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Seroprevalence and risk factors associated with *Haemophilus parasuis* infection in Tibetan pigs in Tibet

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ABSTRACT

Haemophilus parasuis is the causative agent of Glässer's disease, an important emerging infectious disease, but little is known of *H. parasuis* infection in Tibetan pigs in Tibet. The objective of the present investigation was to examine *H. parasuis* seroprevalence in Tibetan pigs in Tibet, China. Serum samples from 423 Tibetan pigs in Nyingchi, Tibet, China from April to December in 2010 were examined independently for the presence of antibodies against *H. parasuis*. A total of 147 (34.75%, 95% confidence interval [CI] 30.21–39.29) Tibetan pigs were tested positive for *H. parasuis* antibodies by the indirect hemagglutination assay (IHA) using a kit commercially available. 80 of 231 in Nyingchi (34.63%, 95% CI 28.50–40.77) and 67 of 192 in Mainling (34.89%, 95% CI 28.15–41.64) were tested positive, but the difference was not statistically significant ($P > 0.05$, $\chi^2 = 0.003$). The prevalence ranged from 19.72% (95% CI 10.46–28.97) to 75.00% (95% CI 32.57–100) varying in different age groups, with higher prevalence in breeding boars than in piglets, and the difference was statistically significant ($P < 0.05$). The prevalence of *H. parasuis* infection in males (45.03%, 95% CI 37.57–52.49) was significantly higher than that in the female (30%, 95% CI 22.41–37.59) pigs ($P < 0.05$, $\chi^2 = 7.361$). Gender of Tibetan pigs was the main risk factor associated with *H. parasuis* infection. The results of the present survey indicated a wide distribution of *H. parasuis* among Tibetan pigs in Tibet, China and further investigation should better assess circulation of *H. parasuis* in Tibetan pigs. To the best of our knowledge, the present study represents the first report of *H. parasuis* infection in Tibetan pigs in China.

1. Introduction

Haemophilus parasuis is the causative agent of Glässer's disease, which is characterized by the systemic symptom including fibrinous polyserositis, polyarthritis and meningitis (Hoefling, 1991; Oliveira and Pijoan, 2004). This disease is recognized as an important emerging infectious disease that has been paid more and more attention due to the considerable economic losses it caused to the pig industry worldwide (Cai et al., 2005; Rapp-Gabrielson et al., 2006). By far, fifteen different serovars have been recognized using traditional serotyping, and large numbers of the isolated non-typeable *H. parasuis* strains have been assessed the heterogeneity at the molecular level (Kielstein and Rapp-Gabrielson, 1992; Olvera et al., 2006). In China, a large number of field isolates have been studied previously (Cai et al., 2005; Li et al., 2009; Chu et al., 2011) and serovars 4 and 5 were considered the most prevalent *H. parasuis* strains (Cai et al., 2005). However, no information is available regarding *H. parasuis* infection in Tibetan pigs, China.

Tibetan pig is a local pig breed most distributed in the southeastern Tibet Plateau and the surrounding areas (with an average altitude of more than 3000 m). The pig is mainly fed by the free-range system combined with the drylot husbandry. Under the special breeding system, this animal is characterized by disease resistance, easy breeding and carcass lean high-quality. Its meat is delicious with high proteins, tender texture and rich amino acids, and is an important source of income for Tibetans.

In the present investigation, the indirect hemagglutination assay (IHA) was used to investigate the most prevalent serovars 4, 5

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Table 1
Prevalence of *Haemophilus parasuis* infection in pigs in China.

Province	No. tested	Positive (%)	Test method[a]	Time tested (year)	Reference
Fujian	1104	18.00	LAT	2005	Lin et al. (2006)
	200	6.50	IHA	2008	He et al. (2010)
	231	11.00	LAT	UN[b]	Zheng (2006)
Guangxi	305	4.59	PCR	2006–2010	Ma et al. (2010)
	86	30.20	PCR	2009–2010	Xuan et al. (2011)
Hubei	332	32.50	IHA	2010	Wan et al. (2010)
	450	21.70	IHA	UN	Gao et al. (2009)
Jiangxi	150	44.00	IHA	UN	Zhao et al. (2009)
Ningxia	403	25.31	IHA	UN	Bai et al. (2010)
Heilongjiang	400	48.75	ELISA	UN	Luo et al. (2010)
Qinghai	639	25.19	IHA	UN	Lu et al. (2009b)
	209	24.40	IHA	UN	Lu et al. (2009a)
Sichuan	1062	10.00	IHA	2006–2008	Feng et al. (2009)

[a] LAT, latex agglutination test; PCR, polymerase chain reaction; IHA, indirect hemagglutination assay; ELISA, enzyme-linked immunosorbent assay.
[b] UN, unknown.

and 12 of *H. parasuis* in Tibetan pigs in Tibet, China. The prevalences of *H. parasuis* infection in other kinds of pigs in China, which were originally published in local Chinese journals (hardly accessible to international readers), are summarized in Table 1. To the best of our knowledge, this is the first report of the *H. parasuis* seroprevalence in Tibetan pigs.

2. Materials and methods

2.1. The study site

The present study was carried out in Nyingchi and Mainling counties of the Nyingchi Prefecture, southeast Tibetan. This area was geographically isolated from Tibet and Sichuan Provinces by Himalayas, Nyainqentanglha Range and Hengduanshan Mountain and shares borders with Yunnan Province and Qamdo Prefecture in the east and northeast, Naqu Prefecture in the north, Lhasa city in the west, Shannan Prefecture in the southwest, and India and Myanmar in the south. The average elevation of the surveyed area is more than 3000 m above sea level.

2.2. Collection of serum samples

Tibetan pigs are bred in the cage-free system in rural areas. A total of 423 blood samples (including 231 from Nyingchi county and 192 from Mainling county) representing 171 males, 140 females and 112 unknown genders were collected from April to December, 2010. All the operations were guided by the Animal Ethics Committee of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Approval No. LVRIAEC2010-010). Before sample collection, the general characters of Tibetan pigs were examined thoroughly. These blood samples were centrifuged at 3000 × *g* for 5 min. The separated sera were stored at −20 °C until further analysis.

2.3. Serological examination

Antibodies against *H. parasuis* were examined by IHA using a commercially marked kit (Lanzhou Veterinary Institute, Chinese Academy of Agriculture Sciences). The detection procedure was performed according to the manufacturer's instructions. Briefly, each test serum was screened by two-fold serial dilutions starting from 1:2 to 1:1024. In the eighth hole, discard 25 μL mixture to maintain 50 μL system. Positive and negative controls were always included in the same plate. After addition of 25 μL of *H. parasuis* antigen sensitized glutaraldehyde-fixed sheep erythrocytes (serovars 4, 5 and 12) to each well, the plates were shaken gently for 1–2 min and then incubated at 37 °C for 2–3 h. The tests were considered positive for *H. parasuis* antibodies when the sera IHA titers were 1:8 or higher. Dilution of 1:4 was suspicious and should be retested.

2.4. Statistical analysis

Data were statistically analyzed using the procedure of SAS (Statistical Analysis System, Version 8.0). Chi-square analysis was used to compare the difference and association between pairs of interesting measures. Logistic regression models were constructed for each pig population to calculate odds ratios for seropositivity among areas and ages while controlling for gender. All tests were 2-sided, and the level of significant difference was defined as $P < 0.05$.

3. Results

Antibodies against *H. parasuis* were detected in 147 of 423 (34.75%, 95% confidence interval [CI] 30.21–39.29) serum samples by IHA; 80 of 231 in Nyingchi (34.63%, 95% CI 28.50–40.77) and 67 of 192 in Mainling (34.89%, 95% CI 28.15–41.64) were tested positive, but the difference was not statistically significant ($P > 0.05$, $\chi^2 = 0.003$). However, male Tibetan pigs from Mainling county were >2 × more than females to be seropositive (odds ratio [OR] 2.42, 95% CI 0.20–1.19) (Table 2). The same association between male pigs and positive serologic test result was also observed in Nyingchi County.

Statistical analysis revealed that the prevalence of *H. parasuis* in male pigs (45.03%, 95% CI 37.57–52.49) was significantly higher than that in the female pigs (30%, 95% CI 22.41–37.59) ($P < 0.05$, $\chi^2 = 7.361$) (Table 2). The proportion of seropositive animals was not equal among ages ($P < 0.05$, $\chi^2 = 12.614$). The highest seroprevalence was found in breeding boars (75%, 95% CI 32.57–100) and the lowest in piglets (19.72%, 95% CI 10.46–28.97) (Table 3). The antibody titers of the confirmed positive samples were 1:1024 in one, 1:512 in one, 1:256 in three, 1:128 in one, 1:64 in six, 1:32 in seven, 1:16 in 33 and 1:8 in 95 in Tibetan pigs (Table 3).

4. Discussion

H. parasuis can reside as commensal flora in the upper respiratory tract of pigs and is known as an opportunistic microorganism. However, under certain conditions, these organisms can cause disease as the secondary pathogens probably associated with