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

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April 28, 2020

Abstract

In order to analyze the prevalence of cat viral diseases in China, including feline parvovirus (FPV), feline calicivirus (FCV), feline herpesvirus-1 (FHV-1), feline leukemia 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, 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 were 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, FHV-1 were 83.9%, 58.3% and 44.0%, respectively. Meanwhile, the seroprevalences of 39 unvaccinated cats to FPV, FCV, 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 reminds the urgency for more effective vaccines.

KEYWORDS

Feline viral infectious disease, prevalence, serological, molecular detection.

1 INTRODUCTION

In China, pets are increasingly becoming an integral part of people’s lives, while the viral infectious diseases showed great threat to cat health. Felid species are susceptible to all pathogens that infect the domestic cat (Filoni et al., 2012). The infection of Feline herpetovirus type 1 (FHV-1), Feline Panleukopenia Virus (FPV), Feline calicivirus (FCV) in tiger and cheetah have 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 causes rhinotracheitis that also named feline viral rhinotracheitis virus, while the 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, panleukopenia and serious enteric symptom (Yang et al., 2008). The infection of feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) and feline infectious peritonitis virus (FIPV) have 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, leukemia, and anemia (Lutz et al., 2009), and FIV is associated with immune suppression and could be used as an animal model for AIDS research (Bendinelli et al., 1995).
Due to the lack of epidemiology on infectious diseases and seroprevalence of viral pathogens of cat national wide, 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 were 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, Haikou in China from 2016 to 2019 (Figure 1). And 316 blood samples were collected from Tianjin, Beijing, Qingdao, 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, FIV were shown in Table 1. The viral nucleotide was extracted using the viral nucleic acid extraction kit II (Geneaid, Taiwan, China). The nucleotide samples of FIPV, FeLV, FCV and FIV were used as templates in the detection of one-step RT-PCR (TransGen Biotech, China), and the nucleotide samples of FHV-1 and FPV were detected using Premix Taq (Ex Taq Version 2.0 plus dye) (TakaRa, Japan).

Prior to further testing, the blood samples of cats were centrifuged (3000 × g, 15 min) and inactivated (56, 30 min). For neutralization assay, two-fold diluted sera with the range of 1:2 to 1:4,096 were added in 96-well microtiter plates and preincubated with the virus (10^{2} TCID_{50}/well) for 1 h before the addition of F81 cells (2.5x10^4/well). The plates were then incubated at 37 in a humidified atmosphere of 5% CO_{2} for 4-5 days. The titer was calculated based on cytopathic effect by the Reed-Muench method. For haemagglutination inhibition (HI) assay of FPV, the serum was two-fold 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 for 30 min before the equal volume of 1% suspensions of pig red blood cells were added. HI titer was defined as the last dilution that shows completely HI effect after the incubation at 4 for 90 min.

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 106deg55' to 126deg53' and north latitude of 20deg1' to 45.8') were detailed in Table 2, and 79.9% (1060/1326) 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 vary 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 northwest China, which was lower than that in our study. Highly variable prevalence (1%~12.2%) of FeLV infection in cats have been reported 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.

FPV 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 Northeast 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 were 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).

FIV is distributed worldwide, and the prevalence of FIV was 2.5% and 5.4% to 31.1% in North America and Asian (Bande et al., 2012; Eckstrand, Sparger, & Murphy, 2017; Levy et al., 2006; Nakamura et al., 2010; Phillips, Lamont, Konings, Shacklett, & Elder, 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).

FIPV, one of the feline coronavirus (FCoV), 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, 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). Previous study showed a significant higher prevalence rate (74.6%) for FIPV in China (Li et al., 2019) than in our study (0.5%), which may be caused by the limited sampling location.

In all the 1,326 samples, 1,060 (79.9%, 1060/1326) were positive for at least one viral pathogen in nucleotide detection. As shown in Figure 3, 64.34% (682/1060) of cats were infected with single type of virus, and 35.66% (378/1060) 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, it is a challenge for the diagnose 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, were analyzed. 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, 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).

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 titers were settled as 1:40, 1:32, and 1:16 for FPV, FHV-1, and FIV respectively (Reese et al., 2008). As shown in Figure 4, a total of 267 serum samples of vaccinated cats (Feline Rhinotracheitis- Calici-Panleukopenia Vaccine, Killed Virus) were analyzed by HI assay for FPV antibodies 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 to 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 were 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 were investigated in cat population of China, the most widespread virus in cat population is FeLV, followed by FPV, FHV, 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 strains 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.

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Data Availability Statement

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

REFERENCES


**Figure legends**

**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.

**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 were marked in blue and red, respectively.

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

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