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REGULAR ARTICLES

Seroprevalence and risk factors of *Streptococcus ovis* infection in Tibet, China

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Abstract
Sheep septicemic streptococcosis is an acute, febrile, and septic zoonotic disease caused by *Streptococcus ovis*. There are few reports of this disease especailly at high-altitude areas of Tibet, China. A cross-sectional study was conducted at five different counties of Shigatse area (a city in Tibet territory) from 2021 to 2022. Sera from 1458 randomly selected sheep and goats from 50 flocks were tested for antigens of *Streptococcus ovis* using a commercial ELISA kit. The individual animal level prevalence was 6.93% (95% CI: 5.7–8.4), and the flock prevalence level was 84% (95% CI: 70.9–92.8). At the individual animal level, year and management type were significantly associated with seropositivity by binary logistic regression analysis. Prevalence of *Streptococcus* in 2021 significantly differed from 2022 (OR = 2.16, 95% CI: 1.39–3.39). The prevalence of *Streptococcus* varied between management types with herds having higher odds of disease than intensive farms (OR = 2.64, 95% CI: 1.71–4.10). Hence, our study reports for the first time on seroepidemiology and risk factors of *Streptococcus ovis* infection, laying a theoretical foundation for preventing and controlling streptococcosis in Tibet area.

Keywords *Streptococcus ovis* · Serological epidemiology · ELISA · Shigatse area

Introduction

Streptococci are Gram-positive suppurative cocci, which are widely distributed in nature. They are isolated in the digestive tract and nasopharynx of humans and other animals, composing of pathogenic and non-pathogenic types (YAO, H C, et al., 1999). Pathogenic Streptococci can cause suppurative diseases in humans and livestock, such as mastitis, septicemia, equine plague, and human scarlet fever (COLMAN, G, 1990). Streptococcosis is a potentially epidemic disease of sheep. It is an acute febrile infectious disease caused by hemolytic *Streptococci* and usually occurs during winter and spring (WANG, M Q, et al., 2021). The condition is mainly caused via the digestive tract, respiratory tract, and through injured skin. The clinical manifestations in sheep and goats are swelling of the mandibular lymph nodes and associated throat region and fibrinous pneumonia (LI, X Y, et al., 2014).

The local sheep found in Tibet are a unique and rare breed that have lived in the Qinghai-Tibet Plateau region for many decades (SU, Y, et al., 2022). The breed is a dual-purpose breed providing meat and milk as well as contributing to the local economic development of the region. Therefore, safe and effective improvement of Tibetan sheep breeding and ensuring the quality of its by-products (meat, milk, etc.) is of great significance to meet the market needs of the people and high-quality development of animal husbandry (ZHANG, X, et al., 2022). Moreover, sheep streptococcosis perennially perplexes local animal husbandry conditions and green growth in the plateau (FOSTER, G, et al., 2020). There are more than 100 identified species of *Streptococcus*, first reported by Weimann in Germany in 1910 (YANG, S D, et al., 1953). It was reported in China in 1952 and then spread all over the country. Furthermore, with the development of the breeding industry, disease occurrence showed an obvious upward trend, causing great harm to the overall GDP of the country. In recent years, sheep streptococcosis has been

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prevalent every year in pastoral areas of Tibet. In 2017, the incidence of *Streptococcus ovis* (*S. ovis*) in Shannan prefectural city of Tibet was 12.3%, with a fatality rate of 18.6% for the first time (BIANBA, Z M, et al., 2017).

The different species of *Streptococcus*, e.g., *Streptococcus mastidis* (*S. mastidis*) and *Streptococcus equi* (*S. equi*), are the primary pathogens, causing a decrease in milk production and the quality of dairy products with severe economic loss to breeding farms of dairy sheep and goats (ROSA, N M, et al., 2021; VEZINA, B, et al., 2022). According to some researchers, *S. equi* may also cause fibrin polyarthritis in goats (STEWARD, K F, et al., 2017). In previous studies, humans were considered the only biological host of suppurative *Streptococci* (BESSEN, D E, 2009). However, it has occasionally been identified in samples of milk from sheep with mastitis (VELA, A I, et al., 2017). With this in mind, the Tibetan government has taken joint measures (vaccination and destruction of infected animals) in infected areas, but the epidemic still occurs frequently due to the development of animal husbandry in the region. Hence, more information is always required to control the outbreak of the disease. This study was designed to determine the prevalence of potential risk factors for sheep streptococcosis for the very first time in the Tibetan region.

Materials and methods

Study areas and sampling

A study was conducted in five counties of Shigatse prefectural city (Sangzhuzi (E: 89.05°, N: 29.35°), Bailang (E: 89.17, N: 28.95°), Lazi (E: 89.31°, N: 29.26°), Saga (E: 84.47°, N: 29.54°), Zhongba (E: 84.02°, N: 29.76°) from October 2021 to April 2022. A total of 1458 sera samples from non-vaccinated (*Streptococcus*) sheep and goats were collected from 50 flocks. Samples were collected from two types of animal-rearing systems: intensive farms and scattered herds. The intensive farms are managed by the relevant departments of the local government. There are full-time managers and qualified veterinarians working on the farms with experience in disease prevention and control and management. Herders graze free-range their sheep/goats. The areas of Saga and Zhongba are pastoral regions, while Sangzhuzi, Bailang, and Lazi areas are semi-agricultural and semi-pastoral areas. The average altitude of the sampling sites was 4000 m above sea level, where animals are grazed openly all year round, with extra supplementary feeding being provided in winter (Fig. 1).

Sampling quantity and distribution of sheep serum

The sample size was calculated using a design prevalence of 20% with a 95% confidence interval (CI) and 5% precision (SAVILLE, W J, et al., 2004). A total of 720 sheep serum samples were collected in Sangzhuzi (n = 197), Lazi (n = 350), and Bailang (n = 175) areas during 2021, and a further 738 samples were collected in Sangzhuzi (n = 122), Bailang (n = 108), Lazi (n = 147), Saga (n = 201), and Zhongba County (n = 158) areas during 2022. Overall, 1458 samples were collected from 13 intensive farms and 37 scattered herds in the Shigatse area. Among them, 938 samples were assocaited with sheep and goat farms, while 520 were associated with scattered herds (n = 192).

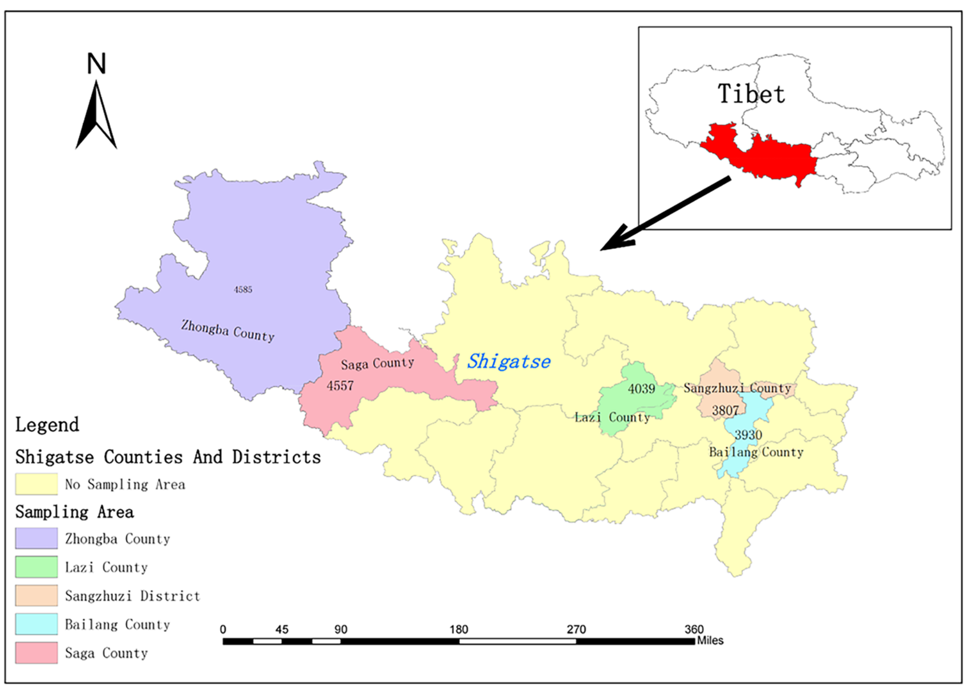

Fig. 1 Map showing the location of the selected counties in Tibet, China

Serological tests

Approximately 5 mL of whole blood was collected from the jugular vein. The samples were kept at room temperature for several hours and then centrifuged at 3000 rpm for 10 min. The serum was then collected in 2 mL centrifuge tubes and stored at −20 °C. The frozen samples were thawed and tested with a commercial ELISA kit for detecting antigens of *Streptococcus ovis* (Batch number: 202107; Shanghai enzyme-linked Biotechnology Co., Ltd.).

Statistical analyses

Data obtained from both the serological test and questionnaire survey was stored in a Microsoft Excel spreadsheet (Microsoft Corp.), and statistical analysis was performed using the statistical software R (R Development Core Team 2022). Chi-square test was used to analyze the association between the presence of serum antibodies against *Streptococcus* and breeds, ages, sex, altitude, sampling years, and management type. The statistical significance was set as $p < 0.05$. In univariate analysis, variables with $p \leq 0.20$ were offered to a multivariate logistic regression analysis. A multivariable logistic regression model was generated by using a backward stepwise process to control for confounding and test for effect modification, and the variables with $p < 0.05$ were retained in the final model. The likelihood ratio test was conducted to evaluate the model and was regarded as the most appropriate model with the smallest AIC (Akaike information criterion) value (BOUKARY, A R, et al., 2013). The model was then assessed by the Hosmer-Lemeshow goodness-of-fit test.

Results

Among all the screened sheep, 6.93% (101/1458, 95% CI: 5.7–8.4) were found as positive for *Streptococcus*, and the flock prevalence of *Streptococcus* was 84% (42/50, 95% CI: 70.9–92.8).

At the individual level, there was no significant difference in the prevalence of *Streptococcus* in different breeds, ages, and sex. The individual prevalence of sheep and goats at the altitude of 4000 m was 5.61%, and 8.79% respectively (Table 1). Statistically, the *Streptococcus* from different altitudes above sea level was significant (OR = 0.62, 95% CI 0.41–0.93; $p < 0.05$). There was a significantly higher animal level prevalence in 2022 (8.29%) than in 2021 (5.54%) (OR = 1.52, 95% CI: 1.02–2.33; $p < 0.05$). Moreover, the prevalence of *Streptococcus* in pastoral households was 10% (95% CI: 7.6–12.9), and at intensive farms was 5.22% (95% CI: 3.9–6.8). A significant difference was noticed in individual positive rates between the two management types (OR = 0.50, 95% CI: 0.33–0.74; $p < 0.01$) (Table 1). In the final model, only the sampling year and the management type had significant differences, among which the odds of *Streptococcus* in 2021 were significantly different from that in 2022 (OR = 2.16, 95% CI: 1.39–3.39). The odds of *Streptococcus* at intensive farms significantly differed from that of *streptococci* in herders (OR = 2.64, 95% CI: 1.71–4.10). The likelihood ratio test, $\chi^2 = 23.14$ ($p < 0.001$), AIC value of the final model, 716.97, and a Hosmer-Lemeshow goodness-of-fit

Table 1 The univariate analysis of risk factors associated with *Streptococcus* seropositivity among sheep and goats in Shigatse area by enzyme-linked immunosorbent assay

Variable	Categories	Prevalence% (95% CI)	OR (95% CI)	*P* value
Age (years)	<3	6.26 (4.3, 8.7)	1.00	0.379
	3–5	7.72 (0.4, 1.5)	0.69 (0.34, 1.39)	
	>5	5.10 (2.2, 9.8)	0.44 (0.17,1.16)	
Sex	Female	6.40 (4.7, 8.5)	1.00	0.4452
	Male	7.42 (5.7, 9.5)	1.08 (0.72,1.63)	
Sampling year	2021	5.54 (4.0, 7.5)	1.00	<0.05*
	2022	8.29 (6.4, 10.5)	1.54 (1.02,2.33)	
Animal type	Goat	5.21 (2.5, 9.4)	1.00	0.3141
	Sheep	7.19 (5.8, 8.8)	1.41 (0.72,2.76)	
Altitude (ASL)	<4000 m	8.79 (6.7, 11.3)	1.00	<0.05*
	>4000 m	5.61 (4.2, 7.4)	0.62 (0.41,0.93)	
Management type	Herder	10.00 (7.6, 12.9)	1.00	<0.01*
	Intensive farm	5.22 (3.9, 6.8)	0.50 (0.33,0.74)	

*$p \leq 0.20$; offered to a multivariable logistic regression model

value ($p = 0.34$), indicated that the model was a good fit of the data (Table 2)

Discussion

China has a long history of raising sheep, regardless of quantity or breed diversity. Tibet region is one of the five major pastoral areas in China, where livestock rearing is an important industry for farmers and herders (ZAMORA, L, et al., 2017). Sheep are considered as important as yaks to farmers and herders in the plateau regions. Different pathogens might affect the growth of the sheep industry, e.g., *Streptococcosis*. There are few studies investigating the prevalence and risk factors for infection with *Streptococci* in sheep. This study provides preliminary information on infection in the Tibet region. The smaller number of studies on the disease has urged researchers to focus more on other potential factors to control the disease's impact. The current study was conducted in five different areas of Shigatse prefectural city. Results showed that the animal level seroprevalence in sheep and goats was relatively high at 6.3%, with the herd level (84%). At the animal level, the seroprevalence detected in the current study was slightly higher than that in Qinghai Province (3.13%) (LI, X Y, MA, R L, WANG, Y P, WANG, S X, FU, Y J and ACKERMANN, K, 2014), although both regions are located in the Qinghai-Tibet Plateau. The difference may be due to the grazing environment, breeds, nutritional status, and sample size. There are few reports on the epidemiology and risk factors for infection with *S. ovis* compared to *S. suis* and *S. equi* in pigs and horses. For example, the prevalence of *S. equi* in Croatia (STRITOF, Z, et al., 2021) and Lesotho (LING, A S, et al., 2011) were 16.5% and 10.1%, respectively. The prevalence of *S. suis* in Tibet was reported to be 60.13% (LAN, L, 2017). This may be related to the different types of livestock breeds in different regions affected by various *Streptococci* species.

From the results of this study, it can be seen that *S. ovis* infection was endemic, with the close connection between Tibet and other provinces in recent years and with the passing of National Highway 318 and National Highway 219 in the sampling area. At the same time, the average elevation of Shigatse is more than 4000 m, the climate is changeable all year round, the temperature difference between day and night, summer, and winter is large, and the grass withering period is long. These provide convenience for the spread and occurrence of *Streptococcus* sheep. Because the sampling site is in an environment of high cold and hypoxia, and the results of this study show that the disease is still widespread in the Shigatse area, *Streptococcus* has adapted to this environment, and its growth and transmission are basically not restricted by this kind of cold and hypoxia. Through the investigation, the seroprevalence in sheep sampled in 2022 (8.29%) was significantly higher than that in 2021 (5.54%, $p < 0.05$). The sampling time was April 2022 and October 2021, which indicated that sheep streptococcosis was more prevalent in early spring than in winter in the Shigatse area. At the same time, Tibetan forage gradually turned green in April, leading to significant diarrhea in sheep. According to some studies, it may decrease sheep resistance, providing favorable conditions for breeding *Streptococcus* in the animals' bodies.

Another finding observed in our study is that the seroprevalence in animals from pastoral herds (10%) was significantly higher than in animals from intensive farms (5.22%). It may be understood that the education level of local herdsmen is generally low, and they know little about the prevention and control of livestock epidemic disease, which may also be one of the reasons for the widespread occurrence of the disease in the local area. Moreover, herders do not have proper supplementary feeding but instead rely on grazing to maintain their nutritional status.

Conclusion

Streptococcus ovis is prevalent in the Shigatse area of Tibet, China. Using a logistic regression model, it was found that only sampling time and management type of Tibetan sheep were risk factors for seropositivity to streptococcus. Therefore, preventive measures such as immunization plans, proper animal housing, monitoring and surveillance, proper carcass disposal, training, and education of farmers regarding the spread of disease, especially during early spring,

Table 2 Multivariable logistic regression analysis of risk factors related to *Streptococcus* seropositivity in sheep and goats

Risk factors	Categories	Estimate	SE (*b*)	OR	95% CI	*p* value
Constant		-3.44	0.23			
Year	2021	0.77	0.23	1.0		<0.001
	2022			2.16	1.39, 3.39	
Management type	Herder	0.97	0.23	2.64	1.71, 4.10	<0.001
	Intensive farm			1.0		

Estimate regression coefficient; *SE*, standard error; *CI*, confidence interval should be strengthened, and also, reasonable supplementary feeding needs to be provided.

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Author contribution MHC, ZJY, and WQX conceived, designed, and coordinated the study. WDJ, YZJ, and PQ made comments on revisions and improvements to this study. MHC did experiments and data analysis. All authors contributed to the data analysis and preparation of the manuscript. All authors read and approved the final manuscript.

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Data availability All data generated or analyzed during this study are included in this published article. The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval Animal care and experiments were carried out in accordance with the criteria set by the Chinese Guidelines for the Care and Use of Laboratory Animals, and all methodologies were supervised and authorized by the Tibet Academy of Agriculture and Animal Husbandry Sciences's Institutional Review Board.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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