Transboundary and Emerging Diseases

SHORT COMMUNICATION

Experimental Infection of Domestic Pigs with African Swine Fever Virus Lithuania 2014 Genotype II Field Isolate

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Keywords:
African swine fever virus, genotype II; dynamics of infection

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Received for publication December 2, 2014

doi:10.1111/tbed.12346

Summary

An experimental infection was conducted to evaluate horizontal transmission, clinical, virological and humoral response induced in domestic pigs infected with African swine fever (ASF) genotype II virus circulating in 2014 into the European Union (EU). Ten naive pigs were placed in contact with eight pigs experimentally inoculated with the Lithuanian LT14/1490 ASF virus (ASFV) responsible for the first ASF case detected in wild boar in Lithuania in January 2014. Clinical examination and rectal temperature were recorded each day. Blood sampling from every animal was carried out twice weekly. Blood samples were examined for presence of ASF virus-specific antibodies and for determining the ASFV viral load. From the obtained results, it was concluded that the Lithuanian ASFV induced an acute disease which resulted in 94, 5% mortality. The disease was easily detected by real-time PCR prior to the onset of clinical signs and 33% of the animals seroconverted. All findings were in accordance with observations previously made in domestic pigs and wild boar when infected with ASF genotype II viruses characterized by a high virulence. One in-contact pig remained asymptomatic and survived the infection. The role of such animals in virus transmission would need further investigation.

The Study

African swine fever (ASF) is a devastating and complex disease of swine, caused by a DNA arbovirus (Arias et al., 2012). Since 2007, the disease is present in East Europe (FAO, 2013). The situation in Russia affecting both wild boar and domestic pigs, with two endemic regions recently described (Gogin et al., 2013), has originated a sporadic spill-over of ASF to the adjacent countries, Ukraine and Belarus (OIE, 2013, 2014a). In earlier 2014, ASF cases in wild boar were reported in Lithuania and Poland, in bordering regions with Belarus (Gallardo et al., 2014). To date (March 2015), nearly 501 ASF cases or outbreaks in wild boar and domestic pigs have been detected in the EU countries Latvia, Lithuania, and Poland. This situation, combined with an uncertain situation in Belarus, represents a permanent risk for ASF spreading into new regions of the EU.

Knowledge about disease dynamics for early detection of ASF is essential in the control of the disease. Therefore, an animal trial has been conducted at the EU Reference Laboratory (EURL) for ASF to obtain a precise description of the clinical, virological and pathological features induced in domestic pigs infected with currently circulating ASFV isolates in the EU. The *in vivo* experiment was conducted under biosafety level 3 conditions at the animal facilities of INIA-CISA, in accordance with the EC Directive 86/609/EEC and with local laws and regulations. Eight 3-month-old European hybrid pigs were inoculated by the intramuscular route with the Lithuanian LT14/1490 ASF virus (10 HAD_{50}/ml), which was isolated from a wild boar case which occurred in January 2014 (Gallardo et al., 2014). At the start of the experiment, ten pigs were housed together with the inoculated pigs as *in*-contact controls. Severity of the disease was expressed by a clinical scoring, obtained by

adding the score of eight clinical signs, recorded daily (Table 1). Paired EDTA-blood and sera samples were collected from pigs twice a week starting at day 3 post-inoculation until death of the animals. Negative control samples were collected at day 0, the day of inoculation. Twenty different types of tissues and organs were obtained from each necropsied animal including; liver, spleen, tonsil, heart, lung, kidney, submandibular, retropharyngeal, inguinal, popliteal, mesenteric, mediastinal, gastro-hepatic, splenic and renal lymph nodes, bone marrow and intra-articular tissues of joints. DNA was extracted from organs homogenates and blood samples using the High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany) and amplified by real-time PCR (Fernández-Pinero et al., 2013). The titre of virus was estimated by inoculation of tissues and blood samples into porcine peripheral blood macrophages (PBM) (OIE, 2014b). Detection of ASFV-specific antibodies was performed in serum using a commercial ELISA (INGEZIM, PPA COMPAC K3, INGENASA, Madrid, Spain) and by Indirect Immunoperoxidase Technique (IPT) validated by the EURL (Gallardo et al., 2012; EURL 2014).

Table 1. List of clinical signs used to create the ASF clinical score

1. Anorexia	0 no abnormality
	1 MILD (reduced eating)
	2 MODERATE (only picking at food)
	3 SEVERE (not eating)
2. Recumbence	0 no abnormality
	1 MILD (Stillness)
	2 MODERATE (Get up only when touched)
	3 SEVERE (Remain recumbent when touched)
3. Skin Haemorrhage/Cyanosis	0 no abnormality
	1 MILD
	2 MODERATE
	3 SEVERE
4. Swelling	0 no abnormality
	1 MILD (Joint swelling)
	2 MODERATE (Lameness, necrotic foci)
	3 SEVERE (Severe lameness, impaired walking)
5. Laboured breathing and/or coughing	0 no abnormality
	1 MILD
	2 MODERATE.
	3 SEVERE
6. Ocular discharge	0 no abnormality
	1 MILD
	2 MODERATE.
	3 SEVERE
7. Digestive findings	0 no abnormality.
	1 MILD (normal diarrhoea<24 h)
	2 MODERATE (diarrhoea, vomiting>24 h)
	3 SEVERE (bloody diarrhoea)
8. Temperature (rectal)	0 = <40
	1 = 40–40.5
	2 = 40.5–41
	2.5 = 41–41.5
	3 = >41.5

An acute, fatal disease was developed in seven of the eight inoculated animals, which died or were euthanized due to the severity of symptoms between 7 and 9 days post-inoculation (dpi). One inoculated pig showed, however, a delayed course of the disease, resembling the same as that seen in in-contact animals, which died or were slaughtered from 14 to 22 days post-exposure (dpe). One in-contact pig remained asymptomatic throughout the experiment and was slaughtered at day 61 (Fig. 1a). Both inoculated and contact animals developed broadly similar clinical patterns, and died or were slaughtered 3–4 days after the appearance of clinical signs. The moribund animal showed mainly a febrile syndrome (fever, mild anorexia, lethargy, weakness and recumbence) (17/18) with increased clinical scores following by a drastic drop in body temperature shortly before death (Fig. 1b). Other related ASF clinical signs were the following: (i) bluish-purple areas and haemorrhages (ecchymosis, petechial) on the ears (13/18) and/or abdomen (6/18), (ii) ocular discharges (11/18), (iii) reddening of the skin (3/18), and (iv) bloody diarrhoea (3/18). Post-mortem examination of animals dead within 22 days revealed enlarged, oedematous and haemorrhagic lymph nodes; particularly renal, gastro-hepatic and mediastinal lymph nodes were more intensely haemorrhagic. Some other lesions included hydropericardium with yellowish fluid, hyperaemic splenomegaly, hepatic congestion and petechial haemorrhages in the renal pelvis and cortex, small and large intestine, gall bladder and urinary bladder. The necropsy of the asymptomatic animal slaughtered at 61 dpe revealed hyperaemic splenomegaly, petechiae in the lungs and moderately enlarged and haemorrhagic lymph nodes.

In seven of the eight inoculated pigs, ASF genome virus was first detected in blood by PCR (Fernández-Pinero et al., 2013) at 3.75 $\pm$ 1.4 dpi. In agreement with the clinical signs, one inoculated pig did not show viremia until 14 dpi following the same pattern that the naturally infected pigs in which the ASF genome virus was first detected at 13.7 $\pm$ 2.8 dpe (Fig. 2a). Maximum titres, ranging between $10^{6.4}$ and $10^{8.7}$HAD$_{50}$/ml, were recorded at 6 dpi or 14 dpe in inoculated and in-contact animals, respectively. ASFV genome was also detected in all tested organs and tissues. The asymptomatic animal showed intermittent and weak peaks of viraemia at 17, 34 and 38 dpe (Fig. 2b), and virus genome was detected in the limit of the detection in nine of the 20 collected tissues including the bone

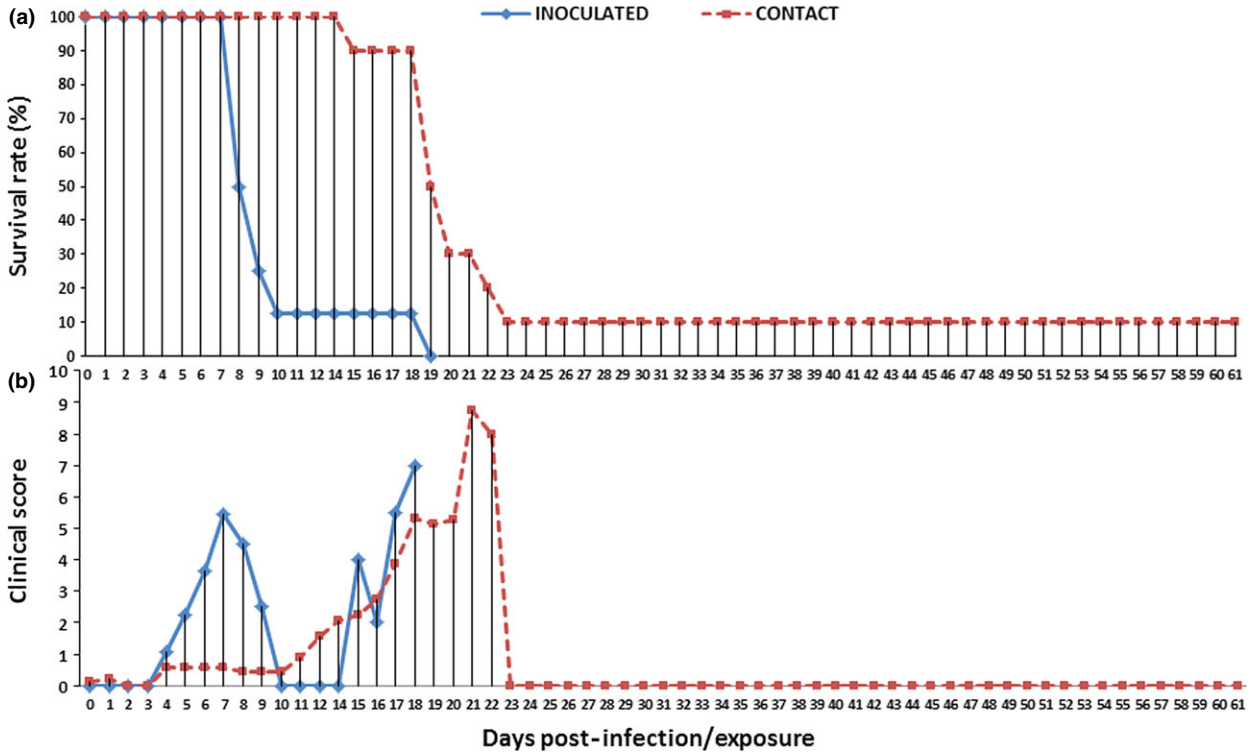

Fig. 1. Survival (a) and clinical scores (b), in the Lithuania genotype II ASFV inoculated (♦) and contact (■) groups. Means per day post-inoculation (dpi) are shown for all inoculated and contact animals.

marrow, articular tissues, spleen, lung and the splenic, renal and submandibular lymph nodes. No virus could be isolated in samples from this animal after three passages in PBM cells. ASFV-specific antibodies were detected by ELISA in two in-contact animals (11%) at day 18 post-exposure. Using the IPT, six animals (33%, one inoculated/ five in-contacts) yielded positive results between 17 and 21 dpi/dpe (Table 2). No antibody response was observed in the survivor pig.

Conclusions

Several experiments have been performed to characterize virulence and clinical appearance of infections by genotype II ASFV isolates circulating in east Europe in wild boar and domestic pigs (Gabriel et al., 2011; Blome et al., 2012, 2013; Guinat et al., 2014; Vlasova et al., 2014). However, experimental evidence on the clinical aspects of ASF caused by ASFV isolates circulating in the EU (2014) in domestic pigs is not known. In accordance with previous observations, the type and pattern of clinical signs induced by the Lithuanian isolate in domestic pigs were consistent with the acute form of ASF caused by virulent viruses, which resulted in 94.5% mortality. Upon the primary infection, clinical signs associated with a febrile syndrome were developed after an incubation period of 4–5 days with the appearance of dead or moribund animals from 7 to 9 dpi. An average delay of 12–14 days was observed in the in-contact pigs resulting in a severe disease or fatalities mainly from 18 to 22 dpe. The disease was easily detected by PCR in blood samples prior to the appearance of the clinical signs and from the tested organs at necropsy. ASF antibodies were detected in 33% of the animals, all of them at 17–18 dpi/dpe. One animal survived the infection showing weak and intermittent peaks of viraemia, and DNA could be detected in tissues, although virus isolation could not be achieved. On this regard, similar features of infection in absence of detectable clinical signs have been described in naturally infected suids in Africa (Penrith et al., 2004; Okoth et al., 2013). The potential virus transmission that may result would need further investigation.

Our findings also suggest that recognition of related ASF clinical symptoms in an industrial holding should be suspected when sudden deaths of few animals occur, representing the first evidence of the disease. ASF should therefore be included in the differential diagnosis of such events particularly taking into account the epidemiological situation. The subsequent wave of infection of pigs in the pen vicinity would require about 12–14 days for first clinical symptoms (usually fever) in the newly infected pigs.

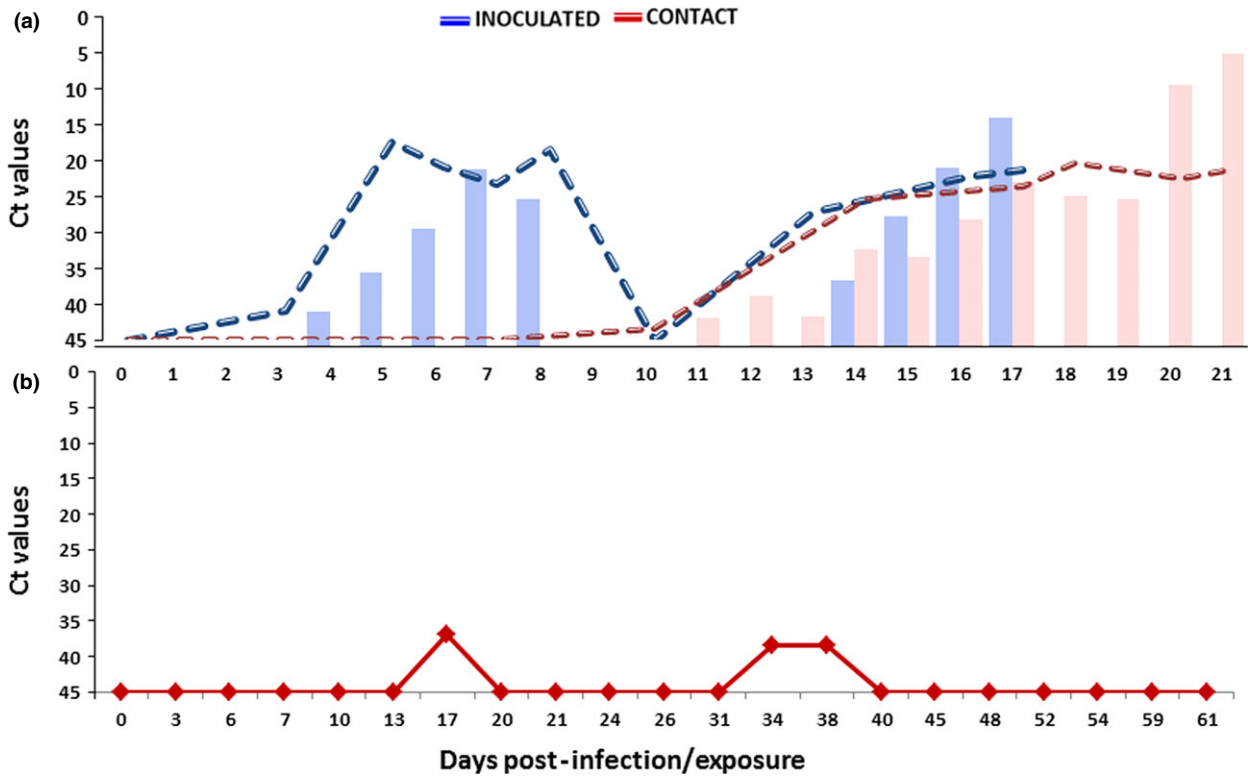

Fig. 2. (a) Viraemia determined using the real-time PCR in the group of the inoculated (—) and in-contact animals (—) overlapped with the clinical score represented by bars. Means are presented per day post-inoculation (dpi); (b) Viraemia determined using the real-time PCR in the survivor animal slaughtered at 61 dpi.

Table 2. ASF positive antibody detection

		Days post-infection/exposure								
Animal identification	Antibody technique	0	3	7	10	14	17	18	20	21
Contact pig 2	*IPT*	NEG	NEG	NEG	NEG	NEG	NEG	NS[a]	NEG	POS
	ELISA	NEG	NEG	NEG	NEG	NEG	NEG	NS[a]	NEG	NEG
Inoculated pig 6	*IPT*	NEG	NEG	NEG	NEG	NEG	NEG	POS		
	ELISA	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
Contact pig 10	*IPT*	NEG	NEG	NEG	NEG	NEG	NEG	POS		
	ELISA	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
Contact pig 11	*IPT*	NEG	NEG	NEG	NEG	NEG	POS	POS		
	ELISA	NEG	NEG	NEG	NEG	NEG	NEG	POS		
Contact pig 12	*IPT*	NEG	NEG	NEG	NEG	NEG	NEG	POS		
	ELISA	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
Contact pig 15	*IPT*	NEG	NEG	NEG	NEG	NEG	NEG	POS		
	ELISA	NEG	NEG	NEG	NEG	NEG	NEG	POS		

[a]Not sampled.
Comparative IPT and ELISA results of serum samples obtained from seroconverted pigs. Light grey cells indicate initial positive ASFV genome detection result.

A prompt diagnosis would be very valuable to prevent further spreading to other farms. This dynamics of infection has been recently corroborated in a large industrial holding in Lithuania (EC, DG-SANCO, 2014).

Acknowledgements

The study was funded by the European Union Reference laboratory for ASF (grant n° UE- LR PPA/03) and by the

EU project, ASFORCE 'Targeted Research Effort on African Swine Fever' (KBBE.2012.1.3-02, 311931). We thank the technical assistance of Alicia Simón, Covadonga Pérez and Elena Martín in performing laboratory diagnostic techniques, and the practical contributions working at the animal facilities and the pathology department at INIA-CISA, especially Antonia Gonzalez and Lourdes Peña.

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