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Analysis of spread of endogenous retroviruses (*PERVs)* of subtypes A and C in genomes of pigs of Ukrainian breeds and their correlation with fat deposition in carcasses

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Abstract

The article presents the results of the analysis of the frequency of PERV retroviruses of subtypes A and C in populations of pigs of Ukrainian and foreign breeds.

Analysis of the genome of retroviruses (PERV) subtypes A and C in animals of the studied breeds revealed their different frequency. Animals without both subtypes of the virus were present in all study groups. The highest relative numbers were observed in the group of wild pigs (86%), the lowest – in the groups of Poltava meat and Pietren breeds.

The hypothesis of an increase in the level of fat deposition in pigs with retrovirus (PERV) during domestication of pigs was investigated. Integration of the virus caused a mutation in the genes responsible for adipopexis, which led to an increase in fat in the carcass and can be isolated by selection during breeding.

However, we did not find a clear correlation in pigs of different productivity between the level of fat deposition in pig carcasses and the presence of PERV-A and PERV-B in their genome of modern breeds of different productivity.

The distribution of PERV of two subtypes in pigs of Ukrainian breeds determined in the study provides information on the possibility and expediency of using each of them for xenotransplantation. On the other hand, this information can be the basis for the selection of founding breeds to create pig lines free from the endogenous retroviral genome.

Key words: porcine endogenous retrovirus *(PERVs)*, genome, pigs of Ukrainian breeds, fat in carcasses

Introduction

Xenotransplantation of organs from pigs to humans is associated with the potential risk of infection of the recipient by endogenous retroviruses (porcine endogenous retrovirus (*PERVs*), which are constituent parts of the genome of pigs (Kimsa et al., 2014). Three subtypes of *PERV* are known as *PERV-A, PERV-B* and *PERV-C*. Types A and B, in addition to pig cell lines, can infect some human cell lines in vitro, and *PERV-C* can only replicate in pig cells (Harrison et al., 2004). However, information has recently appeared regarding the formation of recombinant viruses *PERV-A/C* that are capable to infect human cells and demonstrate high-titled (Hering et al., 2009)

replication, which is evidence of infective competence of *PERV-A/C*. The International Association of Xenotransplantatologists has developed specific guidelines for use in pig cell transplantation in clinical trials: careful screening of the original pig herd for *PERV*, selection of animals with low expression of *PERV-A, PERV-B* and, most importantly, identifying pigs which are not carriers of *PERV* (Nikitin et al., 2008).

The analysis of *PERV* in various breeds of domestic pigs showed a high frequency of revealing *PERV* of types A and B in the genomes of the vast majority of animals tested, but it is quite common for pigs with negative *PERV-C* (Yudin et al., 2011). This is particularly true for pigs of the Aboriginal breeds and wild boars. According to the (Nikitin et al., 2010) hypothesis, *PERV* spreading in the domestic pig population arose from their domestication process, which was accompanied by an increase in fat deposition of animal carcasses. Molecular genetic testing, done to detect pigs with endogenous retrovirus of subtypes C and A, will allow the selection of donor animals with reduced infection capacity.

The aim of the work was to investigate the spread of endogenous retroviruses of A and B subtypes in the genomes of pigs, bred in Ukraine, and to establish possible correlation between the level of fat deposition in carcasses of pigs and the presence of *PERV-A* and *PERV-B* in their genome.

Trail site and research material

The entire research was carried out at the Genetics Laboratory of the Institute of Pig Breeding and Agroindustrial Production of NAAS of Ukraine.

Samples of venous blood and bristles from animals of the main livestock were selected for the research in the State Enterprise "Decabrist Experimental Farm" of the Institute of Pig Breeding and Agroindustrial Production of NAAS (SE EF "Decabrist" of the Institute of Pig Breeding and Agro-Industrial Production of NAAS, Poltava region). Pigs of the Myrgorod breed (M), n = 40, Poltava meat breed (PM), n = 10 animal units ("Derkul" stud-farm, Luhansk region and "Streletskiy" stud-farm), pigs of the Ukrainian spotted steppe (USS), n = 20 animal units (Askania-Nova, Kherson oblast); the Vietnamese potbellied breed (V), n = 10: big Large White (LW), n = 20 (SE EF "Gontarivka" of the Livestock Institute of NAAS, village Gontarivka, Vovcharsk district, Kharkiv region); The Ukrainian meat breed (UM), n = 22 animal units (DNA Bank of the Genetics Laboratory of the Pig Breeding Institute and Agroindustrial Production of NAAS); Wild Pigs (WP), n = 7 and Pietren (P), n = 20 (Hoenheimsk University Experimental Station, Germany) pigs of the Landras breed (L), n = 20 animal units (LLE «Bread», Lozovsk district, Kharkiv region).

The research to find an association between presence of DNA *PERV-C* and *PERV-A* in the genome of pigs and fat depth was carried out on animals of the Mirgorod breed, 40 animal units. The fat depth was determined to be between 6 and 7 vertebrae of a live-animal by means of a fat measuring instrument.

All research animals were pre-tested for g.1843C >T mutations in the gene of the ryanodine receptor 1 (RYR1), related to stress sensitivity of pigs and defects of meat (Cobanovic et al., 2019) and had a genotype g.1843CC, a variant of the mutant allele was absent.

Extraction of DNA from blood samples were done by means of Chelex-100 chelating resin (5% solution of Chelex-100) (Walsh P.S. et al., 1991).

Retroviruses *PERV* and *RYRI* were analysed using PCR-RFLP (Glazko V. I. et al., 2001). Locus specific amplification was carried out according to the following scheme: preparation of the reaction mixture (25 mcl) of the following composition: 2.5 mcl of the universal 10x PCR buffer, 1 mcl of the direct F-primer (5 mcM), 1 mcl of the reverse R (5 mcM), 0.1 mcl Taq – DNA-polymerase (*Thermus aquaticus*) ("thermoscientific", Lithuania), 19.4 mcl of the deionized water and 1 μl of DNA matrices according to the recommendations to *RYRI* (Fujii et al., 1991), *PERV-C* (Guo et al., 2014), *PERV-A* (Seong-Lan et al., 2012) and *α- Actin* (Cailu, 2005).

The reaction was carried out in thermocycler "Tertsyk 2" ("DNA-technology", Russia). 25 mcl of the mineral oil was put on the reaction mix-

ture. Amplification program: 95 °C – 2 min 35 cycles: 95 °C – 30 seconds, (burning primers) °C – 30 seconds, 72 °C – 3 min, 72 °C – 5 min.

The structure of the primers, the annealing temperature of the primers, the length of the amplicons and restriction fragments are shown in Table 1.

The amplified sequences of the *RYRI* gene were hydrolyzed according to a general scheme: the reaction mixture had a capacity of: 10 x restriction buffer (optimized for this enzyme) – 2.5 mcl, H_2O – 7.3 mcl, *HhaI* restriction endonuclease – 0.2 mcl and 15 mcl of PCR product. Reaction mixture was incubated in the thermostat at 37 °C for 3 hours (Fujii J. et al., 1991).

Electrophoresis of restriction products was carried out in 2% agarose gel in a tris-borate electrophoretic buffer.

The gels were colored with an ethidium bromide solution (0.5 μg/ml) for 10 minutes followed by repeated washing in distilled water. The DNA fragments were visualized in UV light.

The photo documentation was done on the digital camera Canon Power Shot IS - S3.

Population genetic characteristics were calculated with the help of Excel 2007 with the subsequent construction of graphs and tables.

Results and Discussion

The detection of retrovirus genomes in the genetic material of animal cells provides for PCR amplification of the DNA fragment of viruses. Moreover, the criterion of correct execution of PCR is, as a rule, amplification of fragment of one of genes of cell "internal structure" in the DNA sample of an animal. In our work it was a gene *α-Actin*, a fragment of which 516 bp in size, was found on the electropherogram in each of the samples studied and served as an internal positive control of amplification in the form of a duplex PCR, Figure 1 and 2.

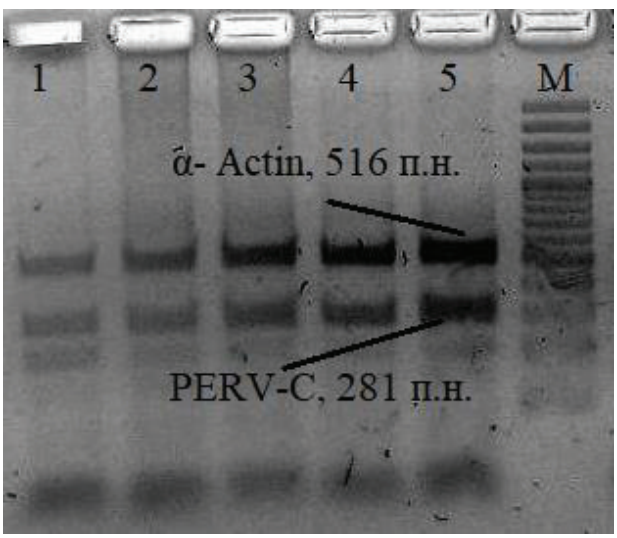

Fig. 1. Electrophoresis in 2% agarose gel of products multiplex *PERV-C – α-Actin* (LAPC). M is a molecular mass marker (50 bp DNA Ladder (from 50 to 500bp)), 1-5 are products multiplex *PERV-C – α-Actin* (LAPC). DNA are of pigs of the Myrgorod breed

Table 1. Structure of primers, temperature of primers, length of the amplicons and restriction fragments

Gene	Structure of primers	Amplicon / t °C of annealing	Restriction fragments
PERV-C	F: 5/ - CTGACCTGGATTAGAACTGG -3/ R: 5/ - ATGTTAGAGGATGGTCCTGG -3/	281 bps / 65 °C	-
PERV-A	F: 5/ - TCCGTGCTTACGGGTTTTAC -3/ R: 5/ - TTGCCAATCTTTCCATCTCC -3/	224 bps / 60 °C	-
α- Actin-	F: 5/- CGCCATGTGTGACGAAGACGAGACC -3/ R: 5/- CACGTACATGGCGGGCACGTTGAAG -3/	516 bps / 62.5 °C	-
RYRI	F: 5/ - GTGCTGGATGTCCTGTGTTCCCT -3/ R: 5/ - CTGGTGACATAGTTGATGAGGTTTG -3/	137 bps / 68.5 °C	TT-137, TC-137, 4, 53, CC-84, 53

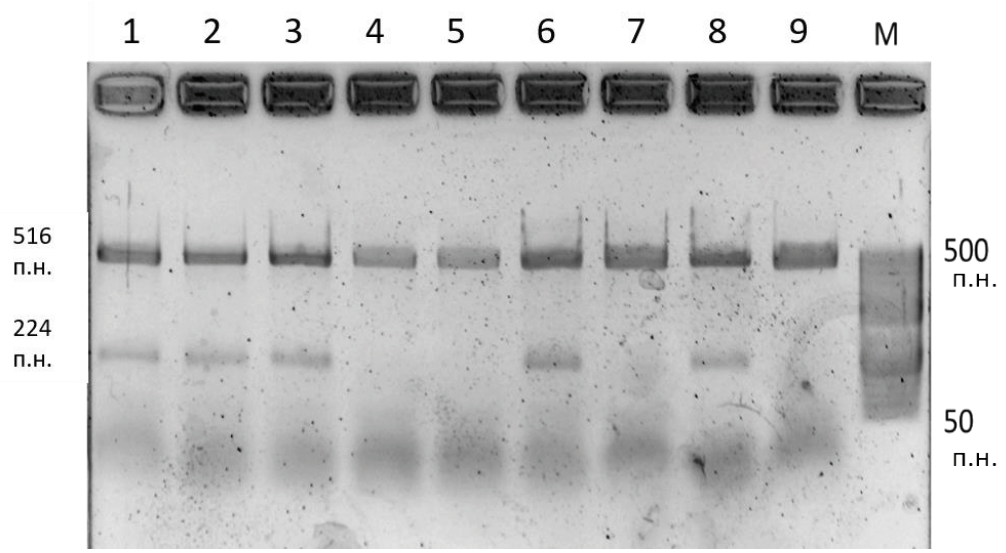

Fig. 2. Electrophoresis in 2% agarose gel of products multiplex *PERV-C – α-Actin* (LAPC). M is a molecular mass marker (50 bp DNA Ladder (from 50 to 500bp)), 1-3, 6, 8 are products multiplex *PERV-C – α-Actin* (LAPC). DNA are of pigs of the Myrgorod breed

The genomes of retroviruses *PERV-C and PERV-A* in pigs of different breeds and directions of productivity were analyzed.

The carriers of retrovirus *PERV-C* were not found in the sub-population of wild pigs and this fact is due to the data (Nikitin et al., 2010) about their absence among pigs of «wild type», (Fig. 3).

The lowest frequency of carriers of retrovirus *PERV-C* genomes were characterized by sub-populations of pigs of the Ukrainian meat breed, Myrgorod and Pietren breeds. At the same time, *PERV-C* virus was found in all tested pigs of the Vietnamese pot-bellied breed. A high prevalence of this retrovirus subtype was observed in the Poltava meat breed, Ukrainian spotted steppe, Landras and Large White pigs, *PERV-C* genome was present in half of these animals. Our data, in general, confirm the results obtained by other

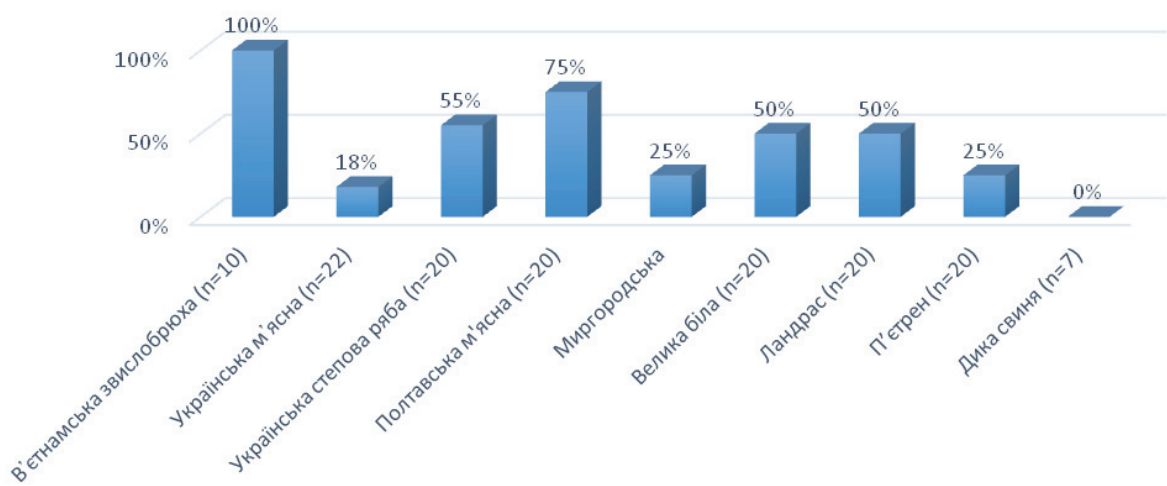

Fig. 3. Distribution of retrovirus *PERV-C* in samples of pigs of domestic and foreign breeds

authors on the *PERV-C* spreading in the population of fatty pigs (Nikitin et al., 2010) – in the Vietnamese pot-bellied and Ukrainian spotted steppe breeds, which belong to the breeds of the identified line of productivity, the frequency was 100% and 55% respectively. It should be noted, however, that the difference between the frequencies of *PERV-C* found in the tested subpopulations was not always statistically confirmed, figure 3.

The analysis of viruses *PERV-A* genome in the subpopulations of pigs of the tested breeds also found different frequencies of these viruses. (Fig. 4). For example, *PERV-A* virus had a relatively low frequency in wild boar, pigs of the Landras, and Ukrainian spotted steppe breeds had a relatively low frequency while all pigs of the Vietnamese pot-bellied breed were the carriers of the virus. Its proportion in Poltava meat breed reached 90%, somewhat less it was in sub-populations of the Myrgorod and large white breeds.

It is notable that wild pigs were the least frequent carriers of both subtypes of the virus. On the contrary, in the Vietnamese pot-bellied breed all animals carried both genomes. Viruses *PERV* of both subtypes were also found with high rates in genomes of animals with subpopulations of the Poltava meat breed.

In general, the analysis of the presence of *PERV* viruses of subtypes *C* and / or *A* in the genome of pigs led to this result. Animals free of both subtypes of the virus were in all of the groups studied (Table 2). The highest relative their number was observed in the group of wild pigs (86%), the lowest one – in the groups of Poltava meat and Pietren. This result is partly coherent with the statements that increased fat deposition in domestic pigs compared to wild ones or early domesticated forms is a genetic anomaly, caused by disruption of the gene structure of the quantitative features because of retrovirus *PERV* insertion. However, the breeding of pigs for the improvement of meat qualities, as shown by the results of the analysis of the Poltava meat breed and Pietren (ultra-meat breed), did not lead to a reduction in the frequency of genomes of retroviruses *PERV* of both subtypes in their populations (Nikitin et al., 2010). At the same time, in other meat breeds – Landras and Ukrainian meat the proportion of animals free of the genomes of both viruses is significantly higher, reaching 23% and 35%, respectively. The proportion of such pigs in the Ukrainian spotted steppe breed with enough fat deposition is at the same level (30%). It can be assumed that in the breeding process aimed at reducing the fat deposition of the carcasses and increasing the yield of the meat, other loci of the genome are attracted, and not only those where the integration of viral DNA takes place. It is known that the sign of fat deposition in an animal is polygamous and depends on the action of many genes.

Table 2. Pigs with retrovirus *PERV* of A and C types

Breed of pigs	With two types		Absolutely free	
	proportion of the general number	%	proportion of the general number	%
Myrgorod breed (M) / Миргородська	6 / 40	15	9/40	23
Vietnamese pot-bellied (V) / В'єтнамська звислобрюха	10/10	100	0/10	-
(Ukrainian meat) / Українська м'ясна	3/22	14	5/22	23
Ukrainian spotted steppe (USS) / Українська степова ряба	2/20	10	6/20	30
Poltava meat (PM) / Полтавська м'ясна	15/20	75	1/20	5
Large white (LW) / Велика біла	9/20	45	6/20	30
Landras (L) / Ландрас	2/20	10	7/20	35
Pietren (P) / П'єтрен	3/20	15	1/20	5
Wild pig (WP) / Дика свиня	0/7	-	6/7	86

In general, our data fit in the context of the hypothesis of increasing the frequency of pigs, in the genome of which retrovirus *PERV* was present, during their domestication. This statement was done due to the analysis of the genomes of pigs with subpopulations of wild breed and numerous modern breeds. However, the date obtained in the research, were not in the favor of the correlation between the frequency of *PERV* spreading in the breeds and the direction of their productivity, and hence the fat deposition of the carcase. However, this does not preclude its existence at the individual level within the separate subpopulation. The latter can be verified in an associative analysis carried out in a group of animals examined by one of the main fat deposition indicators, the depth of the dorsal fat.

Statistically confirmed differences between subpopulations $p < 0.001$ – V/UM, V/P, V/M; $p < 0.01$ – V/USS, V/LW, V/L, V/WP, UM/PM, PM/M, PM/P, PM/WP; $p < 0.05$ – V/PM, UM/USS, USS/WP, M/WP, LW/WP, L/WP.

Statistically confirmed differences between subpopulations $p < 0.001$ – V/USS, V/L, USS/PM, P/L; $p < 0.01$ V/L, V/WP, UM/L, USS/M, USS/P, L/P, PM/WP; $p < 0.05$ – V/UM, V/LW, V/P, UM/WP, PM/M, PM/LW, LW/L, P/WP.

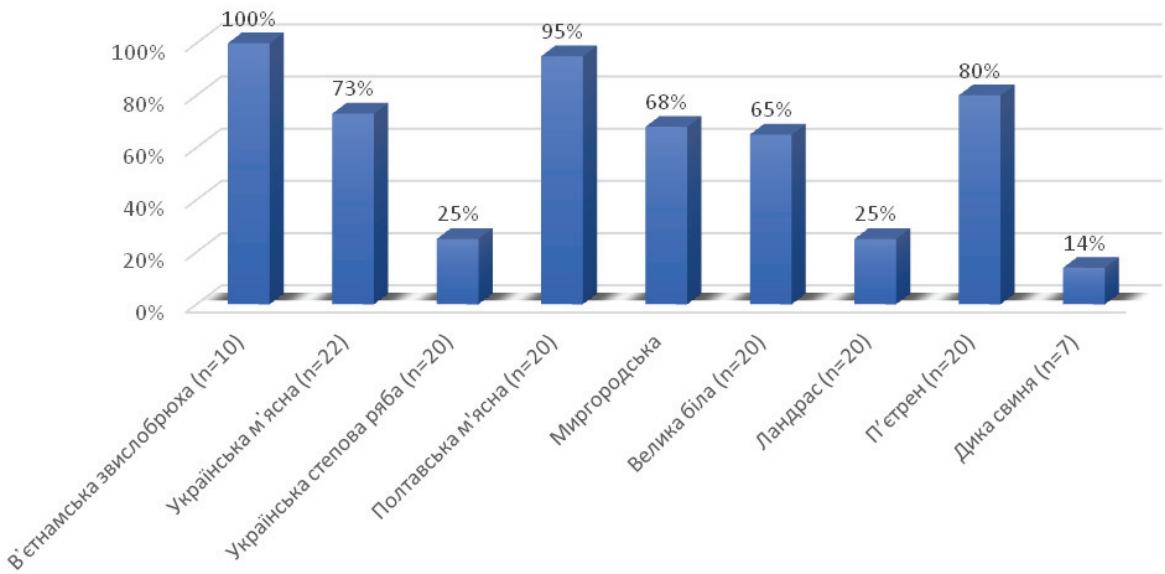

Fig. 4. Spreading of retrovirus *PERV-A* in samples of pigs of domestic and foreign breeds

Table 3.

presence of viruses in the animal genome	n (number of a group)	Fat depth, mm	p
PERV-C$^+$	10 (I)	33.00 ± 1.96	0.553
PERV-C$^-$	30(II)	31.97 ± 2.57	
PERV-A$^+$	27(III)	31.33 ± 0.78	0.083
PERV-A$^-$	13(IV)	34.08 ± 1,51	
PERV-A$^+$ *чи C*$^+$	31(V)	31.97 ± 1.72	0.527
PERV-A$^-$*/C*$^-$	9(VI)	33.11 ± 1.59	
PERV-A$^+$ + *C*$^+$	6(VII)	30.83 ± 1.23	0.510
PERV-A$^-$*/C*$^-$	8(VIII)	32.50 ± 1.28	

The association analysis was carried out on a group of pigs of the Myrgorod breed. Its aim was to establish a correlation between the presence of DNA of retrovirus *PERV* in the animal genome and the depth of the dorsal fat. The results of the analysis are presented in Table 3.

It is worth noticing that among animals with *PERV*-C the fat depth ranged from 26 to 45 mm in the Rusalka family pigs. Among the pigs with absence of *PERV-C*, the fat depth ranged from 25 mm in the animal № 1010 from the Zorka family to 40 mm in the pig № 154 from the Laskava family. Thus, there are considerable limits of the variation of the studied feature even within one family, which indicates the formal referring of animals to a certain family and the absence of directed breeding within genealogical families and their genetic heterogeneity.

The analysis of the association of the fat depth in the Myrgorod breed with the *PERV-C, PERV-A* presence in their genome did not reveal the statistically confirmed results. There is only a definite trend towards such an association to *PERV-A*. In this case, pigs that did not have *PERV-A* in their genome had larger fat depth. It is possible that if the number of experimental animals increases, this association will be statistically confirmed. But this is in contradiction with the assumption of a positive correlation between the presence of a virus in the genome and the level of fat deposition of the carcasses.

The results of our study confirm the hypothesis of increased domestication of pigs in the genome of the retrovirus PERV. Other authors report that the microevolution processes associated with the PERV carrier frequency had two main dimensions, or vectors: the fat deposition variation vector and the minus selection vector (definition of commercial characteristics). Thus, PERV can cause changes in the physiology of pigs. The authors suggest that in the first case, *PERVs* label loci that affect the rate of fat deposition, and in the second case, they are associated with loci that control some commercial and adaptive traits (Nikitin et al., 2010).

The integration of PERV caused a mutation in QTL fat deposition, which led to an increase in carcass fat, and this feature could be picked up by selection in the breeding process. However, there is no clear correlation between the spread of the virus in modern breeds of different productivity. There is also no correlation between one of the main indicators of carcass fat deposition and the presence of PERV DNA in the animal's genome.

Conclusions

The prevalence of PERV of two subtypes in pigs bred in Ukraine determined in the study provides information on the possibility and expediency of using each of them for xenotransplantation. However, we did not find a clear correlation in pigs of different productivity between the level of fat deposition in pig carcasses and the presence of *PERV-A* and *PERV-B* in their genome of modern breeds of different productivity.

On the other hand, this information can be the basis for the selection of founding breeds to create pig lines free from the endogenous retroviral genome. In addition, the absence or low prevalence of PERV in wild boar and aboriginal breeds is an additional important argument for their conservation and use.

Compliance with Ethical Standards

All international, national and / or institutional principles of animal care and use were observed.

Conflict of interests

The authors state that there is no conflict of interest.

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