Analysis of risk factors of Leishmania infection in dogs in Gansu Province, China

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Abstract

Background Gansu is a province with the most serious prevalence of canine Leishmaniasis in China, ranked the first in the number of new cases occurring each year. In order to understand the risk factors of visceral Leishmaniasis in dogs in endemic areas of Gansu province, we conducted an investigation on the risk factors of canine Leishmaniasis in 2 endemic counties from 2018 to 2019, and analyzed the related factors of local CVL infection, so as to provide a basis for exploring new prevention and control methods suitable for Leishmaniasis in China. Methods Leishmaniasis of canine origin was detected in Diebu county and Wenxian County, Gansu province. The risk factors of Leishmania infection in dogs were analyzed by USING IBM SPSS23.0 Pearson$\chi 2$ test and multiple Logistic regression. Results a total of 537 domestic dogs were investigated in the endemic area. The positive rate of Leishmania protozoa nested PCR was 41.15% (221/537). Univariate analysis showed that there were statistically significant differences in age, height, weight, breed, kennel presence, hair length, custody style, physical condition, exercise status, nail symptoms and other variables with the positive rate of nested PCR ($P < 0.05$).The results of multivariate analysis showed that there was a significant correlation between dog ownership [OR=3.051; 95%CI =(0.965 ~ 9.645), dog ownership [OR=1.974; 95%CI =(1.199 ~ 3.252), dog breed [OR=1.819; 95%CI =(0.999 ~ 3.311)] and hair length [0.053 ~ 0.203]. Conclusions there are a large number of asymptomatic infected dogs in Diebu county and Wenxian County of Gansu Province. The infection rate of local short-haired and pure-bred dogs is high. In order to reduce the risk of canine infection, the publicity and education on prevention of canine Leishmaniasis should be strengthened, so as to reduce the risk of human and animal co-contracting visceral Leishmaniasis.

Background

Visceral Leishmaniasis is caused by Leishmania donovani or L. infantum, protozoan parasites that are transmitted to human and animal hosts by the bite of phlebotomine sand flies[1]. Dogs are the main urban reservoirs of L. infantum and represent the major source of contagion for the vectors by virtue of the high prevalence of infection and intense cutaneous parasitism[2, 3].

Canine visceral Leishmaniasis (CVL) is present in approximately 50 countries, mainly in South America and the Mediterranean region[4, 5]. Epidemiological studies in endemic areas have demonstrated that the prevalence of infection as determined by molecular techniques (50–80%) is higher than the seroprevalence (10–30%)[6, 7]. A cohort study conducted by Oliva et al. (2006) showed that most of the study animals were polymerase chain reaction (PCR)-positive months before seroconversion[8]. The CVL infection rate depends on several factors including the length of the transmission season, the vector density, the susceptibility of the dog population, dog behavior, the degree of exposure to vectors, and dog owners' attitudes toward prevention[9, 10]. Preventing the expansion and urbanization of zoonotic visceral Leishmaniasis (ZVL) requires that the risk factors associated with human and canine infection be identified. Some cross-sectional serological surveys have suggested that canine susceptibility to infection is associated with dog size, fur length, age, and housing conditions [11, 14]. During a previously reported cross-sectional study carried out in an urban area of Brazil (Belo Horizonte, southeastern Brazil), They evaluated the prevalence of and risk factors associated with L. infantum infection in dogs, as identified by means of a molecular test (polymerase chain reaction-restriction fragment length polymorphism, PCR-RFLP)[15], and demonstrated that dogs with PCR positive for L. infantum showed approximately twice the risk of seroconversion as those that were PCR negative[16]; Some foreign studies have evaluated the risk factors associated with L. infantum infection in dogs, however few have used a risk factors in China.

Gansu is a province with the most serious prevalence of canine Kala Azar in China, ranked the first in the number of new cases occurring each year. Dogs with visceral leishmaniasis are the primary source of infection; patients are mostly infants and young children; the transmission medium is peri-wild Phlebotomus chinensis [17, 18]. Following the Wenchuan earthquake in 2008, natural factors and the ecological environment have changed, and the large number of stray dogs have become the source of infection of Kala Azar; as a result, the epidemic situation has recovered and showed a tendency to spread to non-prevalent regions. A previous survey shows that a lot of dogs with latent infection exist in Kala Azar epidemic areas in Gansu Province[19, 20]. In order to understand the risk factors of visceral Leishmaniasis in dogs in endemic areas of Gansu province,

we conducted an investigation on the risk factors of canine Leishmaniasis in 2 endemic counties from 2018 to 2019, and analyzed the related factors of local CVL infection, so as to provide a basis for exploring new prevention and control methods suitable for Leishmaniasis in China.

2. Materials and methods

2.1 Site investigation

Xinglong Village, Zhongzhai Town, Wenxian County, Longnan City, and Luoda Village, Luoda Town, Diebu County, Gannan Prefecture is the visceral leishmaniasis (VL) major epidemic area(Fig. 1), 2 county is located in the mountains, vegetation, rich media suitable for sand fly survival quantity many, there is an important channel of traffic travel, business, trade and destination, season more than outsiders to the spread of CVL, flow is frequent, lead to health and infection risk is very big. In recent years, which have reported VL cases, so we were selected as investigation sites. In April of each year, a questionnaire was used to record the gender, age, height, weight, breed, kennel status, custody mode, health status, appearance characteristics and other information of domestic dogs in the survey village, and all dogs were sampled

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Detection method

Nested PCR was used to detect blood samples with high sensitivity and specificity. DNA was extracted using DNeasy Blood &Tissue Kit from Qiagen, Germany, according to the instructions[21]. The samples were subjected to polymerase chain reaction (PCR) targeting the transcriptional interphase (ITA-1) in vivo of the Leishmanian ribosome, with the target fragment of the expected product being 280–330 bp.The venous blood DNA of the dogs with Leishmania parasite detected by bone marrow puncture smear staining microscopy was used as a positive control. After purification, PCR positive products were sequenced by Codon Code Aligner Software V 3.0.1 (Codon Code Corporation, Dedham, Massachusetts, USA), and the quality of each sequence was evaluated and analyzed. Dog Leishmania infection was identified by Blast nucleotide standard software (Blast). Quality control

The research team and the village doctors at the site of the investigation carried out the field investigation together, and the field observation, measurement, inquiry and record formed the questionnaire. The standardization, logic and integrity of the investigation data were checked and improved on the same day to ensure the quality of the on-site investigation. After the experimental detection, the basic information of dogs, PCR detection results and geographic coordinates were matched one by one using Excel.

Statistical Analysis

EpiData3.1 was used to establish a database, and double entry method was used to input data information and compare and verify error correction. IBM Spss 23.0 was used for statistical analysis. The classified data were described by the number of cases and rate (n(%)), and chi-square test was used, with the test level = 0.05. A multivariable adjusted model was fitted with the variables that were statistically significant at $p < 0.05$ in univariate analyses. A step-by-step backward selection procedure was used to select the variables and to produce the final multivariate regression model. Only adjusted variables showing a significant association ($p < 0.05$) with the occurrence of infection by L. infantum remained in the final model. The strength of association was determined by a hazard ratio and a 95% CI.

Results

Sample PCR detection

The study examined 574 dogs. The positive rate of venous blood PCR was 41.15% (221/537) in 537 dogs in Wenxian county and Diebu County, among which the positive rate was 32.30% (126/390) in Diebu County. The positive rate of dogs in Wenxian county was 64.63% (95/147).All the 37 dogs in Tianzhu County were negative.

Basic Information, Symptoms And Signs Of The Dog

Among the 537 dogs in the endemic area, 61.26% were males (329/537) and 38.73% were females (208/537).The canine age distribution was 6 to 180 months, the mean canine age was 45.77 months (standard deviation SD2.707), the median (interquartile range IQR) was 36 months, and the canine age was 88.83% (477/537) from 12 to 84 months. The height of dogs ≤ 40 cm accounted for 59.59% (320/537).Dogs weighing less than 10Kg accounted for 66.10% (355/537).Mixed-breed dogs account for 86.22% (463/537) and purebred dogs for 13.78% (74/537).Captive rearing accounted for 95.90% (515/537) and free range rearing accounted for 4.10% (22/537).The 502 dogs were in good physical condition, and the symptoms and signs (obvious emasculation, skin and mucous injury, local hair loss, and nasal tip ulceration) were none (93.48% (502/537).There were 513 active dogs, accounting for 95.53% (513/537).Only 17 dogs (3.16%) had one or more symptoms and signs of Leishmaniasis, and 29 dogs (5.40%) had only one symptom of toenail growth (Table 1).

According to the frequency distribution and results of univariate analysis in Table 1, variables with $P < 0.05$ were statistically significant. Among them, there were significant differences between the 12 variables of dogs' sex, age, height, weight, breed, kennel presence, hair length, style of confinement, physical condition, exercise status, mucosal signs and toenail symptoms and those of dogs with positive Leishmentan infection.

Analysis Of Relevant Influencing Factors

Statistically significant variables in Table 1 were included in the multivariate Logistic regression analysis, and the results showed that the four variables were the risk factors for the transmission of Leishmaniasis infection in dogs. (Table 2)

Table 1
Analysis of the infection rate of Leishmania parasite in domestic dogs in Gansu province in 2019

Variable		Classification	Number of dogs	Positive number (%)	The OR and 95% CI		χ^2	*P*
Domestic dogs basic situation	**Gender**	The male	329	146 (44.38)	0.707	0.495–1.010	3.642	0.056
		The female	208	75 (36.06)				
	Age	Puppies: ≤2 years old	105	61 (58.10)	0.424	0.275–0.655	15.467	0.000
		Adult: > 2 years old	432	160 (37.04)				
	Height	Small: ≤40 cm	320	111 (34.69)	1.936	1.361–2.752	13.676	0.000
		Big: > 40 cm	217	110 (50.69)				
	Weight	Small: ≤10 kg	355	135 (38.03)	1.460	1.017–2.095	4.228	0.040
		Big: > 10 kg	182	86 (47.25)				
	Varieties	pure	74	46 (62.16)	2.704	1.630–4.484	15.640	0.000
		The hybrid	463	175 (37.80)				
	Presence of kennels	There are	393	128 (32.57)	3.775	2.527–5.640	44.598	0.000
		There is no	144	93 (64.58)				
	Hair length	Long hair	84	72 (85.71)	0.082	0.043–0.155	81.641	0.000
		Short hair	453	149 (32.89)				
	Captive way	Bolt to raise	515	204 (39.61)	5.183	1.883–14.269	12.357	0.000
		grazing	22	17 (77.27)				
Domestic dogs clinical symptoms	**Physical condition**	Good: Ilium, vertebrae, ribs not visible	502	186 (37.05)	0.371	0.331–0.415	53.534	0.000
		Medium: Ilium, vertebrae, ribs visible	35	35 (100.00)				
	Motion state	active	513	203 (39.57)	4.581	1.788–11.737	11.883	0.001
		indifference	24	18 (75.00)				
	Appearance characteristics	No clinical symptoms	527	215 (40.80)	2.177	0.607–7.806	1.494	0.222

Variable		Classification	Number of dogs	Positive number (%)	The OR and 95% CI		χ^2	*P*
		With signs of wasting	10	6 (60.00)				
	Signs of the mucous membrane	No eye secretion	534	218 (40.82)	0.408	0.369–0.452	4.314	0.038
		There are eye secretions	3	3 (100.00)				
	Hair logo	No hair loss	532	217 (40.79)	5.806	0.645–52.305	3.145	0.076
		Systemic/localized hair loss	5	4 (80.00)				
	Dermal mark	No skin abnormalities	530	217 (40.94)	1.923	0.426–8.679	0.749	0.387
		Note tip ulceration/erythema/nodule	7	4 (57.14)				
	Toenail symptoms	Toenail is normal	506	192 (37.94)	4.581	1.788–11.737	37.292	0.000
		Nails grow	31	29 (93.55)				

Table 2
Analysis of factors of Leishmania infection in domestic dogs

The variable name	B	S.E.	Wald χ2	P	The OR	95% CI	
						The lower limit	ceiling
Constant term	19.644	6148.102	0.000	0.997	0.000		
Captive way	1.115	0.587	3.607	0.058	3.051	0.965	9.645
Presence of kennels	0.680	0.255	7.140	0.008	1.974	1.199	3.252
The breed of dog	0.598	0.306	3.828	0.050	1.819	0.999	3.311
Hair length	2.265	0.342	43.802	0.000	0.104	0.053	0.203

Discussion

The survey took place in the rural areas of Wen xian county and Diebu County, Gansu Province. The main purpose of the villagers' dogs was to guard the homes and nursing homes, and most of the dogs were tied up in the corners of the homes. There are a lot of dogs and very few livestock kept in the villages in study area, so dogs are an easy source of blood meal for sandflies. The survey raises the dog the parasite infection is tied to the dog three times higher risk may be due to putting the dog is tied to the dog range is bigger, raises the dog often follow dog owners into the wild fields and mountain stream, often free access to the village inside and outside activities, and Gansu Leishmaniasis media is sand fly mainly wild habitat the sand fly, so put the dog activity increases the chances of the sand fly, resulting in an increased risk of infection[22, 23]. Dogs without kennels were 1.9 times more at risk of infection than dogs with kennels, again due to the greater risk of infection in the open air.

The risk of CVL infection in purebred dogs in this study was 1.8 times higher than that in mongrels (95%CI0.999-3.311).This is consistent with the finding by Solano-Gallego et al that hybrids are a protective factor, that they are less likely to be infected,

that hybrids have developed a degree of resistance, and that pure-bred dogs are at risk[24]. Our study also found that short-haired dogs are more susceptible to infection than long-haired dogs, and there is a significant difference, which is consistent with the results that short-haired dogs have a significantly higher probability of infection than long-haired dogs[25]. In fact, sand fly bite dogs were more likely to choose the face with less hair, around the nostril, muzzle edge, and perianal area. Long-haired dogs in the survey sites were never trimmed, and many long-haired dogs had their face covered with fur, so short-haired dogs were more likely to be bitten and infected by sandflies[26].

In the univariate analysis, the age, height and weight of the dogs were statistically significant. There was a significant difference in Leishmania infection rates between puppies and adults, and we found a higher risk of Leishmania infection in puppies younger than 2 years of age[27]. And the greater the height and weight of the dog, the greater the exposure, and the higher the risk of Leishmania infection. According to foreign studies, Leishmania infection is more easily acquired in dogs under 3 years of age[28]. Puppies are more susceptible to infection due to congenital immaturity, a lack of internal immune barriers, malnutrition and the sand fly's affinity for this group[29]. We found that although the age, height, weight and other factors in the univariate study were statistically significant, these factors were not the decisive factors affecting infection in the regression model because of the correlation strength.

Cvl-related symptoms do not actually occur immediately after Leishmania infection in dogs, which is called asymptomatic infection. In this study, a large percentage of infected dogs showed no obvious signs and symptoms, but asymptomatic dogs may exhibit a high parasite load and play an important role in CVL transmission[30, 31]. Over time, the physical condition, activity, mucosal signs, nail growth and other indicators of asymptomatic infected dogs changed[32]. Our study found that almost all dog owners did not actually know about CVL and were unaware that mucosal signs, toenail growth, etc., were symptoms of CVL development, thus neglecting the care and selection of dogs and allowing the disease to develop. The persistence of this phenomenon may result in more healthy dogs being infected[33]. Therefore, we believe that promoting owners to master basic KNOWLEDGE of CVL and taking active preventive measures and strengthening the management of sick dogs will help to reduce the spread of CVL.

Conclusions

Research results show that the way of captive, kennels, varieties and dog hair length is Gansu research spot dogs infected with the parasite risk factors, the conclusions will help to improve people's understanding of CVL epidemiology, for the dog to a crowd in endemic areas targeted Suggestions, optimize and develop scientific dog management measures so as to reduce the CVL transmission has important significance.

Declarations

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Author’s contributions

YDW was responsible for sample testing, data analysis and paper writing; LF was responsible for research scheme formulation, on-site collection and data sorting; FYwas responsible for on-site sampling coordination and technical guidance; YCM, YJK, LL and Z YF participated in on-site sampling.All authors revised and approved the final manuscript.

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Availability of data and materials

Authors can confirm all relevant data are included in the article and materials are available on request from the authors.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Gansu provincial Center for Disease Control and Prevention (protocol no. 001/2018). Owners of dogs participating in the project were informed of the research objectives and were required to sign an informed consent form before sample and data collection.

Consent for publication

Not applicable.

Competing interests

The authors have declared that no competing interests exist.

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Figures

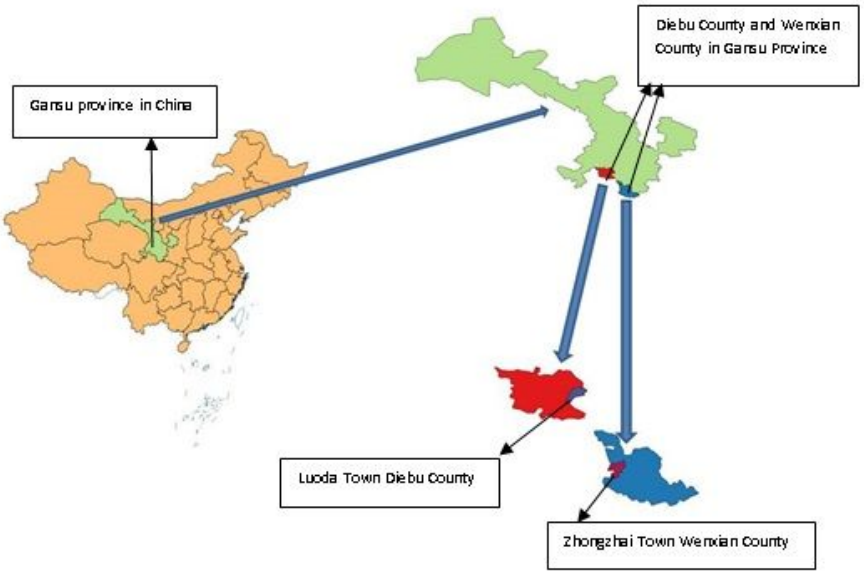

Figure 1

Location of the study area in China and geographic distribution of canine Leishmaniosis Seroprevalence in villages at different areas in the Gansu provincial. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.