

# Seroprevalence of *Dirofilaria immitis* in Cats from Liaoning Province, Northeastern China

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**Abstract:** The present study was performed to investigate the seroprevalence and risk factors for *Dirofilaria immitis* infection in cats from Liaoning province, northeastern China. From October 2014 to September 2016, sera of 651 cats, including 364 domestic cats and 287 feral cats (332 females and 319 males) were assessed. They were tested for the presence of *D. immitis* antigen using SNAP Heartworm RT test kit. In this population, the average prevalence was 4.5%. Age and rearing conditions (feral or domestic) were found to be associated with the prevalence of *D. immitis*. The prevalence was significantly higher in feral cats compared with domestic cats (8.4% vs 1.4%,  $P < 0.01$ ). There was no significant difference between males and females (4.7% vs 4.2%,  $P > 0.05$ ), but older cats ( $\geq 3$  years old) showed a statistically higher prevalence compared with younger cats ( $< 3$  years old) in feral populations (16.8 vs 2.4%,  $P < 0.01$ ), while the difference between the age groups was not statistically significant in domestic cats (2.4% vs 0.51%,  $P > 0.05$ ), all these results suggest that outdoor exposure time may be one of the most important factors for *D. immitis* prevalence in cats. Results reveal that *D. immitis* are prevalence in domestic and feral cats in northeastern China, which indicates that appropriate preventive measures should be taken to decrease the incidence of feline heartworm disease in Liaoning province, northeastern China.

**Key words:** *Dirofilaria immitis*, seroprevalence, cat, Liaoning province

*Dirofilaria immitis*, the etiologic agent of canine heartworm disease, is commonly found in the pulmonary artery and right ventricle of dogs and other animals. It could infect dogs, cats, and various wild mammals, with increased incidence in temperate and tropical areas [1]. The heartworm parasite is transmitted by various mosquito species, including *Aedes*, *Anopheles*, and *Culex* [2,3]. After mosquitoes feed on an infected microfilaremic reservoir host, the mosquitoes act as intermediate hosts, carrying infectious “larvae 3” (L3) stage larvae, which could be transmitted to a dog or a cat [4].

Although *D. immitis* is most commonly found in dogs and other canids, the importance of *D. immitis* in cats is an increasingly recognized problem, alluded to as early as 15 years ago [5]. In addition, the prevalence of heartworm disease in other

species has increased in the past 10 years, with the geographic range of heartworm infection also significantly increased in the canine and feline species [6]. *D. immitis* has been frequently detected in cats in many countries, including the United States [7], Canary Islands [8], Malaysia [9], Korea [10], Portugal [11], and Italy [12]. In China, several studies have reported the prevalence of *D. immitis* in dogs, 12.7% in Shenyang city and 24.0% in Dandong city, northeastern China [13,14], 13% in Henan province and 31.2% in Xian city, central China [15,16]. The prevalence is 13.5% in southern China, such as Nanchang, Fuzhou, Guangzhou, Shenzhen, Nanning [17]. 61.3% in Chongqing [18], the seroprevalences of *D. immitis* were also found in Beijing (0.33%, 1/300) and Shanghai (3.3%, 1/30) [19]. Moreover, the seroprevalence in Taipei city and mountain aboriginal districts were 13.8% and 12.1% in domestic dogs [20]. The occurrence of *D. immitis* suggested that the dog population in China is at potential risk for *D. immitis*.

However, there are very few published epidemiological data regarding feline heartworm prevalence in China. The prevalence of *D. immitis* in cats was 3.04% in Gansu province,

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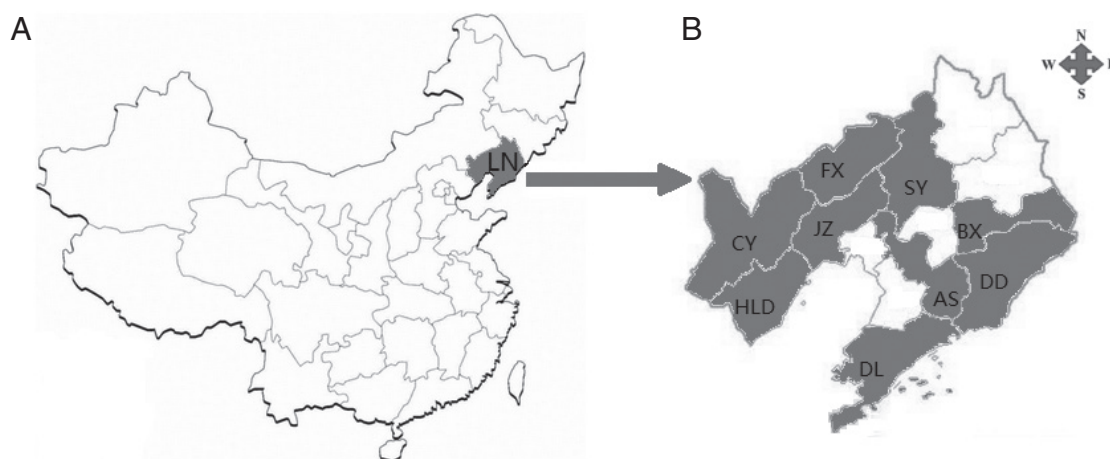
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northwestern China [21], and 1.93% in stray cats collected from Liaoning, Jilin, Heilongjiang, and Shandong province, eastern China [22], which is the only report to date. Therefore, it is very important to collect information on the prevalence of *D. immitis* in cats. The purpose of this study was to determine the seroprevalence of *D. immitis* in cats and analyze its related risk factors in Liaoning province, northeastern China.

Liaoning province is located in the northeastern of mainland China, covering an area of 148,000 km<sup>2</sup> and a population of 43.91 million approximately. Its geographical position is at east longitude 118°53'-125°46' and at north latitude 38°43'-43°26'. The area has a continental monsoon climate, winter is longer than other seasons, and summer is warm. The average annual temperature is 7-11°C, with a mean annual rainfall of 600-1,100 mm. There are 14 provincial cities distributed in Liaoning province, with the city of Shenyang as its capital (Fig.1). Nine cities including Shenyang (41°8'N, 123°38'E), Dalian (38°92'N, 121°62'E), Anshan (41°1'N, 122°98'E), Benxi (41°3'N, 123°73'E), Dandong (40°13'N, 124°37'E), Jinzhou (41°13'N, 121°15'E), Huludao (40°77'N, 120°83'E), Chaoyang (41°58'N, 120°42'E), and Fuxin (42°N, 121°65'E), located in the northern, western, central, southern, and eastern parts of Liaoning province, were selected for sample collection for this study.

From October 2014 to September 2016, a total of 651 blood samples were collected, including 364 domestic cat samples (collected from pet owners) and 287 feral cat samples (collected from humane cat traps) from Shenyang (29 domestic and

53 feral), Dalian (24 domestic and 40 feral), Anshan (42 domestic and 19 feral), Benxi (48 domestic and 28 feral), Jinzhou (50 domestic and 17 feral), Huludao (39 domestic and 20 feral), Chaoyang (53 domestic and 25 feral), Fuxin (36 domestic and 17 feral) and Dandong (43 domestic and 68 feral) in Liaoning province, northeastern China. All operations were handled in strict accordance with the Good Animal Practice requirements of the Animal Ethics Procedures and Guidelines of the People's Republic of China. This study was approved by the Animal Ethics Committee of Jilin University (IACUC approval no. 20140901). The domestic cats for sample collection were selected according to the following criteria. First, cats had never been moved outside of the study area according to the owners. Second, animals were more than 6 months old. Third, animals must have no past history or diagnosis of heartworm infection. Finally, owners were willing to participate in this survey and authorized it. Meanwhile, the feral cats were collected from humane cat traps as follows. Humane cat traps containing food and water were set in shaded areas and checked the next morning. Trapped cats were transported to the Small Animal Hospital at Jilin University or Eastern Liaoning University. Then, they were anaesthetized, and a clinical exam was performed on each cat. A complete record of each cat was kept, including gender (male, female), age (<3 or ≥3 years), rearing condition (domestic or feral) and other demographic data. The ages of the feral cats were estimated according to the teeth and bones of the layer [23]. Approximately 3-5 ml blood samples were obtained and centrifuged at



**Fig. 1.** Geographic distribution of the sampling site in Liaoning province, northeastern China. (A) Liaoning province (LN) is located in the northeastern part of the mainland China. (B) Shaded areas are sampling locations for the present study. SY, Shenyang; DL, Dalian; AS, Anshan; BX, Benxi; DD, Dandong; JZ, Jinzhou; HLD, Huludao; CY, Chaoyang; FX, Fuxin.

**Table 1.** The analysis of risk factors for *D. immitis* prevalence between domestic and feral cats in Liaoning province (northeastern China)

Variable	Samples (n)	Positive (n)	Prevalence (%)	OR	95% CI	P-value
Gender						
Male	319	15	4.7	1		NA
Female	332	14	4.2	1.12	0.53-2.35	>0.05
Age						
<3 years (0.5-3 years)	363	6	1.7	1		NA
≥3 years (3-12 years)	288	23	8	0.19	0.08-0.44	<0.01
Rearing condition						
Domestic	364	5	1.4	1		NA
Feral	287	24	8.4	0.15	0.06-0.36	<0.01
Total	651	29	4.5			

NA, not applicable.

3,500 g for 10 min, and sera were stored at -20°C in airtight vials until tested. All samples were tested to detect *D. immitis* using a commercial antigen detection kit (SNAP Heartworm RT, IDEXX Laboratories, Westbrook, Maine, USA) according to the manufacturer's instructions. For the statistical analysis, the outcome variable was the prevalence of *D. immitis*, and the independent variables were gender (male, female), age (<3 years, ≥3 years), and rearing condition (domestic or feral). Seroprevalence with the odds ratio (OR), 95% confidence intervals (CI) and P-value (P) were calculated.

The prevalence is 4.45%, and 29/651 tested samples were antigen positive samples. Since the presence of as few as 2 adult heartworms can cause cardiac enlargement and a severe acute respiratory syndrome in felines [24], an infected cat may have low production of heartworm antigens and antibodies. Therefore, the actual rate of infection may be higher than the test results indicated. The association of *D. immitis* prevalence and risk factors (gender, age and rearing condition) from positive cats were analyzed. There was no significant difference between the male and female cats (15/14, 4.7%/4.2%,  $P > 0.05$ , OR 1.12, 95% CI 0.53-2.35). The prevalence of *D. immitis* was obviously difference between cats aged ≥3 years and <3 years (23/6, 8.0%/1.6%,  $P < 0.01$ , OR 0.19, 95% CI 0.08-0.44), and the prevalence of *D. immitis* in feral cats was significantly higher than in domestic cats (24/5, 8.4%/1.4%,  $P < 0.01$ , OR 0.15, 95% CI 0.06-0.36). One possible reason is that domestic cats spent more time indoors as pets than feral cats, so feral cats could be exposed for a longer time to mosquitoes, with a consequently higher chance of being bitten by the mosquitoes [25,26]. The prevalence of *D. immitis* in different rearing conditions by age and gender group between domestic cats and feral cats is shown in Table 1. No significant difference in *D. immitis* prevalence was found in male and female cats (4.7%

vs 4.2%,  $P > 0.05$ ), which is in agreement with other studies, in stray and pet cats (3.3% vs 2.7%,  $P > 0.05$ ) in northwestern China [21], in stray cats (2.6% vs 2.6%,  $P > 0.05$ ) in south Korea [27], and in with or without outdoor access cats (0.4% vs 0.4%) in the United States and Canada [28], but this result differs from Kramer and Genchi, in which the male cat prevalence was significantly higher than that in female cats (19.0% vs 13.0%,  $P < 0.01$ ) [29]. Domestic cats aged ≥3 years and <3 years showed no significant differences in prevalence ( $P > 0.05$ ). However, the prevalence of *D. immitis* in older feral cats was significantly higher compared to that in younger feral cats ( $P < 0.01$ ). There is one existing report that older dogs were more likely to be infected with *D. immitis* [30], possibly because the older dogs were more likely to be exposed to mosquitoes for a longer time, with a consequently higher chance of being bitten, which might be the same reason for cats.

There are many surveys about the prevalence of *D. immitis* in dogs, but only few reports about the prevalence of *D. immitis* in cats in China and neighboring countries. The prevalence of *D. immitis* in stray and pet cats was 3.0% (11/362) in Gansu province, northwestern China [21], and 1.9% (22/1,141) in Stray cats in Liaoning, Jilin, Heilongjiang, and Shandong province, eastern China [22]. The only report about the seroprevalence of *D. immitis* is 4.6% (34/746) in pet cats in Bangkok and vicinities, Thailand [31]. A survey of *D. immitis* infection among stray cats in Korea using nested PCR showed that 6.0% (14/235) were positive for *D. immitis* [10], and 2.6% (4/145) among stray cats in Gyunggi province in South Korea [27]. In Japan, a prevalence of *D. immitis* varying from 0.5 to 9.5% for stray cats and from 3.0 to 5.2% for house cats that has been examined since 1957 to 1998 [32]. However, no *D. immitis* was detected from 170 blood samples of domestic and stray cats in Malaysia [9]. There are increasing numbers of cats in

China in the last 3 decades along with raising public health problems in view of cats can transmit a number of zoonotic pathogens, Epidemiological data of *D. immitis* is necessary to control and prevent feline dirofilariosis.

In conclusion, the study confirms the presence of *D. immitis* in domestic and feral cats and that the prevalence was significantly higher in feral cats, especially older feral cats. These results suggest that outdoor exposure time is one of the most important factors for *D. immitis* infection in cats. Therefore, the present study suggests that prophylaxis against the parasite is advisable to decrease the incidence of feline dirofilariosis in Liaoning Province, northeastern China.

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## CONFLICT OF INTEREST

We have no conflict of interest related to this study.

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