpe. All *in-contact* animals developed antibodies from 19.5 ± 3.6 dpe which persisted until the end of the study period with IPT titres >1:163,840.

To assess the virus presence in tissues, four pigs were euthanized at 25 dpe (PW16), 45 dpi (PW17) and 101 dpe (PW15 and PW18). The ASFV genome was detected by PCR in all tissues (100%) at 25 dpe, and in 19/21 tissues (90.4%) at 45 dpi. Infectious ASFV was recovered from 16 (76.1%) and 6 (31.6%) tissues at 25 and 45 dpe/dpi respectively. It is noteworthy that virus genome was

TABLE 2 ASFV detection in tissues determined by real-time PCR in the inoculated (IP) and in the in-*contact* animals (CP) infected with the non-HAD Lv17/WB-Rie1 (PW13 to PW18) or with the HAD Latvia Lv17/WB-Zieme3 (P23 and P24)

	IPPW13 d126pi		CPPW14 d126pe		CPPW15 d101pe		CPPW16 d25pe		IPPW17 d45pi		CPPW18 d101pe		IPP23 d12pi		CPP24 d16pe	
ID domestic pig/tissue identification	**Ct**	**Result**	**Ct**	**Result**	**Ct**	**Ct**	**Ct**	**Ct**	**Ct**	**Result**	**Ct**	**Result**	**Ct**	**Result**	**Ct**	**Result**
Liver	No ct	NEG	**36.66**	**POS**	No ct	NEG	**32.83**	**POS**	No ct	NEG	No ct	NEG	**24.61**	**POS**	**18.47**	**POS**
Lung	No ct	NEG	**38.38**	**POS**	No ct	NEG	**32.79**	**POS**	**34.31**	**POS**	No ct	NEG	**25.88**	**POS**	**21.54**	**POS**
Kidney	No ct	NEG	**36.7**	**POS**	No ct	NEG	**36.18**	**POS**	No ct	NEG	No ct	NEG	**26.61**	**POS**	**26.09**	**POS**
Heart	No ct	NEG	**38.79**	**POS**	No ct	NEG	**35.95**	**POS**	**31.37**	**POS**	No ct	NEG	**28.95**	**POS**	**20.63**	**POS**
Spleen	No ct	NEG	**36.75**	**POS**	No ct	NEG	**31.49**	**POS**	No ct	NEG	No ct	NEG	**23.8**	**POS**	**26.79**	**POS**
Tonsil	**34.6**	**POS**	**37.16**	**POS**	No ct	NEG	**34.04**	**POS**	**29.77**	**POS**	No ct	NEG	**22.89**	**POS**	**17.72**	**POS**
Renal LN[a]	**38**	**POS**	No ct	NEG	No ct	NEG	**27.56**	**POS**	**29.14**	**POS**	No ct	NEG	**24.15**	**POS**	**18.77**	**POS**
Retropharyngeal LN	No ct	NEG	No ct	NEG	**36.23**	**POS**	**30.49**	**POS**	**32.62**	**POS**	No ct	NEG	**22.35**	**POS**	**18.73**	**POS**
Gastrohepatic LN	No ct	NEG	**38.75**	**POS**	No ct	NEG	**35.02**	**POS**	**33.53**	**POS**	No ct	NEG	**25.68**	**POS**	**20.18**	**POS**
Mesenteric LN	No ct	NEG	No ct	NEG	No ct	NEG	**33.41**	**POS**	**36.4**	**POS**	No ct	NEG	**22.69**	**POS**	**19.11**	**POS**
Mediastinal LN	No ct	NEG	No ct	NEG	**37.03**	**POS**	**34.42**	**POS**	**35.72**	**POS**	No ct	NEG	**27.38**	**POS**	**22.41**	**POS**
Inguinal LN	**36.6**	**POS**	No ct	NEG	**36.43**	**POS**	**26.78**	**POS**	**31.65**	**POS**	No ct	NEG	**24.7**	**POS**	**19.09**	**POS**
Submandibular LN	**37.2**	**POS**	No ct	NEG	**35.67**	**POS**	**29.93**	**POS**	**33.62**	**POS**	No ct	NEG	**22.74**	**POS**	**21.24**	**POS**
Splenic LN	No ct	NEG	No ct	NEG	**38.77**	**POS**	**33.83**	**POS**	**34.4**	**POS**	No ct	NEG	**24.15**	**POS**	**22.34**	**POS**
Popliteal LN	No ct	NEG	No ct	NEG	**35.18**	**POS**	**29.81**	**POS**	**29.86**	**POS**	No ct	NEG	**21.83**	**POS**	**18.57**	**POS**
Bone marrow	No ct	NEG	No ct	NEG	No ct	NEG	**35.99**	**POS**	**32.55**	**POS**	No ct	NEG	**25.62**	**POS**	**25.08**	**POS**
Diaphragm	No ct	NEG	No ct	NEG	No ct	NEG	**30.39**	**POS**	**35.36**	**POS**	No ct	NEG	**30.5**	**POS**	**27.43**	**POS**
Front left AC	No ct	NEG	No ct	NEG	No ct	NEG	**27.35**	**POS**	**32.48**	**POS**	No ct	NEG	**27.85**	**POS**	**27.06**	**POS**
Front right AC	No ct	NEG	No ct	NEG	**37.67**	**POS**	**26.28**	**POS**	**29.43**	**POS**	No ct	NEG	**27.64**	**POS**	**27.07**	**POS**
Back left AC	No ct	NEG	No ct	NEG	**39.19**	**POS**	**25.07**	**POS**	**33.87**	**POS**	No ct	NEG	**24.84**	**POS**	**23.7**	**POS**
Back right AC[b]	No ct	NEG	No ct	NEG	**38.81**	**POS**	**27.05**	**POS**	**34.25**	**POS**	No ct	NEG	**25.96**	**POS**	**28.69**	**POS**

ns = not sample. In grey is indicated a positive non-HAD-ASFV result after three passages in PBM cells, red indicated the presence of the HAD Latvia Lv17/WB-Zieme3 ASFV, whereas non-shadow cells indicate a negative virus isolation result

[a]Lymph node. [b]Articular cartilage.

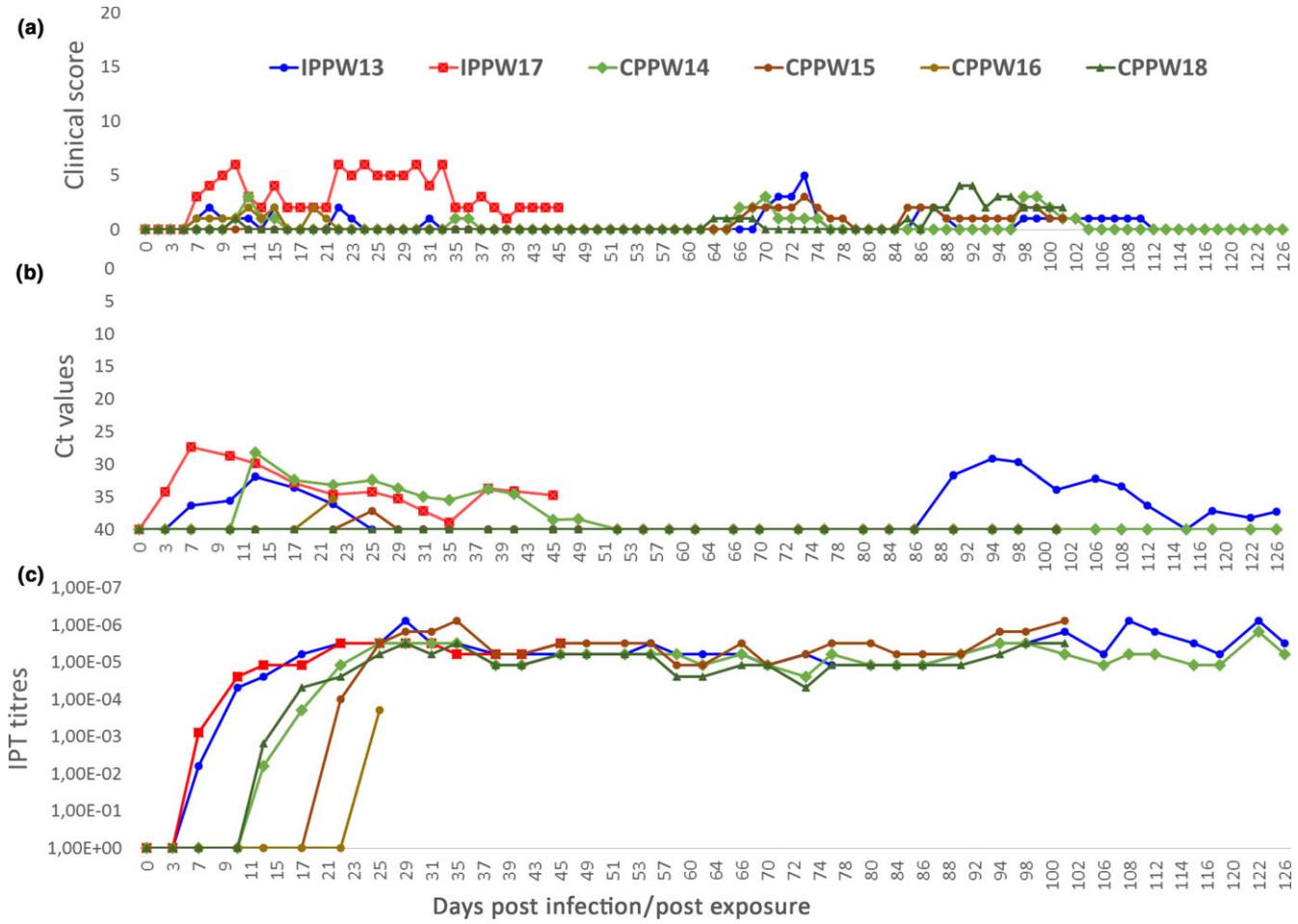

FIGURE 1 Clinical score (a), viremia determined by real-time PCR (b) and antibody titre by IPT (c) in the group of inoculated (IP) and in-contact (CP) pigs infected with the non-HAD Lv17/WB/Rie1 isolate. To assess the virus presence in tissues, PW16, PW17 were euthanized at 25 dpe and 45 dpi, respectively, and PW15 and PW18 were euthanized at 101 dpe [Colour figure can be viewed at wileyonlinelibrary.com]

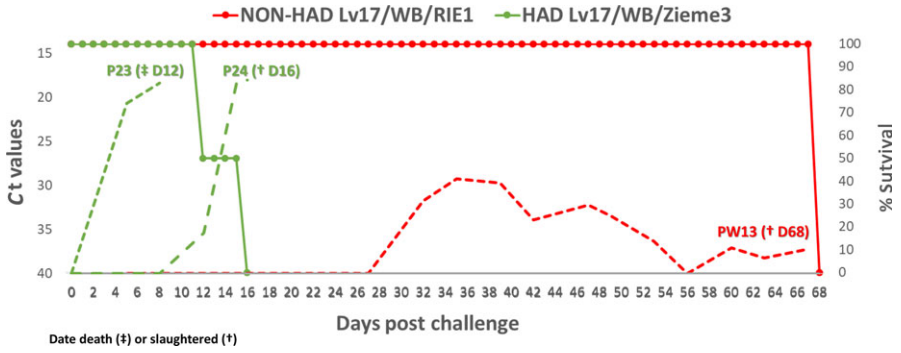

FIGURE 2 Survival rate overlapped with viremia determined by real-time PCR after challenge exposure done at 58 days post infection to the virulent HAD-ASFV Lv17/WB/Zieme3. Naïve pig P23 was i.m. inoculated and P24 was used as in-contact naïve animal [Colour figure can be viewed at wileyonlinelibrary.com]

detected in 9/21 (42%) tissues of the in-contact pig PW15 at 101 dpe, although ASFV could only be recovered from the retropharyngeal and submandibular lymph nodes, yielding titre value (>10^9TCID$_{50}$/mL). In contrast, all tissues of PW18 were negative (Table 2).

To mimic the field conditions, the non-HAD-infected pigs PW13 and PW14 were split in a separate box at 58 dpi/dpe and mixed with two naive pigs (seeder pig) (P23 and P24). Of these, P23 was i.m. inoculated with 10 TCID$_{50}$ of the HAD-ASFV Lv17/WB/Zieme3, a haemadsorbing virus isolated from a dead wild boar in Latvia,

February 2017, with high virulence characteristics. The seeder P24 was used as *in-contact* animal. Both inoculated (P23) and in-contact naïve pigs (P24) showed acute clinical signs of ASF and died or were euthanized at 12 dpi and 16 dpe respectively (Figure 2). By contrast, the two pigs previously infected with the non-HAD Latvian isolate remained completely healthy and aviraemic during the month-long study period. At 33 days post challenge (dpc), PW13 was detected to be PCR positive in blood until euthanasia at 126 dpi/68 dpc (Figure 2) with a sporadic shedding of the virulent ASFV at 36 dpc (titre 6.81×10^5 HAD_{50}/mL). The presence of ASFV was demonstrated by PCR in all tested tissues in the naïve animals (P23 and P24), whereas only 4/21 (33.3%) tissues resulted PCR positives from animals initially infected with, or exposed to, the non-HAD virus (PW13 and PW14 respectively) euthanized at 126 dpi/68 dpc. The HAD-ASFV was exclusively isolated from the tonsil of the PW13, whereas the non-HAD-ASFV could not be isolated (Table 2).

3 | CONCLUSION

This study describes the ASFV Lv/17/WB/Rie1strain, a non-HAD attenuated genotype II ASFV isolated in Europe, Latvia 2017. The sequence analysis of the *EP402R* gene, coding the CD2-like protein responsible for the ASFV distinctive HAD phenomenon, revealed a single adenosine deletion that generates a truncated protein (Spanish patent PCT/2018/000069). In Lv17/WB/Rie1 isolate, the non-functional CD2-like protein is responsible of its non-HAD capacity, a feature shared with other naturally attenuated ASFV strains, such as NH/P68 and OURT88/3. In agreement with previous findings using the same mentioned non-HAD-ASFVs (Boinas et al., 2004; Gallardo et al., 2015; Leitão et al., 2001; Sánchez-Cordón et al., 2017), pigs intramuscularly infected with Lv/17/WB/Rie1 ASFV developed nonspecific clinical signs, and in some cases remained asymptomatic, showing intermittent and weak viremia and a high antibody response. Furthermore, 2 months following the primary infection with Lv17/WB/Rie1, the two pigs exposed were fully resistant to challenge with a virulent HAD Latvian ASFV. Similarly, protection induced by oronasal and intramuscular immunizations of pigs with the non-HAD low virulence field ASFV genotype I isolates have been previously described (Boinas et al., 2004; Gallardo et al., 2015; Leitão et al., 2001; Sánchez-Cordón et al., 2017). Even though the number of animals was quite small, these results suggest the potential use of Lv17/WB/Rie1 as a target for live attenuated vaccines development, as it occurs with NH/P68 and OURT88/3 non-HAD-ASFVs. Further experimental studies with this non-HAD virus may provide new insights on mechanisms of protective immunity to ASFV.

Finally, this study illustrates the natural evolution of the ASFV including the emergence of less virulent forms over the time, as it has occurred in other geographic regions where ASF has been present for long time (Arias, Jurado, Gallardo, Fernández-Pinero, & Sánchez-Vizcaíno, 2018).

ACKNOWLEDGEMENTS

We are grateful for the technical contributions of our colleagues from the National Reference Laboratories for ASF in Latvia. We are especially thankful to Elena Martín, Covadonga Perez and Alicia Simón for their technical assistance. Additionally, we are appreciative to all the staff at the INIA-CISA who work in the animal facilities department including, Miguel Angel Sánchez and Victor Manuel Sánchez Ramirez, as well as Antonia Gonzalez from the pathology department. The study was funded by the European Union reference laboratory for ASF (grant no. UE-1 LR PPA/03) and by the INIA (projects RTA2015-00033-C02-01, AT2015-002). Dr Gallardo is the laboratory research coordinator of the European and Food and Agricultural Organization of the United Nations Reference Laboratory for African swine fever at CISA-INIA. Her research work mainly focuses on molecular characterization, epidemiology and ASF diagnosis.

ORCID

Carmina Gallardo https://orcid.org/0000-0003-3293-306X
Marisa Arias https://orcid.org/0000-0001-6044-2968

REFERENCES

Arias, M., Jurado, C., Gallardo, C., Fernández-Pinero, J., & Sánchez-Vizcaíno, J. M. (2018). Gaps in African swine fever: Analysis and priorities. *Transboundary and Emerging Diseases*, *65*(Suppl 1), 235–247. https://doi.org/10.1111/tbed.12695

Bastos, A. D., Penrith, M. L., Cruciere, C., Edrich, J. L., Hutchings, G., Roger, F., … Thomson, R. G. (2003). Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Archives of Virology*, *148*, 693–706. https://doi.org/10.1007/s00705-002-0946-8

Boinas, F. S., Hutchings, G. H., Dixon, L. K., & Wilkinson, P. J. (2004). Characterization of pathogenic and non-pathogenic African swine fever virus isolates from Ornithodoros erraticus inhabiting pig premises in Portugal. *Journal of General Virology*, *85*(Pt 8), 2177–2187. https://doi.org/10.1099/vir.0.80058-0

Borca, M. V., Kutish, G. F., Afonso, C. L., Irusta, P., Carrillo, C., Brun, A., … Rock, D. L. (1994). An African swine fever virus gene with similarity to the T- lymphocyte surface-antigen Cd2 mediates hemadsorption. *Virology*, *199*(2), 463–468. https://doi.org/10.1006/viro.1994.1146

Carrascosa, A. L., Bustos, M. J., & de Leon, P. (2011). Methods for growing and titrating African swine fever virus: Field and laboratory samples. *Current Protocols in Cell Biology Chapter 26*, Unit 26. 14.

Fernandez-Pinero, J., Gallardo, C., Elizalde, M., Robles, A., Gomez, C., Bishop, R., … Arias, M. (2013). Molecular diagnosis of African swine fever by a new real-time PCR using universal probe library. *Transboundary and Emerging Diseases*, *60*(1), 48–58. https://doi.org/10.1111/j.1865-1682.2012.01317.x

Gallardo, C., Fernandez-Pinero, J., Pelayo, V., Gazaev, I., Markowska-Daniel, I., Pridotkas, G., … Arias, M. (2014). Genetic variation among African swine fever genotype II viruses, eastern and central Europe. *Emerging Infectious Diseases*, *20*(9), 1544–1547. https://doi.org/10.3201/eid2009.140554

Gallardo, C., Nieto, R., Soler, A., Pelayo, V., Fernández-Pinero, J., Markowska-Daniel, I., … Arias, M. (2015). Assessment of African swine fever diagnostic techniques as a response to the epidemic outbreaks

in Eastern European Union Countries: How to improve surveillance and control programs. *Journal of Clinical Microbiology* *53*(8):2555. https://doi.org/10.1128/JCM.00857-15

Gallardo, C., Nurmoja, I., Soler, A., Delicado, V., Simón, A., Martin, E., … Arias, M. (2018). Evolution in Europe of African swine fever genotype II viruses from highly to moderately virulent. *Veterinary Microbiology*, *219*, 70–79. https://doi.org/10.1016/j.vetmic.2018.04.001

Gallardo, C., Soler, A., Nieto, R., Sánchez, M.A., Martins, C., Pelayo, V., … Arias, M. (2015). Experimental transmission of African Swine Fever (ASF) low virulent isolate NH/P68 by surviving pigs. *Transboundary and Emerging Diseases* *62*(6):612–622. https://doi.org/10.1111/tbed.12431

Ge, S., Li, J., Fan, X., Liu, F., Li, L., Wang, Q., … Wang, Z. 2018. Molecular characterization of African Swine Fever virus, China, 2018. *Emerging Infectious Diseases* *24*(11):2131–2133. https://doi.org/10.3201/eid2411.181274

Guinat, C., Gogin, A., Blome, S., Keil, G., Pollin, R., Pfeiffer, D. U., & Dixon, L. (2016). Transmission routes of African swine fever virus to domestic pigs: Current knowledge and future research directions. *The Veterinary Record*, *178*(11), 262–267. https://doi.org/10.1136/vr.103593

Leitão, A., Cartaxeiro, C., Coelho, R., Cruz, B., Parkhouse, R. M., Portugal, F., … Martins, C. L. (2001). The non-haemadsorbing African swine fever virus isolate ASFV/NH/P68 provides a model for defining the protective anti-virus immune response. *Journal of General Virology*, *82*(Pt 3), 513–523. https://doi.org/10.1099/0022-1317-82-3-513

Rodríguez, J. M., Yáñez, R. J., Almazán, F., Viñuela, E., & Rodriguez, J. F. (1993). African swine fever virus encodes a Cd2 homolog responsible for the adhesion of erythrocytes to infected-cells. *Journal of Virology*, *67*(9), 5312–5320.

Rowlands, R. J., Michaud, V., Heath, L., Hutchings, G., Oura, C., Vosloo, W., … Dixon, L. K. (2008). African swine fever virus isolate, Georgia, 2007. *Emerging Infectious Diseases*, *14*(12), 1870–1874. https://doi.org/10.3201/eid1412.080591

Sánchez-Cordón, P. J., Chapman, D., Jabbar, T., Reis, A. L., Goatley, L., Netherton, C. L., … Dixon, L. 2017. Different routes and doses influence protection in pigs immunised with the naturally attenuated African swine fever virus isolate OURT88/3. *Antiviral Research* *138*:1–8. https://doi.org/10.1016/j.antiviral.2016.11.021

World Organisation for Animal Health, 2018. African swine fever in Belgium. Immediate notification REF OIE 27948, Report Date: 14/09/2018, Country: Belgium [cited 2018 September 14].

Zani, L., Forth, J. H., Forth, L., Nurmoja, I., Leidenberger, S., Henke, J., … Blome, S, 2018. Deletion at the 5'-end of Estonian ASFV strains associated with an attenuated phenotype. *Scientific Reports* *8*(1):6510. https://doi.org/10.1038/s41598-018-24740-1

How to cite this article: Gallardo C, Soler A, Rodze I, et al. Attenuated and non-haemadsorbing (non-HAD) genotype II African swine fever virus (ASFV) isolated in Europe, Latvia 2017. *Transbound Emerg Dis*. 2019;00:1–6. https://doi.org/10.1111/tbed.13132