Received: 21 April 2020 | Revised: 25 May 2020 | Accepted: 2 June 2020

DOI: 10.1111/tbed.13667

RAPID COMMUNICATION

Transboundary and Emerging Diseases WILEY

Molecular and serological investigation of cat viral infectious diseases in China from 2016 to 2019

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Funding information
Luoyang Heluo Talent Plan

Abstract

In order to analyse the prevalence of cat viral diseases in China, including feline parvovirus (FPV), feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV) and feline infectious peritonitis virus (FIPV), a total of 1,326 samples of cats from 16 cities were investigated from 2016 to 2019. Collectively, 1,060 (79.9%) cats were tested positive for at least one virus in nucleotide detection, and the positive rates of cat exposure to FeLV, FPV, FHV-1, FCV, FIV and FIPV were 59.6%, 19.2%, 16.3%, 14.2%, 1.5% and 0.5%, respectively. The prevalence of FHV-1 and FPV was dominant in winter and spring. Cats from north China showed a higher positive rate of viral infection than that of cats from south China. The virus infection is not highly correlated with age, except that FPV is prone to occur within the age of 12 months. In the serological survey, the seroprevalences of 267 vaccinated cats to FPV, FCV and FHV-1 were 83.9%, 58.3% and 44.0%, respectively. Meanwhile, the seroprevalences of 39 unvaccinated cats to FPV, FCV and FHV-1 were 76.9% (30/39), 82.4% (28/34) and 58.6% (17/29), respectively. This study demonstrated that a high prevalence of the six viral diseases in China and the insufficient serological potency of FCV and FHV-1 remind the urgency for more effective vaccines.

KEYWORDS

feline viral infectious disease, molecular detection, prevalence, serological

1 | INTRODUCTION

In China, pets are increasingly becoming an integral part of people's lives, while the viral infectious diseases show great threat to cat health. Felid species are susceptible to all pathogens those infect the domestic cat (Filoni et al., 2012). The infection of feline herpetovirus type 1 (FHV-1), feline panleukopaenia virus (FPV) and feline calicivirus (FCV) in tiger and cheetah has been reported in China (Chen, 2013; Gao et al., 2003; Qiu et al., 2000). FCV and FHV-1 are the main viral pathogens of upper respiratory tract infection in cats, and FHV-1 causes rhinotracheitis that also named feline viral rhinotracheitis virus, while FCV often causes stomatitis, gingivitis and circumscribed lesions of the tongue. FPV infections are highly contagious in all members of the family *Felidae*, resulting in high mortality, panleukopaenia and serious enteric symptom (Yang et al., 2008). The infection of feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV) and feline infectious peritonitis virus

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(FIPV) has been reported in China (Chang, de Groot, Egberink, & Rottier, 2010; Cong et al., 2016; Pan, Wang, & Wang, 2018). The infection of FeLV and FIV in domestic cats would cause clinical disease worldwide (Arjona et al., 2007), in which, FeLV is mainly associated with lymphoma, leukaemia and anaemia (Lutz et al., 2009), and FIV is associated with immune suppression and could be used as an animal model for acquired immunodeficiency syndrome research (Bendinelli et al., 1995).

Due to the lack of epidemiology of cat infectious diseases and seroprevalence of viral pathogens nationwide, the aim of the study was to investigate the prevalence of FHV-1, FeLV, FPV, FCV, FIV and FIPV of cat in China. Nucleotide detection of 1,326 samples collected from Harbin, Shenyang, Hohhot, Tangshan, Tianjin, Beijing, Langfang, Shijiazhuang, Qingdao, Zhengzhou, Luoyang, Hefei, Chongqing, Taizhou, Guangzhou and Haikou was conducted from 2016 to 2019, together with the serological analysis of the infection of FCV, FHV-1 and FPV.

2 | MATERIALS AND METHODS

The swab samples of eye, nose and anal from 1,288 clinically diseased cats, tissues from 17 died cats and ascites from 21 cats were collected from Harbin, Shenyang, Hohhot, Tangshan, Tianjin, Beijing, Langfang, Shijiazhuang, Qingdao, Zhengzhou, Luoyang, Hefei, Chongqing, Taizhou, Guangzhou and Haikou in China from 2016 to 2019 (Figure 1). And 316 blood samples were collected from Tianjin, Beijing, Qingdao and Zhengzhou. The study was approved by the Animal Care and Ethics Committee of National Research Center for Veterinary Medicine, and conventional animal welfare regulations and standards were taken into account.

The primers for PCR test of FIPV, FeLV, FCV, FHV-1, FPV and FIV were designed and shown in Table 1. The viral nucleotide was extracted using the viral nucleic acid extraction kit II (Geneaid). The nucleotide samples of FIPV, FeLV, FCV and FIV were used as templates in the detection of one-step RT-PCR (TransGen Biotech,

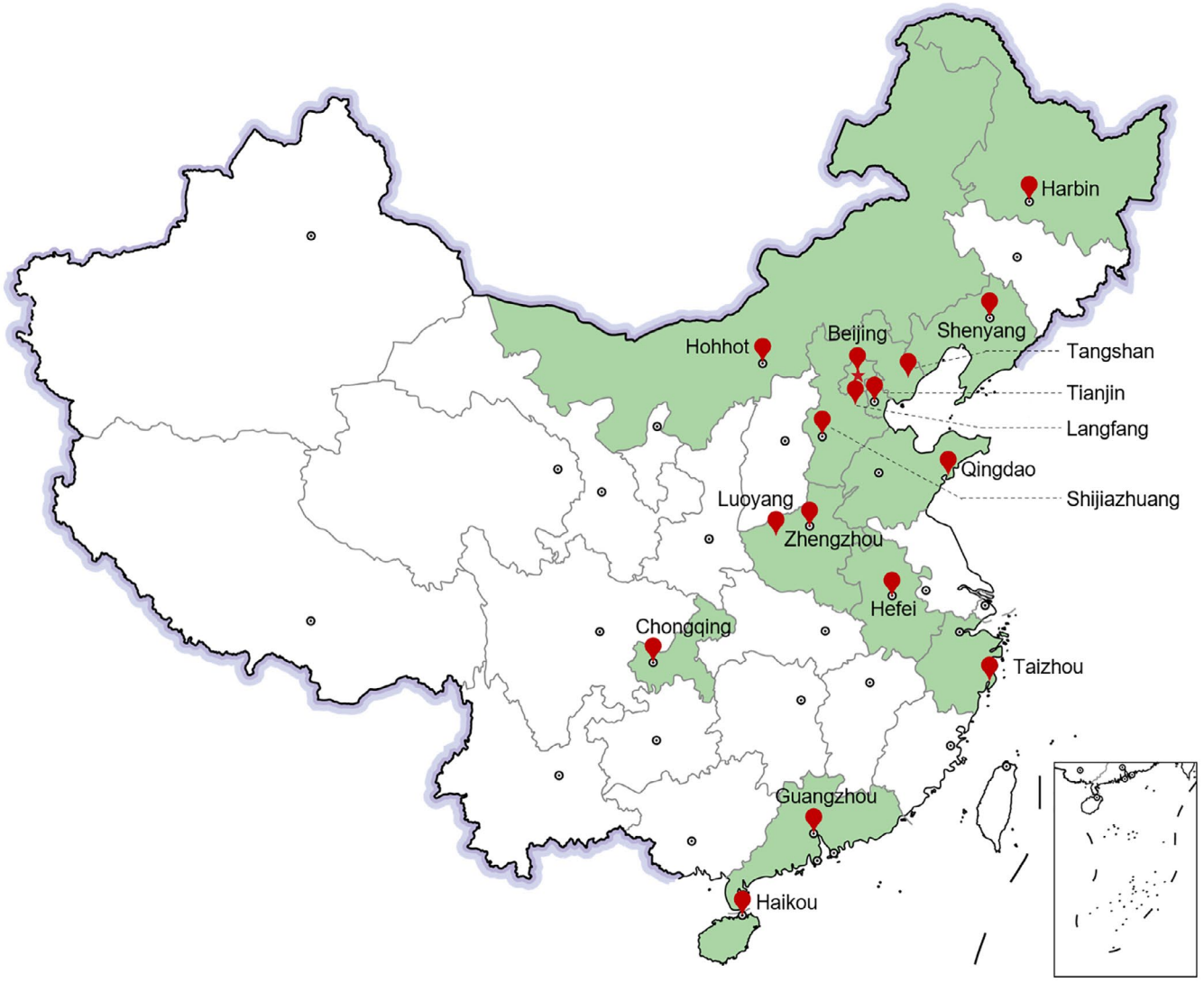

FIGURE 1 Locations of sample collection. The area of light green in the map represents the sampled provinces, and 16 sampled cities are marked in red

China), and the nucleotide samples of FHV-1 and FPV were detected using Premix Taq (Ex Taq Version 2.0 plus dye; Takara). Positive (virus stock from cell culture) and negative controls (H_2O) were included in the test samples.

Prior to further testing, the blood samples of cats were centrifuged (3,000 *g*, 15 min) and inactivated (56°C, 30 min). For neutralization assay, twofold diluted sera with the range of 1:2 to 1:4,096 were added in 96-well microtitre plates and preincubated with the virus (10^2 $TCID_{50}$/well) for 1 hr before the addition of F81 cells (2.5×10^4/well). The plates were then incubated at 37°C in a humidified atmosphere of 5% CO_2 for 4–5 days. The titre was calculated based on cytopathic effect by the Reed–Muench method. For haemagglutination inhibition (HI) assay of FPV, the serum was twofold diluted from 1:2 to 1:4,096 and mixed at the equal volume of 0.025 ml with FPV (8 HA unit per sample), and then incubated at 37°C for 30 min before the equal volume of 1% suspensions of pig red blood cells was added. HI titre was defined as the last dilution that shows completely HI effect after the incubation at 4°C for 90 min.

TABLE 1 The PCR primers for nucleotide detection of six feline viruses

Primer	Sequences (5′–3′)	Product (bp)	Tm[a] (°C)
FHV-1-F	GACGTGGTGAATTATC	288	56
FHV-1-R	CAACTAGATTTCCACCAGGA		
FIPV-F	ATTATGTTGCACTACAAA	353	58
FIPV-R	ACACATAAGCATTAATGT		
FeLV-F	CCAGAATGAGGGGAACA	212	58
FeLV-R	CGTGCCTGACATATAGC		
FCV-F	TTCGGCCTTTTGTGT	664	56
FCV-R	ATTGAACACATCAATAGATC		
FPV-F	AAAGAGTAGTTGTAAATAA	681	56
FPV-R	TATATCACCAAAGTTAGTAG		
FIV-F	CTAGGAGGTGAGGAAGTT	224	56
FIV-R	GCTTGTTGTTCTTGAGTT		

[a]'Tm' represents annealing temperature.

3 | RESULTS AND DISCUSSION

A total of 1,326 samples collected from 16 cities (Figure 1) geographically located from the north to the south of China (within the east longitude of 106°55′ to 126°53′ and north latitude of 20°1′ to 45.8′) are detailed in Table 2, and 79.9% (1,060/1,326) of cats were exposed to at least one pathogen of the six viruses.

In details, the positive rates of cat exposure to FeLV, FPV, FHV-1, FCV, FIV and FIPV were 59.6%, 19.2%, 16.3%, 14.2%, 1.5% and 0.5%, respectively (Figure 2). The highest ratio of FeLV infection may be caused by lacking the utilization of FeLV vaccine in China. The prevalence varies widely depending on the geographical location (Gleich, Krieger, & Hartmann, 2009). A previous report showed a FeLV-positive ratio of 11.33% in Gansu Province of north-west China, which was lower than that in our study. Highly variable prevalence (1%–12.2%) of FeLV infection in cats has been reported

TABLE 2 The detailed information of samples

Cities	No. of samples	Basic information of the samples				
		No. of tissue	No.. of ascites	No. of eye, nose and anal swab	No.. of vaccinated cat	No.. of unvaccinated cat
Tianjin	545	4	20	521	73	49
Luoyang	238	13	1	224	/	/
Haikou	154	0	0	154	39	13
Beijing	118	0	0	118	49	1
Shijiazhuang	44	0	0	44	24	2
Zhengzhou	38	0	0	38	2	6
Chongqing	38	0	0	38	30	3
Qingdao	29	0	0	29	17	2
Guangzhou	28	0	0	28	1	/
Langfang	26	0	0	26	/	/
Harbin	20	0	0	20	3	/
Hefei	13	0	0	13	9	0
Taizhou	10	0	0	10	9	/
Shenyang	9	0	0	9	/	/
Hohhot	9	0	0	9	3	1
Tangshan	7	0	0	7	3	1
Total	1,326	17	21	1,288	262	78

Note: '/' represents no background of immunity in all the samples collected in particular cities.

worldwide (Bande et al., 2012; Chang et al., 2010; Dorny et al., 2002; Gates, Vigeant, & Dale, 2017; Malik et al., 1997). The geographical location, sample quantity and populations may contribute to the variation (Chang et al., 2010). The prevalence of FeLV is 52.5% in male and 40.1% in female in this study, which is consistent with the results of a higher prevalence in male than female in previous reports (Gleich et al., 2009; Levy, Scott, Lachtara, & Crawford, 2006; Norris et al., 2007). The positivity of FeLV infection was higher in male cats than that in female cats, which might attribute to the greater risk of bite wounds in males caused by higher aggressivity.

Feline parvovirus is clinically important in cats with high mortality. Here, the FPV positivity in China is 19.2% from 2016 to 2019, which is lower than that (37.1%) in a previous report of north-east China from 2016 to 2017 (Niu et al., 2018). Yet, little is known about the prevalence of FPV of cat in other parts of China and other countries.

As the main pathogens of upper respiratory tract infection in cats (Binns et al., 2000), the prevalence of FHV-1 and FCV (16.3% and 14.2%, respectively) in our study was lower than that reported in Beijing (26.3% and 46.3%, respectively) (Xu et al., 2017) and in Switzerland (20% and 45% in suspect population, respectively) (Berger et al., 2015).

Feline immunodeficiency virus is distributed worldwide, and the prevalence of FIV was 2.5% and 5.4%–31.1% in North America and Asia (Bande et al., 2012; Eckstrand, Sparger, & Murphy, 2017; Levy et al., 2006; Nakamura et al., 2010; Phillips et al., 1992), while it is higher than that in our study (1.5%). Different detection methods may contribute to the difference. The false positive of antibody tests may own to maternal antibodies or previous FIV vaccination, as the maternal antibodies may persist for 6 months or longer (Barr, Pough, Jacobson, & Scott, 1991).

Feline infectious peritonitis virus, one of the feline coronaviruses (FCoVs), is the pathogen of FIP, a fatal, immune-mediated disease in wild and domestic cats. FCoVs are classified into two serotypes, type I and type II. There are two types of FCoVs, feline enteric coronavirus (FECV) and FIPV, which classified based on pathogenicity. FECVs produce mild enteric infections (Pedersen, 1983), and can be converted into FIPV, and they exist in both serotypes I and II (Tekes & Thiel, 2016). The previous study showed a significant higher prevalence rate (74.6%) for FIPV in China (Li et al., 2019) than that in our study (0.5%), which may be caused by the limited sampling location.

In all the 1,326 samples, 1,060 (79.9%, 1,060/1,326) were positive for at least one viral pathogen in nucleotide detection. As shown in Figure 3, 64.34% (682/1,060) of cats were infected with single type of virus, and 35.66% (378/1,060) of cats were mixed infection. The high complexity of mixed infection, more than 10 kinds of mixed infection patterns as detected, indicated the complexity of viral infectious diseases of cats, and it is a challenge for the diagnosis of cat infectious diseases in China.

In our study, the correlation between positive rate of cats and specific factors, including age, weather and geographical location, was analysed. The prevalence of FeLV, FHV-1, FPV and FIV was highly seasonal (Table 3). The prevalence of FHV-1, FPV and FIV in cold seasons (spring and winter) was higher than that in warm seasons (summer and autumn), while an opposite pattern of the prevalence in FeLV could be found. The positive rates of FPV, FHV-1 and FIV infections of cats in north China (FPV, 21.2%; FHV-1, 18.4%; FIV, 1.9%) were higher than that in south China (FPV, 10.7%; FHV-1, 7.5%; FIV, 0.0%), except that the FeLV showed an opposite pattern of infection (north China, 51.8%; south China, 92.5%). As shown in Table 4, the prevalence of FCV was the same in north and south China (14.2%). FPV infection was age-related in cats (age < 12 months), while the prevalence of FeLV, FHV-1 and FCV was age-irrelevant (Table 5), which varies from previous reports in New Zealand, where the susceptibility of FeLV decreased significantly with age (Luckman & Gates, 2017).

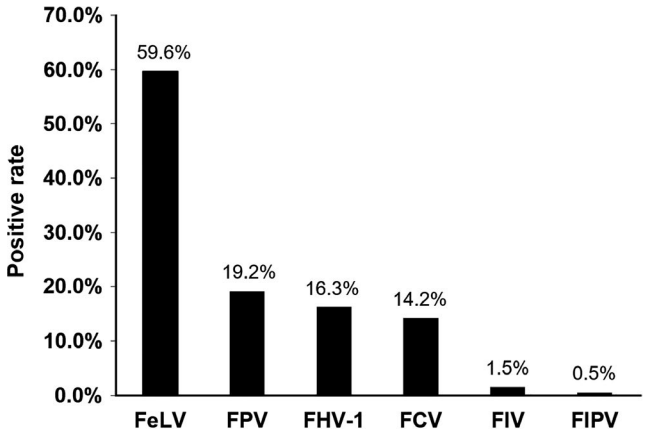

FIGURE 2 Positive rates of FHV-1, FeLV, FPV, FCV, FIV and FIPV of cats in nucleotide detection

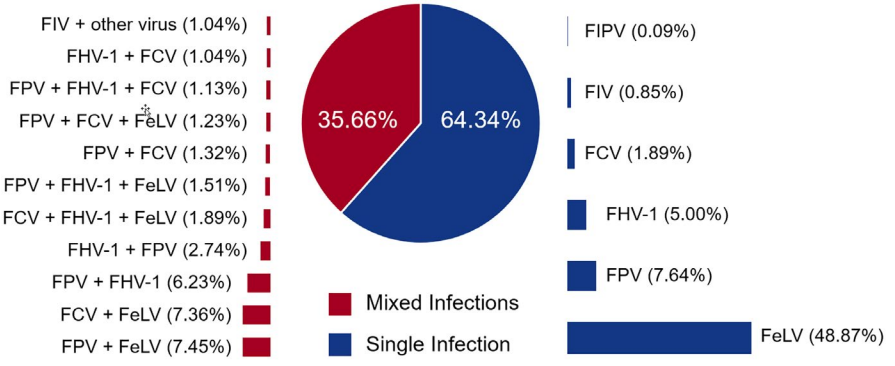

FIGURE 3 Infection patterns of cat viral diseases. The proportion of single and mixed infections patterns was marked in blue and red, respectively

TABLE 3 Prevalence of infectious viruses (FHV-1, FeLV, FPV, FCV, FIV and FIPV) in different seasons

Seasons	No. of cats tested	No. of positive cats (%)					
		FeLV	FPV	FHV-1	FCV	FIV	FIPV
Spring	366	103 (28.1%)	98 (26.8%)	104 (28.4%)	56 (15.3%)	9 (2.5%)	0 (0%)
Summer	222	208 (93.7%)	24 (10.8%)	16 (7.2%)	36 (16.2%)	4 (1.8%)	3 (1.4%)
Autumn	497	338 (68.0%)	81 (16.3%)	42 (8.5%)	51 (10.3%)	2 (0.4%)	1 (0.2%)
Winter	241	141 (58.5%)	51 (21.2%)	54 (22.4%)	45 (18.6%)	5 (2.1%)	3 (1.2%)

TABLE 4 Prevalence of main feline virus infection in north and south China

Area	No. of the tested cats	No. of positive cats (%)				
		FeLV	FPV	FHV–1	FCV	FIV
North China	1,073	556 (51.8%)	227 (21.2%)	197 (18.4%)	152 (14.2%)	20 (1.9%)
South China	253	234 (92.5%)	27 (10.7%)	19 (7.5%)	36 (14.2%)	0 (0.0%)

TABLE 5 Prevalence of main feline virus infection in different ages

Age	No. of the tested cats	No. of positive cats (%)			
		FeLV	FPV	FHV–1	FCV
1–6 months	364	236 (64.9%)	83 (22.8%)	66 (18.1%)	48 (13.2%)
7–12 months	190	111 (58.4%)	40 (21.1%)	35 (18.4%)	33 (17.4%)
>12 months	228	148 (64.9%)	23 (10.1%)	40 (17.5%)	28 (12.3%)

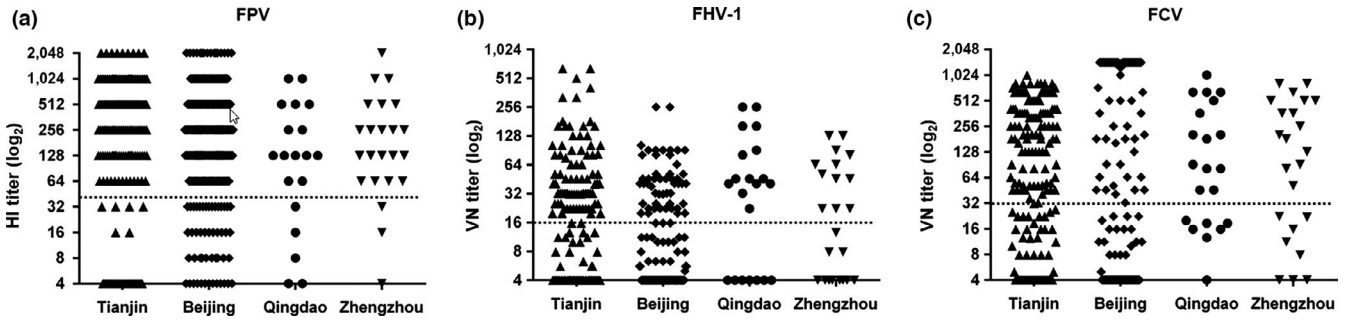

FIGURE 4 The viral HI and VN titres of cat serum from four cities of China. (a) HI titres of cat serum samples against FPV. (b) VN titres of cat serum samples against FHV-1. (c) VN titres of cat serum samples against FCV

TABLE 6 The seroprevalences of FHV-1, FCV and FPV in vaccinated and unvaccinated cats

Cats	Seroprevalences (positive/total)		
	FHV-1	FCV	FPV
Vaccinated	44.0% (88/200)	58.3% (151/259)	83.9% (224/267)
Unvaccinated	58.6% (17/29)	82.4% (28/34)	76.9% (30/39)

A total of 316 serum samples of cats were collected from four cities of China (Beijing, Tianjin, Qingdao and Zhengzhou). Antibodies against FPV were detected by HI assay, and FCV and FHV-1 antibodies were detected by virus neutralization (VN) assay. The protective titres were settled as 1:40, 1:32 and 1:16 for FPV, FCV and FHV-1, respectively (Reese et al., 2008). As shown in Figure 4, a total of 267 serum samples of vaccinated cats (feline rhinotracheitis–calici-panleukopaenia vaccine, killed virus) were analysed by HI assay for FPV antibody detection, and the protective rate was 83.9% (224/267).

As shown in Table 6, the seroprevalences of FCV and FHV-1 were 58.3% (151/259) and 44.0% (88/200) by VN assay, respectively. The lower seroprevalences in vaccinated cats indicated an insufficient potency based on current commercial vaccines against the challenge of wild strains of FPV, FCV and FHV-1. The abroad commercial vaccines utilized in China could not resist the

infection of the domestic wild strains. In the 39 serum samples of unvaccinated cats, the seroprevalences of FHV-1, FCV and FPV were 58.6% (17/29), 82.4% (28/34) and 76.9% (30/39), respectively (Table 6), higher than that reported in Milan (37.1%, 85.4% and 45.7%, respectively) (Dall'Ara et al., 2019). Another study in Beijing found that 47.6% of cats were FHV-1-positive by ELISA, but the vaccination status of these cats was unknown (Wang et al., 2014). The prevalence of FHV-1 and FPV in Costa Rica was 71.9% and 92.8%, while only 25% and 16.5% of them were previously vaccinated with FHV-1 and FPV, respectively (Blanco, Prendas, Corte, Jimenez, & Dolz, 2009). The seroprevalences of FHV-1, FCV and FPV were higher than the antigen-positive rates, which might be caused by the recovery of cat and then the undetectable of the antigens of FHV-1, FCV and FPV.

In conclusion, six main viral infectious diseases of cat were investigated in China, and the most widespread virus in cat population is FeLV, followed by FPV, FHV-1, FCV, FIV and FIPV. The complexity of cat mix infection in China suggested the big challenge for the diagnosis and treatment of these diseases. Our data also revealed that insufficient potency by immunization of current commercial vaccines could not give full protection to wild strain infections of FHV-1, FPV and FCV in China, demonstrating the urgency of improvement of immune strategies and the development of new vaccines.

ACKNOWLEDGEMENT

This work is supported by Luoyang Heluo Talent Plan (Dr. Kegong Tian).

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supporting information.

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How to cite this article: Liu C, Liu Y, Qian P, et al. Molecular and serological investigation of cat viral infectious diseases in China from 2016 to 2019. *Transbound Emerg Dis*. 2020;00:1–7. https://doi.org/10.1111/tbed.13667