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Journal of General Virology

Research Article

Characterization of mammalian orthoreoviruses isolated from feces of pigs in Zambia

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Table S1. Genome organization of Zambian MRV strains

Segment	Genome length (bp)	The position of ORF	Protein
L1	3,854	19-3,822	λ3 (RNA-dependent RNA polymerase)
L2	3,915	13-3,882	λ2 (core-spike protein)
L3	3,901	14-3,841	λ1 (major core protein)
M1	2,304	14-2,224	μ2 (minor core protein)
M2	2,203	30-2,156	μ1 (outer capsid protein)
M3	2,241	19-2,184	μNS (non-structural protein)
S1	1,433	14-1,396	σ1 (cell attachment protein)
S2	1,331	19-1,275	σ2 (major core protein)
S3	1,198	28-1,128	σNS (non-structural protein)
S4	1,196	33-1,130	σ3 (outer capsid protein)

Table S2. Identity comparison of S1 segment and σ1 protein among Zambian strains, MRV prototypes and other representative strains

MRV Strain		Zambian strain			T1						T2								T3						T4
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Zambian strain	**1**. 85		*97.6*	*97.6*	*59.2*	*58.2*	*58.7*	*58.6*	*57.0*	*57.1*	*61.0*	*64.8*	*62.8*	*85.2*	*61.9*	*64.2*	*64.5*	*63.7*	*48.6*	*49.4*	*52.1*	*49.5*	*49.5*	*49.4*	*46.6*
	2. 96	**95.6**		*97.3*	*59.5*	*57.4*	*59.5*	*58.7*	*57.9*	*58.2*	*61.1*	*65.1*	*63.1*	*84.9*	*61.9*	*64.2*	*64.7*	*64.4*	*48.9*	*49.3*	*50.2*	*49.5*	*50.0*	*49.9*	*48.6*
	3. 117	**96.0**	**96.5**		*59.3*	*59.5*	*59.9*	*59*	*57.3*	*58.2*	*61.8*	*65.3*	*63.3*	*85.2*	*62.1*	*64.1*	*65.4*	*63.9*	*50.4*	*48.8*	*50.6*	*50.2*	*48.9*	*51.3*	*48.3*
Type 1 serotype	**4**. T1L	**48.6**	**49.3**	**49.3**		*91.8*	*91.7*	*73.3*	*72.4*	*74.1*	*59.4*	*58.3*	*61.2*	*59.3*	*60.2*	*60.5*	*60.6*	*60.9*	*49.4*	*50.3*	*50.9*	*48.9*	*50.3*	*50.3*	*52.7*
	5. WIV8	**49.5**	**50.2**	**50.2**	**96.5**		*96.2*	*73.9*	*72.3*	*74.7*	*58.9*	*58.8*	*60.5*	*58.2*	*58.8*	*60.9*	*59.6*	*58.7*	*48.7*	*51.0*	*51.4*	*50.7*	*49.6*	*47.6*	*50.6*
	6. HB-B	**50.0**	**50.6**	**50.6**	**96.3**	**98.5**		*74.9*	*73.3*	*74.5*	*59.4*	*58.3*	*59.8*	*59.2*	*59.1*	*60.0*	*60.2*	*59.7*	*49.4*	*49.8*	*52.7*	*49.8*	*50.7*	*49.0*	*48.5*
	7. MRV00304	**49.5**	**50.4**	**50.2**	**77.8**	**78.5**	**78.5**		*92.9*	*88.0*	*58.1*	*58.1*	*59.3*	*58.7*	*59.1*	*60.0*	*61.6*	*59.1*	*49.9*	*48.6*	*48.1*	*49.3*	*50.1*	*49.4*	*47.8*
	8. SHR-A	**48.0**	**49.3**	**48.6**	**74.4**	**74.6**	**74.8**	**91.7**		*87.2*	*58.7*	*57.8*	*58.0*	*57.9*	*58.0*	*59.4*	*60.7*	*58.5*	*48.3*	*51.1*	*50.2*	*51.8*	*52.5*	*50.7*	*50.0*
	9. SI-MRV06	**50.4**	**51.5**	**51.3**	**79.1**	**79.3**	**79.1**	**89.3**	**85.5**		*58.3*	*58.9*	*59.8*	*59.5*	*58.6*	*60.4*	*61.7*	*59.3*	*48.3*	*46.5*	*50.4*	*47.8*	*49.8*	*47.8*	*49.6*
Type 2 serotype	**10**. T2J	**58.0**	**59.3**	**60.4**	**50.1**	**50.9**	**51.1**	**50.1**	**49.6**	**50.7**		*62.3*	*62.8*	*63.2*	*69.5*	*63.0*	*61.2*	*64.2*	*50.0*	*50.5*	*50.1*	*51.0*	*52.1*	*51.0*	*51.2*
	11. TRALAU2004	**59.6**	**60.7**	**61.4**	**45.4**	**45.9**	**46.1**	**44.2**	**42.8**	**46.2**	**57.6**		*65.4*	*67.4*	*62.0*	*66.0*	*64.4*	*64.6*	*48.5*	*50.8*	*50.4*	*49.6*	*47.9*	*50.4*	*49.2*
	12. T2W	**60.4**	**60.8**	**61.5**	**50.1**	**50.3**	**50.3**	**48.3**	**47.8**	**50.7**	**58.6**	**58.9**		*64.0*	*65.8*	*66.2*	*65.3*	*94.4*	*50.5*	*50.0*	*49.4*	*47.6*	*49.8*	*51.3*	*50.1*
	13. MORV/47Ma	**85.8**	**86.0**	**87.3**	**50.8**	**51.7**	**52.1**	**51.7**	**51.3**	**53.2**	**61.5**	**63.2**	**64.6**		*63.1*	*66.3*	*67.2*	*63.6*	*51.2*	*47.1*	*48.0*	*49.4*	*50.4*	*49.7*	*50.7*
	14. SI-MRV07	**58.0**	**58.6**	**59.3**	**52.1**	**52.8**	**52.8**	**50.4**	**48.5**	**50.7**	**73.3**	**55.4**	**58.3**	**61.7**		*64.4*	*62.1*	*65.2*	*47.4*	*48.2*	*49.2*	*47.3*	*49.9*	*50.4*	*48.3*
	15. CMR-HP55	**65.2**	**65.4**	**65.8**	**53.3**	**54.2**	**54.0**	**52.0**	**49.2**	**53.7**	**61.3**	**63.0**	**61.0**	**68.4**	**62.0**		*64.3*	*66.2*	*48.2*	*49.1*	*49.0*	*47.5*	*48.5*	*47.9*	*49.9*
	16. BYD1	**63.6**	**64.3**	**65.4**	**52.7**	**53.1**	**53.5**	**51.8**	**51.1**	**54.4**	**59.8**	**60.3**	**61.9**	**69.3**	**59.6**	**64.8**		*66.2*	*49.6*	*49.3*	*52.3*	*47.2*	*49.4*	*49.6*	*50.7*
	17. 729	**64.1**	**65.0**	**65.6**	**52.8**	**52.6**	**52.6**	**50.8**	**50.4**	**53.9**	**61.9**	**62.5**	**92.8**	**68.0**	**61.9**	**64.7**	**65.4**		*47.6*	*48.0*	*49.6*	*49.8*	*48.9*	*48.3*	*50.3*
Type 3 serotype	**18**. T3D	**26.2**	**26.2**	**26.7**	**25.3**	**24.8**	**25.1**	**13.7**	**16.8**	**23.7**	**27.9**	**25.6**	**25.6**	**28.3**	**26.8**	**29.1**	**25.3**	**26.6**		*78.5*	*80.6*	*85.4*	*85.6*	*85.0*	*71.0*
	19. BM-100	**25.3**	**24.8**	**25.5**	**24.8**	**24.6**	**25.1**	**24.6**	**23.3**	**23.7**	**27.0**	**24.0**	**26.3**	**27.1**	**27.5**	**27.5**	**26.0**	**26.6**	**85.0**		*88.1*	*79.5*	*79.7*	*78.2*	*70.5*
	20. SI-MRV01	**25.6**	**25.1**	**25.8**	**24.4**	**24.4**	**24.8**	**23.0**	**23.2**	**23.4**	**25.2**	**24.2**	**25.6**	**27.6**	**26.3**	**27.5**	**25.5**	**26.4**	**88.3**	**91.2**		*81.5*	*81.6*	*80.2*	*71.9*
	21. MPC/04	**26.0**	**26**	**25.8**	**24.8**	**24.8**	**24.8**	**16.5**	**15.9**	**23.4**	**28.1**	**23.7**	**24.9**	**28.0**	**26.8**	**28.9**	**25.7**	**26.0**	**91.8**	**87.0**	**90.7**		*94.5*	*93.1*	*70.8*
	22. GD-1	**26.7**	**26.7**	**27.1**	**24.6**	**24.6**	**24.6**	**16.7**	**16.0**	**23.4**	**27.5**	**24.6**	**25.1**	**28.3**	**26.3**	**29.1**	**25.5**	**26.2**	**92.7**	**87.6**	**90.7**	**96.2**		*93.6*	*70.9*
	23. ZJ2013	**26.0**	**26.2**	**26.5**	**24.6**	**24.2**	**24.4**	**16.0**	**15.3**	**24.1**	**28.1**	**24.0**	**24.4**	**28.5**	**26.6**	**28.9**	**25.3**	**25.7**	**91.4**	**84.8**	**87.9**	**92.7**	**94.5**		*70.5*
Type 4 serotype	**24**. T4N	**27.3**	**26.8**	**27.0**	**25.9**	**26.2**	**26.2**	**23.1**	**23.1**	**22.8**	**28.1**	**23.6**	**24.1**	**27.0**	**26.2**	**28.7**	**28.7**	**26.6**	**68.3**	**66.8**	**68.9**	**70.1**	**70.1**	**67.5**	

Nucleotide identities of S1 segment are in the top right triangle in italics, and amino acid identities of σ1 protein are in the bottom triangle in bold text. The red-boxed areas indicated the identity among Zambian strains and type 2 MRV strains. Abbreviation: T1, Type 1 serotype; T2, Type 2 serotype; T3, Type 3 serotype; T4, Type 4 serotype; T1L, serotype 1 Lang strain; T2J, serotype 2 Jones strain; T3D, serotype 3 Dearing strain; T4N, serotype 4 Ndelle strain; nt, nucleotide identity; aa, amino acid identity.

Table S3. Primer sets used in this study

Primer	Sequence (5'-3')	Target gene	Reference
1607F	CARMGNCGNSCHMGHTCHATHATGCC	L1	[46]
2608R	TAVAYRAAVGWCCASMHNGGRTAYTG		
2090F	GGBTCMACNGCYACYTCBACYGAGCA		
2334R	CDATGTCRTAHWYCCANCCRAA		
REOL3F	CAGTCGACACATTTGTGGTC	L3	[47]
REOL3R	GCGTACTGACGTGGATCATA		
MRV-L1-5'R	GCACATACACGTCATCAGGGCCTTG	L1	This study
MRV-L1-3'F	ATGTCAAGCTGCTCCCCAAAGATGG		
MRV-L2-5'R	ACCTCGTATTTCAACACGCGGAG	L2	
MRV-L2-3'F	TCCTGATATGCGTGAGTGGGCAG		
MRV-L3-5'R	CTGGTCGAGGTTTGTATTGCTCTCG	L3	
MRV-L3-3'F	CCATCGCTGGACCAGACAAGCAC		
MRV-M1-5'R	CAACATTGTCCGGTATCGCTGAAGG	M1	
MRV-M1-3'F	GTATCTGCTAAGGCGGTGATCAAGG		
MRV-M2-5'R	ATCCTTCGAGGTCATTCGCCGCAAC	M2	
MRV-M2-3'F	GGATTGCATTGGCACAAGGAGTC		
MRV-M3-5'R	GGCATGAGCTGGTGAAATTACTCCAGAG	M3	
MRV-M3-3'F	CGGAATTGGTGAGTACCATCACC		
MRV-M3-3'F2	ATGAATGGAAAGCAGGCACGAGAG		
MRV-S1-5'R	GTTCGCTTGCAGCCACTCTATTGC	S1	
MRV-S1-3'F	CAGGATGGGATACTGGTGCCACC		
MRV-S2-5'R	GAAATGGGATCTGGAGAGCACCAC	S2	
MRV-S2-3'F	GACGGTGTCATGACTCAAGCTCAAC		
MRV-S3-5'R	CGTTCCAGGTTCCTCTGTCCAAGC	S3	
MRV-S3-3'F	CGATGAGACTTTGGACTCGATCGG		
MRV-S3-3'F2	CGATGAGACTCTGGGCTCGATCG		
MRV-S4-5'R	GTAATCCTGTTCGCGCAACTGCTG	S4	
MRV-S4-3'F	ACGTGGGGTTTTGAGAAGATCAGG		

Table S4. Reference sequences used in this study

Strain	Genbank accession number									
	L1	L2	L3	M1	M2	M3	S1	S2	S3	S4
T1/human/USA/Lang	M24734	AF378003	AF129820	AF461682	AF490617	AF174382	M35963	L19774	M18389	X61586
T1/human/USA/Wash.D.C/clone62/1957	-	-	-	-	-	-	-	-	U35356	-
T1/human/NLD/Netherlands 84/1984	-	-	-	AX428872	-	-	-	-	-	-
T1/pig/CHN/SHR-A/2011	JX415466	JX415467	JX415472	JX415468	JX415471	JX415474	JX415469	JX415465	JX415470	JX415473
T1/bat/CHN/WIV8/2011	KT444562	KT444563	KT444564	KT444565	KT444566	KT444567	KT444568	KT444569	KT444570	KT444571
T1/bat/ITA/BatMRV1-IT2011/2011	KT900695	KT900696	KT900697	KT900698	KT900699	KT900700	KT900701	KT900702	KT900703	KT900704
T1/bat/SLO/SI-MRV06/2009	MG457118	MG457119	MG457120	MG457121	MG457122	MG457123	MG457124	MG457125	MG457126	MG457127
T1/mink/CHN/HB-A/2013	KC462149	KC462150	KC462151	KC462152	KC462153	KC462154	KC462155	KC462156	KC462157	KC462158
T1/mink/CHN/HB-B/2013	-	-	-	-	-	-	KF013855	-	KF013856	-
T1/cattle/USA/MRV00304/2014	KJ676379	KJ676380	KJ676381	KJ676382	KJ676383	KJ676384	KJ676385	KJ676386	KJ676387	KJ676388
T2/human/USA/Jones	M31057	AF378005	AF129821	AF124519	M19355	AF174383	M35964	L19775	M18390	X60066
T2/human/NLD/Netherlands 84/1984	-	-	-	-	-	-	AY862138	-	-	-
T2/human/CAN/T2W/1997	-	-	-	-	-	-	DQ220017	DQ220020	DQ220018	DQ220019
T2/human/FRA/MRV2Tou05/2005	GU196306	GU196307	GU196308	GU196309	GU196310	GU196314	GU196315	GU196311	GU196312	GU196313
T2/human/CHN/BYD1/2006	DQ664184	DQ664185	DQ664186	DQ664187	DQ664188	DQ664189	DQ312301	DQ664190	DQ664191	DQ318037
T2/human/CMR/CMR-HP55/2014	MH933769	MH933770	MH933771	MH933772	MH933773	MH933774	MH933775	MH933776	MH933777	MH933778
T2/human/SLO/SI-MRV07/2017	MG999576	MG999577	MG999578	MG999579	MG999580	MG999581	MG999582	MG999583	MG999584	MG999585
T2/pig/AUT/729/1998	JN799426	JN799427	JN799428	JN799423	JN799424	JN799425	JN799419	JN799420	JN799421	JN799422
T2/bat/SLO/SI-MRV05/2008	MG457108	MG457109	MG457110	MG457111	MG457112	MG457113	MG457114	MG457115	MG457116	MG457117
T2/bat/CHN/WIV3/2011	KT444572	KT444573	KT444574	KT444575	KT444576	KT444577	KT444578	KT444579	KT444580	KT444581
T2/bat/CHN/RpMRV-YN2012/2012	KM087105	KM087106	KM087107	KM087108	KM087109	KM087110	KM087111	KM087112	KM087113	KM087114
T2/rodent/GER/TRALAU2004/2004	JX204738	JX204739	JX204740	JX204741	JX204742	JX204743	JX204737	JX204744	JX204745	JX204746
T2/rodent/HUN/MORV/47Ma/06/2006	KX384846	KX384847	KX384848	KX384849	KX384850	KX384851	KX384852	KX384853	KX384854	KX384855
T3/human/USA/Dearing	HM159613	HM159614	HM159615	HM159616	HM159617	HM159618	EF494441	HM159620	HM159621	HM159622
T3/human/USA/Colorado/1996	-	-	-	-	-	-	AY302467	-	-	-
T3/human/SLO/SI-MRV01	KF154724	KF154725	KF154726	KF154727	KF154728	KF154729	KF154730	KF154731	KF154732	KF154733
T3/pig/CHN/GD-1/2012	JX486057	JX486058	JX486059	JX486060	JX486061	JX486062	JX486063	JX486064	JX486065	JX486066
T3/pig/CHN/ZJ2013/2013	KY419120	KY419121	KY419122	KY419123	KY419124	KY419125	KY419126	KY419127	KY419128	KY419129
T3/pig/USA/FS-03/2014	KM820754	KM820755	KM820756	KM820757	KM820758	KM820759	KM820760	KM820761	KM820762	KM820763
T3/pig/USA/BM-100/2014	KM820744	KM820745	KM820746	KM820747	KM820748	KM820749	KM820750	KM820751	KM820752	KM820753
T3/pig/ITA/224660-4/2015	KX343200	KX343201	KX343202	KX343203	KX343204	KX343205	KX343206	KX343207	KX343208	KX343209
T3/bat/GER/342/2008	JQ412755	JQ412756	JQ412757	JQ412758	JQ412759	JQ412760	JG412761	JG412762	JG412763	JG412764
T3/rodent/FRA/clone9/1961	-	AF378007	-	-	-	-	-	-	-	-
T3/masked palm civet/CHN/MPC/04/2004	GQ468266	GQ468267	GQ468270	GQ468268	GQ468269	GQ468271	GQ468272	GQ468273	GQ468274	GQ468275
T3/cattle/USA/Maryland/clone18/1961	-	AF378008	-	-	-	-	-	-	U35358	-
T4/rodent/CMR/Ndelle	AF368033	-	-	-	AF368034	-	AF368035	AF368036	-	AF368037

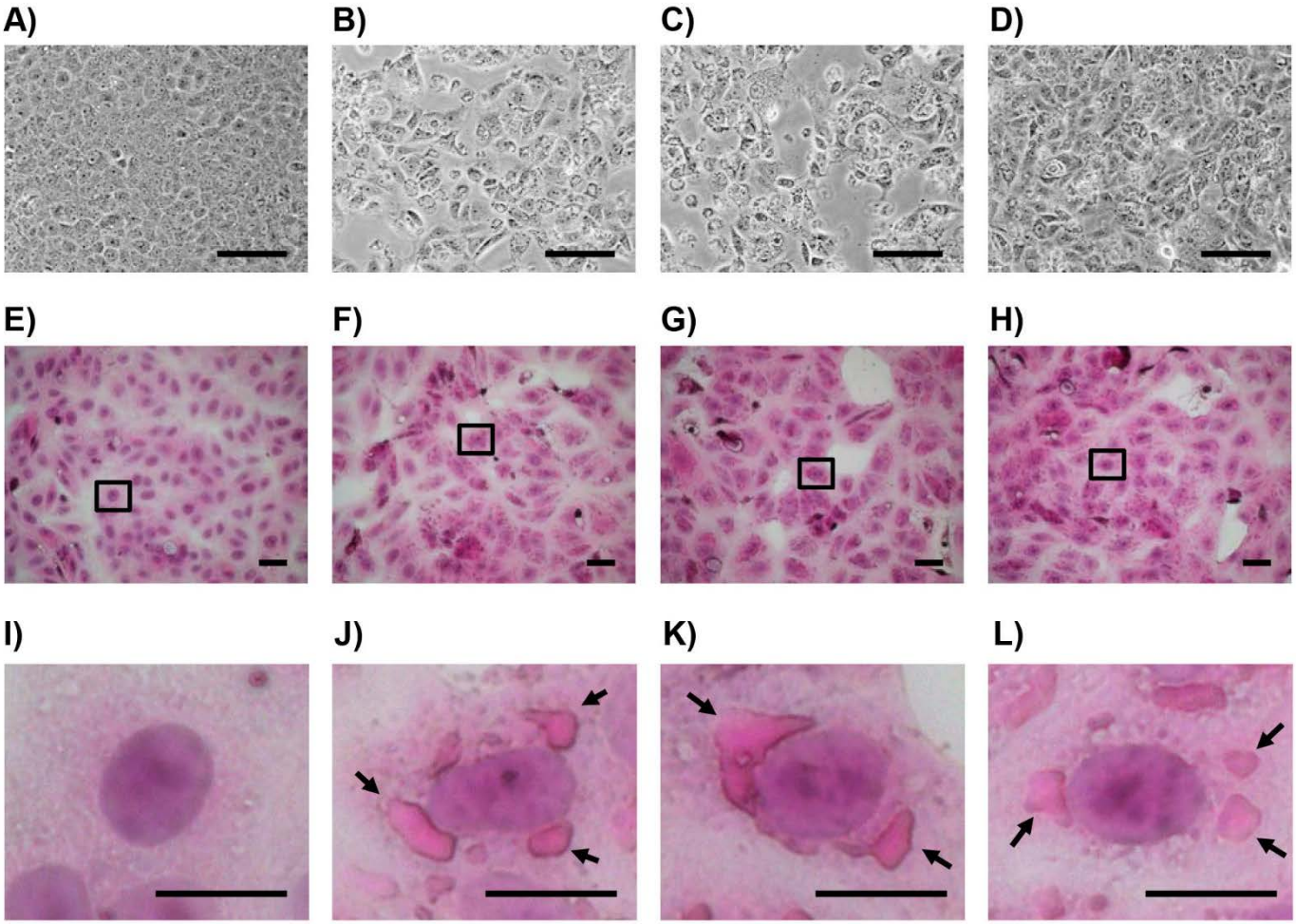
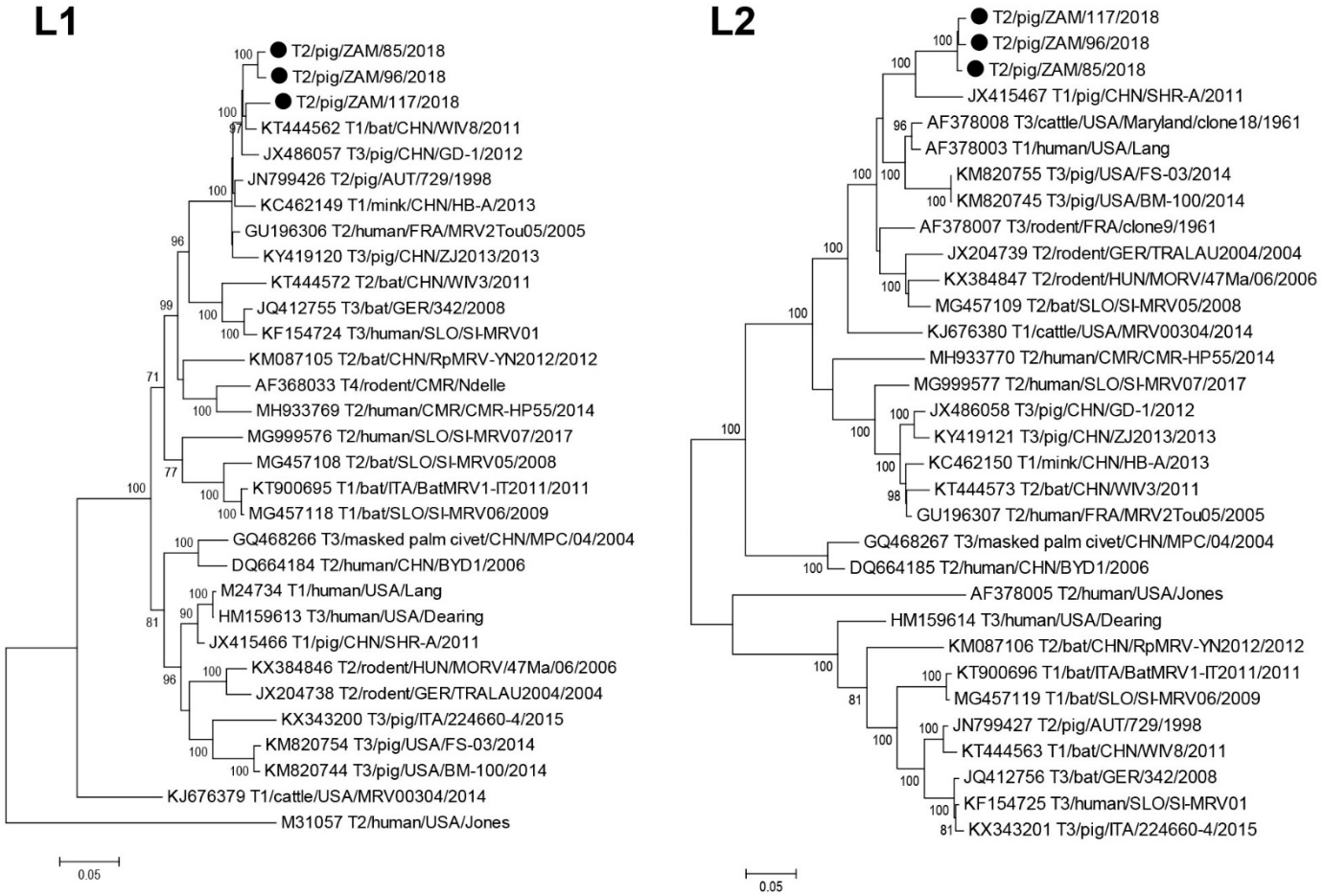


Fig. S1. Isolation of mammalian orthoreoviruses (MRVs) from fecal samples of pigs. (A-D) Phase-contrast images of mock-infected (A), MRV-85–infected (B), MRV-96–infected (C) and MRV-117–infected Vero E6 cells (D) at 24 h post-infection (hpi). The cells infected with MRV-85, -96 and -117 exhibited cytopathic effects. Scale bar, 100 µm. (E-L) Hematoxylin-eosin staining images of mock-infected (E), MRV-85–infected (F), MRV-96–infected (G) and MRV-117–infected Vero E6 cells (H) at 16 hpi. Scale bar, 20 µm. Figure S1I, J, K and L are the magnified image of the boxed area in Figure S1E, F, G and H, respectively. Eosinophilic inclusion bodies were observed in the cytoplasm of MRV-infected cells (black arrows). Scale bar, 10 µm.


L1
T2/pig/ZAM/85/2018
T2/pig/ZAM/96/2018
T2/pig/ZAM/117/2018
KT444562 T1/bat/CHN/WIV8/2011
JX486057 T3/pig/CHN/GD-1/2012
JN799426 T2/pig/AUT/729/1998
KC462149 T1/mink/CHN/HB-A/2013
GU196306 T2/human/FRA/MRV2Tou05/2005
KY419120 T3/pig/CHN/ZJ2013/2013
KT444572 T2/bat/CHN/WIV3/2011
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HM159613 T3/human/USA/Dearing
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KX384846 T2/rodent/HUN/MORV/47Ma/06/2006
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KX343200 T3/pig/ITA/224660-4/2015
KM820754 T3/pig/USA/FS-03/2014
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JX204739 T2/rodent/GER/TRALAU2004/2004
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MG999577 T2/human/SLO/SI-MRV07/2017
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KY419121 T3/pig/CHN/ZJ2013/2013
KC462150 T1/mink/CHN/HB-A/2013
KT444573 T2/bat/CHN/WIV3/2011
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GQ468267 T3/masked palm civet/CHN/MPC/04/2004
DQ664185 T2/human/CHN/BYD1/2006
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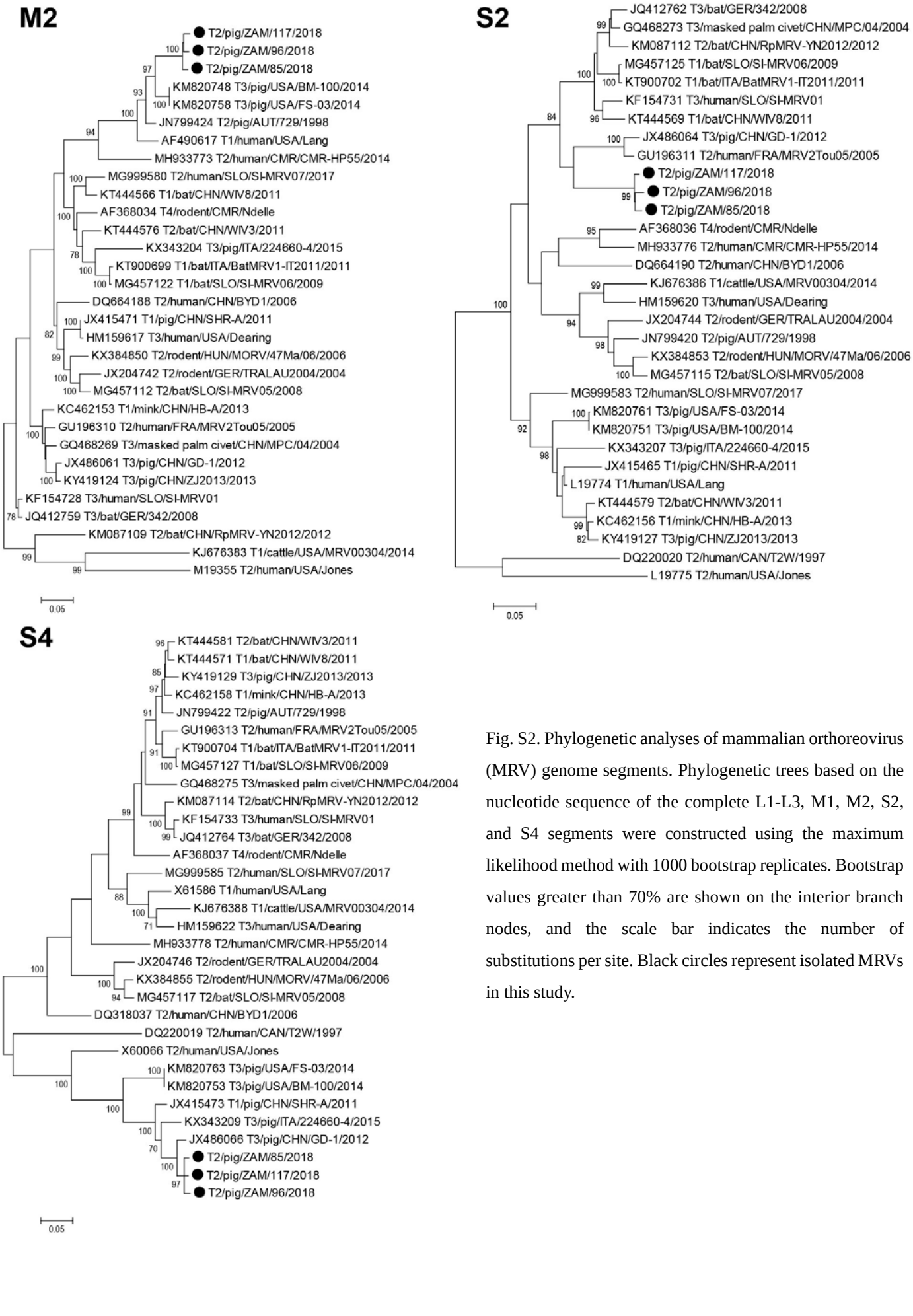


Fig. S2. Phylogenetic analyses of mammalian orthoreovirus (MRV) genome segments. Phylogenetic trees based on the nucleotide sequence of the complete L1-L3, M1, M2, S2, and S4 segments were constructed using the maximum likelihood method with 1000 bootstrap replicates. Bootstrap values greater than 70% are shown on the interior branch nodes, and the scale bar indicates the number of substitutions per site. Black circles represent isolated MRVs in this study.