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Research on the adaptive changes of the virome in Meriones meridianus at laboratory-adaption process

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Abstract

The *Meriones meridianus*, a widely distributed rodent across arid and semi-arid desert regions of Eurasia, hosts diverse pathogens and holds potential as an experimental animal. This study focused on the adaptive changes in the virome carried by the *M. meridianus* during the laboratory domestication, and the key factors driving the changes in the virome. Metagenomic sequencing of lung, liver, and intestinal tissues from wild (W), captive-bred (CBG), and laboratory-bred (LBG) of M. meridianus was performed, followed by comparative analysis of viral carriage. A total of 48 viruses spanning 12 families were detected, Picobirnaviridae and Herpesviridae were the main types of viruses constituting the virome, accounting for 39.58% and 16.67% of the total number of virus, respectively. The numbers of total virus species remained constant across generations, significant tissues-specific differences emerged. Viral traits differed markedly between W and domesticated (CBG/LBG) hosts, and shared virus abundance varied. Virome diversity and evenness increased progressively with domestication, while dominance declined. The intestinal tissue harbored the highest viral richness and abundance, followed by liver and lung. and the intestinal viromes strong positive correlated with total viromes in three generations. This approach demonstrated that during the transition from wild to laboratory animals, the virome of *M. meridianus* had significantly evolved due to environmental stress in captivity. The main pathway for these changes was fecal-oral transmission through the digestive tract, highlighting that maintaining a high-quality rearing environment was crucial for the successful domestication of *M. meridianus* as laboratory animals.

Introduction

The interaction between host microbiomes (particularly gut microbiota) and physiological, behavioral, and metabolic disease-related phenotypic traits in humans and animals has become a focal point in biomedical research. Breakthroughs and applied advancements have been achieved in areas such as host-microbe coevolution theory[1], microbial metabolic regulatory networks[2], and microbiome-based intervention strategies[3]. Notable examples include novel mechanisms by which gut microbiota regulate the host neuroendocrine system via the "gut-brain axis"[4] and the successful application of fecal microbiota transplantation in treating metabolic syndromes[5]. Thus, analyzing the fundamental principles of biological phenomena through the lens of host-associated microbiomes holds significant value for basic medical research, disease prevention, and laboratory animal science, spanning fields from biomedical studies to disease management, animal husbandry, and experimental applications.

The midday gerbil (*M. meridianus*), one of the most widely distributed rodents in arid and semi-arid regions of Eurasia, exhibits remarkable adaptability and serves as a natural host for multiple critical pathogens, including plague, Lyme disease, leishmaniasis, tick-borne relapsing fever, and brucellosis. Recent genomics research has revealed distinct adaptive structural features in its genome evolved through co-evolution with these pathogens, confirming its significant potential and broad application prospects in laboratory animal research[6]. Consequently, the domestication and experimental utilization of this rodent species have gained widespread recognition within the scientific community, with systematic breeding programs and experimental studies having been gradually initiated as early as the 1980s[7–11]. Researchers have systematically established its physiological and biochemical parameters[7–9], characterized its gut bacterial communities[10], and successfully bred in conventional animal facility[11]. Studies using this species in animal models for hydatid disease (caused by Echinococcus granulosus)[12] and hepatitis E virus (HEV) infection[13] have demonstrated its robust pathological responsiveness.

However, current research lacks comprehensive virome surveillance data during its laboratory-adaption process, highlighting the need to establish viral monitoring and quality assessment systems for this experimental animal. Importantly, the virome profile and its dynamic evolution during the transition from wild to laboratory-adapted populations provide a critical window to longitudinally understand host-microbe-environment interactions. This study investigated the composition and variation of the virome in different bred generation from wild to captive-bred generation, then to laboratory-bred generation, aiming to elucidate how and why the virome shifts during laboratory adaptation. The findings would not only inform the development of viral surveillance and test standards for this species but also contribute to the theoretical framework for studying environment-microbe-host interplay.

Materials and methods

Samples

The Wild (W),Captive-bred generation (CBG) and Laboratory-bred generation(LBG)of *M. meridianus* were captured alive in the Junggar Basin by the research team. While the CBG were domesticated for one year under conventional animal breeding conditions at the Laboratory Animal Center in Center for Disease Control and Prevention of Xinjiang, LBG were domesticated to the fifth generation at the same center. Three adult *M. meridianus* were selected from each of the above three generations, and lung, liver, and intestinal tissues were aseptically taken and stored in liquid nitrogen for later use.

Metagenomic Sequencing

Three individuals of W, CBG, and LBG of *M. meridianus* were selected. Tissues of the same organ from each group were pooled together. DNA and RNA were extracted using kits(Vazyme, Nanjing, China), respectively. High-throughput sequencing was performed on the extracted DNA and RNA samples using the Illumina Novaseq 6000 sequencing platform for paired-end sequencing.

Bioinformatics Analysis and Viral Sequence Annotation

High-throughput sequencing data underwent quality control using FastQC v0.11.9[14] and Trimmomatic v0.32[15] to remove low and medium-quality reads. The cleaned reads were then assembled and spliced using the Trinity program (Version 2.5). Viral nucleotide and amino acid sequences were identified by aligning the assembled contigs against reference databases using BLASTn[16] and BLASTx[17]. Contigs with significant viral sequence similarity (BLAST e-

value < 1e-5) were retained. Based on the alignment results, taxonomic lineage assignment (annotation) was performed on these viral contigs. Bowtie v1.3.1 was used to align the original sequencing reads back (alignment) to the viral contigs to determine the number of reads supporting the assembly of each viral contig[18]. Viral sequence information was subsequently compiled. The entire process described above was automated and executed using the High-throughput Sequencing Data Virus Automatic Identification Software v1.0 (Copyright holders: Wang Jun; Wang Siyuan; Deng Fei) on a Linux platform. All identified viral sequences were manually verified and corrected based on NCBI BLAST comparison results.

Statistical Analysis

The Kolmogorov-Smirnov test was employed to assess the normality of the virus species and abundance data. For normal distribution data, the mean ± standard deviation was calculated to describe the distribution characteristics, and one-way analysis of variance (ANOVA) was conducted for inter-group comparisons, otherwise, the Quartile method was used, and the non-parametric Kruskal-Wallis H test was applied for inter-group differences. For the analysis of composition ratios, the chi-square test was used when the data met the required assumptions; otherwise, Fisher's exact test was employed. The Spearman correlation coefficient was utilized to evaluate the relationships between variables. All statistical analyses were performed using SPSS version 26.0. The diversity, evenness, and dominance indices of the viral community structure were calculated using Microsoft Excel 2016, while principal component analysis (PCA) was conducted in R version 4.4.2.

Results

Virome of M. meridianus

3.1.1 virus species Composition

High-throughput sequencing technology was applied to analyze 27 tissue samples (lung, liver, and intestine) from 9 gerbils across three generations: wild (W), Captive-bred generation (CBG), and Laboratory-bred generation (LBG). A total of 78.6 GB of sequencing data were generated, yielding 54,418 viral sequences after quality control, alignment, and bioinformatic analysis. These sequences were classified into 48 virus species spanning 12 families, as detailed in Table 1. The distribution and relative abundance of the 48 virus species across the three generations and three tissue types were illustrated in Fig. 1.

Figure 1 The virome composition of three generations of M. meridianus

Note

The vertical axis represent all virus species after removing Unclassified and Retroviridae viruses, and the code represent the sequence numbers of viruses sorted by the first letter of their respective families. The horizontal axis indicate three generations of *M. meridianus* and its different organs. Different colors represent the total number of viruses carried by *M. meridianus* from various tissues during different generations. The three columns for each time period represent the number of viruses carried by lung, liver, and intestinal tissues, respectively. The red circles inside the graph indicate the amount of virus, with varying shades and sizes representing different quantities.

W represent viruses in wild life; CBG represent viruses in Captive-bred generation; LBG represent viruses in Laboratory-bred generation; W + CBG represent viruses shared by W and CBG; W + LBG represents viruses shared by W and LBG; CBG + LBG represent viruses shared by CBG and LBG; W + CBG+LBG represent viruses shared by W, CBG and LBG;

TPM means virus abundance.

Table 1
Virome family composition of *M. meridianus* across wild (W), captive-bred generation (CBG), and laboratory-bred generation (LBG)

Viridae	Number of virus species	Percentage of virus species in each generation (number of species)		
		W	CBG	LBG
Herpesviridae	8	62.50(5)	75.00(6)	75.00(6)
Picobirnaviridae	19	31.58(6)	57.89(11)	68.42(13)
Alloherpesviridae	2	50.00(1)	50.00(1)	0.00(0)
Flaviviridae	2	50.00(1)	50.00(1)	100.00(2)
Mimiviridae	2	100.00(2)	0.00(0)	0.00(0)
Paramyxoviridae	2	50.00(1)	100.00(2)	100.00(2)
Hepeviridae	2	0.00(0)	100.00(2)	0.00(0)
Parvoviridae	2	0.00(0)	50.00(1)	50.00(1)
Picornaviridae	3	3.33(1)	66.67(2)	0.00(0)
Phycodnaviridae	3	33.33(1)	0.00(0)	66.67(2)
Caliciviridae	1	0.00(0)	0.00(0)	100.00(1)
Poxviridae	2	50.00(1)	50.00(1)	100.00(2)
total	48	19	27	29

As shown in Table 1, among the 48 virus species, Picobirnaviridae exhibited the highest diversity with 19 species, accounting for 39.58% (19/48) of the total virus species. Herpesviridae accounted for 8 species (16.67%, 8/48), while the remaining 10 families together included 21 species (43.75%, 21/48). Both at the family and species levels, CBG and LBG had significantly more virus species (27 and 29 species, respectively) than the W, which had 19 species). However, all three generations shared the same 12 viral families. The increase in viral diversity was primarily driven by Picobirnaviridae, which expanded by 5 and 7 species in CBG and LBG, respectively, relative to W. Statistical analysis revealed no significant differences in virus species numbers among the three generations ($\chi^2 = 4.675$, $P = 0.097 > 0.05$).

An analysis of virus species composition across three generations showed that 30 out of 48 total virus species (62.50%, 30/48) were unique to their respective generations. The CBG and LBG populations exhibited significantly higher numbers of unique virus species (12 and 12 species, respectively) compared to the W(6 species). Only 9 virus species (18.75%, 9/48) were shared across all three generations.

Regarding tissue viral distribution, intestinal tissues had significantly more virus species than lung or liver tissues. In the W, 73.68% (14/19) of virus species were detected in intestinal tissues, while this proportion increased to 81.48% (22/27) in CBG and 75.86% (22/29) in LBG. The number of virus species shared or unique to lung, liver, and intestinal tissues across generations were detailed in Table 2. Statistical analysis confirmed significant differences in virus species composition among the three generations (Fisher's exact test, $P = 0.000 < 0.01$).

Table 2
Distribution of virus species across lung, liver, and intestinal tissues in three generations of *M. meridianus*

Periods	Total	Number of virus species in tissues			Number of shared virus species			Number of Shared virus species Across Tissues			Number of Tissue-Specific virus species		
		Lung	Liver	Intestine	W-CBG	W-LBG	CBG-LBG	CBG-Lu-In	LBG-Lu-Li	LBG-Li-In	Lung	Liver	Intestine
W	19	4	7	14	1	3	0	0	0	0	0	2	4
CBG	27	7	9	22	1	0	5	1	0	0	1	2	8
LBG	29	8	12	22	0	3	5	0	1	1	1	1	8

Notes: W-CBG represent viruses shared by W and CBG; W-LBG represent viruses shared by W and LBG; CBG-LBG represent viruses shared by CBG and LBG; CBG-Lu-In represent viruses shared by Lung and Intestine in CBG; LBG-Lu-Li represent viruses shared by Lung and Liver in LBG; LBG-Li-In represent viruses shared by Liver and Intestine in LBG.

3.1.2 Biological Characteristics of the Virome composition

The 48 viruses were divided into three groups according to whether they were shared by three generations, uniqued to the wild, or presented in the domesticated generations. Comparative analyses were conducted on the distribution of these three types of viruses in the organs of *M. meridianus*, as well as their biological properties such as virus families and the living environments and transmission modes of the viruses. The results were presented in Tables 3.

Table 3
Biological characteristics of viruses carried by the three generations of *M. meridianus*

Periods	Total	Number of virus species per organ			Existence environment			Mode of transmission		Virus families		
		Lung	Liver	Intestine	human/nature	nature	human	touch	bite	Herpesviridae	Picobirnaviridae	Flaviviridae
W + CBG+LBG	9	6	6	7	9	0	0	9	2	3	3	1
W	6	0	2	4	4	2	0	6	0	2	1	0
CBG + LBG	29	6	7	23	27	0	2	29	1	3	13	1

Notes: W + CBG+LBG represent viruses shared by the three generations; W represent viruses unique to W; CBG + LBG represent viruses shared by CBG and L

As shown in Table 3, the 9 species of viruses shared by the three generations were all widely existing virus types in humans and nature environments, and their transmission modes can all spread directly through multiple pathways such as fecal-oral or respiratory transmission. Among them, 2 viruses [*Avian orthoavulavirus* 1(40) and *Pestivirus A*(4)] can also be transmitted through vector bites. These 9 viruses have consistent environmental presence and transmission methods with the 29 viruses detected only in CBG and LBG .Among the 6 viruses unique to W, 4[*Ateline gammaherpesvirus* 3(33),*Paramecium bursaria Chlorella virus NE-JV-1*(3),*Mimivirus U306*(38),*Mimivirus sp. SH*(39)] primarily exist in humans or other natural environments, while the other 2[*Cyprinid herpesvirus* 3(29) and *Limnipivirus A*(45)] have only been detected in wildlife in natural environments, highlighting key differences from the previous two types. In terms of the detected organs, the distribution ratios of the 9 viruses shared by the three generations in the lung, liver, and intestine were basically consistent, at 66.67% (6/9), 66.67% (6/9), and 77.78% (7/9) respectively, which was different from the distribution of viruses unique to W or present in both CBG and LBG in the three organs. The richness of virus species were consistently and significantly lower in lung and liver tissues compared to the intestine across both W and (CBG + LBG) groups. The ratios of lung and liver virus species to the total number of viruses in W were 0 (0/6) and 33.33% (2/6) respectively, while those in the intestine was 66.67% (4/6). In (CBG + LBG), the ratios for the lung and liver were 20.69% (6/29) and 24.13% (7/29) respectively, and for the intestine was 79.31% (23/29). However, there were no statistically significant difference among the three groups (Fisher = 5.225, P = 0.230>0.05). In terms of virus classification, the 9 viruses shared by the three generations belonged to 4 families, with 3 viruses each in Herpesviridae and Picobirnaviridae, both accounting for 33.3% (3/9). The 6 viruses unique to W also belonged to 4 families, with 2 in Herpesviridae, 1 in Picobirnaviridae, and the other 3 viruses in 2 families. The viruses in (CBG + LBG) showed significant diversity, with 29 viruses belonged to 11 families, among which Picobirnaviridae was the main type, accounting for 44.83% (13/29), followed by Herpesviridae, accounting for 10.34% (3/29), and the other 13 viruses belonged to 9 families. There were significant differences in the biological characteristics of viruses (organ distribution, virus family, living environment, and transmission mode) between W and (CBG + LBG) groups (Kruskal-Wallis Test, P = 0.016 < 0.05), and the differences in virus environmental distribution between W and (CBG + LBG) were statistically significant (Kruskal-Wallis Test, P = 0.020 < 0.05).

3.2 TPM Analysis of Viruses Carried by *M. meridianus*

3.2.1 TPM of Viruses in the Three Generations

After normality testing (Kolmogorov-Smirnov test), the TPM values of viruses in W, CBG, and LBG of *M. meridianus* did not follow a normal distribution. Therefore, the TPM of viruses in the three generations were statistically analyzed using quartiles. The total TPM of viruses in W, CBG, and LBG were 14.417, 37.551, and 29.734, respectively. Significant within-group variation was observed across(CBG and LBG) domestication generations (Kruskal-Wallis H = 8.353, P = 0.015 < 0.05).Specifically, there were statistically significant differences between W and CBG (H=-18.812, P = 0.019 < 0.05) and between W and LBG (H=-21.187, P = 0.008 < 0.01), while there was no statistically significant difference between CBG and LBG (H = 2.375, P = 0.767>0.05),shown in Table 4.

Table 4 Composition of virus TPM and species in the three generations of M. meridianus

	W			CBG			LBG		
Groups	TPM percentage (%) (TPM)	TPM $\bar{x}\pm s$	Virus species code*	TPM percentage (%) (TPM)	TPM $\bar{\chi}\pm s$	Virus species code	TPM percentage (%) (TPM)	TPM $\bar{\chi}\pm s$	Virus species code
0~25	2.87 (0.415)	0.083±0.035	**4**、**6**、10、23、***39***	8.63(3.240)	0.463±0.148	**6**、***9***、***22***、***26***、***28***、***32***、**37**	6.84(2.034)	0.254±0.145	***1***、10、***13***、***18***、***24***、***25***、35、**41**
26~50	12.80 (1.846)	0.369±0.130	16、**34**、35、**36**、***45***	16.07 (6.034)	0.862±0.092	**14**、**15**、***17***、***21***、23、**34**、***46***	13.47 (4.006)	0.572±0.131	***2***、***5***、16、***19***、***43***、***44***、***48***
51~75	20.72 (2.987)	0.597±0.084	***3***、***8***、**20**、***33***、***38***	29.76 (11.175)	1.596±0.433	**4**、***7***、***8***、**30**、**36**、**41**、***47***	25.37 (7.543)	1.078±0.180	**6**、***11***、**14**、**15**、**34**、**37**、**40**
76~100	63.60 (9.169)	2.292±1.069	**12**、***29***、**31**、**40**	45.54 (17.101)	2.850±0.246	**12**、**20**、***27***、**31**、**40**、***42***	54.32 (16.151)	2.307±0.528	**4**、**8**、**12**、**20**、**30**、**31**、**36**
sum	14.417			37.551			29.734		

As shown in Table 4, the 9 shared virus species conserved across three generations of *M. meridianus* (indicated by red bold font virus species code) exhibited TPM values of 10.426 (W), 18.522 (CBG), and 17.071 (LBG), respectively. These core viruses constituted 72.32% (10.426/14.417) of the total virome in W, 49.32% (18.522/37.551) in CBG, and 57.41% (17.071/29.734) in LBG, represented the dominant components of the virome across all generations of *M. meridianus*. (Fig. 2A). In addition to the 9 shared virus species, W had 10 virus species, of which 6 were unique to W (Black bold italic virus species code). The remaining 4 virus species (black font virus species code) were shared: *Rat picobirnavirus*(23)was shared by CBG, while *Feline picobirnavirus*（10）,*Picobirnavirus 504*（16）and *Human betaherpesvirus 6B*（35）were shared by LBG. Among the 6 unique virus species in the W, the 4 unique viruses *Paramecium bursaria Chlorella virus* NE-JV-1 (3), *Cyprinid herpesvirus* 3 (29), *Ateline gammaherpesvirus* 3 (33), and *Mimivirus U306* (38) had a TPM that accounts for 66.13% (2.639/3.991) of the total TPM of the 10 virus species, making them the main types of the 6 unique viruses in W. In CBG,12 unique virus species (black bold italic virus species code) and 5 virus species shared by LBG (blue bold font virus species code), the TPM of 17 virus species,accounted for 47.95% (18.005/37.55） to CBG total TMP. Among them, 4 unique species [*Bovine picobirnavirus* (7), *Orthohepevirus* C (27), *Macaca fascicularis chapparvovirus* (42), and *Rosavirus B* (47)], and two species shared by LBG[*Macacine betaherpesvirus* 3 (30) and *Human respirovirus* 1 (41)] collectively represented 61.06% (10.993/18.005 TPM) of this subgroup, formed major components of CBG's virome. LBG also had 12 unique virus species (black bold italic virus species code) and 5 virus species shared by CBG (blue bold font virus species code), the TMP of 17 virus species,contributed 37.04% (11.013/29.734TPM) to LBG total TMP. Among them, 1 unique species [*Fox fecal picobirnavirus* (11)] and 4 shared species [*Mongoose picobirnavirus* (14), *Otarine picobirnavirus* (15), *Monodontid alphaherpesvirus* 1 (37), and *Macacine betaherpesvirus* 3 (30)], accounted for 60.13%(6.622/11.013TPM) of the 17 virus species, making them the main types of the virome in LBG (Fig. 2B).

Figure 2 The values of TPM analysis of major viruses in the three generations of M. meridianus

3.2.2 Abundance(TPM), Diversity, Evenness, and Dominance of the Shared Virus Species in the Three Generations

There were 9 virus species shared among the three generations, W, CBG, and LBG, and the total TPM of these shared viruses was lowest in W at 10.426, while it was relatively consistent in CBG and LBG, being 18.522 and 17.071 respectively. The average abundance of these shared viruses in W, CBG, and LBG were 1.158 ± 1.238, 2.058 ± 0.893, and 1.897 ± 0.663 respectively. There was no statistically significant difference among the three groups (ANOVA, F = 1.993, df = 2, P = 0.158)(Fig. 3).

Figure 3 The abundance (TPM) of spectrum among 9 shared virus species across three generations

As shown in Fig. 3, there were 9 shared virus species among the three generations W, CBG, and LBG. Among them, the abundance of *Avian orthoavulavirus* 1 (40) and *Human gammaherpesvirus* 4 (31) showed a decreasing trend across the three generations, maked them the main viruses in W, accounted for 64.08% (6.681/10.426) of the 9 viruses, while the TPM of the other 7 viruses was 3.745, accounting for 35.92% of the total TPM. In CBG, the TPM volatility of the 9 shared viruses was lower than that in W (standard deviation: 0.893 vs 1.238). Except for the two main virus species *Avian orthoavulavirus* 1 (40) and *Human gammaherpesvirus* 4 (31), the TPM of *Marmot picobirnavirus* (12) had increased by 92.59% (2.962/1.538) compared to W. The total TPM of these three virus species accounted for 47.70% (8.835/18.522) of the total TPM of the 9 viruses in CBG. The variability of TPM for shared virus was lower in LBG (standard deviation: 0.663). *Avian orthoavulavirus* 1 (40) had a TPM value equivalent to 51.86% of that in CBG (1.468/2.829), maked it a minor virus in the LBG where it accounted for 8.60% of the total TPM of the 9 shared viruses in LBG(1.468/17.072). *Human gammaherpesvirus* 4 (31) and *Marmot picobirnavirus* (12) were major viruses in LBG, together accounted for 33.68% of the total TPM of the 9 viruses(5.750/17.071), Additionally, apart from the three major shared viruses, the remaining 6 viruses showed differences across different domestication generations (F = 8.769, df = 2, P = 0.003), Further analysis of intergroup differences revealed that the total TPM of the W was lower than that of the CBG, with a statistically significant difference (Tukey, P = 0.007). It was also lower than that of the LBG, with a statistically significant difference (Tukey, P = 0.006). The TPM of the other 6 viruses was also significantly increased compared to W. The average TPM of CBG increased by 3.39 times (1.615/0.368), and the average TPM of LBG increased by 4.47 times (1.642/0.368).

The diversity and evenness of the TPM of the 9 shared viruses in the three generations W, CBG, and LBG were 1.662, 2.081, and 2.136, and 0.756, 0.947, and 0.972, with dominance of 0.323, 0.164, and 0.175 respectively. Diversity and evenness showed progressive increases, while dominance demonstrated an overall declining trend (Fig. 4).

Figure 4 Diversity, evenness, and dominance of the abundance of the 9 shared viruses in the three generations of M. meridianus

Shifts in structural parameters (diversity, evenness, dominance) of the 9 shared virus species across the three generations indicated that while *Human gammaherpesvirus* 4 (31) persisted as a dominant virus throughout the three generations, the abundance changes of the other 8 viruses had an impact on the viral community structure. Moreover, the changes in the virome of CBG and LBG were consistent.

3.2.3 TPM Analysis of Viruses in Different Organs

Among the three generations W, CBG, and LBG, intestinal tissue consistently exhibited the highest TPM, being 12.082, 33.172, and 22.212 respectively, with an average of 22.489 ± 8.612. The liver tissue was next, with values of 6.870, 10.176, and 7.837 respectively, with an average of 8.294 ± 1.388. The lung tissue had the lowest TPM, with values of 2.075, 5.438, and 7.665 respectively, and an average of 5.059 ± 2.298. Quartile-based analysis of TPM in different organ tissues across three generations was presented in Table 5.

Table 5
Composition of virome and virus TPM in different organs across the three generations

Statistical Methods	W				CBG				LBG			
	Total	Lung	Liver	Intestin	Total	Lung	Liver	Intestin	Total	Lung	Liver	Intestin
Number of virus species	19	4	7	14	27	7	9	22	29	8	12	22
TotalTPM	14.417	2.075	6.869	12.082	37.551	5.438	10.176	33.172	29.734	7.665	7.837	22.212
TPMMedian	0.522	0.439	0.629	0.523	1.024	0.592	0.932	6.017	0.959	0.788	0.503	0.860
0 ~ 25%(TPM)	0.415	0.123	0.028	0.264	3.24	0.070	1.334	3.171	2.034	0.510	0.087	1.466
26 ~ 50%(TPM)	1.846	0.272	0.395	1.574	6.034	0.626	1.809	5.689	4.006	1.279	1.168	2.927
51 ~ 75%(TPM)	2.987	0.605	1.579	2.358	11.175	1.485	2.355	9.956	7.543	2.026	2.046	6.622
76 ~ 100%(TPM)	9.169	1.075	4.867	7.886	17.101	3.257	4.679	14.357	16.151	3.850	4.355	11.198

As shown in Table 5, intestinal dominanced in both virus species richness and TPM across three generations of M. meridianus, accounted for 73.68% (14/19), 81.48% (22/27), and 75.86% (22/29) of the total number of virus species in the three organs (lung, liver, and intestine) of each generation, and 83.80% (12.082/14.417), 88.34% (33.172/37.551), and 74.70% (22.212/29.34) of the total TPM of viruses in the three organs respectively. The differences in the number of virus species and total TPM carried in the lung and liver were not significant. From the quartile-based abundance analysis, intestinal TPM across interquartile ranges(0 ~ 25%, 26 ~ 50%, 51 ~ 75%, and 76 ~ 100%) were 1.634 ± 1.193, 3.397 ± 1.712, 6.312 ± 3.110, and 11.147 ± 2.642 respectively, which were significantly higher than those in the lung and liver tissues (Kruskal-Wallis test, H = 8.000, P = 0.018<0.05). There was a statistically significant difference in virus TPM between the lung and intestine (H=-2.746, P = 0.018<0.05).

Kruskal-Wallis analysis revealed significant differences in viral abundance (TPM) across organs within each generation W, CBG, and LBG (H = 15.243, P = 0.001; H = 28.738, P<0.001; H = 25.317, P<0.001). The within-group test indicates that in all three generations, there was no significant difference between the total virus TPM and the intestinal virus TPM, and no significant difference in TPM between the lung and liver viruses too. In the W, there was also no significant difference in TPM between the liver and intestinal viruses. However, there were significant differences in TPM between the other organs and between each organ and the total virome TPM, as shown in Table 6.

Table 6
Analysis of variance in virus TPM among different organs across the three generations

W	Total-Lung	Total-Liver	Total-Intestine	Lung-Liver	Lung-Intestine	Liver-Intestine
	H = 29.688, ***P = 0.000	**H = 23.396, **P = 0.005**	**H = 9.667, P = 0.247**	**H=-6.292, P = 0.451**	**H=-20.021, *P = 0.017**	**H=-13.729, P = 0.100**
CBG	H = 42.240, ***P = 0.000	H = 37.229, ***P = 0.000	H = 8.281, P = 0.386	H=-5.010, P = 0.600	H=-33.958, ***P = 0.000	H=-28.948, **P = 0.002
LBG	H = 42.865, ***P = 0.000	H = 38.052, ***P = 0.000	H = 14.417, P = 0.142	H=-4.812, P = 0.624	H=-28.448, **P = 0.004	H=-23.635, *P = 0.016
Note:*$P \leq 0.05$,**$P \leq 0.01$,***$P \leq 0.001$						

Spearman correlation analysis revealed strong positive associations between total virome abundance and intestinal viral abundance across three generations of M. meridianus (correlation coefficients are 0.818, 0.939, and 0.829 respectively).

After Kruskal-Wallis tests in virome TPM among different generations in three tissues, there were no significant difference across generations in lung and liver tissues (H = 1.758, $P = 0.415$; H = 1.340, $P = 0.512$); there was a significant difference across generations in intestinal tissue (H = 6.182, $P = 0.045$). Among them, the virus TPM in the intestinal tissue of W was significantly lower than that in CBG (H=-18.167, $P = 0.016$), while there were no statistical difference between W and LBG and between CBG and LBG (H=-13.208, $P = 0.080$; H=-4.958, $P = 0.512$).

3.3 Principal Component Analysis of the Virome in M. meridianus

Using the KMO and Bartlett tests, the KMO values for the total virus abundance (Total-TPM) and the intestinal virus abundance (Intestine-TPM) data from the three types of organ tissues across the three generations of *M. meridianus* were 0.70 and 0.72 respectively, the Bartlett values were 38.757 ($P < 0.001$) and 46.971 ($P < 0.001$) respectively. These results indicate that both sets of data had strong collinearity and were suitable for principal component analysis. The principal component analysis showed that the cumulative contribution rates of PC1 and PC2 for Total-TPM and Intestine-TPM were 86.39% and 87.37% respectively. Therefore, both sets of data retained PC1 and PC2 for composition analysis. The principal component scores of all factors (the TPM of each virus) are shown in S1 Table.

Factors with values greater than |0.5| in the PC1 and PC2 principal components of Total-TPM and Intestine-TPM were statistically analyzed, and the results are shown in Fig. 5.

Figure 5 Principal Component Analysis of the Total and Intestinal Virome in the Three Generations of M. meridianus

Note

the code represent the sequence numbers of viruses sorted by the first letter of their respective families.

According to the Principal component scores of PC1 and PC2, among the 9 virus species shared by W, CBG, and LBG, 7 viruses had principal component scores greater than 1.0 in the total virome of *M. meridianus*, while 6 viruses exceed this threshold in the intestinal virome. These viruses were relatively concentrated in the high-value regions of PC1 and PC2 on the principal component analysis plot, indicating that they significanly influnced the composition of both the total and intestinal viromes, constituting the dominant groups within these viral profiles. They were: *Pestivirus A* (4), *Chicken picobirnavirus* (8), *Marmot picobirnavirus* (12), *Picobirnavirus sp.* (20), *Human gammaherpesvirus* 4 (31), *Human alphaherpesvirus* 2 (36) and *Avian orthoavulavirus* 1 (40). In addition, most of the unique virus species in the three generations of *M. meridianus* influenced the composition of the virome, though their impact were relatively minor. In the unique virome of the three generations, there were 6,11 and 11 viruses respectively, with principal component scores ranging from 0.5 and 1.0 in absolute values. These accounted for 100.00%, 91.67%, and 91.67% of the unique species in each generation. In the intestinal virome, the proportions were 100.00% (5/5), 90.00% (9/10), and 77.78% (7/9) respectively. These viruses were relatively concentrated on the left side of the principal component analysis plot, near the origin PC1 and PC2. Therefore, although the principal component scores of the unique viruses in each generation of *M. meridianus* were lower than those of the shared species cross the three generations, the large number of unique viruses still had a significant impact on the diversity of the virome. It could also be seen from Fig. 5 that there was a high degree of consistency in the composition of the total viome and intestinal virome of *M. meridianus*, whether it was viruses with principal component scores greater than 1.0 or those with scores ranging from 0.5 to -1.0. Among the former, 6 out of 9 viruses are the same, with a consistency of 66.67% (6/9). Among the latter, 20 out of 36 viruses are the same, with a consistency of 55.56% (20/36). Therefore, the virome composition of the three generations of *M. meridianus* was highly similar to the intestinal virome composition.

Discussion

Extensive research have explored the complex interactions among hosts, microbes, and the environment. On one hand, numerous studies using epidemiological analyses, such as geographic population comparisons[19] and lifestyle intervention analyses[20], have identified significant correlations between human intestinet microbiota composition and the prevalence of metabolic diseases. For instance, the spatial coupling of the Bacteroidetes/Firmicutes ratio with obesity phenotypes[21] highlights the interactive relationship between host behavior/disease and host-associated microbiomes. Furthermore, metabolomic studies had elucidated molecular mechanisms underlying host-microbe coevolution, such as the "cross-feeding" mechanism through which intestine commensals regulate host energy metabolism via short-chain fatty acid (SCFA) production[22]. On the other hand,

longitudinal experimental biological models had demonstrated that environmental changes could reshape intestine microbial communities and subsequently influence host behavior. A notable example is the induction of anxiety-like behavioral changes in germ-free mice following colonization with specific microbial consortia[23]. Collectively, these findings underscore the complexity of host-microbe-environment interactions and their underlying mechanisms, emphasizing the need for additional foundational data to deepen our understanding.

Although existing studies had encompassed diverse host systems, including humans, livestock, poultry, model rodents (mice, rats), and wild rodents, research on microbial communities remains imbalanced. A substantial body of literature focused on bacteria, while studied on viruses, Rickettsia, Chlamydia, and other microbes remain scarce[24]. The main reason for this phenomenon is the limitation of research methods: the cultivability and independent metabolic traits of bacteria facilitate metabolomic and in vitro interaction studies[2], whereas the strict host dependency of viruses necessitates reliance on metagenomics[25] and single-cell sequencing technologies[26]. Thus, investigating host-microbe-environment interactions at the finer resolution of virology is essential.

This study, by analyzing the dynamic evolution of the virome during the W, CBG and LBG of M. meridianus, revealed patterns of viral community shifts across different breeding stages under environmental pressures. These findings would provided critical virome-level evidence to refine the theoretical framework of host-microbe interactions in the future.

First, from a qualitative perspective of virome composition, this study revealed two distinct patterns in the viral diversity across three generations of M. meridianus. One pattern showed that approximately 50% (9/19) of wild life viruses persisted into subsequent CBG and LBG. The other pattern highlighted that each generation harbored a unique subset of viruses, with 31.6%, 44.4%, and 41.4% of virus species being generation-specific, respectively. These findings indicated both shared and unique virome characteristics across breeding generations.

Notably, both of CBG and LBG all exhibited a significantly higher number of virus species compared to the wild - a trend contrasting with the typical reduction in microbial diversity observed during the domestication of most laboratory animals[27]. This anomaly may stem from two factors. First, M. meridianus is a highly adaptable species, widely distributed in arid ecosystems and naturally resilient to diverse environmental microbes, including viruses. Second, the CBG and LBG populations in this study were housed in a conventional animal facility, where there was a risk infection from opportunistic viruses due to potential contamination from humans, feed, or bedding in laboratory environments.

The analysis of viral biological attributes across the three generations supported these hypotheses. Significant environmental-driven differences in viral composition were observed, with CBG and LBG populations displaying higher viral diversity compared to the wild. Notably, Picobirnaviridae viruses—primarily inhabiting the intestinal tract—were significantly more prevalent in these two generations (44.83% vs 16.7%). This disparity indirectly suggested that novel viral acquisition in two generations might occur through fecal-oral transmission route.

Second, from a quantitative perspective of viral abundance, the viral communities across the three generations exhibited distinct differences. These variations align with the observed patterns of shared and unique virus species previously described.

Among the 9 core virus species shared across generations, among which, Avian orthoavulavirus 1(40), Human gammaherpesvirus 4 (31), and Marmot picobirnavirus(12) were dominated. However, the remaining 6 core species showed significant abundance differences between domestication stages. From wild to domestication, the abundance levels of major species in each generation showed a decreasing trend in their proportion of the total viral abundance, with W, CBG and LBG being 66.13%, 47.95%, and 37.04%, respectively. The emergence of new virus species during the two domestication periods increased viral community diversity and evenness while reducing dominance, leading to a trend of further enhanced commonality among shared viruses. On the other hand, endemic species, although the main factor for the differences in viral communities at various domestication stages of the M. meridianus, re-established viral communities adapted to the new breeding environment as the number of domestication generations increased. This new viral community was consistent with the intestinal viral community, further proving that the establishment of new viral communities in M. meridianus domestication generations is achieved through the fecal-oral transmission route and is closely related to the breeding environment.

Third, principal component analysis (PCA) of the virome revealed distinct contributions of shared and unique virus species community structure across three generations. Among the 9 core virus species conserved in all generations, 7 exhibited principal component scores ≥ 1.0, showed that different generation of M. meridianus all have an important influence on the composition of the viral community, while the generation-specific viruses exhibit relatively minor influence with principal component scores < 1.0. This suggested that while the laboratory-adapted environment drives partial restructuring of the virome during LBG and CBG, the observed patterns of viral composition might also reflected inherent host-specific traits. However, the potential correlations between these virome characteristics and host factors-such as intestine microbiota composition or behavioral adaptations-remain were uncleared and warranted further investigation.

Authors' contributions Guoyu Zhao and Abulimiti Moming: conceived and designed the study, analyzed the data, and drafted the manuscript.Huahua Zhang: participated in data analysis. Fang Li: analyzed the data.Siyuan Wang, Jian Xiao, Shen Shi, Jiangling Yuan, Ran An, Tao Luo: involved in field surveys. Haiyan Wu, Fei Deng, and Yujiang Zhang:contributed to the study design and edited the manuscript.

Declarations

Conflict of interest

All authors declare that they have no conflict of interest.

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Animal Welfare and Ethics Committee of Xinjiang Uygur Autonomous Region Center for Disease Control and Prevention

Supporting information

S1 Table. Composition of virome and virus TPM in different generations

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Author Contribution

Guoyu Zhao and Abulimiti Moming: conceived and designed the study, analyzed the data, and drafted the manuscript.Huahua Zhang: participated in data analysis. Fang Li: analyzed the data and prepared figures .Siyuan Wang, Jian Xiao, Shen Shi, Jiangling Yuan, Ran An, Tao Luo: involved in field surveys. Haiyan Wu, Fei Deng, and Yujiang Zhang:contributed to the study design and edited the manuscript.

Data Availability

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

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Figures

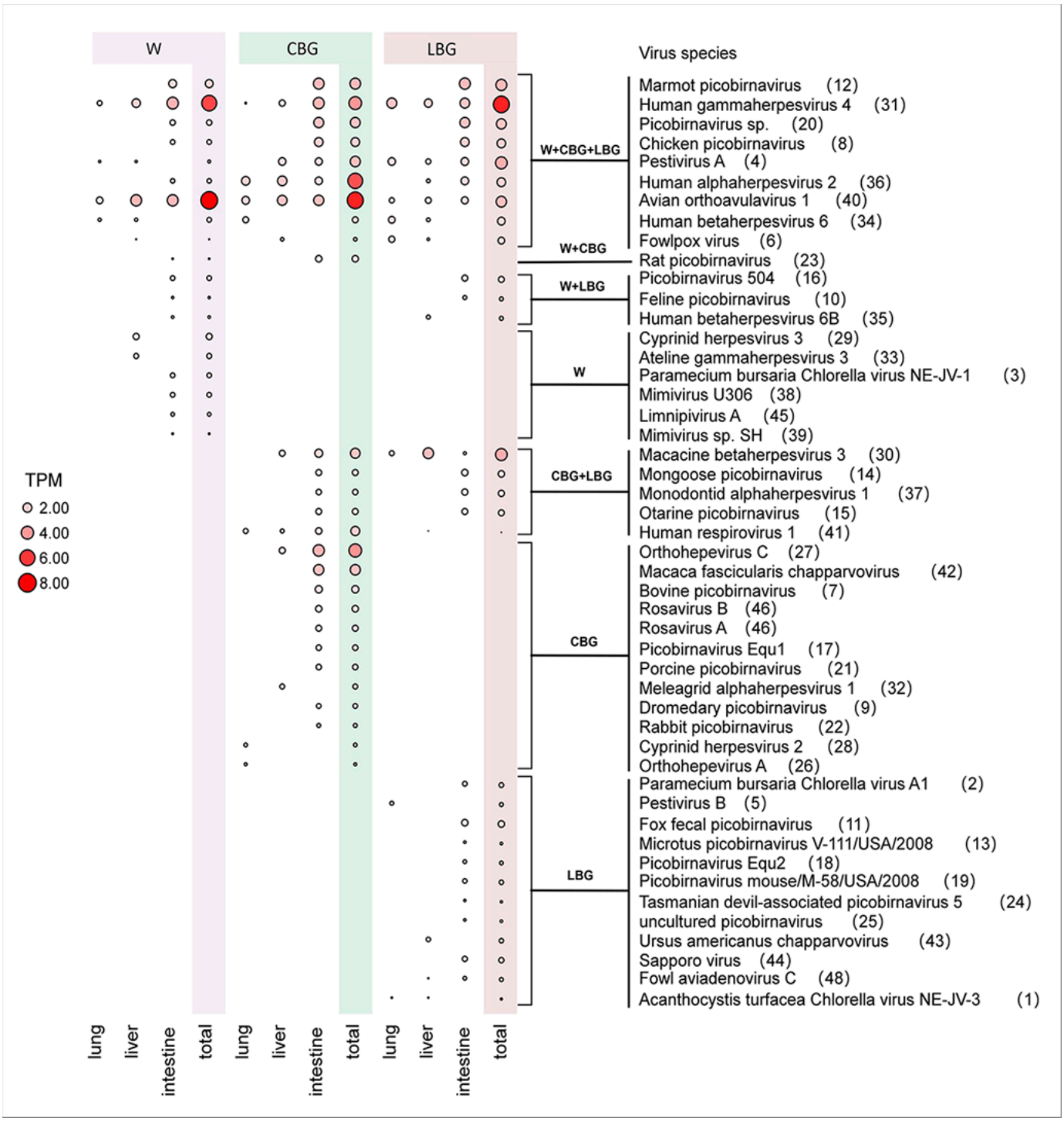
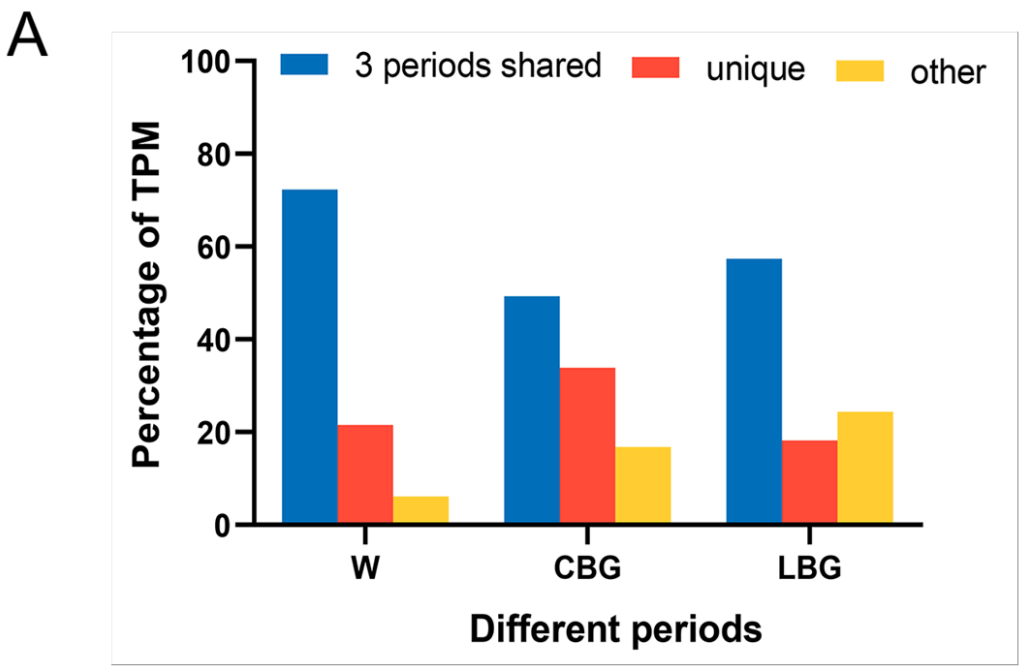


Figure 1

The virome composition of three generations of *M. meridianus*

Note: The vertical axis represent all virus species after removing Unclassified and Retroviridae viruses, and the code represent the sequence numbers of viruses sorted by the first letter of their respective families. The horizontal axis indicate three generations of *M. meridianus* and its different organs. Different colors represent the total number of viruses carried by *M. meridianus* from various tissues during different generations. The three columns for each time period represent the number of viruses carried by lung, liver, and intestinal tissues, respectively. The red circles inside the graph indicate the amount of virus, with varying shades and sizes representing different quantities.



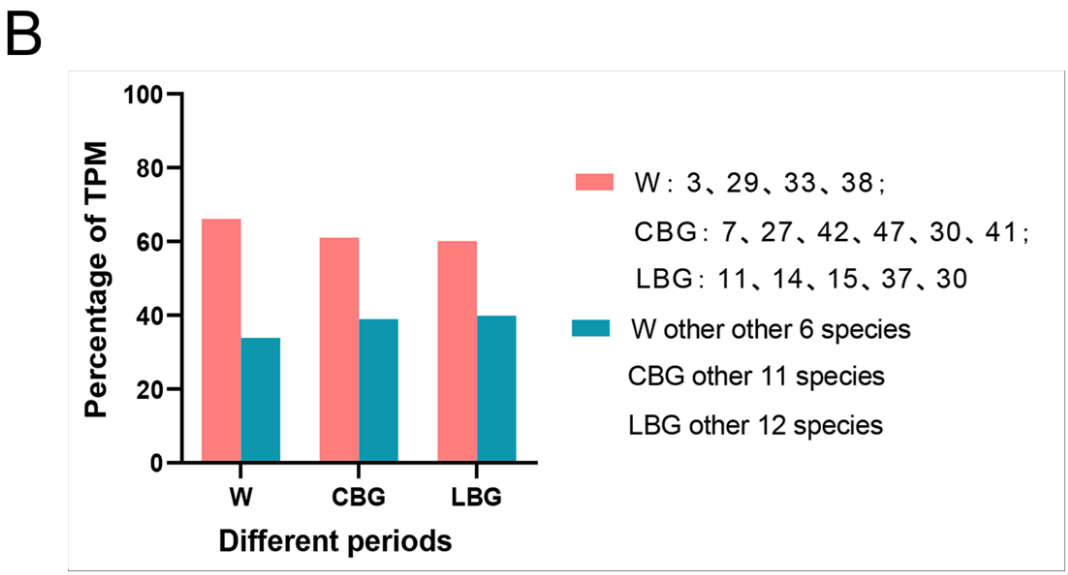


Figure 2

The values of TPM analysis of major viruses in the three generations of *M. meridianus*

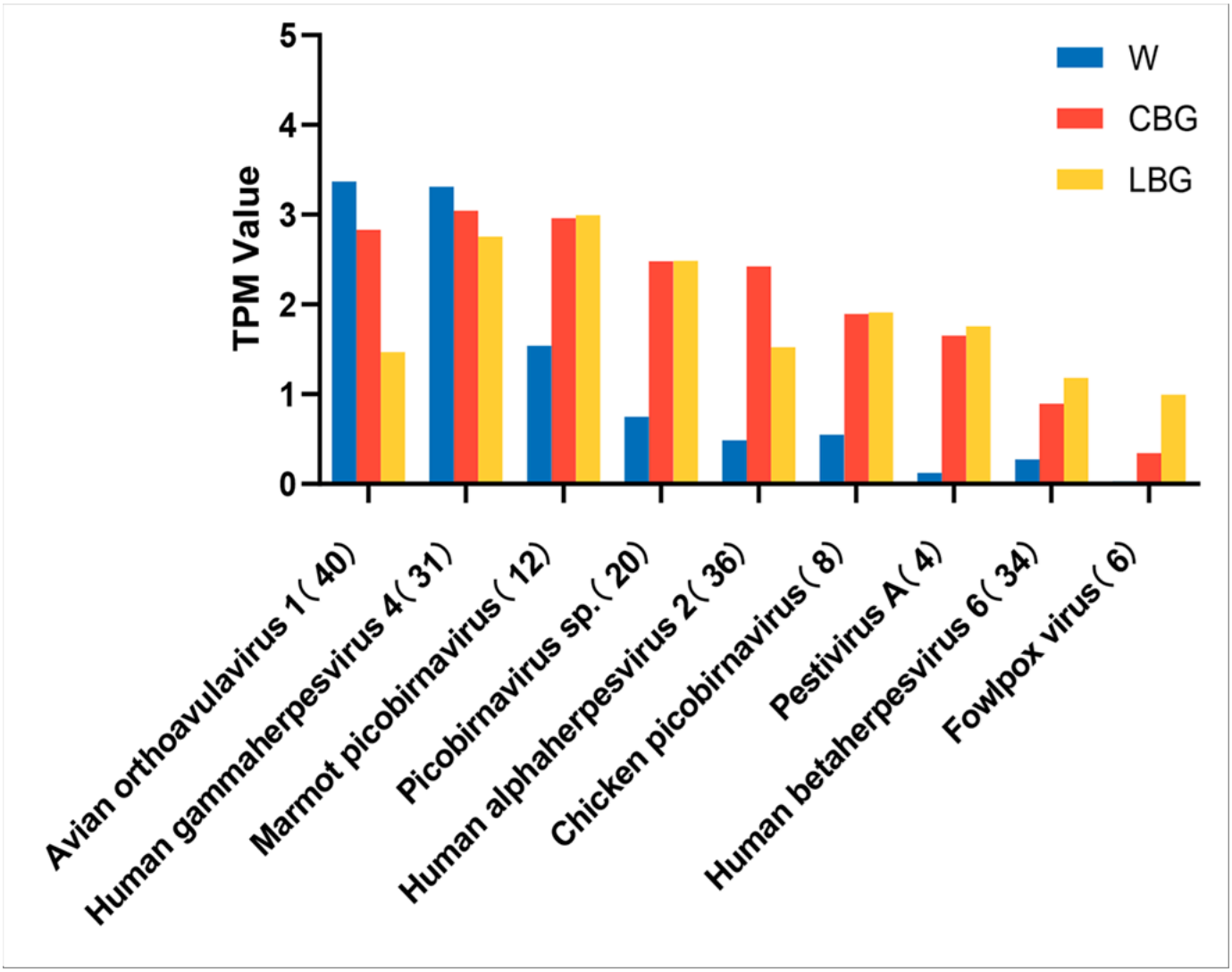


Figure 3

The abundance (TPM) of spectrum among 9 shared virus species across three generations

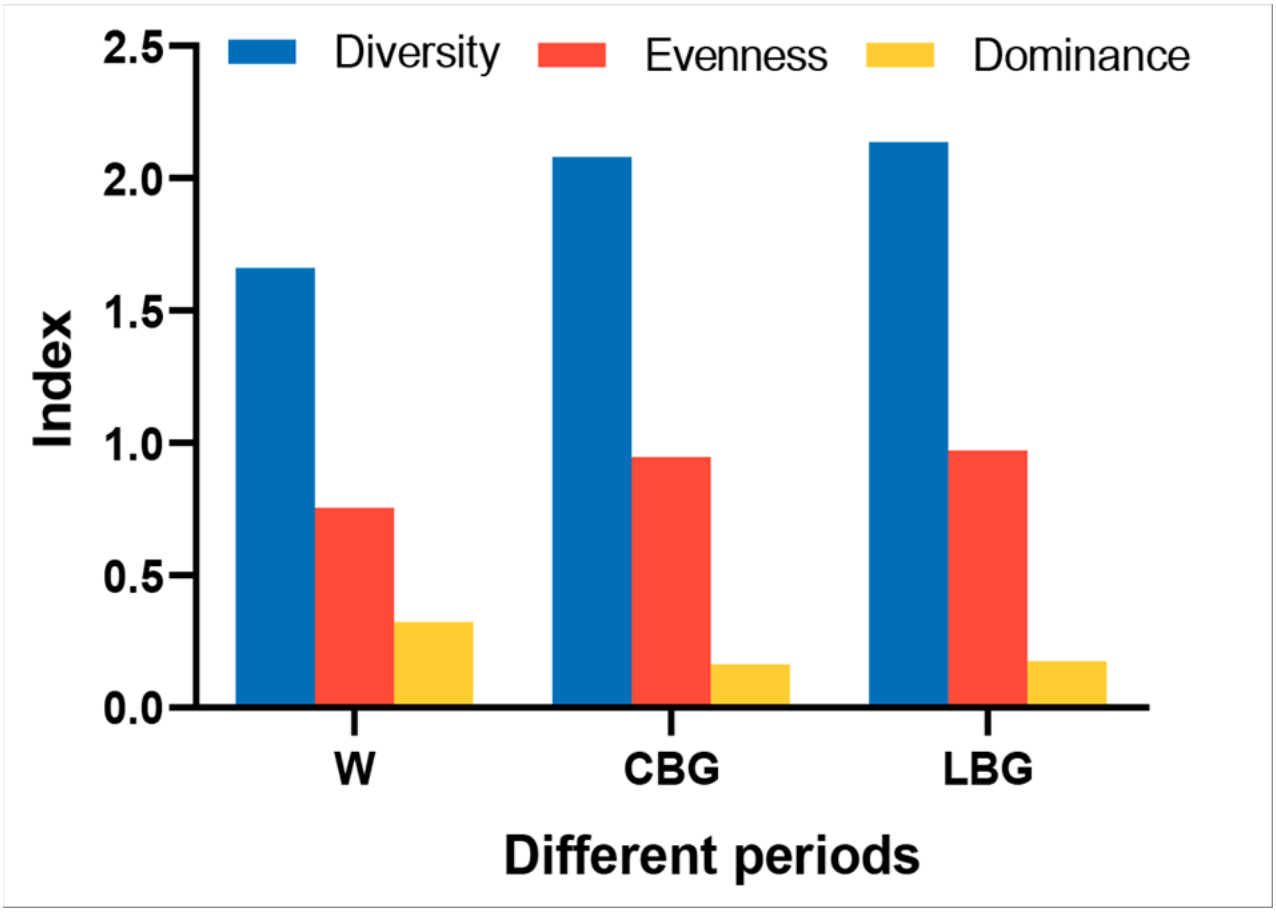


Figure 4

Diversity, evenness, and dominance of the abundance of the 9 shared viruses in the three generations of *M. meridianus*

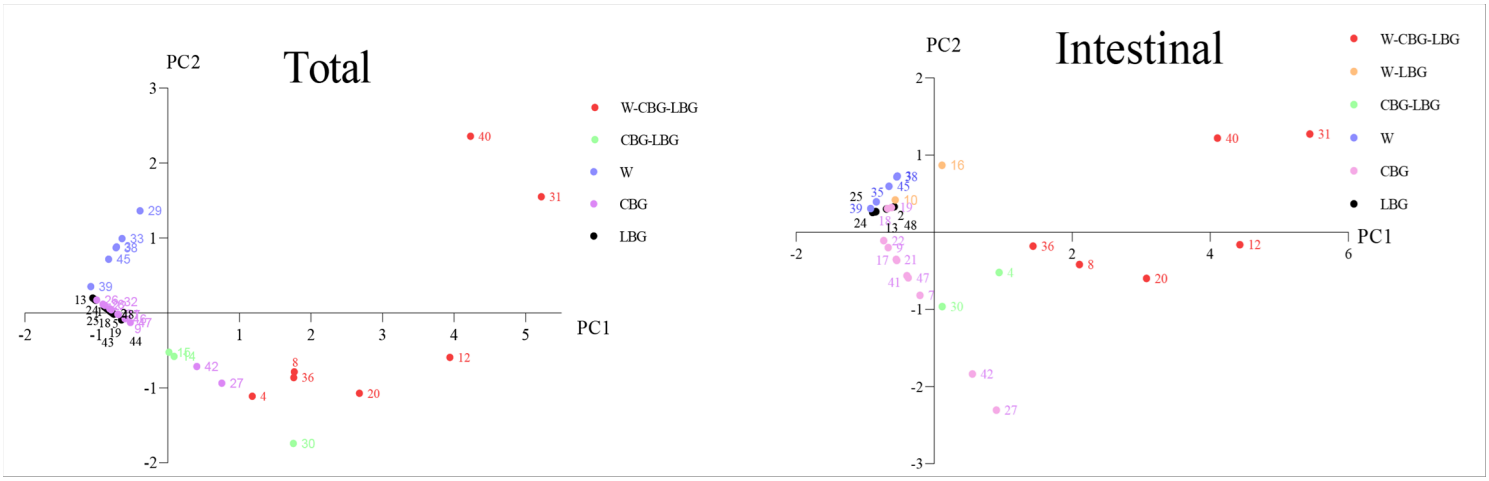


Figure 5

Principal Component Analysis of the Total and Intestinal Virome in the Three Generations of *M. meridianus*

Note:the code represent the sequence numbers of viruses sorted by the first letter of their respective families.

Supplementary Files

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- TableS1.xlsx