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Article

Porcine Lymphotropic Herpesvirus (PLHV) Was Not Transmitted During Transplantation of Genetically Modified Pig Hearts into Baboons

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Abstract

Porcine lymphotropic herpesviruses -1, -2, and -3 (PLHV-1, PLHV-2, and PLHV-3) are gammaherpesviruses that are widespread in pigs. These viruses are closely related to the human pathogens Epstein-Barr virus (EBV) and Kaposi sarcoma-associated herpesvirus (KSHV), both of which are known to cause severe diseases in humans. To date, however, no definitive association has been established between PLHVs and any disease in pigs. With the growing interest in xenotransplantation as a means to address the shortage of human organs for transplantation, the safety of using pig-derived cells, tissues, and organs is under intense investigation. In preclinical trials involving pig-to-nonhuman primate xenotransplantation, another porcine herpesvirus—porcine cytomegalovirus, a porcine roseolovirus (PCMV/PRV)—was shown to be transmissible and significantly reduced the survival time of the xenotransplants. In the present study, we examined donor pigs and their respective baboon recipients, all of which were part of preclinical pig heart xenotransplantation studies, for the presence of PLHV. PLHV-1, PLHV-2 and PLHV-3 were detected in nearly all donor pigs; however, no evidence of PLHV transmission to the baboon recipients was observed.

Keywords: porcine lymphotropic herpesviruses (PLHV); xenotransplantation; pig; baboon; virus safety; PCR; porcine cytomegalovirus/porcine roseolovirus (PCMV/PRV)

1. Introduction

Xenotransplantation using cells or organs from genetically modified pigs is progressing toward clinical application. Following numerous successful preclinical trials involving the transplantation of pig organs into non-human primates, first transplantations of pig hearts and kidneys into human patients have been performed [1]. In addition to challenges such as immune rejection and physiological incompatibility, the risk of transmitting porcine microorganisms remains a significant hurdle [2]. The transmission of porcine cytomegalovirus, a porcine roseolovirus (PCMV/PRV), to the first human recipient of a pig heart [3] highlighted the need for highly sensitive methods to detect porcine viruses in donor pigs and effective strategies to prevent such transmissions.

Porcine lymphotropic herpesviruses -1, -2, and -3 (PLHV-1, PLHV-2, and PLHV-3), also called suid gammaherpesvirus 3, 4, and 5 (SuHV-3, SuHV-4, and SuHV-5) are viral species of Macavirus genus, Gammaherpesvirinae subfamily within the Herpesviridae family. SuHV-3/PLHV-1, SuHV-4/PLHV-2 were first reported by Ehlers et al. in 1999 [4]. Later, another suid gammaherpesvirus was found with considerable genomic differences relative to PLHV-1 and PLHV-2, and named suid gammaherpesvirus 5 (porcine lymphotropic herpesvirus 3—PLHV-3) [5]. For the sake of simplicity,

we will stick to the designations PLHV-1, PLHV-2, and PLHV-3. For the sake of completeness: suid herpesvirus 1 (SuHV-1), also known as pseudorabies virus, causes Aujeszky’s disease in both wild and domestic swine. SuHV-1 has a broad host range and causes disease in distantly related mammals such as sheep, dogs, cattle, mink, and puma [6,7]. Pigs are considered to be the only the natural host and reservoir for SuHV-1 in nature, all other infection were the result of trans-species transmissions. SuHV-2 is the above mentioned PCMV/PRV [8].

Under natural conditions there were no reports that PLHV-1, PLHV-2, and PLHV-3 represent primary pathogens of pigs, or co-factors in other viral infections [9]. Despite their high prevalence, PLHVs’ relevance for the pig industry appears low. Their transmission occurs mainly horizontally, but vertical transmission is possible [10,11].

PLHV-1 has been associated with a form of post-transplantation lymphoproliferative disorder (PTLD) in immunosuppressed pigs undergoing experimental allogeneic bone marrow transplantations [12–14]. The clinical symptoms of experimental porcine PTLD, such as fever, lethargy, anorexia, high white blood cell count and palpable lymph nodes, are similar to those of human PTLD, a serious complication of solid human organ and allogeneic bone marrow transplantation linked to Epstein-Barr virus (EBV) (human herpesvirus -4, HHV-4) [14].

PCR-based methods using primers and probes which were located in the DNA polymerase gene and in the gene encoding the glycoprotein B and immunological methods (Western blot analysis, ELISA) to detect PLHV based on recombinant fragments of the gB protein were established and the viruses were found in numerous pig breeds [15–30] (Table 1). When the ELISA was performed seropositivity ranged from 38% (piglets), to 90% (gilts) and 100% (breeding sows and pigs for slaughter) [30]. The presence of high antibody titers in newborn piglets, which decline over time, indicates the transfer of virus-specific antibodies via colostrum from infected mother sows [30]. This pattern has also been observed in cases involving PCMV/PRV [31].

Table 1. Prevalence of PLHV-1, -2, and -3 in pigs.

Pig breed	Detection of virus using real-time PCR			Western blot analysis	Reference
	PLHV-1	PLHV-2	PLHV-3		
	Number of positive animals / number of tested animals				
Göttingen minipigs 1	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (0%)	Morozov et al., 2016 [15]
Göttingen minipigs 2	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	Plotzki et al., 2016 [16]
Göttingen minipigs 3	2/11 (18%)	2/11 (18%)	2/11 (18%)	n.t.	Krüger et al., 2019 [17]
Göttingen minipigs with DPS ^a	0/7 (0%)	0/7 (0%)	1/4 (25%)	n.t.	Jhelum et al., 2023 [18]
Aachen minipigs	0/18 (0%)	5/18 (28%)	2/18 (16%)	0/10 (0%)	Plotzki et al., 2016 [19]
Mini LEWE	0/10 (0%)	0/10 (0%)	0/10 (0%)	n.t.	Halecker et al., 2021 [20]
Indigenous Greek black pigs	12/21 (57%)	15/21 (71%)	21/21 (100%)	n.t.	Jhelum et al., 2024 [21]
Greek pigs with erythema multiforme	5/5 (100%)	1/5 (20%)	4/5 (80%)	n.t.	Halecker et al., 2022 [22]
German slaughterhouse pigs 1	2/36 (6%)	0/36 (0%)	10/36 (28%)	7/36 (19%)	Plotzki et al., 2016 [16]
German slaughterhouse pigs 2	10/10 (100%)	0/10 (0%)	10/10 (100%)	n.t.	Jhelum et al., 2024 [23]
Large White x Landrace	12/22 (55%)	2/22 (9%)	n.t.	n.t.	Mueller et al., 2004 [24]

Farm animals, Poland	11/45 (24%)	n.t.	n.t.	n.t.	Cybulski et al. 2025 [25]
Farm animals, Brazil	12/19 (63%)	n.t.	n.t.	n.t.	Dall Agnol et al., 2020 [26]
Italien pigs	50/168 (30%)	19/168 (11%)	7/168 (7%)	n.t.	Franzo et al., 2021 [27]
German pigs, lung spleen	21/27 (78%) 20/34 (59%)	11/27 (41%) 9/34 (26%)	16/27 (59%) 21/34 (62%)	n.t.	Chmielewicz et al., 2003 [5]
Italien pigs, blood	16/20 (80%)	4/29 (20%)	13/20 (65%)		
Farm pigs, Ireland	25/32 (78%)	7/32 (22%)	37/65 (60%)	n.t.	McMahon et al., 2006 [28]
Wild boars, Brazil	48/50 (96%)	28/50 (56%)	22/50 (44%)	n.t.	Porto et al., 2021 [29]

^a dippity pig syndrome.

PLHVs are closely related to alcelaphine herpesvirus type 1, AIHV-1, and ovine herpesvirus type 2, OvHV-2, two gammaherpesviruses, which are apathogenic in their natural hosts but cause serious lymphoproliferative diseases in other species [32]. Accordingly, PLHV may be apathogenic in pigs but pathogenic in other species, including humans. Here, we demonstrate that although PLHV-1, PLHV-2 and PLHV-3 were present in the donor pigs, they were not transmitted to the baboon recipient of the pig heart.

2. Results

2.1. Presence of PLVH in Donor Pigs for Heart Xenotransplantation

Eleven donor pigs and the corresponding baboon recipients were tested for PLHV-1, PLHV-2 and PLHV-3. The immunosuppressive regimen was based on a costimulation blockade targeting the CD40/CD40L pathway, as previously described in detail [33]. For induction therapy, animals additionally received a B cell-depleting monoclonal antibody and a T cell-directed immunomodulatory agent. Maintenance therapy consisted of continued co-stimulation blockade in combination with an antiproliferative compound and corticosteroids. PCMV/PRV from the donor pig was transmitted to some of the baboons. The presence of PCMV/PRV (in some pigs and baboons at very high virus loads) was the reason of a short survival time of these transplants [34] (Table 2). Whereas only three donor pigs were PCMV/PRV positive, all pigs were positive tested for PLHV-1 or -2, but none of them were positive for PLHV-3. To test for PLHV-1, PLHV-2 and PLHV-3 two conventional PCR methods, one detecting PLHV-1 and PHLV-2 and the other PHLV-3 were performed. Although PLHV-1/2 were found in all donor pigs, no virus was found in the recipient baboons [34] (Table 2).

Table 2. Testing donor pigs and recipient baboons for PLHV-1, -2, -3 and PCMV/PRV [34].

Animal	ID number	Transplant survival days	PCMV/PRV real-time PCR	PLHV-1,-2 PCR	PLHV-3 PCR
Pig 5528			-	++	-
Baboon J	17186	90	-	-	-
Pig 5415			-	+++	-
Baboon K	17187	50	-	-	-
Pig 5420			-	+++	-
Baboon L	17290	90	-	-	-
Pig 5623			++	+	-
Baboon M	17188	10	++	-	-
Pig 5803			-	+++	-
Baboon O	17493	195	-	-	-

Pig 5807			-	+++	-
Baboon N	17491	182	-	-	-
Pig 6249			++	+++	-
Baboon P	17494	15	+++	-	-
Pig 6253			+	++	-
Baboon Q	17492	27	++	-	-

2.2. Real-Time PCR-Based Detection of PLHV in Donor Pigs

To investigate the situation in greater detail, real-time PCR assays were developed and employed to detect PLHV-1, PLHV-2, and PLHV-3. Samples were collected from the liver and spleen of three new donor pigs (7649, 7654, and 7687), as well as from tissues of the corresponding baboon recipients (A, B and C). These animals had survival times over 100 days, with exception of baboon B with only one day. The tissues analyzed included the liver and spleen of the baboons—except for baboon B, for which no tissues were available—and the left and right ventricles of the explanted pig hearts retrieved post-mortem (Table 3). Importantly, one donor pig, animal 7649, tested negative for all three PLHV viruses. This pig was the only one among the 11 animals tested to be completely free of PLHV. In contrast, pig 7654 was positive for PLHV-2 in both the liver and spleen. Pig 7687 was found to be co-infected with PLHV-1 and PLHV-3, with both viruses present in both organs.

None of the PLHV viruses were detected in any of the transplanted baboons or in the explanted pig hearts. This indicates that PLHV-1, PLHV-2, and PLHV-3 were not transmitted to the baboons. Although the presence of PLHV might be expected in the transplanted pig hearts—given the viral detection in the donor liver and spleen—the viral load was probably below the detection threshold of our assay.

The detection limit of the real-time PCR method used was 1 copy per 100 ng of total DNA for all three PLHV targets [35]. PCMV/PRV was not detected in any of the donor pigs, the transplanted baboons, or the explanted pig hearts.

To assess the presence of pig cells in baboon tissues (microchimerism), we performed a Short Interspersed Nuclear Elements (SINE) PCR and detected pig-specific sequences in all baboon tissue samples (Table 3). This indicates that pig cells were present in all analyzed baboon organs. These findings are consistent with previous results obtained using the same SINE PCR assay [36], as well as in a case of transplantation of a PCMV/PRV-positive heart, with immunohistochemical analyses employing a PCMV/PRV-specific antibody [42]. The high load of SINE sequences in the right and left ventricles is not surprising, as the tissue is of pig origin. What is unexpected, however, is the low load of SINE sequences in the ventricles explanted from baboon A—levels even lower than those found in the baboon’s liver and spleen. This discrepancy suggests that incorrect material may have been submitted for testing.

However, if any of the pig cells present in the baboon tissues were positive for PLHV, their number was likely below the detection threshold of our assay, and thus the viruses could not be detected.

The results clearly indicate that all three PLHV, PHLV-1, PHLV-2 and PHLV-3, were not replicating in the transplanted pig heart and were not infecting baboon cells.

Table 3. Screening of donor pigs and transplanted baboons for PLHV-1, PLHV-2 and PLHV-3 using real-time PCRs.

Animal	Organ tested	PCMV/PRV	PLHV-1		PLHV-2		PLHV-3		SINE	
		V real-time PCR ct	real-time PCR ct	copy number	real-time PCR ct	copy number	real-time PCR ct	copy number	real-time PCR ct	copy number
Pig 7649	Spleen	n.d.	n.d.		n.d.		n.d.		n.t.	
	Liver	n.d.	n.d.		n.d.		n.d.		n.t.	
Baboon A	Spleen	n.d.	n.d.		n.d.		n.d.		16.86	10 ⁶
	Liver	n.d.	n.d.		n.d.		n.d.		23.12	10 ⁴
	RV ^a	n.d.	n.d.		n.d.		n.d.		28.48	10 ³
	LV ^b	n.d.	n.d.		n.d.		n.d.		28.00	10 ³
Pig 7654	Spleen	n.d.	n.d.		26.38	10 ³	n.d.		n.t.	
	Liver	n.d.	n.d.		26.94	10 ³	n.d.		n.t.	
Baboon B	RV	n.d.	n.d.		n.d.		n.d.		7.05	10 ¹⁰
	LV	n.d.	n.d.		n.d.		n.d.		7.06	10 ¹⁰
Pig 7687	Spleen	n.d.	27.51	10 ³	n.d.		35.13	10 ¹	n.t.	
	Liver	n.d.	28.51	10 ²	n.d.		35.17	10 ¹	n.t.	
Baboon C	Spleen	n.d.	n.d.		n.d.		n.d.		19.55	10 ⁶
	Liver	n.d.	n.d.		n.d.		n.d.		20.64	10 ⁵
	RV	n.d.	n.d.		n.d.		n.d.		7.2	10 ¹⁰
	LV	n.d.	n.d.		n.d.		n.d.		6.54	10 ¹⁰

^a, right ventricle of the explanted pig heart; ^b, left ventricle of the explanted pig heart; n.d., not detected by PCR; n.t., not tested.

3. Discussion

We demonstrate that although PLHV-1, PLHV-2 and PLHV-3 were present in the donor pigs, these viruses were not transmitted to the non-human primate recipients of the pig hearts. This contrasts sharply with the case of PCMV/PRV, which was consistently transmitted to recipients regardless of the species (baboon or cynomolgus monkey), the transplanted organ (kidney or heart), or the transplantation method (heterotopic or orthotopic) [34,38–40]. In every instance, PCMV/PRV transmission significantly reduced graft survival. Notably, in orthotopic heart transplantations, survival time dropped from 195 days to fewer than 20 days [34]. PCMV/PRV was identified as the cause of multiorgan failure, altered cytokine profiles, and coagulation abnormalities [34]. The virus was also transmitted to the first human patient who received a pig heart transplant in Baltimore, where it likely contributed to the patient’s death [3]. Importantly, PCMV/PRV transmission occurred even when the virus was undetectable in the donor pig by PCR due to its latent state—this was observed both in a baboon case [41] and in the first human xenotransplantation patient [3]. Although PCMV/PRV does not infect human or non-human primate cells in vitro and does not affect healthy humans in contact with pigs or consuming undercooked pork, it causes disease specifically in the context of xenotransplantation [42].

When Mueller et al. [24] conducted transplantations of thymokidneys, kidneys, and hearts from Large White/Landrace cross-breed pigs transgenic for human decay-accelerating factor, as well as from Massachusetts General Hospital (MGH) miniature swine, they found that 78% of the donor pigs were positive for PLHV-1 and 25% for PLHV-2. All donor animals tested positive for PCMV/PRV. In the recipient baboons, the authors observed strong replication of PCMV/PRV in the explanted pig organs. However, no increase in PLHV-1 viral load was detected in xenografts that were PLHV-1–positive. PLHV was primarily localized to lymphoid tissues. Despite immunosuppression and the presence of xenogeneic immune responses, neither PLHV-1–positive xenotransplants nor those negative at baseline showed signs of PLHV-1 activation. The authors suggested that the lack of PLHV replication may be due to the absence of a sufficient number of target cells capable of supporting PLHV-1 replication within solid-organ xenografts.

When Issa et al. [43] transplanted organs from pigs transgenic for human decay-accelerating factor or from alpha-1,3-galactosyltransferase gene-knockout miniature pigs into baboons, PLHV-1 DNA was detected in peripheral blood mononuclear cells (PBMCs) of 6 out of 10 transplanted baboons. However, there was no evidence of productive PLHV-1 infection, as viral loads in the serum did not increase over time, even with prolonged transplant survival. The authors concluded that the presence of viral DNA likely resulted from persistent pig cell microchimerism rather than active viral replication. While PCMV/PRV can be effectively eliminated from source animals through early weaning of piglets [44,45], this strategy failed to exclude PLHV-1 [44]. This difference underscores the distinct mechanisms of infection and latency between PCMV/PRV and PLHV-1, two unrelated herpesviruses.

In our first experiment, summarized in Table 2, PLHV-3 was not detected in any of the donor pigs, and no distinction was made between PLHV-1 and PLHV-2. In contrast, in the second experiment (Table 3), all three PLHV types were specifically differentiated and detected. Notably, this experiment marked the first time a PLHV-free animal was identified in our facility, as well as the first documented case of co-infection with PLHV-1 and PLHV-3 (Table 3).

Co-infections with two or even all three PLHV types are not uncommon. In a screening of 21 indigenous Greek black pigs, six animals were infected with all three viruses, and the remaining animals were infected with two [21]. Among them, six pigs carried PLHV-1 and PLHV-3, while nine were infected with PLHV-2 and PLHV-3 [21]. In contrast, all 10 German slaughterhouse pigs tested were positive for PLHV-1 and PLHV-3, but not for PLHV-2 [23]—the same combination found in animal 7687.

The absence of PLHV in the explanted pig hearts after prolonged survival times of 166 and 196 days (Table 3) indicates that the virus present in the donor pig was not replicating in the xenotransplant, even under conditions of immunosuppression and extended survival.

Regarding the genetic relatedness of the three PLHV viruses, PLHV-3 is significantly more distantly related to PLHV-1 and PLHV-2 than PLHV-1 and PLHV-2 are to each other [5].

As mentioned in the introduction, the gene content of porcine lymphotropic herpesviruses (PLHVs) is highly similar to that of alcelaphine herpesvirus 1 (AlHV-1), associated with wildebeests, and ovine herpesvirus 2 (OvHV-2), associated with sheep [5,31,46]. These viruses cause malignant catarrhal fever (MCF), a lymphoproliferative and inflammatory disease that is typically fatal in susceptible species. PLHVs are also related to bovine lymphotropic herpesvirus (BLHV), which is associated with bovine leukemia [47], and caprine herpesvirus 2 (CprHV-2), which has been linked to chronic disease in sika deer [48,49]. While BLHV is believed to contribute to the pathogenesis of bovine leukemia virus (BLV) infection [47], and CprHV-2 is implicated in chronic illness in cervids [50], AlHV-1 and OvHV-2 are well adapted to their natural hosts—wildebeests and sheep, respectively—and remain apathogenic in those species. However, when transmitted to non-natural hosts such as cattle or deer, they can cause MCF [32]. Therefore, the potential for PLHVs to cause disease in xenotransplantation recipients cannot be excluded.

PLHV-1, -2, -3 are also related to the human gammaherpesviruses Epstein-Barr-Virus (EBV or human herpesvirus 4, HHV-4) and the human Kaposi sarcoma-associated herpesvirus (KSHV, or human herpesvirus 8, HHV-8). Moreover, they are related to EBV and KSHV in terms of B cell tropism, and sequence similarity of conserved genes [4,5,51]. PLHV-3 was found in the permanent porcine B cell line L23 and two other lymphoma cell lines, 1B2 and 2F8 [5,52]. EBV is associated with a post-transplantation lymphoproliferative disorder (PTLD) occurring in the setting of iatrogenic immune suppression following hematopoietic or solid organ transplant [53,54]. A similar disorder was observed in PLHV-1 positive minipigs after experimental allogeneic bone marrow transplantations [12–14]. In the genome of PLHV-1 three genes encoding for putative immediate-early gene (IE) transactivators (ORF50, ORF6/BZLF1h, ORF57) were found which are homologous to (i) ORF50 and BRLF1/Rta, (ii) K8/K-bZIP and BZLF1/Zta and (iii) ORF57 and BMLF1 of HHV-8 and EBV [55], demonstrating the relationship of these viruses.

Several herpesviruses contain open reading frames that encode viral G protein-coupled receptors (vGPCRs), which they acquired from the host. BILF1 is the vGPCR encoded by EBV, it is molecule with immunoevasive properties associated with MHC-I cell surface downregulation [56–58], thereby preventing recognition of EBV-infected cells by CD8⁺ T cells [59]. BILF1 has not only an immunoevasive properties, but also acts as an oncogene, inducing tumorigenesis through activation of G α i-dependent signaling [60,61]. Signaling induced by BILF1 results in activation of nuclear factor κ -B (NF- κ B) and nuclear factor of activated T cells (NFAT) transcription factors, and inhibition of forskolin-induced transcription of cyclic AMP-responsive elements (CRE) [60,61]. The BILF1 orthologues from PLHV1-3 are similar to EBV-BILF1 regarding cell surface localization, constitutive internalization and ability to downregulate MHC-I as well as upstream signaling resulting in G α i-mediated constitutive activation [62]. However, PLHV1-3 BILF1 differed in downstream signaling and activation of NF- κ B and NFAT transcription factors compared to EBV-BILF1. Interestingly, PLHV1- and PLHV2-BILF1 increased NF- κ B activity to comparable levels as EBV-BILF1, whereas PLHV3-BILF1 did not induce NF- κ B activation. PLHV1-BILF1 was found upregulated in lymphatic tissue from diseased miniature pigs with PTLTD [62]. This data suggests that like EBV-BILF1, PLHV1-3-BILFs could have cell transforming properties.

Given these findings and given that PLHV-1 transactivators are capable of upregulating EBV and HHV-8 promoters [63], it is advisable to use PLHV-free donor pigs for xenotransplantation. However, eliminating PLHV-1 to -3 from donor herds poses significant challenges. Early weaning has proven ineffective [44], there are currently no antiviral treatments or vaccines available, and although cross-placental transmission of PLHV is extremely rare, it remains possible [11]. Nonetheless, as demonstrated in this study, PLHV-negative animals—such as pig 7649—do exist and can be selectively bred for use in xenotransplantation.

4. Materials and Methods

4.1. Animals and Tissue Samples

Pigs 5528, 5415, 5420, 5623, 5803, 5807, and 6249, all of which were triple genetically modified, served as heart donors for baboons J, K, L, M, O, N, P, and Q, as previously described [33] (Table 2). Donor pigs were screened using blood samples, while formalin-fixed tissues were used for testing the baboon recipients [34].

Similarly, pigs 7649, 7654, and 7687, which were also triple genetically modified (α 1,3-galactosyltransferase-knockout and expressing human CD46 and thrombomodulin), were used as heart donors for baboons A, B, and C. Orthotopic pig-to-baboon cardiac xenotransplantation using continuous non-ischemic preservation and pharmacological growth inhibition was performed as previously described in detail [33]. The immunosuppressive regimen was based on a costimulation blockade targeting the CD40/CD40L pathway, also as previously detailed [33]. For induction therapy, animals additionally received a B cell-depleting monoclonal antibody and a T cell-directed immunomodulatory agent. Maintenance therapy consisted of continued costimulation blockade in combination with an antiproliferative compound and corticosteroids.

4.2. Blood Collection

Blood sampling from adult sows was performed without sedation under manual fixation. Whole blood was drawn from the jugular vein with single-use needles (Ehrhardt Medizinprodukte, Geislingen, Germany) into lithium heparin and serum Monovettes (Sarstedt, Nümbrecht, Germany). Blood from the baboons was taken using a central venous catheter.

4.3. Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

For the isolation of PBMCs, the heparin blood was dispensed on Pancoll human (PAN-Biotech GmbH, Aidenbach, Germany), centrifuged at at 900 x g for 25 min at 21°C and the isolated PBMCs were washed twice with phosphate-buffered saline at 350 x g for 10 min at 21°C.

4.3. DNA Isolation

Formalin-fixed tissues from baboons J; K; L; M; O; N; P; and Q were cut into small pieces, and DNA was isolated according to the manufacturer’s instructions using QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) [34].

DNA was isolated from purified PBMCs and frozen tissue samples according to the manufacturers’s instructions using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). DNA concentrations were determined using a NanoDrop ND-1000 (Thermo Fisher Scientific Inc. Worcester, MA. USA).

4.4. PCR and Real-Time PCR

Pigs and baboons listed in Table 2 were tested using a conventional PCR detecting PLHV-1 and PLHV-2 and a conventional PCR detecting PLHV-3 (Table 4) as described [5,34,64]. PCMV/PRV was screened using a real-time PCR [34]. Animals listed in Table 3 were tested using real-time PCRs with specific primers and probes. The sensitivity to detect PCMV/PRV (sensitivity 10 copies/100 ng DNA), PLHV-1 (1 copy/100 ng DNA), PLHV-2 (1 copy/100 ng DNA), PLHV-3 (1 copy/100 ng DNA) was described previously [21]. The primers and probes are listed in Table 4. All protocols were performed using the SensiFAST Probe No-ROX Kit (Meridian Bioscience, Cincinnati, OH, USA) in a reaction volume of 16 µL plus 4 µL (100 ng) of DNA template. Duplex real-time PCRs were performed, testing simultaneously the viral gene of interest and porcine/human glyceraldehyde-3-phosphate-dehydrogenase (p/hGAPDH) as an internal control. The functionality of the PCRs was verified using virus-specific gene blocks containing the sequence of the primers and the probe [20]. Real-time PCR reactions were carried out using a qTOWER3 G qPCR cyclers (Analytik Jena, Jena, Germany) and the real-time PCR conditions as previously described [21].

Table 4. Primers and probes.

Primers Used for PCR	Sequence 5'-3'	Nucleotide Position	Accession Number	Reference
Primers used for PCR				
PLHV-1,-2 (747) fw	CAYGGTAGTATTTATTCAGACA	21,146–21,167	AF478169.1	Ehlers B., 2002 [64]
PLHV-1,-2 (747) rev	GATATCCTGGTACATTGGAAAG	21,488–21,467		
PLHV-3 (905) fw	ACAAGAGCCTTAGGGTTCCAAACT	13,472–13,495	AY170316.1	Chmielewicz et al., 2003 [5]
PLHV-3 (905) rev	GTGTCCAGTGTGTAATGGATGCC	13,727–13,704		
Primers and probes used for real-time PCR				
pGAPDH fw	ACATGGCCTCCAAGGAGTAAGA	1083–1104	NM_001206359.1	Duvigneau et al., 2005 [65]
pGAPDH rev	GATCGAGTTGGGGCTGTGACT			
pGAPDH probe	CCACCAACCCCAGCAAGAGCACGC-BHQ1	1188–1168 1114–1137		
hGAPDH fw	GGCGATGCTGGCGCTGAGTAC	3568–3587	AF261085	Behrendt et al., 2009 [66]
hGAPDH rev	TGGTTCACACCCATGACGA	3803–3783		
	HEX-	3655–3678		

hGAPDH probe	CTTCACCACCATGGAGAAGGCTGGG-BHQ1			
PCMV/PRV fw		45206 –		
PCMV/PRV	ACTTCGTCGCAGCTCATCTGA	45226		
rev	GTTCTGGGATTCCGAGGTTG	45268 –	AF268039	Mueller et al., 2002 [10]
PCMV/PRV	6FAM-CAGGGCGGCGGTCGAGCTC-	45249		
probe	BHQ!	45247 - 45229		
PLHV-1 (1125) fw	CTC ACC TCC AAA TAC AGC GA	14808-14827		
PLHV-1 rev	GCT TGA ATC GTG TGT TCC ATA G	14880-14859	AF478169	Chmielewicz et al., 2003 [5]
PLHV-1 probe	6FAM-CTG GTC TAC TGA ATC GCC GCT AAC AG-TAMR	14829-14854		
PLHV-2 (1155) fw	GTC ACC TGC AAA TAC ACA GG	10814-10833		
PLHV-2 rev	GGC TTG AAT CGT ATG TTC CAT AT	10887-10865	AY170314	Chmielewicz et al., 2003 [5]
PLHV-2 probe	6FAM-CTG GTC TAC TGA AGC GCT GCC AAT AG-TAMRA	10835-10860		
PLHV-3 /210s) fw	AAC AGC GCC AGA AAA AAA GG	11999-12018		
PLHV-3 (210as) rev	GGA AAG GTA GAA GGT GAA CCA TAA AA	12064-12039	AY170315.1	McMahon et al., 2006 [28]
PLHV-3 (210) probe	6-FAM CCA AAG AGG AAA ATC-MGB	12022-12036		
PRE-1 fwd	5' GACTAGGAACCATGAGGTTGCG 3'			
PRE-1 rev	5' AGCCTACACCACAGCCACAG 3'	37–58		Walker et al., 2003 [67]
PRE-1 probe	5' FAM-TTTGATCCCTGGCCTTGCTCAGTGG-BHQ1 3'	61–85 151–170	Y00104	

5. Conclusions

PLHV-1, PLHV-2, and PLHV-3 are widely distributed among pigs, including those bred specifically for xenotransplantation. These viruses can be readily detected using PCR-based methods. Although PLHV-1, PLHV-2 and PLHV-3 have been identified in donor pigs, no transmission to transplanted baboons has been observed to date. Nevertheless, it is recommended to use virus-free animals, as some related gammaherpesviruses have been shown to cause severe disease following trans-species transmission.

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Abbreviations

The following abbreviations are used in this manuscript:

AIHV-1	Alcelaphine herpesvirus type 1
DPS	Dippity pig syndrome
EBV	Epstein–Barr virus
HHV-4, HHV-8	Human herpesvirus 4, 8
IE	Immediate-early genes
KSHV	Kaposi sarcoma-associated herpesvirus
NF-κB	Nuclear factor κB
NFAT	Nuclear factor of activated T cells
OvHV-2	Ovine herpesvirus type 2
PBMCs	Peripheral blood mononuclear cells
PCMV/PRV	Porcine cytomegalovirus, a porcine roseolovirus virus
PLHV-1, PLHV-2, PLHV-3	Porcine lymphotropic herpesviruses -1, -2, and -3
SINE	Short Interspersed Nuclear Elements
SuHV-1, SuHV-2, SuHV-3, SuHV-4, SuHV-5	Suid herpesviruses 1, 2, 3, 4, and 5
vGPCRs	Viral G protein-coupled receptors

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