

Prevalence of *Dirofilaria immitis* infection in dogs in Henan province, central China

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Abstract – The heartworm *Dirofilaria immitis* is the causative agent of cardiopulmonary dirofilariasis in dogs and cats, and also infects humans. However, there has been no study on dirofilariasis in dogs in central China. From March 2015 to February 2016, sera from 1176 randomly selected household dogs from Henan province, central China were examined for *D. immitis* antigen using the Canine Heartworm Antigen Test Kit. The overall seroprevalence of *D. immitis* in dogs in Henan province was 13% (155/1176). The prevalence was significantly higher in older dogs and dogs kept outdoors, compared to the younger ones and those sheltered indoors. No significant difference of prevalence was observed between sexes. The results suggest that the risk of exposure to *D. immitis* in dogs is high in Henan, and prophylaxis against the parasite is advisable to decrease the incidence of canine dirofilariasis in this region.

Key words: *Dirofilaria immitis*, Prevalence, Dog, Central China.

Résumé – Prévalence de l'infection par *Dirofilaria immitis* chez les chiens dans la province du Henan, Chine centrale. Le Nématode *Dirofilaria immitis* est l'agent causal de la dirofilariose cardiopulmonaire chez les chiens et les chats, et infecte aussi les humains. Cependant, il n'y a pas de rapport sur la dirofilariose chez les chiens dans le centre de la Chine. De mars 2015 à février 2016, les sérums de 1176 chiens domestiques choisis au hasard dans la province du Henan, en Chine centrale, ont été examinés pour l'antigène de *D. immitis* en utilisant le kit Canine Heartworm Antigen Test. La séroprévalence globale de *D. immitis* chez le chien dans la province du Henan était de 13 % (155/1176). La prévalence était significativement plus élevée chez les chiens âgés et les chiens abrités en plein air, par rapport aux plus jeunes et ceux abrités à l'intérieur. Aucune différence significative de prévalence n'a été observée entre les sexes. Les résultats suggèrent que le risque d'exposition à *D. immitis* chez les chiens est élevé dans le Henan, et il est conseillé d'utiliser la prophylaxie contre le parasite pour diminuer l'incidence de la dirofilariose canine dans cette région.

Introduction

The causative agent of cardiopulmonary dirofilariasis, *Dirofilaria immitis* (heartworm), affects domestic dogs, cats, and various wild mammals, with increasing incidence in temperate and tropical areas [9, 12, 16]. As mosquito-borne zoonotic pathogens, heartworms can also be transmitted to humans, where they cause diseases such as pulmonary dirofilariasis and subcutaneous dirofilariasis [5, 19]. Adult *D. immitis* worms can survive for seven years or more in dogs, usually producing chronic inflammatory vascular disease [19].

In addition, the simultaneous death of groups of adult worms can trigger an acute disorder characterized by the exacerbation of inflammatory reactions and the occurrence of serious thromboembolism that put the life of the infected dogs at immediate risk [14].

There are many reports on the prevalence of *D. immitis* infection in dogs from various countries, including Korea [3], Iran [10], Poland [11], Portugal [1], Costa Rica [18], and Hungary [2]. In recent years, studies of *D. immitis* infection in dogs have been undertaken in different regions of China (Table 1 [6–9, 13, 20–22]). These reports show that canine

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Table 1. Prevalence of *Dirofilaria immitis* infection in dogs in China.

| Localities | Year of sampling ^A | No. tested | Positive (%) | Method ^B | Reference |
|--------------|-------------------------------|------------|--------------|--------------------------------------|-----------|
| Taipei | 1998–1999 | 664 | 89 (13.4) | ELISA | [6] |
| Taiwan | 1993–1997 | 2065 | 803 (38.9) | Necropsy and microscopic examination | [22] |
| Heilongjiang | 1996–2004 | 178 | 2 (1.1) | Necropsy | [21] |
| Changchun | <2007 | 62 | 32 (51.6) | ELISA and microscopic examination | [8] |
| Guizhou | <2010 | 300 | 15 (5.0) | Immunochromatographic strip | [7] |
| Dandong | 2003–2010 | 886 | 213 (24.0) | PCR and microscopic examination | [9] |
| Kunming | 2010–2011 | 30 | 2 (6.7) | ELISA | [20] |
| Chongqing | 2010–2011 | 30 | 3 (10.0) | ELISA | [20] |
| Nanchang | 2010–2011 | 30 | 3 (10.0) | ELISA | [20] |
| Fuzhou | 2010–2011 | 50 | 6 (12.0) | ELISA | [20] |
| Guangzhou | 2010–2011 | 60 | 9 (15.0) | ELISA | [20] |
| Shenzhen | 2010–2011 | 80 | 15 (18.8) | ELISA | [20] |
| Nanning | 2010–2011 | 30 | 4 (13.3) | ELISA | [20] |
| Shenyang | 2009–2012 | 528 | 67 (12.7) | ELISA | [13] |

^A Years of sampling are listed as published in the references. In cases where this information was not available, the year listed here is the year when the study was published, as indicated by “<”.

^B ELISA: enzyme-linked immunosorbent assay; PCR: polymerase chain reaction.

dirofilariasis is endemic in China. However, there has been no study on dirofilariasis infection in dogs in Henan, central China.

Adult worms of *D. immitis* reside in pulmonary arteries and the right ventricles, resulting in production of blood-circulating microfilariae in dogs as natural hosts [12]. Because dogs with a low worm burden are usually asymptomatic, primary diagnostic screening by detecting blood microfilariae or circulating heartworm antigens is necessary prior to treatment [17]. However, due to occult infection in some cases, antigen testing is considered the most sensitive diagnostic method [17]. Therefore, in the present study, we studied the seroprevalence of *D. immitis* infection in domestic dogs in central China for the first time and evaluated the main risk factors associated with exposure to *D. immitis* in this area.

Materials and methods

Ethical statement

The study was reviewed and approved by the Ethics Review Committee of the Xinxiang Medical University (Reference No. 2015016).

Study site

The study was conducted in Henan province, located in the central part of mainland China (Fig. 1), and covering an area of 167,000 km² and a population of approximately 106.01 million. Its geographical position is at east longitude 110°21′–116°39′ and at north latitude 31°23′–36°22′. The Yellow River passes through central Henan. The area has a continental monsoon climate, with four distinctive seasons. The average annual temperature is 12.1–15.7 °C, with a mean annual rainfall of 532.5–1380.6 mm. As shown in Figure 1, there are 17 provincial cities distributed in Henan province, with the city of Zhengzhou as its capital. Five cities

including Anyang (35°13′–36°22′ N, 113°37′–114°58′ E), Sanmenxia (33°31′–35°05′ N, 110°21′–112°01′ E), Zhengzhou (34°16′–34°58′ N, 112°42′–114°13′ E), Xinyang (31°46′–31°52′ N, 114°01′–114°06′ E), and Shangqiu (33°43′–34°52′ N, 114°49′–116°39′ E), located in the northern, western, central, southern, and eastern parts of Henan province, were selected for sample collection.

Sample collection

A total of 1176 blood samples of domestic dogs were collected from these five cities in Henan province between March 2015 and February 2016. Dog owners were asked for details of the animals’ age, sex, rearing condition, and medical history using a structured questionnaire. Blood samples were centrifuged and sera were recovered and transferred to 1.5 mL Eppendorf tubes. All sera were then stored at –80 °C until testing for circulating *D. immitis* antigens.

Test for *D. immitis* antigens

In order to identify *D. immitis* infection, all serum samples were analyzed for the *D. immitis* antigens using the Canine Heartworm Antigen Test Kit (IDEXX Laboratories, Westbrook, ME, USA) according to the manufacturer’s instructions. When testing samples from dogs with more than two adult female worms, sensitivities and specificities of this kit are 94% and 98% for *D. immitis*, respectively [4].

Statistical analysis

Statistical analysis was performed using SPSS 20 software for Windows (SPSS Inc, Chicago, IL, USA). Statistical analyses of *D. immitis* prevalence in different variables were performed by the χ^2 -test. The differences were considered statistically significant if $p < 0.05$.

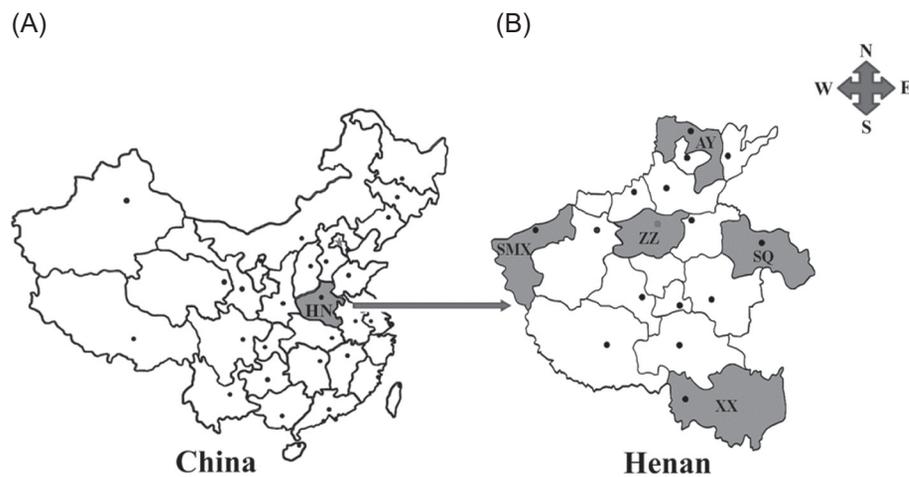


Figure 1. Geographic distribution of the sampling sites in Henan province, China used in this study. A: Henan province (HN, shadowed areas) is located in the central part of the mainland China. B: Shadowed areas are the sampling locations for the present survey. AY: Anyang; SMX: Sanmenxia; ZZ: Zhengzhou; XX: Xinyang; SQ: Shangqiu.

Results and discussion

As shown in Table 2, the overall recorded seroprevalence of *D. immitis* in dogs in Henan province, central China was 13.18% (155/1176). Compared with other provinces or cities in China, it was lower than the values of 51.6% in dogs in a study performed in Changchun [8], 38.9% in Taiwan [22], 24.0% in Dandong [9], and 18.8% in Shenzhen [20], similar to those observed in Shenyang (12.7%) [13] and Nanning (13.3%) [20], but higher than those observed in Heilongjiang (1.1%) [21], Guizhou (5.0%) [7], Kunming (6.7%), Chongqing (10.0%), Nanchang (10.0%), and Fuzhou (12.0%) [20]. The differences in prevalence of *D. immitis* among these regions may be due to differences in ecological and geographical factors, detection methods used, survey periods, sample sizes, and breed of dog populations in these areas. In the present study, the seroprevalence of *D. immitis* in males was 14.26% (88/617) and in females 11.99% (67/559) (Table 2). Although the seroprevalence in males was higher than in females, the difference was not significant ($p > 0.05$). This is in agreement with previous studies [9, 13].

A significant difference was observed in prevalence of *D. immitis* between dogs sheltered in different conditions [9, 13]. In this study, the seroprevalence of *D. immitis* infection in outdoor dogs (16.32%) was significantly higher than that in indoor dogs (9.54%; $p < 0.01$). A similar higher prevalence was also observed in outdoor dogs by Hou et al. [9] and Liu et al. [13]. The possible reason is that dogs outdoors had a greater chance of being bitten by mosquitoes [9, 13]. In the present study, the highest prevalence of infection (22.11%) was detected in six-year-old or older dogs, followed by intermediate prevalence (12.22%) in the 3–6 year age group, while the prevalence found in dogs in the ≤ 3 year age group was 6.92% (Table 2). The prevalence of *D. immitis* infection in dogs increased significantly ($p < 0.05$) with the increase in age. The difference in prevalence with respect to age coincides with other studies

Table 2. Seroprevalence of *Dirofilaria immitis* infection in dogs in Henan province, central China.

| Variable | No. examined | No. positive | Prevalence (%) |
|--------------------------|--------------|--------------|---------------------|
| Region | | | |
| Anyang | 224 | 40 | 17.86 ^{bc} |
| Sanmenxia | 235 | 23 | 9.79 ^a |
| Zhengzhou | 256 | 19 | 7.42 ^a |
| Xinyang | 242 | 28 | 11.57 ^{ab} |
| Shangqiu | 219 | 45 | 20.55 ^c |
| Sex | | | |
| Male | 617 | 88 | 14.26 |
| Female | 559 | 67 | 11.99 |
| Rearing condition | | | |
| Indoor | 545 | 52 | 9.54 ^a |
| Outdoor | 631 | 103 | 16.32 ^b |
| Age (years) | | | |
| ≤ 3 | 318 | 22 | 6.92 ^a |
| 3 ~ 6 | 573 | 70 | 12.22 ^b |
| ≥ 6 | 285 | 63 | 22.11 ^c |
| Total | 1176 | 155 | 13.18 |

Values bearing a different superscript letter (a–c) within a column differ significantly from one another ($p < 0.05$).

where higher prevalence was observed in the adult group than in the juvenile one [10, 13, 15]. These findings suggest that the risk of exposure to *D. immitis* increases with age.

Conclusions

In conclusion, a high prevalence of *D. immitis* infection (13%) was found in domestic dogs in Henan, central China. Therefore, lifelong chemoprophylaxis is needed to prevent canine dirofilariosis. Monitoring the prevalence of this particular nematode among domesticated dogs is also important because it poses a serious health risk to humans.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

- Alho AM, Landum M, Ferreira C, Meireles J, Goncalves L, de Carvalho LM, Belo S. 2014. Prevalence and seasonal variations of canine dirofilariasis in Portugal. *Veterinary Parasitology*, 206(1–2), 99–105.
- Bacsadi A, Papp A, Szeredi L, Toth G, Nemes C, Imre V, Tolnai Z, Szell Z, Sreter T. 2016. Retrospective study on the distribution of *Dirofilaria immitis* in dogs in Hungary. *Veterinary Parasitology*, 220, 83–86.
- Byeon KH, Kim BJ, Kim SM, Yu HS, Jeong HJ, Ock MS. 2007. A serological survey of *Dirofilaria immitis* infection in pet dogs of Busan, Korea, and effects of chemoprophylaxis. *Korean Journal of Parasitology*, 45(1), 27–32.
- Courtney CH, Zeng Q. 2001. Comparison of heartworm antigen test kit performance in dogs having low heartworm burdens. *Veterinary Parasitology*, 96(4), 317–322.
- Falidas E, Gourgiotis S, Ivopoulou O, Koutsogiannis I, Oikonomou C, Vlachos K, Villias C. 2016. Human subcutaneous dirofilariasis caused by *Dirofilaria immitis* in a Greek adult. *Journal of Infection and Public Health*, 9(1), 102–104.
- Fan CK, Su KE, Lin YH, Liao CW, Du WY, Chiou HY. 2001. Seroprevalence survey of *Dirofilaria immitis* infection among domestic dogs in Taipei City and mountain aboriginal districts in Taiwan (1998–1999). *Veterinary Parasitology*, 102(1–2), 113–120.
- He G, Tian W, Wang P, Wang W, Han J, Jian C, An Y. 2010. Colloidal gold immunochromatographic strip for rapid detection of *Dirofilaria immitis*. *Animal Husbandry & Veterinary Medicine*, 42(6), 89–91 (in Chinese).
- Hou H, Zhang X, Li J, Gong P. 2007. Development of indirect ELISA for the detection of canine dirofilariasis. *Journal of Pathogen Biology*, 2(1), 35–40.
- Hou H, Shen G, Wu W, Gong P, Liu Q, You J, Cai Y, Li J, Zhang X. 2011. Prevalence of *Dirofilaria immitis* infection in dogs from Dandong, China. *Veterinary Parasitology*, 183(1–2), 189–193.
- Khedri J, Radfar MH, Borji H, Azizzadeh M, Akhtardanesh B. 2014. Canine Heartworm in Southeastern of Iran with review of disease distribution. *Iranian Journal of Parasitology*, 9(4), 560–567.
- Kramer F, Schaper R, Schunack B, Polozowski A, Piekarska J, Szwedko A, Jodies R, Kowalska D, Schupbach D, Pantchev N. 2014. Serological detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi sensu lato* and *Ehrlichia canis* antibodies and *Dirofilaria immitis* antigen in a countrywide survey in dogs in Poland. *Parasitology Research*, 113(9), 3229–3239.
- Lee AC, Montgomery SP, Theis JH, Blagburn BL, Eberhard ML. 2010. Public health issues concerning the widespread distribution of canine heartworm disease. *Trends in Parasitology*, 26(4), 168–173.
- Liu C, Yang N, He J, Yang M, Sun M. 2013. Prevalence of *Dirofilaria immitis* in dogs in Shenyang, Northeastern China. *Korean Journal of Parasitology*, 51(3), 375–377.
- McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L. 2008. Heartworm disease in animals and humans. *Advances in Parasitology*, 66, 193–285.
- Montoya-Alonso JA, Carreton E, Simon L, Gonzalez-Miguel J, Garcia-Guasch L, Morchon R, Simon F. 2015. Prevalence of *Dirofilaria immitis* in dogs from Barcelona: validation of a geospatial prediction model. *Veterinary Parasitology*, 212(3–4), 456–459.
- Montoya-Alonso JA, Carreton E, Morchon R, Silveira-Viera L, Falcon Y, Simon F. 2016. The impact of the climate on the epidemiology of *Dirofilaria immitis* in the pet population of the Canary Islands. *Veterinary Parasitology*, 216, 66–71.
- Nelson CT, McCall JW, Rubin SB, Buzhardt LF, Dorion DW, Graham W, Longhofer SL, Guerrero J, Robertson-Plouch C, Paul A, Executive Board of the American Heartworm Society. 2005. 2005 Guidelines for the diagnosis, prevention and management of heartworm (*Dirofilaria immitis*) infection in dogs. *Veterinary Parasitology*, 133(2–3), 255–266.
- Rojas A, Rojas D, Montenegro VM, Baneth G. 2015. Detection of *Dirofilaria immitis* and other arthropod-borne filarioids by an HRM real-time qPCR, blood-concentrating techniques and a serological assay in dogs from Costa Rica. *Parasites & Vectors*, 8, 170.
- Simon F, Siles-Lucas M, Morchon R, Gonzalez-Miguel J, Mellado I, Carreton E, Montoya-Alonso JA. 2012. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. *Clinical Microbiology Reviews*, 25(3), 507–544.
- Sun M, Zhuo W, Guo S, Liao S, Shi D, Liu J, Cheng Z, Liu Y, Niu X, Wang S, Yang D. 2012. Serological survey of canine dirofilariasis in Chongqing, Kunming, Nanchang, Fuzhou, Guangzhou, Shenzhen, and Nanning in Southern China. *Veterinary Parasitology*, 185(2–4), 225–228.
- Wang CR, Qiu JH, Zhao JP, Xu LM, Yu WC, Zhu XQ. 2006. Prevalence of helminthes in adult dogs in Heilongjiang Province, the People's Republic of China. *Parasitology Research*, 99(5), 627–630.
- Wu CC, Fan PC. 2003. Prevalence of canine dirofilariasis in Taiwan. *Journal of Helminthology*, 77(1), 83–88.

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