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The seroprevalence of canine dirofilariosis in dogs in the eastern coastal areas of China

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ABSTRACT

Background and objectives: The present study was carried out to assess the presence of canine Dirofilaria immitis infection in pet dogs in China.
Materials and methods: From October 2018 to November 2019, a total of 216 sera were collected from pet hospitals in Shandong, Jiangsu, Shanghai, Zhejiang, and Fujian regions of Eastern China. The sera were tested by using a commercial canine heartworm antibody ELISA test kit.
Results: 70.8% of the pets had significant clinical symptoms resembled to heartworm infection; the overall dirofilariosis positivity found was 12.5% (27/216); Significant positive rates differences were observed between symptomatic and asymptomatic dogs ($P < 0.05$) (i.e. 15.7% and 4.7% respectively).
The prevalence of infection in Shandong Province (15.5%) was the highest among the surveyed areas, but the difference among the geographic regions was not statistically significant ($P > 0.05$). Furthermore, the prevalence detected in summer (28.2%) was significantly higher than in other seasons ($P < 0.05$). In addition, no significant difference was observed between male and female sex ($P > 0.05$).
Conclusions: Altogether, these results suggest that an epidemic of dirofilariosis exists in eastern coastal China, as such preventive measures should be taken to control the spread of dirofilariosis to reduce the risk of human and pet infection with heartworm.

1. Introduction

Canine Heartworm Disease (CHD) is a serious cardiopulmonary disease that can cause vascular and pulmonary damages and even death for parasitized animals in the absence of preventive measures and appropriate treatments [1]. The disease is caused by *Dirofilaria immitis* (*D. immitis*), a mosquito-transmitted nematode with worldwide distribution that affects dogs, as main host but also cats, ferrets, and several other wild carnivores (e.g., foxes, jackals, and wolves) [2]. Different culicid mosquitoes of the genera *Culex*, *Aedes*, and

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Anopheles play a vectors role for CHD, some of which are commonly detected close to urban and sub-urban environments [3]. Humans may act as accidental hosts of *D. immitis,* which can cause pathological lesions, such as ocular, subcutaneous and pulmonary disorders. Some reports demonstrated that the risk of infection by *D. immitis* in humans is tied to the prevalence in the canine [3,4], suggesting that dirofilariosis is becoming a serious threat to human and veterinary public health.

One of the main factors that influences the prevalence of *D. immitis* is the climate change proceeds that has caused an increase the populations of mosquito, shortened the development of infective stages and lengthened the transmission season [5,6]. Another key factor is the traveling pet, which may serve as carriers for *D. immitis* and introduce these parasites to non-endemic countries [7,8].

The clinical cases of CHD are generally divided into three developing stages. The first stage is often without any or with very mild symptoms. The second stage, the dog usually shows mainly a chronic cough, dyspnea, mild anaemia and vomit. The third stage, the affected dogs with a large worm burden, present cough, vomit, anaemia, tachycardia, tachypnoea and syncopes [9,10].

In China, Sun *et al.* (1999) conducted a canine dirofilariosis serological survey of 310 domestic dogs in Chongqing, Kunming, Nanchang, Fuzhou, Guangzhou, Shenzhen, and Nanning and found that 42 of the cases (13.5%) were seropositive for dirofilariosis [11]. The prevalence of *D. immitis* in dogs were also found in Chongqing (61.3%) and Xian city (31.2%) [12,13]. The heartworm antigen positive rate in Beijing was 0.33% detected by canine SNAP 4Dx test kit [14]. Wang *et al.* (2019) found that the prevalence of dirofilariosis in dogs was 5.3% in Hainan Island/Province and 1.5% in Shanghai [15]. Moreover, the seroprevalence of *D. immitis* in Taipei city were 13.8% in domestic dogs detected by a commercial ELISA kit [16]. In Hong Kong a novel species of *Dirofilaria* has been found in dogs and humans detected by PCR, and the novel *Dirofilaria* species detected in dogs' but none of the cats' blood samples [17].

So far, one of the main factors that influences the prevalence of *Dirofilaria* is the climate change proceeds that has caused an increase the populations of mosquito, shortened the development of infective stages and lengthened the transmission season [5,6]. Another key factor is the traveling pet, which may serve as carriers for *D. immitis* and introduce these parasites to non-endemic countries [7,8].

Nevertheless, current published serological data in China are far from adequate regarding the effects of heartworm disease on dogs and human, especially in southeast China where there are more residents and pets, which means potentially higher heartworm rates and needs more public health concerns.

The present study was carried out to elucidate the current state of CHD by antibody detecting ELISA among domestic dogs in Shandong, Jiangsu, Shanghai, Zhejiang, and Fujian in southeastern China, and the factors that may affect the prevalence of canine dirofilariosis were also analyzed. The presented results could provide an improved understanding of canine heartworm epidemiology in the southeastern coastal regions of China.

Fig. 1. The historical prevalence data in past reports and the new prevalence data in this study of canine *D. immitis* in dogs of China. N: northern latitude, E. east longitude. Locations of the five coastal regions in this study.

2. Materials and methods

2.1. Study areas

The study was carried out in four Provinces (Shandong, Jiangsu, Zhejiang, and Fujian) and one city (Shanghai) in the southeastern coastal areas of China. The geographical locations of these areas in China are shown in Fig. 1. The Environmental conditions of five coastal areas throughout the year including their longitudes, latitudes, altitude, temperature, and relative humidity are recorded in Table 1.

2.2. Animal sampling

Blood samples were collected from October 2018 to November 2019 from 216 owned dogs (103 males and 113 females). The pet dogs were selected from the local pet clinics in five coastal areas of China. Notably, 70.8% of these dogs were symptomatic (including anemia, cough, dyspnea, anorexia, diarrhea, fever, or cardiac failure), while 29.2% of the dogs were asymptomatic (showing no clinical signs). A complete record, including sex, age, location, season of sampling, and health status (symptomatic/asymptomatic), was collected for each enrolled dog. The data were acquired from pet owners or medical records. During the three months prior to blood collection, the dogs in the current study were neither on prophylaxis nor treated with anthelmintics. The blood samples were collected from the cephalic or saphenous veins of the dogs, placed into sterile plain test tubes, left to clot at room temperature for 20–30 min, and centrifuged at $1000\times g$ for 15 min. The separated sera were stored at -20 °C until the tests were performed.

2.3. Heartworm antibody test

Canine *D. immitis* infection was identified using a canine heartworm antibody ELISA test kit (specificity: 94% and sensitivity: 96%) from Shanghai Jining Biotech Co., Ltd. (China) according to the recommendations of the manufacturer. Positive and negative control sera were placed in the ELISA kit. Briefly, a 96-well ELISA plate was coated with *D. immitis*-specific antigens. After incubating the diluted serum sample (1:100) in the test well and sub-sequent washing, a conjugate was added. The plate was washed again and a chromogenic enzyme substrate was then added. The optical density (OD) at 450 nm was read using a photometer (Bio-Rad, Hercules, California). The dogs were designated as either *D. immitis* positive or *D. immitis* negative. All sample testing procedures and result determination were conducted according to the manufacturer's instructions.

2.4. Statistical analysis

Test validity:$\overline{x}$ (positive control) ≥ 1.00,$\overline{x}$ (negative control) ≤ 0.20. Cut off $= \overline{x}$ (negative control) $+0.15$. The sera were considered negative for canine dirofilariosis if the OD of the sample was $<$ the cut-off value and positive if the OD of the sample was $\geq$ the cut-off value according to the recommendations of the manufacturer.

The effects of risk factors on *D. immitis* status were investigated using an exact binomial confidence interval (CI) of 95%. A chi-square ($\chi 2$) goodness-of-fit test was performed. Differences were considered statistically significant if $P < 0.05$. Statistical analysis was implemented using SPSS version 17.0 [18].

3. Results

Among the 216 serum samples in this study, the overall seroprevalence of *D. immitis* infection in dogs was 12.5% (27/216) in the five areas of China (Table 2, Fig. 2A). Briefly, the prevalence rate of *D. immitis* was 15.5% (9/58) in Shandong, 12.8% (5/39) in Jiangsu, 8.5% (3/35) in Shanghai, 10.6% (5/47) in Zhejiang, and 13.5% (5/37) in Fujian, respectively (Table 2, Fig. 2D). The prevalence in Shandong Province (15.5%) was the highest among the surveyed areas, but the difference among the geographic regions was not statistically significant ($P > 0.05$).

Furthermore, of the 216 dogs sampled, 70.8% showed significant clinical symptoms, including anemia, cough, dyspnea, anorexia, diarrhea, fever, and cardiac failure. The positive rates to *D. immitis* were 15.7% in symptomatic and 4.7% in asymptomatic dogs (Table 2, Fig. 2E). Significant differences were found between symptomatic and asymptomatic animals ($P = 0.027$, OR $= 3.721$, 95% CI $= 1.078$–12.841).

The prevalence rates were 8.7% (9 out of 103) in males and 15.9% (18 out of 113) in females (Table 2, Fig. 2B). The prevalence rate

Table 1
Environmental conditions of five coastal areas throughout the year.

Area	Longitude (E, range)	Latitude (N, range)	Altitude (m, about)	Temperature (∘C, average)	Relative humidity (%, average)
Shandong	114°47.5′-122°42.3′	34°22.9′-38°24.0′	316	14	70
Jiangsu	116°21′-121°56′	30°45′-35°08′	25	17.5	70.3
Shanghai	120°52′-122°12′	30°40′-31°53′	3	18.5	75
Zhejiang	118°01′-123°10′	27°02′-31°11′	226	17.8	70.3
Fujian	115°50′-120°40′	23°33′-28°20′	300	22	77

Table 2
Seroprevalence of canine dirofilariosis in eastern coastal areas of China determined by ELISA.

Factor	Category	No. Tested	No. Positive (%)	Odds ratio (95%CI)	P[a]
Total		216	27 (12.5)		
Sex					
	Males	103	9 (8.7)	1	
	Females	113	18 (15.9)	0.505 (0.216–1.18)	0.11
Age (year)					
	<1	42	3 (7.1)	1	
	1–5	90	14 (15.5)	0.418 (0.113–1.54)	0.179
	≥5	84	10 (11.9)	0.569 (0.148–2.19)	0.407
Location					
	Shandong	58	9 (15.5)	1	
	Jiangsu	39	5 (12.8)	1.249 (0.385–4.05)	0.711
	Shanghai	35	3 (8.5)	1.959 (0.493–7.79)	0.333
	Zhejing	47	5 (10.6)	1.543 (0.480–4.96)	0.465
	Fujian	37	5 (13.5)	1.176 (0.361–3.82)	0.788
Health status					
	Symptomatic*	153	24 (15.7)	1	
	Asymptomatic	63	3 (4.7)	3.721 (1.078–12.841)	0.027
Season					
	Spring (Mar.–May)	72	6 (8.3)	1	
	Summer (Jun.–Aug.)	46	13 (28.2)	0.231 (0.080–0.662)	0.004
	Autumn (Sep.–Oct.)	50	7 (14.0)	0.558 (0.176–1.774)	0.318
	Winter (Dec.–Feb.)	48	1 (2.1)	4.273 (0.498–36.673)	0.152

Note: *Symptomatic included anemia, cough, dyspnea, anorexia, diarrhea, fever, cardiac failure, and others.
[a] P analyzed by Pearson's chi-square test for independence.

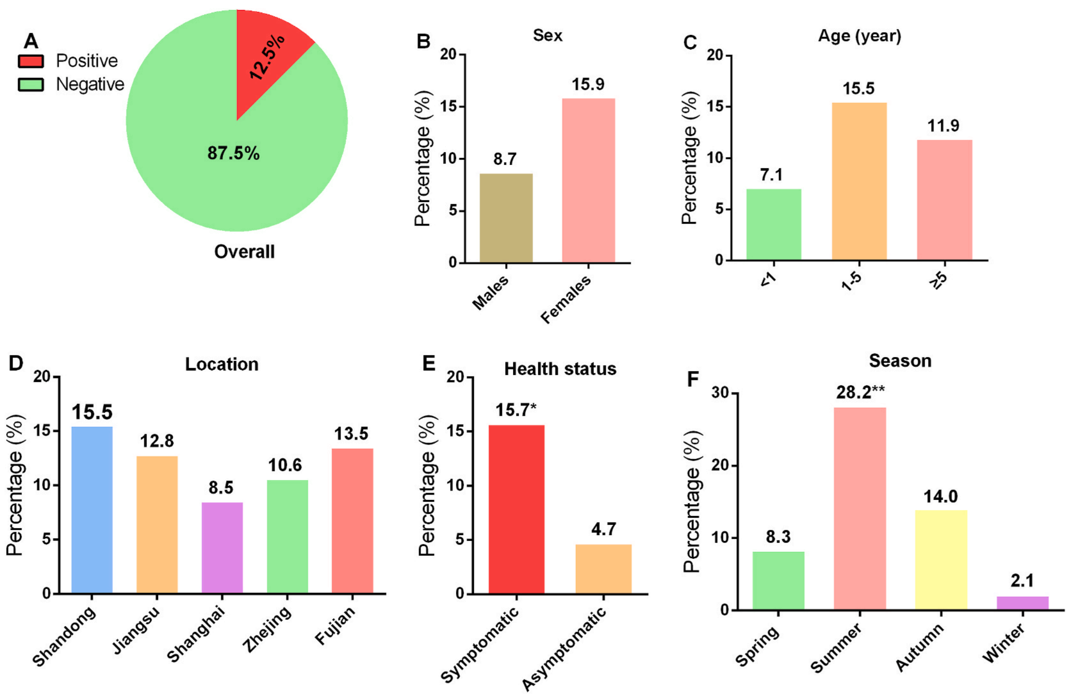

Fig. 2. The overall seroprevalence of canine *D. immitis* in southeastern coastal regions of China, and potential risk factors that may affect the prevalence of canine dirofifilariosis. **A.** The overall seroprevalence of canine *D. immitis* in southeastern coastal regions of China. **B.** The seroprevalence of *D. immitis* in dogs of different sex. **C.** The seroprevalence of *D. immitis* in dogs of different ages. **D.** The seroprevalence of *D. immitis* in dogs of different location. **E.** The seroprevalence of *D. immitis* in dogs of different health status. **F.** The seroprevalence of *D. immitis* in dogs of different season. *$P < 0.05$, **$P < 0.01$.

value was higher in females than in males, but no significant difference in terms of sex was observed among the infected dogs ($P = 0.11$, OR = 0.505, 95% CI = 0.216–1.18). Additionally, the highest prevalence (15.5%) of *D. immitis* was observed between the ages of 1 and 5 years, followed by intermediate prevalence (11.9%) in ≥5 years, and 7.1% in <1 year (Table 2, Fig. 2C). However, no significant difference was observed in the prevalence of *D. immitis* in different age periods ($P > 0.05$).

Regarding to the risk factor season (Table 2, Fig. 2F), the positive rate to *D. immitis* was the highest (28.2%) in summer (June–August), followed by 14.0% in autumn (September–October), 8.3% in spring (March–May), and 2.1% in winter (December–February). Also, the seroprevalence in summer (28.2%) was significantly higher than that in other seasons ($P = 0.004$, OR = 0.231, 95% CI = 0.080–0.662).

4. Discussion

Dirofilariasis is a zoonotic disease caused by *D. immitis*, which can be transmitted by mosquitos and spread worldwide. A meta-analysis (2020) reported that the prevalence of *D. immitis* in dogs was 7.57% in Africa, 10.45% in Europe, 11.60% in America, 12.07% in Asia, and 22.68% in Australia [19], respectively. In the present study, the overall canine heartworm prevalence rate in five coastal areas of China was 12.85%, which is slightly higher than that meta-analysis report in Asia. This result may be attributed to the fact that this study chose coastal areas where mosquitoes like to inhabit [20]. Among the coastal areas, Shandong Province's seroprevalence (15.5%) was lower than the 24.0% rate found in dogs performed in Dandong [21]; it was similar to the 15.0% reported by Sun *et al.* for Guangzhou [11] but higher than those observed in Shenyang (12.7%) [22], Heilongjiang (1.1%) [23], and Henan (13.0%) [24]. The prevalence (8.5%) in Shanghai was the lowest among the surveyed areas in this study, but higher than the previous study surveyed in Shanghai (1.5%) [15], illustrating that the prevalence of canine heartworm in Shanghai has increased in recent years. However, differences in detection methods can also cause large discrepancies. The result of *D. immitis* antigen detection test could underestimate the real prevalence in those geographical regions with low infection rates, because low worm burdens in dogs could lead to a negative antigen test. However, other differences in geographical factors, survey periods, sample sizes, ages, and breeds cannot be excluded for the divergence in the prevalence of *D. immitis* across these regions.

Clinical symptoms of respiratory system can mostly be observed in heartworm-infected dogs. In mild or early infections, occasional cough and exercise induced dyspnea may be noted. In more severe and chronic cases, dyspnea, exercise intolerance, anaemia, or right-side heart failure signs can occur [9,19]. In this study, significant difference was observed in prevalence of *D. immitis* between symptomatic dogs and asymptomatic dogs. Lu *et al.* (2017) indicated that dogs having symptoms of cough or dyspnea were associated with an increased risk of heartworm infection [25].

In the present study, no significant difference prevalence of *D. immitis* was found between male and female dogs, which is in agreement with other previous surveys [24,26]. However, Montoya *et al.* (1998) indicated that heartworm infection has significant difference between male and female dogs in Gran Canaria [27]. Higher prevalence rates in males can be associated with the fact that more male dogs kept outdoors to defend safety and property in Gran Canaria, leading male dogs more likely to be bitten by mosquitoes.

Age is one of the risk factors for canine *D. immitis* infection. The present study indicated that the highest seroprevalence (15.5%) of *D. immitis* was observed between the ages of 1 and 5 years, but no significant difference found among different age periods. On the contrary, other reports demonstrated a significantly higher prevalence of heartworm in older dogs than in younger dogs [24,27,28]. Wang *et al.* (2016) found that the risk of exposure to *D. immitis* increases with age, especially in dogs from the $\geq$ 6-year-old age group [24]. This discrepancy may be due to differences in heartworm detection methods, the heartworm antibodies was applied to assess the prevalence of *D. immitis* in our study. Specifically, the immune system of young dogs is immature and the degradation of immune function of old dogs can lead to low antibody levels after *D. immitis* infection.

Temperature is an important factor for the establishment and the prevalence of *D. immitis* infection in an area. Considering that *D. immitis* is transmitted by different species of culicid mosquitoes, the life cycle of vectors and larval development of the parasite depend on temperature and humidity [29,30]. In the current study, significant difference was observed in prevalence of *D. immitis* between different seasons as showed in Table 2 and Fig. 2F. The prevalence of heartworm in summer is much higher than in other seasons. A survey also reported that the risk period for *D. immitis* transmission is seasonal in Hungary and peaks in summer [31]. The five coastal areas of China chose in this study is located in a subtropical monsoon climate or temperate monsoon climate zone, its hot and humid environment in the summer season (June–September) facilitates the extrinsic incubation of *D. immitis* in mosquitoes, which made dogs have greater chances of exposure to mosquitoes [20,32].

Nothing is perfect, and neither is this study. There are some limitations. First, sample size was not large enough to guarantee the accurately representative of regional prevalence, which may result in over- or underestimation of prevalence rates. Second, *D. immitis* worms were not collected and echocardiography was not performed for dirofilariosis definitive diagnosis, which may lead to a false-negative or false-positive results. Third, because all dogs in our study were from pet hospitals, our study can't answer the question of heartworm prevalence in stray dogs. Fourth, molecular techniques were not employed in the detection of dirofilariosis. These limitations will be improved in our future studies.

5. Conclusion

The present study demonstrates the presence of canine *D. immitis* infection and confirms the significant circulation of dirofilariosis in domestic dogs in the eastern coastal areas of China. Even though our study has certain limitations, it can still enrich the prevalence data of canine heartworm and provide reference for clinical veterinarians. That is, veterinarians should continue to emphasize the importance of annual *D. immitis* testing and year-round use of chemoprophylaxis. Furthermore, preventive measures, such as control of parasite reservoirs, are highly recommended. These measures will contribute to the control of canine dirofilariosis and prevention of zoonotic infections.

Ethics statement

The present study was submitted to and approved by the Animal Welfare Committee of Sichuan Agricultural University (approval number SYXK2019-187). The samples were collected and handled in accordance with the good animal practices required by the Animal Ethics Procedures and Guidelines of the People's Republic of China.

Author contribution statement

Dongjie Cai: Analyzed and interpreted the data; Wrote the paper.
Bin Tian: Performed the experiments.
Yongxia Liu: Performed the experiments.
Mujeeb Ur Rehman: Wrote the paper.
David Ranucci: Wrote the paper.
Fabrizia Veronesi: Analyzed and interpreted the data.
Antonio Varcasia: Analyzed and interpreted the data.
Wanzhong Jia: Contributed reagents, materials, analysis tools or data.
Jianzhu Liu: Conceived and designed the experiments.

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Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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