Table 1: Factors associated with pooled sero-prevalence of PRV infection among pigs in China

Factors		Study numbers	Tested numbers	Positive numbers	Positive rate (%) (95 CI)	P value
Region	Northeastern China	7	5703	1284	22.5 (21.4-23.6)	*P*<0.001
	Northern China	5	10740	4257	39.6 (38.7-40.6)	*P*<0.001
	Northwestern China	8	12738	3125	24.5 (23.8-25.3)	*P*<0.001
	Central and Southern China	27	83202	24744	29.7 (29.4-30.1)	*P*<0.001
	Eastern China	38	96339	34534	35.8 (35.6-36.2)	*P*<0.001
	Southwestern China	22	43800	7211	16.5 (35.4-36.3)	Reference
Feeding pattern	Free range farm	8	5396	2272	42.1 (40.8-43.4)	*P*<0.001
	Small farm	9	12218	4194	34.3 (33.5-35.2)	*P*<0.001
	Middle pig farm	9	16215	5771	35.6 (33.6-35.1)	*P*<0.001
	Intensive pig farm	10	18621	3558	19.1 (18.6-19.7)	*P*<0.001
	Breeding pig farm	1	414	19	4.2 (2.2-6.1)	Reference
Season	Spring	6	5026	1176	23.4 (22.2-24.6)	*P*<0.05
	Summer	6	7060	2102	29.8 (28.7-30.8)	P<0.001
	Autumn	6	8262	1763	21.3 (20.5-22.2)	Reference
	Winter	6	8142	2304	28.3 (27.3-29.3)	P<0.001
Developmental stage	Piglets	12	4353	1102	25.3 (24.0-26.6)	*P*<0.001
	Nursery pigs	13	5965	2159	36.1 (34.9-37.4)	*P*<0.001
	Growing-Finishing Pigs	11	4539	1151	25.4 (24.1-26.6)	*P*<0.001
	Gilts	10	4546	1507	33.2 (31.8-34.5)	*P*<0.001
	Boars	10	2258	447	19.8 (18.2-21.4)	Reference
	Reproductive Pigs	15	15153	5049	33.3 (32.6-34.1)	*P*<0.001

Note: The statistics of different groups were analyzed using the SPSS 22.0 (IBM Corp), A p value < 0.05 was considered as statistically significant. Additionally, odd

ratios (ORs) with 95% confidence intervals (95CI) based on likelihood ratio statistics were analyzed.

Table 2: Pooled epidemiology of PRV infection among pigs with different clinical signs in China

Clinical signs	Study numbers	Tested numbers	Positive numbers	Positive rate (%) (95 CI)	P value
Nervous symptoms	1	84	63	75.0 (65.7-84.3)	*P*<0.001
Diarrhea	4	1251	460	36.8 (34.1-39.5)	*P*<0.001
Reproductive failure	2	1739	1025	58.9 (34.5-39.1)	*P*<0.001
Other symptoms	18	26325	3014	11.5 (58.4-59.5)	*P*<0.001
Health	2	11838	180	1.5 (1.3-1.7)	Reference

Note: The statistics of different groups were analyzed using the SPSS 22.0 (IBM Corp). A p value < 0.05 was considered as statistically significant. Additionally, odd ratios (ORs) with 95% confidence intervals (95CI) based on likelihood ratio statistics were analyzed.

Table 3: Clinical signs and pathological characteristics of different PRV-infected species in China

Species	Case numbers	Clinical signs	Pathological characteristics	Reference
Cattle/cow	10	Pruritus, nervous symptoms, being excited and manic, etc.	Leptomeningeal hyperemia, consolidation of lung lobes, etc.	(Chen et al., 2020)
Dog	12	Pruritus, hypersalivation, broken winded, etc.	Endocardial and thymic hemorrhage, pulmonary hemorrhage and/or congestion, etc.	(Zhang et al., 2015b)
wolf	1	Pruritus, vomiting, quadriplegia, etc.	hemorrhagic spots and edema in the meninges Hemorrhagic spots and necrosis in the liver, etc.	(Lian et al., 2020)
Goat/sheep	11	Pruritus, nervous symptoms, muscle spasm, etc.	Leptomeningeal hyperemia, consolidation of lung lobes, etc.	(Zhang et al., 2016)
fox	2	Pruritus, vomiting, broken winded, etc.	Sugillation in the lung, hemorrhage in the spleen, thymus and liver, etc.	(Jin et al., 2016)
Mink	9	Pruritus, diarrhea, muscle spasm, etc.	Hemorrhage in the thymus and submandibular lymph node, liver and spleen tumefaction, etc.	(Liu et al., 2014)
Nyctereutes	3	Pruritus, vomiting, etc.	Not mentioned	(Liu et al., 2016)

Table 4: Updated information of human PRV infection cases in China

Date	Interval after injury	Age/Sex	Occupation	Injured at work	symptoms	Diagnostic methods	Treatment	Reference
NA	4 days	43/male	Veterinarian	Yes	Fever, headache, tonic-clonic seizures and coma	NGS; PCR and ELISA methods	Acyclovir treatment for 2 weeks; antibiotics	(Yang et al., 2019)
3.18.2018	5 days	50/male	Pig slaughterer	Yes	Fever, headache, visual disturbance, coma	NGS	IVIG treatment for 5 days, Glucocorticoids, antiviral and other treatments	(Yang et al., 2019)
3.3.2018	7 days	50/female	Pork cutter	Yes	Fever, coma, respiratory failure, seizure	NGS		(Yang et al., 2019)
3.27.2018	7 days	43/male	Sick pig handler	Yes	Fever, extremity tremors, respiratory failure	NGS		(Yang et al., 2019)
4.23.2018	10 days	59/male	Pork cutter	Yes	Fever, seizures, respiratory failure	NGS		(Yang et al., 2019)
4.26.2018	NA	50/male	Pork cutter	NA	Fever, seizure, respiratory failure	NGS		(Yang et al., 2019)
NA	6 days	59/male	Swineherd	Yes	Fever, seizures, tonic-clonic seizures and coma	NGS, ELISA method	Antimicrobial therapy, penciclovir and foscarnet sodium treatment for 17 days	(Zheng et al., 2019)
3.1.2019	14 days	44/male	Pork cutter	Yes	Fever, seizure, tonic-clonic seizures and coma	NGS and PCR methods	Acyclovir, dexamethasone and other treatments	(Wang et al., 2020)
NA	NA	44/male	Sick pig handler	NA	Fever, seizures, visual loss	NGS and PCR methods	Acyclovir and other treatments	(Wang et al., 2019)
6.14.2017	3 days	46/female	Swineherder	Sewage expose	Fever, visual impairment, headaches	NGS, real-time PCR and PCR	NA	(Ai et al., 2018)

						methods		
12.14.2017	4 days	55/male	Pork cutter/cooker	NA	Fever, headaches, cough, coma	NGS (CSF)	NA	[33]
12.14.2017	NA	51/man	Pork cutter/cooker	NA	Fever, headache, coma, tonic-clonic seizures and death	NGS (CSF)	Acyclovir and other treatments	[33]
11.4.2017	4 days	38/male	Pork cutter/cooker	NA	Fever, headache, coma, tonic-clonic seizures	NA	Acyclovir and other treatments	[33]
11.23.2016	4 days	42/female	Pork cutter/cooker	NA	Fever, tonic-clonic seizures, coma, blindness	NA	Acyclovir and other treatments	[33]
NA	40 days	59/male	Pig farmer	Yes	Fever, weakness, tonic-clonic seizures, respiratory failure	NGS (CSF)	Penciclovir treatment combined with Sodium phosphonate	[34]

Table 5: Various diagnostic approaches developed in China

Target	Methods	Sensitivity and specificity (function)	Reference
PRV antibody	**Blocking ELISA**: targeting to the gB antibody	High sensitivity (80.9%) and specificity (96.4%) compared with the commercial ELISA kit (IDEXX)	(Sun et al., 2018a)
	Indirect ELISA: targeting to the gB antibody	Highly total positive coincidence rate (97.8%) compared with the commercial ELISA kit. and the lowest detection limit was 1: 128 dilution of the positive serum.	(Liu et al., 2019a)
	Indirect ELISA: targeting to the gE antibody	High 88.0% sensitivity and 91.5% specificity compared with the commercial ELISA kit (IDEXX) **(allowing DIVA)**	(Kou et al., 2018)
	Indirect ELISA: targeting to the gE antibody	Total 89.1% positive coincidence rate compared with the commercial ELISA kit (IDEXX) **(allowing DIVA)**	(Zheng et al., 2017)
	DFM: targeting to the gE antibody	Total 74.7% positive coincidence rate compared with the conventional PCR, while which was more sensitive than the later one **(allowing DIVA)**.	(Xu et al., 2017a)
	ICA： targeting to the gE antibody	The lowest detection limit was 1:1280 dilution of the positive serum, and 95.3% positive coincidence rate compared with the commercial ELISA kit (IDEXX) **(allowing DIVA)**	(Lei and Zhang, 2016)
	Liquid chip technology： targeting to the gE antibody	This method could be applied to detect both PRV and PRRSV antibodies with higher sensitivity than the commercial ELISA kits **(allowing DIVA)**	(Xiao et al., 2018)
	IFAT: targeting to the gE antibody	High sensitivity (93.8%) and specificity (91.7%) compared with the commercial ELISA kit (IDEXX) **(allowing DIVA)**	(Zhu et al., 2019)
PRV antigen	**LFA：** targeting to the gB antigen	Detection limit of inactivated PRV antigens were lower than $1\times10^{6.6}$ TCID50/0.1 mL with 86.7% positive coincidence rate compared with the conventional PCR	(Wang et al., 2018b)
PRV DNA	**qPCR:** targeting to the gE gene	The detection limit was 10 copies/μl with high specificity (100%) compared with conventional PCR and commercial kits.	(Wen et al., 2019)

	PCR combined with nucleic acid probe spot hybridization: targeting to the gE gene	The sensitivity was 100 times higher than conventional PCR, being 10 pg/μl and 1 ng/ul, respectively **(allowing DIVA)**	(Wunaerhan et al., 2017)
	DdPCR: targeting to the gE gene	Higher sensitivity (6.1 copies/μl) than qPCR with high specify (96.2%) compared with viral isolation **(allowing DIVA)**.	(Chen et al., 2017)
	RPA: targeting to the gE and gB genes	Both the sensitivities of gE and gB gene were 100 copies/μl, this approach could be used to distinguish PRV wild and attenuated virus with 100% specify compared with qPCR **(allowing DIVA)**.	(Liu et al., 2018a)
	TaqMan qPCR: targeting to the gE and gD genes	The detection limits of gE and gB genes were 12.1 and 39.4 copies, respectively., which was more sensitive than qPCR and conventional PCR **(allowing DIVA)**.	(Lan et al., 2018)
	LAMP: targeting to the gB gene	Showed 100 times higher sensitivity than conventional PCR with the detection limit of 1fg/μl.	(Xu et al., 2017b)
	QPCR: targeting to the gB gene	The detection limit was lower than 1000 copies/μl, showing higher sensitivity than conventional PCR.	(Hua et al., 2019)
	PCR: targeting to the gE, gB and TK genes	This approach could be used to distinguish between PRV wild virus, SA215 and Bartha-K61 vaccine strains with detection limit of 1.8×10^{6} copies/μl **(allowing DIVA)**.	(Jiang et al., 2018)
	QIAxcel CGE: targeting to the gE gene	This method could be applied for the detection of PRV, CSFV, JEV, PCV2, PRRSV, PPV and ASFV, with the detection limit of 4.53×10^{3} copies/μl for PRV **(allowing DIVA)**.	(Wu et al., 2019)
	Multiple PCR: targeting to the gE gene	This method could be applied for the detection of PRV, PCV2 and PPV, with the detection limit of 72 pg/μl for PRV **(allowing DIVA)**.	(Xin et al., 2019)
	Multiple RT-PCR: targeting to the gE gene	The approach could detect the nucleic acids of PRV, PCV2, PPV, PPRSV, JEV, and CSFV with the detection limit pf 10^{-3} ng/μl for PRV **(allowing DIVA)**	(Li et al., 2019)

	NanoPCR: targeting to the gB, gE, and gG genes	This test could be used for the differentiation of wild PRV and gene-deleted vaccine strains with higher sensitivity than the conventional PCR **(allowing DIVA)**	(Ma et al., 2013)
	NGS	This approach could be used for detecting undetermined pathogens with high sensitivity	(Ai et al., 2018)

Table 6 List of genetic modified vaccines against PRV infection

Gene-deleted vaccines	Features	Technologies	Main advantages	References
Single gene-deleted vaccine (inactivated)	gE-deleted	BCA	High safe without virulence reversion; Allowing DIVA with more complete protection than Bartha K61 vaccines	(Wang et al., 2016a)
Double gene-deleted vaccine (inactivated)	gE/gI-deleted	BCA		(Gu et al., 2015)
Single gene-deleted vaccine	gE-deleted	HR	Safe to piglets without visible gross pathological lesions; Effective immune response; Completely provides protection against emerging PRV variants; Allowing DIVA;	(Wang et al., 2014; Wang et al., 2015a)
Double gene-deleted vaccine	gE/gI-deleted	HR		(Tong et al., 2016; Yin et al., 2017)
	gE/US2 deleted	High-temperature passage		(Liang et al., 2017; Wang et al., 2018c)
Triple gene-deleted vaccine	gE/gI/TK-deleted	HR	Safer than double gene-deleted vaccines to piglets and growing pigs; Effective immune response; Completely provides protection against emerging PRV variants; Allowing DIVA;	(Dong et al., 2017; Hu et al., 2015b; Wang et al., 2019; Zhang et al., 2015a)
	gE/gI/TK-deleted	CRISPR/Cas9		(Tang et al., 2016; Zhao et al., 2020)
	gE/gC/TK-deleted	CRISPR/Cas9		(Lin et al., 2020)

Note: HR: Homologous DNA recombination; **DIVA**: Distinction between the infected and vaccinated animals; **CRISPR/Cas9**: Clustered regularly interspaced short palindromic repeats/Cas9.

Table 7 List of live attenuated recombinant vaccines against PRV infection

Insertion sites in PRV genome	Parental PRV strains	Insertion Genes	Function (Animal model)	References
gG gene	HB-98 strain	Porcine IL6 gene and VP2 gene of PPV	Provided partial protection against the virulent PPV and PRV challenges (mice)	(Zheng et al., 2020)
gI gene	gE/gI/TK-deleted SA 215 strain	VP2 gene of PPV	Completely protected pigs against maternal PRV infection and significantly reduced the death rate (1/28) after PPV challenge compared with the control (7/31) (pig)	(Chen et al., 2011)
gG gene	HB-98 strain	Porcine IL18 gene and Cap gene of PCV2	Protected mice against PRV variants infection and significantly reduced the amount of PCV2 viremia (mice)	(Zheng et al., 2015)
Between gE and gI gene	gE/TK-deleted strain	prM and E genes of JEV	Provided 100% and 80% protection against PRV and JEV infection, respectively (mice)	(Qian et al., 2015)
Between gE and gI gene	gE/gI-deleted strain	E2 gene of CSFV	Provided complete protection against maternal PRV and CSFV infection (pig)	(Wang et al., 2015c)
	gE/gI/TK-deleted TJ strain			(Lei et al., 2016)
Between gG and gD gene	gE/gI-deleted JS-2012 strain		Provided complete protection against maternal PRV and CSFV infection without MDAs (pig)	(Tong et al., 2019)
Between gG and US9 gene	gE/gI/TK-deleted TJ strain	Cap gene of PCV2 and E2 gene of CSFV	Only protected pigs against PRV infection (pig)	(Abid et al., 2019)
gG gene	gG deleted strain	SiRNA targeting to the N gene of HP-PRRSV	Safe to pigs and efficiently inhibited HP-PRRSV replication in vivo (pig)	(Cao et al., 2015)
Between gE and gI gene	gE/TK-deleted strain	Bp26 gene of Brucella melitensis	Induced good humoral and cell-mediated immune response in mice (mice)	(Yao et al., 2015)
gG gene	HB-98 strain	SAG1 and MIC genes of Toxoplasma gondii	Induced partial protection against a lethal challenge with Toxoplasma gondii strain (mice)	(Nie et al., 2011)
Between gE and gI gene	gE/gI/TK-deleted TJ strain	SAG1 and MIC genes of FMDV	Significantly increased the survival rate (3/5) after FMDV challenge compared with the control (0/5) (pig)	(Zhang et al., 2011)

Table 8: Different types of compounds with anti-PRV infection activity

Source	Extracts	Mechanism	IC50	CC50	In vitro	In vivo	PRV strain	MOI	References
Resveratrol	Ethanol	Inhibition of viral replication; Inhibition of IKB kinase activation	>262.87 μM	17.17±0.35 μM	√	√	Rong A	0.01	(Chen et al., 2019; Zhao et al., 2017)
Germacrone	Dimethyl sulfoxide	Inhibition of viral replication	233.5 μM for Vero 184.1 μM for PK15	54.51 μM for Vero 88.78 μM for PK15	√	×	Variant PRV Bartha K61 vaccine	0.1-10	(He et al., 2019b)
Isatis indigotica (leaf)	Ethanol	Inhibition of viral replication	226 μg/mL	11 μg/mL	√	×	TNL	100 pfu/well	(Hsuan et al., 2019)
Radix isatidis	Ethanol and water	Inhibition of viral replication; Killing virus directly	Not mentioned	Not mentioned	√	×	MinA	100 $TCID_{50}$	(Tong et al., 2020)
Marine Bacillus S-12-86 lysozyme	water	Inhibition of viral replication	100 μg/mL	0.46 μg/mL	√	×	Attenuated	Not mentioned	(Zhu et al., 2013)
Diammonium glycyrrhizin	Not mentioned	Killing virus directly	1.25 mg/mL	Not mentioned	√	√	Bartha K61 vaccine	10^4 pfu/mL	(Sui et al., 2010)
Vanadium-substituted Heteropolytungstate	DMEM	Killing virus directly	400 μg/mL	5 μg/mL	√	×	Bartha	200 $TCID_{50}$/mL	(Liu et al., 1998)
Graphene Oxide	DMEM	Killing virus directly	Not mentioned	Not mentioned	√	×	HNX variant	0.01	(Ye et al., 2015b)
Ivermectin	Dimethyl sulfoxide	Blocking the nuclear translocation of viral DNA polymerase UL42	Not mentioned	Not mentioned	√	√	Not mentioned	0.01	(Lv et al., 2018)
phosphonoformate sodium	Not mentioned	Inhibition of viral DNA polymerase	480 μg/mL	Nearly 60 μg/mL	√	×	Kaplan	2	(Ren et al., 2011)