Epidemiological investigation of thelaziosis in domestic dogs in Beijing, capital of China from 2018 to 2019

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Research

Keywords: Thelazia callipaeda, Domestic dogs, Vector-borne zoonosis, Epidemiology, Chinese capital of Beijing

Posted Date: October 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-979815/v1

Abstract

Background

The first reported case of thelaziosis in human in the world is located in Beijing, the capital of China, in 1917. With the promotion of urbanization, the development trend of the disease is also gradually urbanized. As the capital of China, Beijing has a permanent population of 21.893 million, and the number of domestic dogs in the city has reached more than 1 million. Therefore, the analysis of the prevalence of domestic dogs thelaziosis in Beijing can not only provide strategies for domestic dogs to prevent and control the disease, but also benefit the risk analysis of local zoonotic parasitic.

Methods

The ophthalmology related cases treated in the animal hospital of China Agricultural University from September 2018 to December 2019 were collected. If the domestic dog carries parasites in its ocular, the parasites will be collected for morphological identification of the species, and finally the species will be confirmed by molecular biology. The data were statistically analyzed to analyze the correlation between risk factors and prevalence rate.

Results

Among 3215 domestic dogs examined, 102 cases were found to have ocular parasitic infection, which was identified as *Thelazia callipaeda* by morphology and molecular biology. The results of risk factor analysis showed that regional, season, field travel history and anthelmintic history had significant effects on the prevalence rate ($P < 0.05$). The regional distribution of cases thelaziosis in domestic dogs showed an obvious cluster distribution phenomenon. The cases with moderate symptoms accounted for the largest proportion regardless of the number of nematodes carried.

Conclusions

The infection of domestic dogs thelaziosis in Beijing was significantly correlated with regional distribution, seasonal distribution, field travel history and anthelmintic history. There was no significant correlation between the severity of ocular clinical symptoms and the number of ocular *T. callipaeda* in domestic dogs infected with thelaziosis.

Background

The *Thelazia callipaeda* (*T. callipaeda*) belongs to the sucking nematode genus of sucking family. It takes *Phortica okadai* as the intermediate host and transmission vector [1, 2]. The definitive host range is very wide, which can be infected by wild mammals, domestic dogs and humans [3]. It is widely

distributed in China, Myanmar, South Korea and other Asian countries, so it is also called Oriental eyeworm [4]. It can parasitize in the ocular conjunctival sac of many mammals such as humans, dogs and cats, resulting in thelaziosis [5]. The damage caused by *T. callipaeda* in the eyes of animals is different. Due to its serrated wrinkled surface and the adsorption of the buccal capsule, it will cause mechanical damage to the ocular tissue. In addition, the metabolites produced by the nematode during the whole life cycle of conjunctival sac parasitism will continue to stimulate the ocular tissue. Clinically, some infected dogs show foreign body sensation and increased ocular secretion, resulting in conjunctival edema, congestion and corneal ulcer, and even secondary glaucoma [6, 7]. Severe cases can also cause blindness.

In 1917, Trimble found human infection cases in Beijing, the capital of China. So far, China has become the first country in the world to report human infection with *T. callipaeda* [8], and also the country with the largest number of cases of the disease [7]. A large number of cases have been reported in other Asian countries such as South Korea and Japan. In recent 20 years, the number of cases in Europe has gradually increased [9, 10, 11, 12]. As an important zoonotic parasitic disease, the epidemiological research on thelaziosis has not been interrupted by both human doctors and veterinarians. From 1917 to 2019, a total of 643 cases of human thelaziosis were recorded in China [13]. The prevalence of infection is closely related to the age, gender, occupation, health condition and population density of the population in the epidemic area [14]. The data showed that the prevalence of infection of children aged 0-5 was the highest, and it had a great relationship with its weakness of avoiding the intermediate host of *T. callipaeda* [14]. The gender is mainly male, which is mainly related to men's frequent outdoor work. The prevalence of infection of outdoor occupation, especially for forest workers, is the highest, which is related to the distribution density of the intermediate host of the nematode [15]. It is generally believed that infected domestic animal (dogs and cats) are the most important reservoir host of *T. callipaeda*, which directly threatens human health [16, 17]. With the increase of social demand for working dogs and the gradual prosperity of China's pet industry, more and more families choose dogs as companion animals, and the number of cases of domestic dogs thelaziosis is increasing year by year [18, 19, 20], which poses a certain threat to dog family members.

Previously, cases of thelaziosis in humans and dogs were mostly reported in areas with poor economic and health conditions and a large number of domestic or wild animals. However, with the promotion of urbanization, the development trend of thelaziosis is also gradually urbanized. As the capital of China (Beijing) has a permanent population of 21.893 million, and the number of domestic dogs in the city has reached more than 1 million. Therefore, the analysis of the prevalence of domestic dogs thelaziosis in Beijing can not only provide strategies for domestic dogs to prevent and control the disease, but also benefit the risk analysis of local zoonotic parasitic.

Methods

Sample collection and preservation

Collect the ophthalmic related cases of domestic dogs treated in the animal hospital of China Agricultural University from September 2018 to December 2019, and record their breed, age, weight, sex, living area, feeding conditions, whether they are infected with *T. callipaeda* and the number of infections, immunization history, insect repellent history, ophthalmic examination and other information. For the case of thelaziosis in domestic dogs, after local anesthesia, take out the nematode body with a sterile cotton swab, put it into a sterile collection tube containing 95% alcohol for fixation, and number the collection tube.

Morphological observation

The morphology of *T. callipaeda* was observed by desktop microscope: the cuticle on the surface of nematode had clear circular fold structure; There are distinct buccal capsule and muscular esophagus in the front; The intestine, anus and rectum can be seen at the back of the nematode body; A large number of discoid larvae or eggs can be seen in the uterus of the female; When the above morphological characteristics are met, it can be preliminarily identified as *T. callipaeda*.

Molecular biology confirmation

We extracted genomic DNA of each nematode from the conjunctival sac of the domestic dogs with the HiPure Tissue & Blood DNA Kit (MAGEN, China). A partial sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox*1; 689 base pairs) was amplified by PCR. Amplicons were purified by a HiPure Gel Pure Micro Kit (MAGEN, China) and sequenced in an ABI3730XL with the BigDyeTr v3.1 Cycle Seq Kit (Applied Biosystems, USA). Amplicon sequences were determined in both directions (GenBank accession nos. MN719910) and genetic analyses performed using available sequences of related nematodes from GenBank and the Global Initiative on Sharing all Influenza Data (GISAID) database (https://www.gisaid.org) [21, 22, 23].

Statistical analysis

The survey results and test data were entered into Microsoft Excel for classification and sorting, and the prevalence rate under different groups was calculated. The frequency and prevalence of infection are reported with 95% confidence intervals (CIs), and the risk factors (region, season, age, sex, breed, body type, living environment, diet, field travel history, immunization history and insect repellent history) were assessed using a Chi-square test. Differences were considered statistically significant at $P < 0.05$.

The geographic location information of the collected cases was analyzed by ArcGIS 10.2 software (http://www.esri.com/software/arcgis). Select the nuclear density analysis module, input the detection results of domestic dogs thelaziosis in each area, adjust the result layer to an appropriate effect map, and then export the nuclear density map. The spatial statistics module is used to analyze the hot spot and spatial autocorrelation.

Results

Overall prevalence

From September 2018 to December 2019, 3215 cases of ophthalmic examination were collected, and 102 domestic dogs infected with ocular parasites were found. After morphological observation, the adults were slender, milky white and cylindrical; At high magnification, transverse lines can be seen in the cuticle of the nematode. There are obvious buccal capsule and muscular esophagus in the front of the nematode, intestines and uterus in the middle, anus and rectum in the tail. The female is 11.2~15.3mm long and 311~359 wide μm; The uterus is full of nematode eggs or larvae, and there are spiral larvae in the uterus near the vulva. The morphological characteristics of the nematode body are consistent with the description of Otranto [9]. The sample was PCR positive for *T. callipaeda* (GenBank access Nos. MN719910), while the blast analysis received a 98% nuclear simplicity to an *T. callipaeda* sequence from a dog from China (GenBank access Nos. AM042555). It can be diagnosed as thelaziosis, and the total prevalence rate is 3.17% (102/3215; 95% CI 2.57%~3.78%) (Table 1). Among 102 infected domestic dogs, 62 cases were binocular infection and 40 cases were monocular infection. A total of 1277 *T. callipaeda* were collected, including 816 in the oculus sinister and 796 in the oculus dexter. The average number of nematodes carried by each domestic dog was 7.80 ± 8.51 in the oculus sinister and 8.00 ± 9.08 in the oculus dexter.

Table 1
Prevalence of thelaziosis infection in domestic dogs from the Beijing, capital of China, 2018-2019

Risk factors	Frequency (thelaziosis cases/ophthalmic related cases)	Prevalence	95% CI	χ2 (*df*)	*P*-value
Region					
Urban area	57/2181	2.61%	1.94%~3.28%	6.902 (1)	0.009
Rural area	45/1034	4.35%	3.11%~5.60%		
Season					
Spring	15/780	1.92%	0.96%~2.89%	14.887 (3)	0.002
Summer	40/936	4.27%	2.98%~5.57%		
Autumn	35/827	4.23%	2.86%~5.60%		
Winter	12/672	1.79%	0.78%~2.79%		
Age					
≤7Y	66/1592	4.15%	3.17%~5.12%	2.241 (1)	0.134
>7Y	36/1176	3.06%	2.08%~4.05%		
Sex					
Male	59/1821	3.24%	2.43%~4.05%	0.062 (1)	0.803
Female	43/1394	3.08%	2.18%~3.99%		
Breed					
Pure breed	88/2753	3.20%	2.54%~3.85%	0.036 (1)	0.850
Half breed	14/462	3.03%	1.47%~4.59%		
Body type					
Small	25/659	3.79%	2.34%~5.25%	1.856 (3)	0.603
Medium	27/836	3.23%	2.03%~4.43%		
Large	41/1479	2.77%	1.94%~3.61%		
Giant	9/241	3.73%	1.34%~6.13%		
Living environment					
House	67/2289	2.93%	2.24%~3.62%	3.530 (2)	0.171
Kennel	12/416	2.88%	1.28%~4.49%		
Free range	23/510	4.51%	2.71%~6.31%		

Risk factors	Frequency (thelaziosis cases/ophthalmic related cases)	Prevalence	95% CI	χ2 (*df*)	*P*-value
Diet					
Commercial food	80/2607	3.07%	2.41%~3.73%	0.485 (1)	0.486
Self-made food	22/608	3.62%	2.13%~5.10%		
Field travel history					
Yes	16/309	5.18%	2.71%~7.65%	4.475 (1)	0.034
No	86/2906	2.96%	2.34%~3.58%		
Immunization history					
Yes	65/2169	3.00%	2.28%~3.71%	0.671 (1)	0.413
No	37/1046	3.54%	2.42%~4.66%		
Anthelmintic history					
Yes	56/2138	2.62%	1.94%~3.30%	6.362 (1)	0.012
No	46/1077	4.27%	3.06%~5.48%		
Total	102/3215	3.17%	2.57%~3.78%	-	-
CI Confidence interval; *df* degree of freedom					

Correlation between regional distribution and prevalence rate

102 cases of thelaziosis were collected from various administrative regions. There were 2181 ophthalmic related cases from urban areas of Beijing (Dongcheng, Xicheng, Chaoyang, Haidian, Fengtai and Shijingshan District), of which 57 cases were clinically diagnosed as thelaziosis, and the prevalence rate was 2.61%; There were 1034 ophthalmic related cases from the rural area of Beijing (Changping, Daxing, Fangshan, Mentougou, Miyun, Pinggu, Shunyi and TongZhou District), of which 45 cases were clinically diagnosed as thelaziosis, and the prevalence rate was 4.35% (Fig. 1a). According to the results of Chi-square test ($\chi2=6.902$, $df=1$, P-value = 0.009), there were significant differences in the prevalence rate in different regions ($P < 0.05$) (Table 1). Regional distribution had a significant effect on the thelaziosis in domestic dogs. The prevalence rate of domestic dogs in the rural area of Beijing was significantly higher than that in the urban area of Beijing. Geographic information analysis showed that the high-density distribution area of domestic dogs thelaziosis is mainly in Haidian District (Fig. 1b), and the hot spots are Changping District, Haidian District and Shijingshan District (Fig. 1c); From the spatial autocorrelation analysis, it can be seen that Haidian and Shijingshan District belong to high–high aggregation areas,

indicating that the number of domestic dogs thelaziosis in these two regions is high, which drives the number of domestic dogs thelaziosis in the surrounding regions to be correspondingly high (Fig. 1d).

Correlation between seasonal distribution and prevalence rate

The collected cases were grouped according to spring from March to May, summer from June to August, autumn from September to November and winter from December to February. The number of ophthalmic related cases from spring to winter is 780, 936, 827 and 672 respectively, of which the number of cases of thelaziosis is 15, 40, 35 and 12 respectively, and the prevalence rates between different seasons are 1.92%, 4.27%, 4.23% and 1.79% respectively. According to the results of Chi-square test ($\chi 2=14.887$, $df=3$, P-value = 0.002), there were significant differences in the prevalence rate in different seasons ($P < 0.05$) (Table 1). The seasonal distribution had a significant effect on the infection of thelaziosis in domestic dogs. The prevalence rates in summer and autumn were significantly different from those in spring and winter, but there was no significant difference between spring and winter, autumn and summer. Combined with the data, the prevalence rate in summer and autumn is significantly higher than that in spring and winter.

Correlation between individual factors and prevalence rate

Age

In this study, 102 cases of thelaziosis were adult domestic dogs over 1 year old, aged between 1 and 15 years. Therefore, the puppies under 1 year old in 3215 ophthalmic related cases were excluded, and 2768 adult domestic dogs ophthalmic related case data were retained for statistical analysis. They were divided into two groups: one group was young to middle-aged dogs (≤ 7 years old), and the other group was older dogs (>7 years old). The number of ophthalmic related cases in the two groups were 1592 and 1176 respectively, and the number of cases of thelaziosis were 66 and 36 respectively. The prevalence rates between different ages were 4.15% and 3.06% respectively. According to Chi-square test ($\chi 2=2.241$, $df=1$, P-value = 0.134), there was no significant difference in the prevalence rate between the two groups ($P > 0.05$) (Table 1), and age had no significant effect on the prevalence rate of thelaziosis.

Sex

Among the 3215 ophthalmic related cases collected in this study, 1821 were male domestic dogs and 1394 were female domestic dogs. Among the thelaziosis cases, 59 were male domestic dogs and 43 were female domestic dogs. The prevalence rates between different sexes were 3.24% and 3.08% respectively. According to Chi-square test ($\chi 2=0.062$, $df=1$, P-value = 0.803), there was no significant difference in the prevalence rate between the two groups ($P > 0.05$) (Table 1), and sex had no significant effect on the prevalence rate of thelaziosis.

Breed

The cases collected in this study were from different breeds of domestic dogs, including 2753 purebred domestic dogs and 462 hybrid domestic dogs. Among the cases of thelaziosis, 88 were purebred domestic dogs and 14 were hybrid domestic dogs. The prevalence rates among different breeds were 3.20% and 3.03% respectively. According to the results of Chi-square test ($\chi2=0.036$, $df=1$, P-value = 0.850), there was no significant difference between the two groups ($P > 0.05$) (Table 1), and the breeds had no significant effect on the prevalence rate of thelaziosis.

Body type

The collected cases were divided into four groups according to their weight: small dogs (<10kg, < 40cm tall), medium dogs (10~25kg, 40~60cm tall), large dogs (26~ 45kg, 61~70cm tall) and giant dogs (> 45kg, > 70cm tall). The number of ophthalmic related cases in each group was 659, 836, 1479 and 241 respectively, and the number of cases of thelaziosis was 25, 27, 41 and 9 respectively. The prevalence rates among different body types were 3.79%, 3.23%, 2.77% and 3.73% respectively. According to the results of Chi-square test ($\chi2=1.856$, $df=3$, P-value = 0.603), there was no significant difference in the prevalence rate among groups ($P > 0.05$) (Table 1), and the body type had no significant effect on the prevalence rate of thelaziosis.

Correlation between feeding management factors and prevalence rate

Living environment

According to the different living environment, the cases collected in this study were divided into three groups: house, kennel and free range. The number of ophthalmic related cases in each group were 2289, 416 and 510 respectively, and the number of cases of thelaziosis were 67, 12 and 23 respectively. The prevalence rates among different living environments were 2.93%, 2.88% and 4.51% respectively. According to the results of Chi-square test ($\chi2=3.530$, $df=2$, P-value = 0.171), there was no significant difference in the prevalence rate among groups ($P > 0.05$) (Table 1), and the living environment had no significant effect on the infection rate of thelaziosis.

Diet

According to the different types of food fed daily, the cases collected in this study were divided into two categories. There were 2607 ophthalmic related cases of commercial dog food and 608 cases of self-made food. The number of cases of thelaziosis in the two groups were 80 and 22 respectively. The prevalence rates between feeding different kinds of food were 3.07% and 3.62% respectively. According to the analysis results of Chi-square test ($\chi2=0.485$, $df=1$, P-value = 0.486), there was no significant difference in the prevalence rate among the groups ($P > 0.05$) (Table 1), The type of diet had no significant effect on the prevalence rate of thelaziosis.

Field travel history

According to the medical history investigation, the collected cases were grouped according to whether there was a field travel history in recent 6 months. The number of ophthalmic related cases with a field travel history was 309, the number of ophthalmic related cases without a field travel history was 2906, and the number of cases of thelaziosis were 16 and 86 respectively. The prevalence rates of each group were 5.18% and 2.96% respectively. According to the analysis results of Chi-square test ($\chi2=4.475$, $df=1$, P-value = 0.034), there were significant differences in the prevalence rates among the groups ($P < 0.05$) (Table 1), the prevalence rate of thelaziosis in domestic dogs with field travel history was significantly higher than that in domestic dogs without travel.

Correlation between history of immune or anthelmintic and prevalence rate

Immunization history

The collected cases were grouped according to whether immunization was carried out in recent one year. There were 2169 and 1046 ophthalmic related cases of complete and incomplete immunization respectively. The number of cases of thelaziosis in the two groups were 65 and 37 respectively, and the prevalence rate was divided into 3.00% and 3.54%. According to the analysis results of Chi-square test ($\chi2=0.671$, $df=1$, P-value = 0.413), there was no significant difference in the prevalence rate between the two groups ($P > 0.05$) (Table 1), Whether the immunization history is complete or not has no significant effect on the prevalence rate of thelaziosis.

Anthelmintic history

The collected cases were divided into groups according to whether they were fed with insect repellents in recent 3 months. The cases treated in internal and external were regarded as treated. The number of ophthalmic related cases treated and not treated were 2138 and 1077 respectively, of which the number of cases of thelaziosis were 56 and 46 respectively, and the prevalence rates were 2.62% and 4.27% respectively. According to the analysis results of Chi-square test ($\chi2=6.362$, $df=1$, P-value = 0.012), there was a significant difference between the two groups ($P < 0.05$) (Table 1), The prevalence rate of thelaziosis in nondeworming domestic dogs was significantly higher than that in deworming domestic dogs.

Correlation between ocular clinical symptoms and number of T. callipaeda **carried**

The carrying amount of monocular *T. callipaeda* was divided into three categories: 1~10, 11~20 and more than 20; According to the results of ocular examination, the symptoms were divided into four categories: asymptomatic, mild symptoms (increased ocular secretion and purulent secretion), moderate symptoms (increased ocular secretion and purulent secretion with conjunctivitis) and severe symptoms (keratitis and corneal ulcer). Among the 102 cases of thelaziosis, 83 cases had oculus sinister infection and 81 cases had oculus dexter infection. A total of 164 cases of thelaziosis were included in the statistics. A total of 111 cases of thelaziosis carried 1~10 nematodes, 35 cases carried 11~20

nematodes, and 18 cases carried more than 20 nematodes. According to the classification of symptoms, it was found that the cases with moderate symptoms accounted for the largest proportion regardless of the number of nematodes carried (Table 2). Therefore, there was no significant correlation between clinical symptoms and the carrying amount of ocular *T. callipaeda*.

Table 2
Correlation between ocular clinical symptoms and Thelazia callipaeda carrying capacity

Ocular clinical symptoms	**Number of monocular *T. callipaeda***		
	1~10	**11~20**	**>20**
	Prevalence		
Asymptomatic	6.31% (7/111)	8.57% (3/35)	5.56% (1/18)
Mild	36.94% (41/111)	14.29% (5/35)	5.56% (1/18)
Moderate	47.75% (53/111)	62.86% (22/35)	66.67% (12/18)
Severe	9.00% (10/111)	14.29% (5/35)	22.22% (4/18)
Mild symptoms, increased ocular secretion and purulent secretion; Moderate symptoms, increased ocular secretion and purulent secretion with conjunctivitis; Severe symptoms, keratitis and corneal ulcer			

Discussion

A total of 3215 cases of ophthalmic examination were investigated. A total of 102 domestic dogs infected with thelaziosis were found. 62 cases were binocular infection and 40 cases were monocular infection. The total infection rate was 3.17%. At the same time, 1277 *T. callipaeda* were collected. The average number of nematodes carried by each domestic dog was 7.80 ± 8.51 in the oculus sinister and 8.00 ± 9.08 in the oculus dexter. Further exploring the relationship between the prevalence rate and various risk factors, it was found that the epidemiological characteristics of thelaziosis were significantly correlated with regional distribution, seasonal distribution, field travel history and anthelmintic history, but not with individual factors (age, sex, breed and body type), living environment, diet and immunization history (Table 1).

Due to the relative lack of epidemiological survey data of cases of domestic dogs thelaziosis in China, compared with the survey results abroad, the total prevalence rate obtained in this test is low. Although it is similar to the prevalence rate (3.8%) and carrying capacity (8.08 ± 9.49) of pet dogs in Portugal reported by Maia [24], it is quite different from the prevalence rate (26.1%, 33.1% and 68.0%) of dogs in Spain reported by Marino [25]. The difference is large, and the main reason may be the differences in sampling areas and sampling objects. The sampling site of this test is Beijing, and the sampling objects are mainly domestic pet dogs. The sampling area of Marino [25] is in the same latitude range as China, but there are many wild animals in this area. Among them, the collection objects with the highest prevalence rate (68.0%) are hounds with high prevalence risk, and there are fruit plantations around the

sampling site. The results of this experiment show that regional distribution and field travel history have a significant impact on domestic dogs infected with thelaziosis. The prevalence rate of domestic dogs in the rural area of Beijing is significantly higher than that in the urban area. The prevalence rate of domestic dogs with field travel history is significantly higher than that without travel, and the proportion of domestic dogs living in the rural with field travel history is higher. It shows that the influence of field travel history on the prevalence rate is of great significance, which may be related to the activities of wild animals, the continuous reproduction of intermediate hosts, ecological environment and other factors.

Thelaziosis can affect a variety of mammals, including wild animals and even economic animals in addition to domestic pets. Therefore, its prevalence rate will be relatively high in areas with wild animal activities [26]. According to the survey data of European countries, in areas with high prevalence rate of dogs, there are more cases of other hosts such as cats and foxes [24, 27]. In European countries that were not endemic before, the geographical distribution of thelaziosis is attributed to the distribution of several wild species, such as wild predators (such as red fox, wolf and badger) and rabbits [28, 29]. In Korea, according to Seo [30], the infection rate of military dogs is as high as 33.5%, 12.4 times that of farm dogs, mainly because military dogs are raised in mountainous areas. At the same time, there are many fruit planting parks around the city, which are suitable for the reproduction and development of intermediate host. There is a report of a dog infection case [21], and there are many strawberry plantations in its living area. Combined with the results of this test, it can be speculated that the increase of the activities of wild hosts and the reproductive activities of intermediate hosts can promote the infection and transmission of thelaziosis. Domestic dogs often exposed to the wild environment are more likely to contact other definitive host and transmission vector, so the prevalence rate is relatively high.

The results showed that the seasonal distribution had a significant effect on the infection of thelaziosis in domestic dogs. The prevalence rate in summer and autumn was significantly higher than that in spring and winter. This result is related to the reproductive law of *P. okadai*. The reproduction, density and biological activity of *P. okadai* vary seasonally according to climatic conditions [31]. Therefore, thelaziosis may occur seasonally. According to the investigation of *P. okadai* in Spain by Marino [25], the number of *P. okadai* captured is the largest when the daily average temperature is 24.5 -26.0 ℃, but no *P. okadai* are captured when the daily average temperature is 8.8 ℃. The density and number of *P. okadai* increase with the increase of temperature, and the proportion of male *P. okadai* (79.65%) is significantly higher than that of female *P. okadai* (20.35%). Generally, only male *P. okadai* feed on animal and human ocular secretions. Therefore, it can be seen that the number of male *P. okadai* increases significantly in summer with high temperature, and the transmission capacity is the largest at this time, so the prevalence rate is the highest in summer and autumn [32]. In addition, the survey data of Marino [25] also show that the proportion of male *P. okadai* is positively correlated with the temperature at the time of capture, that is, with the decrease of temperature, the proportion of female *P. okadai* will gradually increase. Because mature female *T. callipaeda* will continuously release larvae (L1) during the active period, so as to maintain their reproductive cycle, the infected *P. okadai* in the previous year can move again in early spring after overwintering. Therefore, there is still a certain risk of infection in spring and winter.

In this experiment, the prevalence rate of nondeworming domestic dogs was significantly higher than that in deworming domestic dogs. It can be seen that anthelmintic has a certain effect on the prevention of this disease. Different kinds of anthelmintic target different types of parasites, and animal owners have different awareness of regular internal and external anthelmintic. Therefore, the results of this test do not mean that any kind of anthelmintic has preventive effect. Because the investigation of anthelmintic history is largely affected by the subjective judgment of animal owners, the effective components and use frequency of anthelmintic drugs are not counted in this test, and the data with more guiding significance for treatment and prevention need to be further studied.

In this study, the number of monocular *T. callipaeda* and ocular clinical symptoms of 102 cases of domestic dogs thelaziosis were classified and counted respectively. The cases with moderate symptoms (increased ocular secretion and purulent secretion with conjunctivitis) accounted for the largest proportion. Therefore, there was no significant correlation between clinical symptoms and infection intensity (Table 2). In general, because the movement of *T. callipaeda* will directly cause damage to conjunctival sac and other parts, it is reasonable to guess that ocular clinical symptoms will increase with the number of monocular *T. callipaeda*, but the results of this test deny this view. According to the report of hodžić [33], the severity of ocular clinical symptoms has nothing to do with the number of infected nematodes, which is the same as the results of this test. The results of Maia [24] showed that the prevalence rate of dogs with ocular clinical symptoms was significantly higher than that of asymptomatic dogs. However, it is also emphasized that in the early stage of infection, animals usually do not show obvious ocular clinical symptoms, so they may be ignored by owners and veterinarians.

Conclusions

The infection of domestic dogs thelaziosis in Beijing was significantly correlated with regional distribution, seasonal distribution, field travel history and anthelmintic history. There was no significant correlation between the severity of ocular clinical symptoms and the number of ocular *T. callipaeda* in domestic dogs infected with thelaziosis. It suggests that we should reduce the frequency of taking pets to wild rural area in summer. If the domestic dog has a history of field travel, whether there are ocular clinical symptoms or not, regular internal and external anthelmintic should be carried out in time according to the procedure. At the same time, the owner should pay special attention to ocular hygiene and guard against the possibility of zoonotic diseases.

Declarations

Acknowledgements

This work was supported by the animal hospital of China Agricultural University in Beijing. We gratefully acknowledge Zheng Fang, Kaiming Zhang and Wanrong Song for their help with data analysis, and Qinghui Mu for help with manuscript preparation.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article. Sequences obtained during the current study are available in the GenBank database with accession numbers MN719910.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

ZCL, CY and YPJ conceived the study. ZCL and XLT organized the sampling plan. ZCL, NC and XLT were responsible for the collection of cases of domestic dogs thelaziosis used in this study. ZCL obtained the sequence of *Thelazia callipaeda* andanalysed the results. ZCL and CY drafted the manuscript and all authors critically contributed to its final version. All authors read and approved the final manuscript.

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Figures

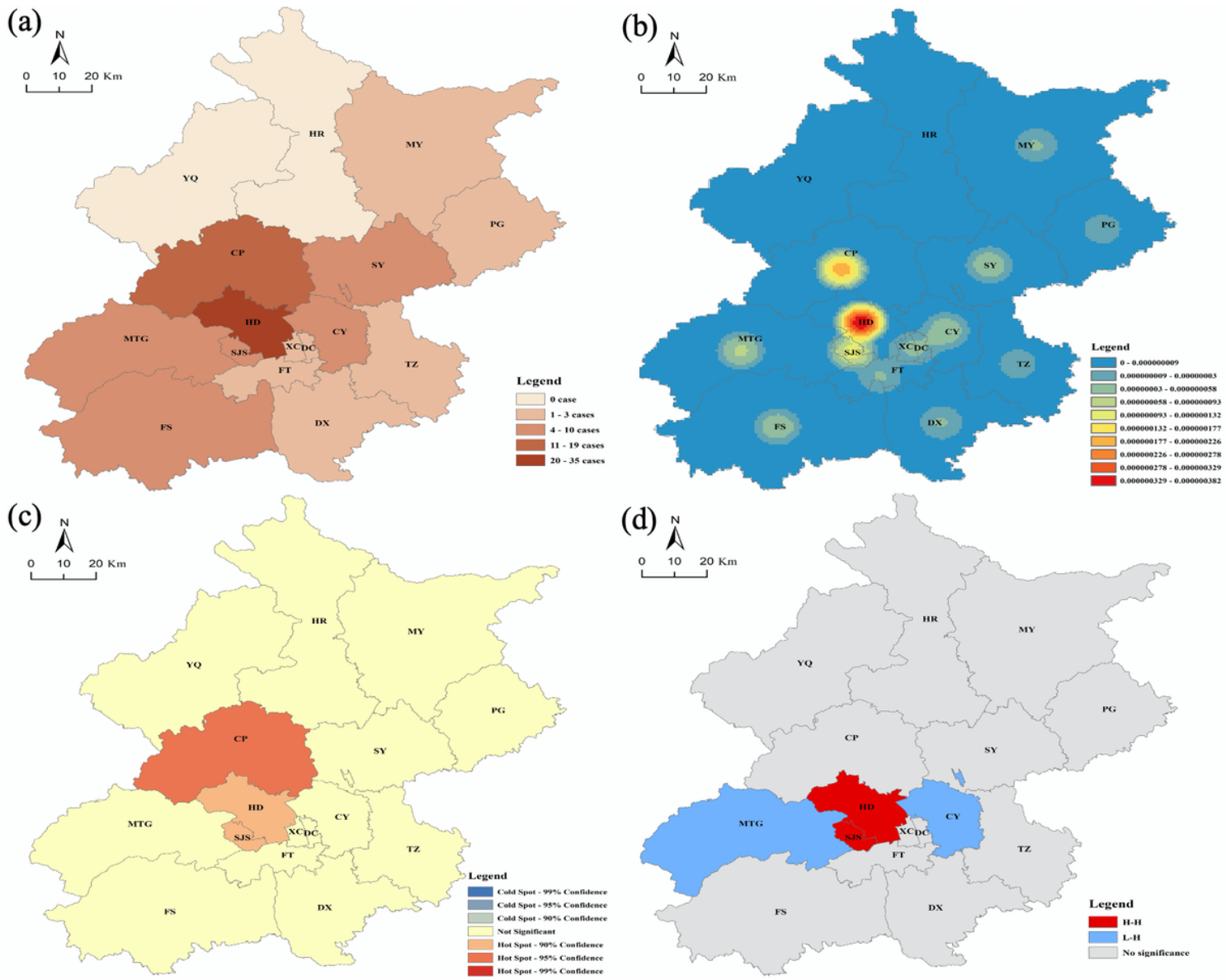

Figure 1

Geographic Information Analysis of domestic dogs thelaziosis in Beijing from 2018 to 2019. a Distribution map; b Nuclear density map; c Hot spot map; d Spatial autocorrelation map. H-H Positive correlation of “high-high”, L-H Positive correlation of “low-high”, DC Dongcheng District, XC Xicheng District, CY Chaoyang District, HD Haidian District, FT Fengtai District, SJS Shijingshan District, CP Changping District, DX Daxing District, FS Fangshan District, MTG Mentougou District, MY Miyun District, PG Pinggu District, SY Shunyi District, TZ TongZhou District, YQ Yanqing District, HR Huairou District

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