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J Vet Res 63, 303-310, 2019
DOI:10.2478/jvetres-2019-0058

REVIEW ARTICLE

African swine fever virus – persistence in different environmental conditions and the possibility of its indirect transmission

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Received: April 25, 2019 *Accepted: August 12, 2019*

Abstract

Since 2007, African swine fever (ASF) has posed a serious threat to the European swine industry. In Poland, the numbers of reported outbreaks in pigs and affected areas grow every year. In 2018, the disease was noted in Western Europe, in Belgium specifically, where several hundred infected wild boars have been detected so far. In 2018, the virus unexpectedly emerged in pig holdings in eastern China, northern Mongolia, Vietnam, and Cambodia, causing worldwide concern about its further spread. Since there is still no vaccine available, the only approach to control the disease is biosecurity. Identification of potential sources of the virus is extremely important in light of its phenomenal survivability. The review summarises the current knowledge about ASFV survivability and resistance to environmental conditions, and discusses the role of indirect contact in spreading the disease.

Keywords: ASFV, stability, survivability, indirect transmission, spreading.

Introduction

African swine fever (ASF) is an infectious disease causing high mortality of pigs, and is notifiable to the World Organisation of Animal Health (OIE). The aetiological agent, African swine fever virus (ASFV), is classified as the sole member of the *Asfarviridae* family and is among the large (200 nm), complex, enveloped double-stranded DNA viruses (11). ASFV affects domestic and wild members of the Suidae family, leading to a wide range of symptoms from chronic or persistent infection to acute haemorrhagic fever, and inflicts up to 100% mortality (18). The main routes for disease transmission are direct contact between susceptible and sick animals or their fluids or excretions, and indirect contact through contaminated feed, pork meat, people, vehicles, or fomites (10, 13, 31). In endemic areas, ASFV also infects soft ticks of the *Ornithodoros* genus, making them actively engaged in the disease epidemiology due to their potential to indirectly transmit the virus to susceptible vertebrate hosts (43). Studies regarding hard ticks (*Ixodes ricinus* and *Dermacentor reticulatus*) as a source of ASFV demonstrated that these parasites facilitate neither virus replication nor tick-to-pig transmission, at least under laboratory conditions; nevertheless, viral DNA can persist in the tick organism up to eight weeks, allowing them to act as mechanical vectors (8). Mellor *et al.* (29) proved that the stable fly, *Stomoxys calcitrans*, is able to mechanically transmit the virus up to 24 h post digestion of ASFV infected blood. Moreover, a recent study conducted by Olesen *et al.* (39) established that infection of pigs might also occur following the oral uptake of flies fed ASFV-infected blood.

ASFV resistance and stability have attracted the interest of numerous investigators over the years (5, 7, 10, 23, 26–28, 41, 42, 47). It has been proved that ASFV shows high resistance to environmental conditions and remains infectious over a long storage time either below 0°C or at 4°C. The curing process of infected meat (a process like that which Parma, Iberian, or Serrano ham undergoes) facilitated survival of ASFV in ham for over a year (28). ASFV can survive many freeze–thaw cycles, and furthermore it is stable at pH levels between 4 and 13 and can survive a temperature of 56°C for over an hour (42).

Due to its high stability, ASFV is able to persist for a long time in contaminated fomites or meat; therefore they could play a role as vehicles for the transboundary or even transcontinental spread of the pathogen. Such a mode of dissemination is one of the most frequent routes of ASFV introduction into territories previously free of it. For example, in 2007 an ASF outbreak in Georgia was caused by improper disposal of contaminated pork meat from a ship at Poti docks. Similar events occurred in history to cause other ASFV introductions, namely to Portugal (1957), Cuba (1971), Brazil (1978), and Belgium (1985) (10, 31). Besides through the negligence by which humans spread the disease transnationally, ASFV is present in an environment because of its long persistence in wild boar carcasses, which can in effect be a virus reservoir. Therefore eradication of the disease is extremely difficult, due to the necessity of actively searching for decaying boar cadavers to make possible the proper disposal of infected carcasses (3).

ASFV resistance to physical treatment

Over a span of many years, numerous experiments were dedicated to ASFV stability. As far back as 1921, Montgomery (30) demonstrated that ASFV is extremely resistant to high temperatures, putrefaction and desiccation. Much later, Coggins (5) evidenced high ASFV resistance to selected chemical (trypsin and EDTA) and physical treatments (freezing/thawing and ultrasonic waves). In the same study he successfully collected viable virus after 1 h incubation at 56°C and one week at 37°C. Plowright and Parker (42) in 1967 showed that storing at 4°C preserves infectivity of viraemic blood for at least 75 weeks and of virus-spiked medium without Ca^{2+} or Mg^{2+} for 61 weeks. At 37°C, medium containing ASFV remained infectious for 11–22 days, but at 60°C it was only for 30 min. It was also shown that the virus is stable over a wide pH range (from pH 3.9 to 13.4) for seven days (in serum-supplemented media) (42). Another study concerning ASFV resistance to temperature was conducted more recently, and partially confirmed previous results. It was shown that the virus was stable at 4, 22, and 40°C and lost only less than 10^1 50% haemadsorption doses (HAD_{50})/mL during 24 h incubation in EMEM. At 50°C, only a small fraction of virus remained infectious, and at 60°C no infectious virions could be detected after only 15 min (47). In summary, ASFV in tissues can survive deep freezing (−70°C) for many years without significant loss of titre, but at −20°C it systematically loses its titre, nevertheless remaining viable for at least 105 weeks (2 years) (42). At 4°C it is also very stable when contained in medium; it remains infectious for at least 61 weeks (1 year and 2 months). In higher temperatures ASFV is inactivated relatively quickly. At 37°C traces of viable virus could be found after 22 days, at 56°C after 1 h, but at 60°C no longer than after 15 min.

Direct transmission

Direct contact between sick and susceptible animals has repeatedly been proved to be an effective transmission route for ASFV (2, 14, 17, 21, 38, 41, 48). Recent experiments conducted with current ASFV European strains showed that viral DNA and/or infective virus might be detected in blood (first detection at 3.75 ± 1.4 dpi) (2, 14, 17, 21, 33, 38, 41, 48), nasal, rectal, and oral fluids (21, 33, 37, 41, 48), and faeces and urine (7, 21, 37, 38) of infected animals. The highest viral loads in blood are recorded between 5 and 27 dpi by intramuscular or intranasal inoculation or 9 and 29 dpi when pig-to-pig contact is investigated, and maximum ASFV titres in blood range from 10^6 to 10^9 HAD_{50}/mL in the acute phase (Table 1).

As regards excretions and secretions, it has been demonstrated that they might contain viable virus (1.6–4.8 $\log_{10}HAD_{50}$/mL) (Table 1) on the day of euthanasia, but in the case of contact animals, the virus was molecularly detected in nasal fluid prior to being evident in blood (38). ASFV survivability in these contaminated excretions depends mainly on temperature; however, in favourable conditions it may retain its viability for long time, increasing the risk of the disease spreading, particularly under low-biosecurity conditions (13).

It has been clearly and repeatedly shown, that solely air contact (without direct physical contact between sick and healthy animals) is sufficient to develop a clinical course of the disease in susceptible pigs (9, 38, 50). Air sampled during experimental infection was consistently molecularly and virologically positive during the first 25–30 days after infection, with virus titres up to $10^{3.2}$ $TCID_{50}$ eq./m^3 (9).

ASFV survival in excretions

As the viable virus has been identified in excretions, it raises the question of how long the virus can survive in them. Montgomery (30) showed as long ago as 1921 that when faeces are stored in the dark at room temperature they remain infective for at least 11 days, but other later studies showed that this time might be extended up to 160 days (13). The most recent investigations indicated that ASFV stability in faeces is much lower than previously thought and depends largely on the temperature. Faeces collected from experimentally inoculated pigs remained infectious for 8 days at 4°C, and for 3–4 days at 37°C. When it comes to urine, it might contain viable virus for up to 15 days at 4°C, 5 days at 21°C, and 2–3 days at 37°C (7).

Table 1. Current Eurasian ASFV strain levels of maximum viraemia in blood and shedding potential in other body fluids after various modes of inoculation

Inoculation mode	ASFV strain	Maximum viraemia			Virus detection in other body secretions/excretions	Reference
		Dose (HAD_{50}/mL)	Titre (log_{10} HAD_{50}/mL)	Dpi		
Intramuscular	Georgia 2007/1	10^2	6–8	5	Nasal fluid: VI+ (10^2–10^4), 5 dpi Rectal fluid: (10–10^2), 6 dpi Urine: PCR+*, VI+ Excretions: PCR+*, VI+ Oral fluid: PCR+*, VI− *1st day of fever	Guinat *et al.* (21), 2014
	Lithuania LT14/1490	10^1	6.4–8.7	6	n/a	Gallardo *et al.* (17), 2015
	Russia K/08/13	5×10^3	6.5–7	7	Nasal fluid: PCR+ Rectal fluid: PCR+	Vlasova *et al.* (48), 2015
		50	6.5–7	9		
	Georgia 2007/1	10^3	7–8	7	n/a	O'Donnell *et al.* (34), 2016
	Odintsovo 02/14	5×10^3	7.56	11	n/a	Elsukova *et al*. (14), 2017
Oral/nasal	Caucasian	2×10^6	Cq: 22–39	5	Oropharyngeal fluid: PCR+, 6 dpi Faecal fluid: PCR+, 5 dpi	Blome *et al.* (2), 2013
	Kashino 04/13	5×10^3	7.5	7	Nasal fluid: PCR+ Rectal fluid: PCR+	Vlasova *et al.* (48), 2015
		50	6.5–7.5	7		
	Boguchary 06/13	5×10^3	6.5–7.5	9		
		50	6.5–7	5		
	Pol/15/Lindholm	2×10^4	~9	6	Nasal fluid: PCR+*, VI+ ($10^{1.6–4.8}$) Rectal fluid: PCR+*, VI+ ($10^{2.8–3}$) Oral fluid: PCR +*, VI− *1st day of fever Viral isolation at day of euthanasia	Olesen *et al.* (38), 2017
	Odintsovo 02/14	10^3	7.45	11	n/a	Elsukova *et al.* (14), 2017
		50	7.45	27		
Contact	Caucasian	n/a	Cq: 20–29	11	n/a	Blome *et al.* (2), 2013
	Georgia 2007/1	n/a	6–8	10	Nasal fluid: VI+ (10–10^2) from 7 dpi, rectal fluid: VI+ (10–10^2) from 12 dpi	Guinat *et al.* (21), 2014
	Kashino 04/13	n/a	6.5–7	15	n/a	Vlasova *et al.* (48), 2015
	Boguchary 06/13	n/a	7	9	n/a	
	Lithuania LT14/1490	n/a	6.4–8.7	14	n/a	Gallardo *et al.* (17), 2015
	Odintsovo 02/14	n/a	7.45–7.66	29	n/a	Elsukova *et al.* (14), 2017
	Pol/15/Lindholm	n/a	~9	12	Nasal fluid: PCR+, VI+ ($10^{1.8–2.8}$) Rectal fluid: PCR+, VI+ ($10^{1.6–8}$) Oral fluid: VI− PCR + in many prior to the PCR+ from blood	Olesen *et al.* (38), 2017

n/a – not applicable, dpi – day post infection, HAD_{50}/mL – haemadsorbing doses per millilitre, PCR+(−) – viral DNA detected (not detected) by real-time PCR, VI+(−) – infectious virus detected (not detected) by virus isolation in cell culture

The study by Olesen *et al.* (37) conducted *in vivo,* was in line with these results and showed that the time span required for inactivation of ASFV in excretions is relatively short: pigs introduced into pens contaminated with faeces and urine one day after the removal of infected pigs succumbed to experimental ASFV infection, although they were not susceptible to infection after three days. However, for the first time it was demonstrated experimentally that exposure of pigs to an environment contaminated with virus-containing excretions might result in infection. Excretions containing viable ASFV should be considered

an important route for virus transmission, particularly within a herd. Moreover, as the infective dose of ASFV is small – estimated at 10^1 HAD_{50}/mL *via* oronasal inoculation – a small amount of infective virus included in excretions might contaminate some fomites like clothes, footwear, equipment, etc. In consequence of this, the probability of virus transmission to other pens or even farms cannot be ruled out.

Indirect contact – contaminated fomites, feed, and drinking water

Since infectious ASFV is secreted and excreted, it therefore easily contaminates the environment, which subsequently may act as a virus source. Numerous epidemiological studies have proved that ASFV can be easily transmitted, either by direct contact or indirectly, *via* swill feed or contaminated fomites like clothes, footwear, equipment, food waste, bedding, etc. The most spectacular example of the disease spreading through fomites is its current epidemics in Europe, where it was introduced by ships containing ASFV-contaminated kitchen wastes used to feed pigs near Poti docks. Subsequently, the disease spread quickly to the neighbouring Caucasian region, then to Eastern Europe, and finally reached European Union countries in 2014 (4). Presently, as a result of that single introduction into Georgia, the disease is prevalent in 13 European countries (excluding Italy, where the disease has a different origin, and the Czech Republic, which has already eradicated the disease) and poses a serious threat to the pig industry of the remaining ones.

In 2018, ASF unexpectedly emerged in eastern Asia where dozens of outbreaks in pigs were reported in China and Mongolia, and in 2019 ASF struck also in Vietnam (35). Molecular characterisation of the intergenic region (IGR) between the I73R and I329L genes revealed a high level of DNA sequence similarity between recent Chinese and Eastern European (IGR II) ASFV isolates, but not between Chinese and Siberian ones (of the Irkutsk 2017 strain) (IGR I) (19, 22, 25). The exact origin of the disease in eastern Asia still remains unknown and needs further investigation; nevertheless a recent phylogenetic analysis indicated that ASFV in China might have had at least two independent introductions due to some level of divergence in nucleotide sequences obtained from cases which occurred far from each other (49). Most of the outbreaks which recently emerged in China were separated by thousands of kilometres, suggesting that the spread of the disease might be associated with contaminated feed. This hypothesis seems to be probable, in particular having regard to the fact that ASFV DNA has been detected in pig feed and feed ingredients like dried pig blood (24, 44, 49). In Europe, besides on backyard farms, the disease has been reported on numerous high-biosecurity operations (12). In Romania, ASFV was introduced into a high biosecurity breeding farm containing up to 140,000 pigs; however, the exact source of the disease has not been determined. Hypothetically it may have originated from contaminated water in the nearby Danube River (3).

Several experimental studies have demonstrated that transmission *via* contaminated feed is possible; nonetheless the knowledge is relatively poor concerning fomite-to-pig transmission (6, 10, 20, 32). In 1969 Colgrove *et al.* (6) effectively infected pigs orally through solid feed contaminated with minced tissues from sick animals. Shortly thereafter it was proved that when consumed by a susceptible pig, ASFV contained in milk led to infection, but it required a relatively high virus load of $10^{5.4}$ HAD_{50}/mL (20). An *in vitro* study published in 2018 demonstrated that artificially ASFV-contaminated feed ingredients had viable virus pathogens for at least 30 days (10). The most recent study conducted by Niederwerder *et al.* (32) determined the minimum infectious dose of ASFV through natural oral exposure *via* drinking and feeding behaviours. The investigation showed that as little as 10^0 $TCID_{50}$ contained in liquid is required to develop successful infection, whereas a dose of 10^4 $TCID_{50}$ is needed in solid, plant-based feed (32). Moreover, the study demonstrated that a liquid diet has a much higher infection probability compared with dried feed. The highest dose tested in liquid (10^4) led to infection of 100% of experimental pigs, while neither of the investigated solid feed doses showed such a high rate of infection (32). Furthermore, the study published by Sindryakova *et al.* (46) in 2016 showed that feed and water contaminated by infectious blood stored at 4°C preserved ASFV viability for 30 and at least 60 days (the duration of the whole study), respectively. On the other hand, storage at room temperature resulted in far shorter ASFV survival: in feed to 1 day and in water to 50 days. Therefore, contaminated feed and water stored at 4°C might pose a risk of infection over at least 30-day and 60-day periods, respectively (46).

Accordingly to these experimental data and recent epidemiological findings in Europe and Asia, long-distance (transboundary and transcontinental) movement of ASFV with contaminated feed and feed ingredients should be considered a possible mode of virus spread, especially within ASF-free areas.

ASFV survival in raw meat and offal

Historically, ASFV introduction into distant disease-free territories has been attributed to the consumption of contaminated pork or pork products (31, 45). Moreover, although prohibited in the EU and in contravention of biosecurity measures, swill feeding is still a common practice all over the world, especially in free-ranging and backyard farms (1). Therefore, contaminated pork presents a possible mode of transmission for ASFV. Heated, cooked, and canned

meat products are generally considered safe as regards any viable pathogen presence, which has been experimentally demonstrated (5, 42, 46). Several experiments provided data concerning ASFV stability in raw and processed meat and other pork products (13, 26). Frozen raw meat and organs provide ASFV viability for periods lasting from 103 to 118 days, but according to Adkin *et al.* (13) ASFV may remain infectious for even up to 1,000 days. In meat stored at 4–8°C, a viable virus could be detected over 84- to 155-day periods (13). Infected spleen samples stored in a refrigerator remained infectious for 204 days, but when buried in soil in June at 8 cm depth it remained so for 280 days. Bone marrow (in boned meat) remained infectious for 180–188 days, skin and fat for 300 days, and offal for 105 days, however the temperature at which these samples were stored was not stated despite its being a key factor for virus survivability (13).

ASFV survival in dry-cured pork

The matter of ASFV survival in products which cannot be heat-treated but are preserved through salting and drying is more complicated than in raw pork (12). The studies regarding ASFV survival in dry-cured processed meat are limited to ham, Spanish and Italian shoulder, loin, smoked pepperoni and salami, pork belly, and corned meat (26–28, 40, 46). Salami and pepperoni might remain infectious up to 30 days (26). Pork belly and loin were demonstrated to still contain viable ASFV after 60 and 83 days, which is longer than the duration of their commercial curing processes (14–21 and 60 days, respectively) but still within the shelf-life of the products. These pork products pose a low potential short-term risk if in swill fed to pigs (40). Corned meat stored at 4–6°C remained infectious for at least 60 days (the study duration), nevertheless the time reduced to 16 days as the temperature increased to room temperature (42). Ripening hams, like Iberian loin (112 days), shoulders (140 days), and Serrano and Parma (respectively 180 and 300–399 days) hams might remain infectious relatively long, but still cease to be within the duration of the curing process, which lasts much longer (13, 27, 28). Therefore the curing time is sufficient to inactivate ASFV and these products should be considered safe.

Indirect contact – arthropods as mechanical vectors

ASFV is a tick-borne virus; therefore ticks as well as pigs may host the virus. Nevertheless, so far only soft ticks of *Ornithodoros* spp. have been found to facilitate virus replication, and they act as the main virus reservoirs in Africa where they participate in the so-called sylvatic ASFV transmission cycle between ticks and wild suids. The geographical distribution of these ticks is limited to Africa and southern Europe (Mediterranean countries). Other ticks belonging to the *Ixodes ricinus* and *Dermacentor reticulatus* species are particularly present in Central Europe and represent a major group of mammalian parasites within this climate zone (8, 16).

Nevertheless, as several cases of ASFV outbreaks have been reported on high-biosecurity farms in Eastern Europe and the Baltic States where the density of infected wild boars was high, the question arises whether arthropods may play a mechanical vector role between wild and domestic pigs. Hard ticks were investigated to determine their competence as an ASFV vector. ASFV was not detected either in the field-collected ticks or ticks fed infectious blood which transmitted the virus to susceptible animals in laboratory conditions (8). ASF virus does not replicate within the tick organisms; however, viral DNA could be detected from six to eight weeks after feeding with infected blood. Therefore, hard ticks may represent only a potential mechanical but not a biological vector in transmission between wild boars and pigs (8).

The stable fly, *Stomoxys calcitrans*, is able to mechanically transmit the virus for 24 h post contact with infected blood, but only by the ingestion route. Moreover, infectious virus survived in these flies for at least two days (29). Olesen *et al.* (36) demonstrated that viable ASFV is present in the bodies of flies fed infectious blood for up to 12 h and DNA could be detected there for three days post feeding. These results indicate that such flies might mechanically transmit the virus to susceptible hosts. The dose contained in only one fly, when ingested, was sufficient to develop clinical signs of the disease in a pig. Nevertheless, spatial separation is likely to constrain the stable fly vector, and transmission within a herd is considered to be more probable than transmission between farms. Therefore, occurrence of indirect transmission of the virus within distant farms *via* ASFV-contaminated flies remains virtually impossible (39, 36). Another study verified the hypothesis whether fly larvae growing in and feeding on carcasses of infected wild boars might be involved in disease spreading. However, despite ASFV DNA presence, it was demonstrated that virus replication within larvae is absent. It was stated that although feeding by wild boars on larvae-ridden carrion was highly improbable, it could not be ruled out, although the researchers contended that blowfly larvae do not play a significant role in ASFV spreading *via* mechanical transmission (15).

Adducing evidence from fieldwork, viral DNA was detected in Poland in stable flies collected from farms during disease outbreaks in pigs as well as in hard ticks (*Dermacentor reticulatus* and *Ixodes ricinus*) collected from the bodies of dead ASFV-positive wild boars (Woźniakowski 2019, unpublished data). This suggests that arthropods cannot be excluded as ASFV mechanical transmission factors; nevertheless this issue needs further investigation.

Conclusions and future research perspectives

Along with increasing globalisation, the introduction of human and animal diseases is going to pose a continuous threat to public and livestock health, trade of animals and their products, and food security. Worldwide, the pig-farming industry is constantly growing in reply to rising demand for pork meat. Nevertheless, this branch of the economy is particularly vulnerable to production decimation because of transmission of various transboundary infectious diseases, amongst which ASF is currently causing the greatest concern. During recent years, ASFV has been spreading towards new areas; however, the most dramatic turn occurred in 2018, when the disease emerged in China, the top pig-farming nation providing half of global pig production. Due to the lack of a safe effective vaccine and the common presence of infected wild boars in particular areas, the only method to control the disease is strict biosecurity measures allied to international cooperation on this matter. Knowledge and epidemiological understanding of how the virus may be introduced into susceptible populations of pigs is crucial to provide awareness to prevent the outbreaks and detect and control them immediately and appropriately when they do occur. Therefore, identification of potential sources and pathways of transmission in regards to ASFV is exceptionally important to prevent further disease spread.

ASFV stability in different environmental conditions was the subject of numerous investigations, but most of them were conducted in the previous century. The virus has been identified as extremely resistant to physical treatment such as high temperatures, putrefaction and desiccation, freezing/thawing, ultrasonic waves, or extreme pH values. Low temperatures, such as the −20°C usually required to preserve pork meat, facilitate virus survival for years. Raw meat and other pork products can provide long ASFV survivability, but the temperature conditions are the main factor directly influencing virus stability. Ripening hams and dry-cured meat products may contain viable virus, nevertheless it depends greatly on the preparation and conservation techniques, which differ widely between regions and countries.

In reference to the disease's transmission, it was proved that ASFV infectivity without a susceptible animal having direct contact with infected blood is rather moderate; nevertheless, transmission only *via* air contact is still possible. Moreover, excretions and secretions are also considered infectious and they may participate in disease spread. Indirect transmission of ASFV by contaminated feed products has been shown to be possible for at least 30 days. Moreover, it was proved that oral uptake of contaminated liquid will more likely lead to infection than uptake of contaminated solid feed. A 10^0 HAD_{50}/mL dose received with liquid is sufficient to induce clinical signs of the disease. Despite scrutiny of the preventive measures which were undertaken, the source of the disease's introduction onto farms has remained unknown in the majority of ASFV outbreaks recently reported in Central Europe. It is suggested that arthropods like flies or ticks may act as mechanical vectors. Laboratory investigations partially confirmed this hypothesis, but infection was possible only *via* ingestion of flies no longer than 24 h after the insects has been fed infected blood. Therefore, while involvement of such arthropods in disease spread seems to be strictly limited, it cannot be ruled out.

As regards ASFV survivability and its indirect transmission, there are still data that are missing, *e.g.* those to result from investigation of the mechanism explaining outbreaks on high-biosecurity farms located near areas where infected wild boars are present but direct contact between them and domestic pigs is not possible. Further studies concerning arthropods' competence to become a mechanical vector for ASFV spread are needed. ASFV susceptibility to various environmental conditions is well studied; nevertheless there is still a lack of knowledge regarding the possibility of virus transmission on the surfaces of certain fomites, *e.g.* fresh grass contaminated with viable ASFV originating from wild boars, which could also elucidate the source of disease at least on part of the farms of Central Europe.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The study was supported by the National Science Centre (grant number UMO-2016/21/D/NZ6/00974) and the KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal - Safe Food" under decision of the Ministry of Science and Higher Education number 05-1/KNOW2/2015.

Animal Rights Statement: Not applicable.

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