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Research Article

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Investigating the Prevalence of Babesia Protozoa in Dogs in Southwestern Fars Province, Iran

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Abstract

Background and aim: The classification of *Babesia* spp. places them in order Piroplasmida within the phylum Apicomplexa, which is primarily transmitted to humans and animals through Ixodid vectors. This parasite invades the erythrocytes of their hosts, leading to various disease symptoms. The present study focuses on determining the prevalence of Babesia infection in the blood of dogs in Southeastern of Fars province, taking into account the favorable geographical and climatic conditions and the abundance of vector ticks in Fars province, Iran.

Materials and Methods: A total of 324 dog collars (132 females and 192 male) were selected from four different cities in Southeastern of Fars province, and categorized based on age, type of use, and sex. The samples were subjected to blood smear test. The obtained results were statistically analyzed using SPSS software and the Chi-square test (X2).

Results: Forty twosamples were infected with Babesia, indicating an infection rate of 12.96%. Among the infected samples, 13.02% were male dogs. Additionally, 12.87% of the infection was observed in female dogs. Finally, 12.71% of infection was observed in Shiraz city (15 sample), 14.94% in Kazerun city (13 sample), 8.92% in Fasa city (5 sample), and 14.28% in Jahrom city (9 sample).

Conclusion: The statistical analysis showed no significant relationship between gender and the level of infection, no significant association was found between the type of use (Pets, guard dogs, herding dogs, and stray dogs) and the level of infection. Furthermore, there was no significant correlation between age and Babesia parasite infection.

Keywords: *Babesia, dog, Hard tick, Fars province, Iran*

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Introduction

Canine babesiosis is recognised as a tick-borne disease, transmitted by a variety of Ixodid vectors around the world. Babesia species are often referred to as piroplasms within the phylum Apicomplexa, a collective term for phenotypically similar protozoan parasites that utilise mammalian erythrocytes in their life cycle (Irwin, 2009; Schnittger *et al.,* 2012; Jacobson, 2006; Irwin, 2005). Over 100 Babesia species have been identified, but only *Babesia canis* and *Babesia gibsoni* have been shown to infect dogs. In dogs, infection by these haemoparasites results in a wide range of clinical presentations; from subclinical disease to serious illness characterised by fever, pallor, jaundice, splenomegaly, weakness and collapse associated with intra- and extravascular haemolysis, hypoxic injury, systemic inflammation (Levine., 1988; Birkenheuer *et al.,* 2005).

Babesia species enter the erythrocytes in the sporozoite stage. Inside the erythrocyte, the protozoa cycle become a trophozoite ring. The trophozoites develop into merozoites, which have a quadrilateral structure coined the Maltese cross shape. Two morphologically distinct forms of the erythrocytic stage in the canine host were recognised in early studies that led to the naming of the larger form, measuring approximately 3–5 µm as *B. canis*, and the smaller (1–3 µm) as *B. gibsoni* (Uilenberg, 2006; Jefferies *et al.,* 2003).

Babesia species is spread through the tick's saliva when it bites. In the larva stage, a tick bites the skin for a blood meal. The tick, if not removed, remains attached for three to four days, with longer feeding periods associated with a greater likelihood of acquiring parasites. This parasite can survive in the tick during different developmental stages of the tick, and as a result, all stages of the tick are potentially infectious. Transmission through congenital transmission (from an infected mother to a newborn) is also possible. Because symptoms may not appear, many women may not be aware that they are infected during pregnancy, and therefore the extent of congenital transmission is currently unknown. Babesia species require competent vertebrates and invertebrates to maintain transmission cycles (Chauvin *et al.,* 2009; Cornillot *et al.,* 2013; Quintão & Ribeiro, 2003).

Because ticks spend most of their lives off the host, they are subject to abiotic environmental conditions and are likely to be affected by climate parameters (Gilbert, 2021). Hot and dry weather interferes with the life cycle of Babesia in the tick (Carret *et al.,* 1999). The findings, highlight the importance of considering geographical and climatic factors in understanding the distribution and transmission of Babesia parasites. Further research is needed to identify the specific Babesia species involved and to explore. Studies have been conducted to investigate the prevalence, species diversity, clinical manifestations, and abundance of Babesia vector ticks in dogs in different parts of Iran. (Jalali *et al.,* 2011; Raki *et al.,* 2014; Hosseinzade Varjoy *et al.,* 2016; Bigdeli *et al.,* 2012; Khanmohammadi *et al.,* 2021; Seyyed Hoseini & Rasouli, 2022; Hosseini & Hamzehali Tehrani, 2022). The present study focuses on determining the level of Babesia infection in the blood of dogs in Southwestern of Fars province, taking into account the favorable geographical and climatic conditions and the abundance of vector ticks in this specific region of Fars province, Iran.

Materials and Methods

Research method

The current descriptive-analytical cross-sectional study was conducted in Southwestern of Fars province with the aim of investigating the prevalence of Babesia protozoa in the blood of dogs in the spring, summer and autumn of 2022. For this purpose, 324 blood samples were collected from cephalic or saphenous veins from dogs in Southwestern of Fars province from four different cities in Southwestern of Fars province (Shiraz 118 sample, Kazerun 87 sample, Jahrom 63 sample, Fasa 56 sample) and they were inserted into the tube containing EDTA anticoagulant and the desired characteristics and variables were recorded. The samples were taken from different breeds, ages of dogs and the characteristics of dogs such as age, sex, and type of dog (Pet, guardian and stray). Dogs were classified according to 3 different indicators: 1- Sex (132 female collars and 192 male collars), 2- Age: it was estimated by dental formula and owner information's and Based on this, the study group was divided into three categories: less than 6 months (85 collars), between six months and one year (93 collars) and older than one year (146 collars), and 3-Usage: based on this, dogs in three groups referred to the

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veterinary clinics of Southwestern of Fars province (58 collars), stray dogs without owner (172 collars) and owned dogs that are kept as herd dogs or guard dogs (94 collars) were categorized.

Blood smear test

A drop of collected blood was placed near the edge of the slide and was prepared by the spreader slide at an angle of about thirty degrees and with an average speed of spreading thin blood on the slide. After a few minutes, when the above-mentioned expansion was dry, it was fixed in methanol for three minutes, and then they were stained with Giemsa dye (Arj Azama co. Iran). Depending on the concentration of Giemsa dye and according to the manufacturer's instructions, slides were placed in a container containing Giemsa dye and stained after about half an hour. At the end, the stained blood slides were placed under a gentle stream of ordinary water to wash off the excess dyes. Prepared blood smears were examined under a microscope (Yaxun model: AK12) and by oil lens with cedar oil and 1000x magnification. All parts of the slide were carefully examined and red blood cells infected with *B. canis* were searched.

Statistical review

Chi-square test and Fisher's exact statistical method was used in order to determine the significant relationship between the prevalence of Babesia and the considered factors such as age, sex, season and the studied area. SPSS 26 software was used for this method.

Results

The analysis revealed that out of the 324 blood samples, 42 samples were infected with *B. canis*, indicating an infection rate of 12.96% (Figure 1). Among the infected samples, 13.02% were male dogs, including 5 samples of stray dogs under 6 months, 8 samples of stray dogs between 6 months and 1 year, 8 samples of guard or herding dogs older than one year, and 12 samples of guard or herding dogs older than one year. Additionally, 12.87% of the infection was observed in female dogs, including 6 samples of stray dogs older than one year and 3 samples of guard or herding dogs older than one year (Chart 1). Finally, 12.71% of infection was observed in Shiraz city (15 sample), 14.94% in Kazerun city (13 sample), 8.92% in Fasa city (5 sample), and 14.28% in Jahrom city (9 sample) (Chart 2). The infection samples were more in male instead of females (25 male sample and 17 female sample) and the infection rate was much higher in stray dogs and dogs with age of one year or older with 15.11% and 16.43% respectively.

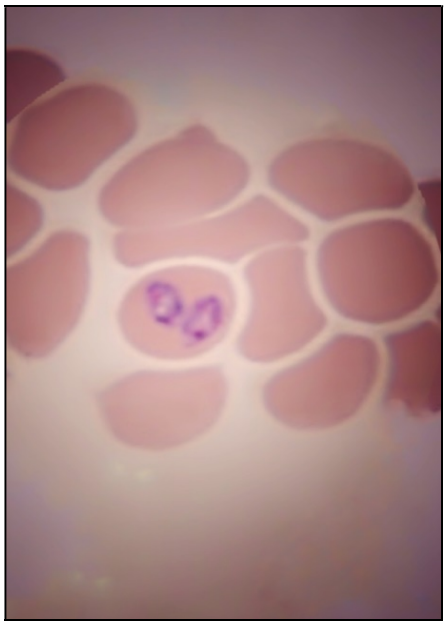

Figure 1. A Positive *B. canis* sample.

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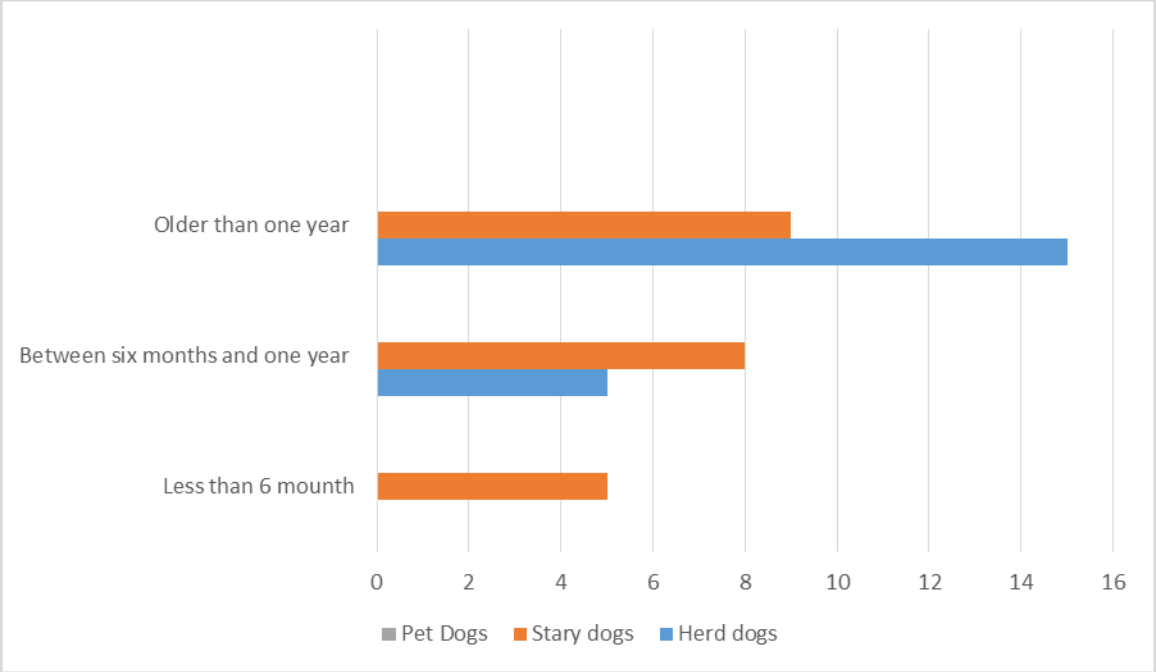

Chart 1. The Prevalence of *B. Canis* by type of Use & Age.

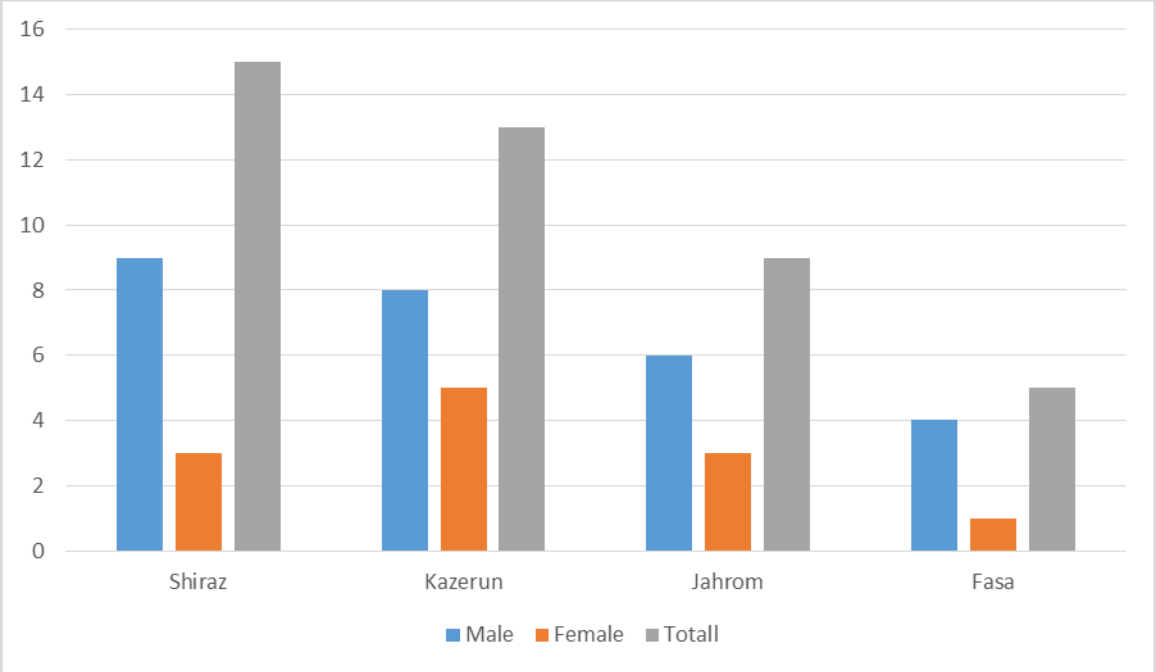

Chart 2. The Prevalence of *B. Canis* by Cities.

Discussion

In the survey conducted in Ahwaz city, among 400 dogs, 15 samples (3.75%) were infected with *B. canis* and showed that from 200 rural dogs, 11 samples (5.5%) and from 200 urban dogs, 4 samples (2%) were positive for *B. canis*. Infection rate was higher in adult dogs 3-6 years-old (4.46 %5 out of 112) compared with young's less than 3 years (3.59 %7 out of 195) and above 6 years (3.85; 3 out of 78). The infection was higher in female dogs (4.29%; 6 out of 140) than males (3.46%; 9 out of 260) and in warm season (5.15%; 12 out of 233) compared with cold season (1.8%; 3 out of 167), nevertheless, there was not significant relationship between sex, age and

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season in urban dogs (P>0.05), but significant difference was revealed between season and infection in rural dog's population (P<0.05). (Jalali *et al.,* 2011). Raki *et al.,* in 2014 showed that the prevalence of *B. canis* DNA were detected by PCR from 9 (7.5%) out of 120 dogs also in this comparative study found that the highest infection rate in young dogs less than two years and a low-frequency of *B. canis* infection in dogs in the Chaharmahal Va Bakhtiari provinces (west of Iran). (Raki *et al.,* 2014). Hosseinzade Varjoy *et al.,* in 2016 showed that 22(18.2%) and 8(6.6%) out of 121 blood samples were positive for Babesia in all study areas in molecular and microscopic survey, respectively. Commercial (28.6%), male (19.2%) and 2> years old (21.1%) dogs had the highest infection rates but no significant differences were found between geographic distribution of infection and obtained data (Hosseinzade Varjoy *et al.,* 2016). Bigdeli *et al.,* in 2012 showed that one herd dog was infected with *B. canis* (0.4% or 1 out of 280) in dogs of South of Iran (Bigdeli *et al.,* 2012). In the survey conducted in Meshkin Shahr County, Northwestern Iran, revealed that four dogs 9.3% (4/43) including one female and three male dogs were infected with Babesia. (Khanmohammadi *et al.,* 2021). In the survey conducted in Isfahan city, in the samples obtained from pet dogs, a molecular prevalence of 21/10% was observed in urban dogs compared to 39/20%. It was reported by comparing the observational method of parasitology 13/6% and 17/9%, in domestic and urban dogs, respectively. This indicates the low prevalence of *Babesia* is in domestic dogs compared to the urban dogs in Isfahan. (Hosseini & Hamzehali Tehrani, 2022). In the survey conducted in Urmia city, Northwestern Iran, 23 (13.9%) positive samples of Babesiosis were identified out of 165 dogs examined for Babesiosis, the highest number belonged to stray dogs with the number of 10 (6.06%) and the lowest number belonged to the commercial dogs with the number of 2 (1.21%) but there was no statistically significant relationship between the level of infection and sex, how dogs were kept (p>0.05) (Seyyed Hoseini & Rasouli, 2022).

In this study, which was conducted on 324 dog blood samples in four different cities in Southwestern of Fars province, the definitive method for detecting infection was the detailed examination of blood spread. The reason for choosing the thin spread for examining blood samples was that, according to the opinion of most researchers, it is the most reliable and simplest way to detect the presence of parasites in red blood cells, so that in addition to determining the type of *Babesia*, the percentage of red blood cell infection can also be determined. In the present study, which was carried out with various objectives, including the knowledge of the approximate level of infection of a relatively limited but diverse statistical population of dogs in Southwestern of Fars province, with the parasitic protozoan *Babesia*, the methods that were previously introduced and used during the stages of the work Expected results were obtained. According to the results of the investigation, among the 324 dog collars studied, the chi-square statistical test did not show a statistically significant difference between the rate of *Babesia* infection in guard dogs or herding dogs and the group of stray dogs. However, the higher infection in stray dogs indicates the presence of ticks in non-urban areas and the possibility of their transmission to dogs. Also, it's obvious that due to the more temperature at Kazerun and Jahrom city the infaction rate was much higher than two other cities.
Data analysis using the Chi-square test showed that there is no significant relationship between gender and the level of infection with a confidence coefficient of 95%. (P value = 0.9257)

Also, there was no significant relationship between the type of use (dogs visiting the clinic, guard dogs, herding dogs, and stray dogs) with the level of infection. (P value = 0.7443)

In addition to the above, there was no significant relationship between age and Babesia parasite infection (P value = 0.7984)

In terms of gender, males with 13.02% of infection had a higher percentage than females (12.87%), but this difference is not significant and cannot be interpreted. Even though there are still limited studies on the relationship between the effect of the parasitic protozoan Babesia according to the sex of the animal, it can be concluded that the infection has no significant relationship with the sex and the parasite shows the ability to infect itself regardless of the host's sex. In terms of the type of use, the group of stray dogs with 25 cases of infection has more cases of infection than guard dogs (15 cases of infection), but because the statistical population of stray dogs under investigation is much larger (172 collars) and approximately triple as compared to were guard dogs

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or herd dogs (94 collars), the percentage of infection in stray dogs (14.53%) is lower than guard dogs (15.95%). The group of dogs referred to the clinic were all free of infection. It is very clear that the existence of this difference indicates the awareness of dog owners about possible dangers and parasitic diseases, as well as the fact that there are significantly fewer intermediate hosts of parasites in urban environments. On the other hand, it seems that the owners of guard dogs or herding dogs have not been able to prevent the entry or growth of parasites in their animals by taking preventive measures and timely and regular use of anti-parasitic drugs. Obviously, the presence of more transporters in rural areas and areas where livestock and agriculture are mostly carried out plays a very important role in infection by hard thicks.

Conclusion

The statistical analysis showed no significant relationship between gender and the level of infection, no significant association was found between the type of use (Pets, guard dogs, herding dogs, and stray dogs) and the level of infection. Furthermore, there was no significant correlation between age and Babesia parasite infection.

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Conflict of Interest

The authors declare no conflict of interest.

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بررسی شیوع تک یاخته بابزیا در سگ های جنوب غربی استان فارس، ایران

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چکیده

زمینه و هدف: طبقه بندی *Babesia* spp. آنها را به ترتیب Piroplasmida در شاخه Apicomplexa قرار می دهد که عمدتا از طریق ناقل های Ixodid به انسان و حیوانات منتقل می شود .این انگل به گلبول های قرمز میزبان حمله می کند و منجر به علائم بیماری می شود. مطالعه حاضر با توجه به شرایط مساعد جغرافیایی و اقلیمی و فراوانی کنه های ناقل در استان فارس، بر تعیین شیوع عفونت بابزیا در خون سگ های جنوب شرقی استان فارس متمرکز است.

مواد و روش‌ها: در مجموع ۳۲۴ قلاده سگ (۱۳۲ ماده و ۱۹۲ نر) از چهار شهر مختلف جنوب شرق استان فارس انتخاب و بر اساس سن، نوع استفاده و جنس طبقه بندی شدند. نمونه ها تحت آزمایش اسمیر خون قرار گرفتند. نتایج به‌دست‌آمده با استفاده از نرم‌افزار SPSS و آزمون کای اسکوئر (X2) مورد تجزیه و تحلیل آماری قرار گرفت.

یافته‌ها: چهل و دو نمونه آلوده به بابزیا بودند که نشان دهنده میزان آلودگی ۱۲/۹۶ درصد است. از بین نمونه‌های آلوده، ۱۳/۰۲ درصد سگ‌های نر بودند. علاوه بر این، ۱۲/۸۷ درصد از عفونت در سگ ماده مشاهده شد. در نهایت ۱۲/۷۱ درصد آلودگی در شهر شیراز (۱۵ نمونه)، ۱۴/۹۴ درصد در شهرستان کازرون (۱۳ نمونه)، ۸/۹۲ درصد در شهرستان فسا (۵ نمونه) و ۱۴/۲۸ درصد در شهرستان جهرم (۹ نمونه) مشاهده شد.

نتیجه‌گیری: تجزیه و تحلیل آماری نشان داد که بین جنسیت و میزان آلودگی رابطه معنی داری وجود ندارد، ارتباط معنی داری بین نوع استفاده (حیوان خانگی، سگ نگهبان، سگ گله و سگ ولگرد) و میزان آلودگی مشاهده نشد. همچنین بین سن و آلودگی انگل بابزیا ارتباط معنی داری مشاهده نشد.

واژه‌های کلیدی: *بابزیا، سگ، کنه سخت، استان فارس، ایران*

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