

Research Article

First report on *Toxoplasma gondii* and *Neospora caninum* Seroprevalence in cats and dogs in Shijiazhuang, China

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Abstract

Objective *Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) are two important protozoan parasites that cause abortion and neurological disorder in many animals worldwide. These are closely correlated in many areas, such as morphology and intermediate hosts, but the definitive hosts differ. Cats are the definitive hosts for *T. gondii*, while dogs, wolves and dingoes are definitive hosts for *N. caninum*. The present study aims to investigate the seroprevalence of *T. gondii* and *N. caninum* in cats and dogs in Shijiazhuang, Hebei province, China.

Methods

A total of 187 dog and 85 cat sera samples obtained from Shijiazhuang were analyzed by enzyme-linked immunosorbent assays (ELISA).

Results

The results revealed that 14.97% (28/187) of the individual serum samples from dogs and 8.24% (7/85) of the individual serum samples from cats are seropositive for *T. gondii*, 4.81% (9/187) of the individual serum samples from dogs and 11.76% (10/85) of the individual serum samples from cats are seropositive for *N. caninum*, and 1.6% (3/187) of the individual serum samples from dogs and 2.35% (2/85) of the individual serum samples from cats are seropositive for mixed infection.

Conclusions

A higher seroprevalence of *T. gondii* and *N. caninum* in stray dogs and cats were found in the present study when

compared to pet dogs and cats, and the difference was significant. To the best of our knowledge, the present study was the first to detect *T. gondii* and *N. caninum* antibodies in dogs and cats in Hebei province, China.

Keywords: *Toxoplasma gondii*; *Neospora caninum*; Seroprevalence; Dogs; Cats.

Introduction

Toxoplasma gondii (*T. gondii*) is an important zoonotic intracellular protozoan parasite, which can affect all warm-blooded mammals and birds throughout the world, including humans [1]. It has been reported that *T. gondii* chronically infects a quarter of the world's population, and causes reproductive, neuromuscular and respiratory disorders, and even death [2, 3]. Felids play a crucial role in the epidemiology of this parasitic disease, because these are the only definitive host that shed and excrete millions of infective oocysts within a short time in their faces [4]. *T. gondii* can be transmitted to many animals and humans through oocysts from cats. Hence, it has been generally suggested that people should protect the environment, and pay attention to health, especially for pregnant mothers. However, as it is known, canines can also spread the parasite. Therefore, the individual seroprevalence of cats and other susceptible animals can be a public health and conservation concern.

Neospora caninum (*N. caninum*) was easily confused with the structurally similar *T. gondii* until 1988, causing neosporosis, with the symptom of abortion and neonatal mortality in cattle-rearing regions worldwide [5]. Studies on *N. caninum* and neosporosis began late, and have been

ignored due to less harm during the first phase. After entering the 21st century, the coverage of scientific research on the parasites has increased. Many canid animals, including dogs, coyotes, gray wolves and dingoes, were found to be the definitive hosts after 1998 [6-9] and a large number of intermediate hosts have been reported in subsequent years, such as chickens, rats, pigs and red foxes [10-13]. Cattle, as one of the intermediate hosts of *N. caninum*, suffers from the vertical transmission of neosporosis, and the cattle industry has been harmed through this manner [14]. It is known that parasites can be maintained and propagated between definitive and intermediate hosts for a long time. Hence, given the close association of dogs and other animals in herds, particularly the relationship between dogs and cattle, many surveys have been carried on *N. caninum* infection in dogs in different regions in the world [15, 16].

Domestic dogs and cats are both important companion animals for mankind, and many families could keep these pets. However, there is still a varied number of stray dogs and cats around communities for various reasons, including cities in the metropolitan area of China, such as Shijiazhuang, Baoding, Qinhuangdao in Hebei, northern China. These individual activities have increased the potential and opportunities for the spread of these two diseases. In Hebei, the epidemiological information on *T. gondii* and *N. caninum* in dogs and cats is limited despite the higher prevalence rates reported in other animals, such as humans, cattle, sheep, raccoon dogs and ostriches [17-21]. In the present study, the seroprevalence of *T. gondii*, *N. caninum* and the co-infection among pet dogs and cats and stray dogs and cats in Shijiazhuang was initially assessed. The data would provide the necessary information and scientific basis for establishing monitoring, and prevention and control strategies for toxoplasmosis and neosporosis.

Materials and Methods

Sera Samples

The study area was Shijiazhuang city in Hebei province, which is located in northern China. The study included samples collected from animal hospitals and homeless-animal shelters throughout the city in 2019. The experimental protocol was approved by the Ethics Committee of Hebei Normal University (2019SC14). All canines and cats in participating clinics were qualified to participate in the present study. A permission was obtained from the owners prior to the collection of samples, and a medical history questionnaire was completed for each subject. A total of 272 blood samples were collected. Among these, 187 samples were from canines and 85 samples were from cats. These serum samples were stored at -80 °C for further analysis.

The serum samples in the survey were individually tested for antibodies to *T. gondii* and *N. caninum* using commercially available indirect enzyme-linked immunosorbent assay (ELISA) kits (Canine Toxoplasmosis ELISA kit, Canine Neosporosis ELISA kit, Cat Toxoplasmosis ELISA kit and Cat

Neosporosis ELISA kit, MEIMIAN, Jiangsu, China), and all operations were performed according to the manufacturer's instructions of these ELISA kits.

Statistical analysis

All statistical analyses were performed using the software SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). χ^2 -test was used to analyze the differences in the prevalence of toxoplasmosis and neosporosis between domestic and stray dogs, and domestic and stray cats and these differences were considered statistically significant when the *P*-value was < 0.05

Results

At the host level, antibodies against *T. gondii* were detected in 28 of 187 dogs (14.97%) and 7 of 85 cats (8.24%). Furthermore, 12 of 114 pet dogs (10.53%) and 16 of 73 stray dogs (21.92%) were positive for *T. gondii* antibodies. Stray dogs had a higher *T. gondii* seroprevalence, when compared to pet dogs, and the difference was statistically significant ($\chi^2=12.770$, $P=0.000352<0.01$). Furthermore, 1 of 51 pet cats (1.96%) and 6 of 34 stray cats (17.65%) were positive for *T. gondii* antibodies. Stray cats had a higher *T. gondii* seroprevalence, when compared to pet cats, and the difference was statistically significant ($\chi^2=6.637$, $P=0.009988<0.01$). However, there was no significant difference between pet dogs and cats, or stray dogs and cats ($P>0.05$, Table 1).

Antibodies against *N. caninum* were detected in 9 of 187 dogs (4.81%) and 10 of 85 cats (11.76%): two pet dogs (1.75%), seven stray dogs (9.59%), two pet cats (3.92%) and eight stray cats (23.53%). Stray dogs had a higher *N. caninum* seroprevalence, when compared to pet dogs, and the difference was statistically significant ($\chi^2=5.965$, $P=0.014593<0.05$). Stray cats had a higher *N. caninum* seroprevalence, when compared to pet cats, and the difference was statistically significant ($\chi^2=7.560$, $P=0.005968<0.01$). However, there was no significant difference between pet dogs and cats, or stray dogs and cats ($P>0.05$, Table 2).

Antibodies against both *T. gondii* and *N. caninum* antibodies were detected in 3 of 187 dogs (1.60%) and 2 of 85 cats (2.35%). It was surprising to find that mixed infection occurred in stray dogs (4.10%, 3/73) and cats (5.88%, 2/34). Table 3 shows the results for the *T. gondii* and *N. caninum* antibodies in the different animal groups.

Discussion

Dogs and cats are two animals closely correlated to daily life at present and are almost never out of sight in the area of human life. However, the various pathogens carried by these animals, such as bacteria, virus, and parasites, are ignored. These pathogens are likely to infect people or other animals in some ways, including food, and to threaten the health of humans and animals [22, 23]. Therefore, there is a need to detect the diseases of locally frequently active animals.

Host	No. of samples	No. of positive	Seroprevalence of <i>T. gondii</i> %	χ^2	P-value
Dog	187	28	14.97	12.770	0.000352**
Pet dog	114	12	10.53		
Stray dog	73	16	21.92		
Cat	85	7	8.24	6.637	0.009988**
Pet cat	51	1	1.96		
Stray cat	34	6	17.65		

**P<0.01, significant difference.

Table 1. *T. gondii* seroprevalence in dogs and cats

Host	No. of samples	No. of positive	Seroprevalence of <i>T. gondii</i> %	χ^2	P-value
Dog	187	9	4.81	5.965	0.014593*
Pet dog	114	2	1.75		
Stray dog	73	7	9.59		
Cat	85	10	11.76	7.560	0.005968**
Pet cat	51	2	3.92		
Stray cat	34	8	23.53		

*P<0.05 and **P<0.01, significant difference.

Table 2. *N. caninum* seroprevalence in dogs and cats

Host	No. of samples	No. of positive	Mixed seroprevalence %
Dog	187	3	1.60
Pet dog	114	0	0
Stray dog	73	3	4.10
Cat	85	2	2.35
Pet cat	51	0	0
Stray cat	34	2	5.88

Table 3. Both *T. gondii* and *N. caninum* seroprevalence in dogs and cats

T. gondii infection, which is a zoonotic parasite, can cause systemic and ocular diseases in infants during pregnancy, and has been categorized as a neglected tropical disease by the World Health Organization [24]. For *N. caninum*, most researchers do not consider that this can infect humans. However, this is greatly endangering cows, sheep, and dogs in the world [5]. A recent study revealed that the infection rate of *T. gondii* is relatively high (7.73%) among the healthy population in Shijiazhuang, Hebei province, China, and that it is probably correlated to pets [25]. *T. gondii* and *N. caninum* are two important protozoan parasites that could be transmitted by cats and dogs. However, to the best of our knowledge, there have been no studies on the infection rate of *T. gondii* or *N. caninum* in dogs and cats. These present findings may provide data support for the above study. Meanwhile, the investigators intended to master the epidemiological dynamics of these two diseases in Shijiazhuang, China during the post-controlling period, and provide a basis for laying down a technological scheme for reducing the rate of toxoplasmosis and neosporosis.

In a survey, the levels of infection in dogs and cats by *T. gondii* were in various degrees in the city. Especially for

stray dogs and cats, the infection rates of *T. gondii* were significantly higher, when compared to pets. Compared to other regions in China, the prevalence of *T. gondii* infection in pet dogs (14.97%) was higher. However, the pet cats (8.24%), stray dogs (21.92%), and stray cats (17.65%) were lower, when compared to those in Zhenjiang, eastern China, in which the infection rates of *T. gondii* in pet dogs, pet cats, stray dogs and stray cats were 13.1%, 20.7%, 38.7% and 28.6%, respectively [26]. Several studies have revealed that the human and animal natural infection of *T. gondii* is correlated to dogs and cats, regardless of whether these were domestic or stray. In Ethiopia and Iran, the infection of *T. gondii* in pregnant women was significantly associated with a risk factor, that is, contact with cats and dogs [24, 27]. In the previous study conducted by the investigators, the infection of *T. gondii* was common in dairy cows in Hebei, and this could not rule out the activity of stray dogs and cats [18]. Similarly, pasture dog and cat access were identified as associated factors for the occurrence of toxoplasmosis in sheep and dairy farms [28, 29]. Dogs and cats that move freely caused the spread of *T. gondii*, increasing the chances of human and other animals contact. In fact, these two animals that carry *T. gondii* have become an important public health

problem, and this has been reported many times [30, 31]. In different populations and regions, different distributions have been reported, such as 40% in stray dogs in Mexico, 19.8% in dogs in Nigeria, 47.1% in dogs and 40% in cats in Iran, 15% in dogs and 6% in cats in South Dakota, and 6.3% in dogs and cats in Korea [14, 28, 32]. Therefore, dogs and cats are beneficial to the transmission of *T. gondii*, both for humans and animals. Hence, attention should be given to this.

Although *N. caninum* could infect many animals, including birds, terrestrial animals, and sea animals, its impacts are uncertain for these animals, except for cows and canine [14, 33, 34]. A recent report revealed that the total prevalence of *N. caninum* infection in the dog population worldwide was measured as 17.14% (15.25-19.10%), which was higher than the prevalence (4.81%) in dogs in Shijiazhuang in the present survey. Furthermore, there was a significant difference detected among countries, and WHO regions and continents and the worst level of infection reached up to 26.59% in Africa. Dogs are a matter of concern to public health officials as a source of *N. caninum* infection [35]. In China, the prevalence differs in different regions and animal groups: 36.8% in sheep and goats in southwest China, 8.99% in raccoon dogs in Shandong, and 20% in dogs in rural northeastern China [16, 36, 37]. However, it is noteworthy that the producers and farms should focus on the potential harm from neosporosis, and establish prevention and control measures as soon as possible, due to this spectacular phenomenon. That is, for the *N. caninum* in the city, there was a 9.59% infection rate for stray dogs and a 23.53% infection rate for cats in the survey, and a 37.39% infection rate for dairy cows in the previous investigation [18, 38, 39]. More importantly, there is a horizontal transmission between dogs and cattle, which poses a concern [16].

From these survey results, the health of humans and animals has been severely affected by the toxoplasmosis and neosporosis in Shijiazhuang city, Hebei province, northern China. This initial study data on these two local parasite diseases in dogs and cats could provide valuable information and warnings.

Conclusion

These present results revealed that there are antibodies to *T. gondii* and *N. caninum* in dogs and cats from Hebei province, northern China. Stray dogs and cats are the two potential risk factors for the widespread presence of *T. gondii* and *N. caninum*. To our knowledge, this is the first serological evidence of *T. gondii* and *N. caninum* infection in pets and stray animals in the area, showing that emphasis should be given on the parasitic diseases of cats and dogs.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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