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First report of *Mycoplasma hyopneumoniae* seroprevalence in Tibetan pigs in Tibet, China

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The present study examined the seroprevalence of *Mycoplasma hyopneumoniae* infection in Tibetan pigs in China for the first time. Serum samples were collected randomly from 423 Tibetan pigs of different categories in Nyingchi county and Mainling County, Tibet, China from April to December 2010, and then tested for the presence of antibodies against *M. hyopneumoniae* by double-sandwich enzyme-linked immunosorbent assay (ELISA). The results showed that 249 (58.86%) Tibetan pigs were tested positive and the seroprevalence ranged from 25.00 to 75.00% in different age type groups. There was a higher seroprevalence in Mainling (78.97%) than in Nyingchi (41.67%). There was no significant difference in *M. hyopneumoniae* seroprevalence between male (59.54%) and female (51.41%) Tibetan pigs. The results of this investigation demonstrated that the Tibetan pigs are highly susceptible to *M. hyopneumoniae*, similar to that of the lowland domestic pigs. With the tendency of Tibetan pigs being bred in more places besides Tibet, the prevention and control of *M. hyopneumoniae* infection in Tibetan pigs should be carried out.

Key words: Tibet, Tibetan pigs, seroprevalence, *Mycoplasma hyopneumoniae,* enzootic pneumonia.

INTRODUCTION

Geographically, Tibet is a plateau region in Asia, north-east of the Himalayas and it is the highest region on earth, with an average elevation of 4,900 m. Due to the largest continuous high elevation ecosystem in the world of Tibet, some characteristical animals, such as Tibetan pigs, are living in this area. Tibetan pigs are mainly distributed in the Tibetan highlands and show striking phenotypic and physiological differences from lowland domestic pigs that have permitted them to adapt to the ultra-living conditions such as hypoxia (Yang et al., , 2011). Tibetan pigs have the ability to adapt to low temperature and poor food quality, and physically possess powerful musculature, with coarse black hair covering the whole body.

Mycoplasma hyopneumoniae plays an important part in the porcine respiratory disease complex (PRDC) as the causative agent of enzootic pneumonia (Thacker et al., 1999). It is a respiratory disease in swine characterized by a chronic dry cough, growth retardationbackward growth, slow onset and decreased feed conversion efficiency (Straw et al., 1989). *M. hyopneumoniae* is widely diffused in areas with pig production (Thacker et al., 1999) and can be transmitted in herds vertically and horizontally (Sibila et al., 2007). As yet, there is no data

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Table 1. Prevalence of antibodies to *Mycoplasma Hyopneumoniae* in different type groups of Tibetan pigs in Tibet, China determined by ELISA Kit (IDEXX Co. Ltd., USA).

Types of pig	Number examined	Number of positive	Prevalence (%)
Breeding boar	4	1	25
Breeding sow	3	2	66.67
Slaughter pig	112	43	38.39
Fattening pig	127	73	57.48
Growing pig	137	100	72.99
Piglet	40	30	75
Total	423	249	58.86

or report about the seroprevalence of *M. hyopneumoniae* among Tibetan pigs. The epidemiology of *M. hyopneumoniae* of lowland domestic pigs and wild boars (Choi et al., 2003; Fano et al., 2005; Vengust et al., 2006; Halli et al., 2012) have been reported in many areas of the world (Choi et al., 2003; Fano et al., 2005; Vengust et al., 2006; Halli et al., 2012) including as well as many regions in China (Xing et al., 2009; Yao et al., 2010; He et al., 2011). The purpose of this survey was to investigate the seroprevalence of *M. hyopneumoniae* in Tibetan pigs in Tibet, China, which would provide base-line data for the prevention and control of *M. hyopneumoniae* infection in Tibetan pigs.

MATERIALS AND METHODS

The preparation of serum samples

Blood samples were collected randomly from 423 Tibetan pigs by professionals veterinarians from the precaval vein (superior vena cava) in Nyingchi Prefecture including Nyingchi county and Mainling county, Tibet, China, during April to December 2010. Serum was separated by centrifugation at 3000 g for 5 min and then frozen at -20°C until further examination.

Serological investigation

Herd Chek Mhyo antibody enzyme-linked immunosorbent assay (ELISA) kit (IDEXX Co. Ltd., USA) was used to examine the antibodies against *M. hyopneumoniae* in accordance to the manufacturer's instructions. Briefly, a 96-well ELISA plate with coating antigen of *M. hyopneumoniae* was prepared and then 100 µl diluted serum samples (1:40) was added to the testing wells of the coated plates prior to mixing them gently. Then the plate was incubated at 25°C for 30 min, and subsequently washed three times. Afterwards, HRP conjugate addition and incubation for 30 min once again were conducted. Chromogenic enzyme substrate was added after the two times of washing with incubation at 37°C for 10 min. The kit has set two wells for each as the positive and negative control sera and another two wells was were treated as blank control at on the same plate.

The optical density (OD) value of each well was read by the microplate reader at the wavelength of 650 nm. Then we analysed the OD value of the plate with software xChek ® (IDEXX Co. Ltd., USA) and classified each sample as positive or negative.

Statistical analysis

Data were statistically analyzed using the procedure of SAS (Statistical Analysis System, Version 8.0). Chi-square analysis was used to analyze the difference in seroprevalence in gender and age type groups of Tibetan pigs. The level of significant difference was defined as $P< 0.05$.

RESULTS

In this study, 423 serum samples were collected from Tibetan pigs in Nyingchi and Mainling, Tibet, China. A total of 249 (58.86%) samples were positive for the presence of antibodies against *M. hyopneumoniae*. The prevalence of antibodies against *M. hyopneumoniae* in different age type groups ranged from 25.00 to 75.00% and the piglets had the highest *M. hyopneumoniae* seroprevalence of *M. hyopneumoniae* infection, followed by growing pigs which had *M. hyopneumoniae* seroprevalence of 72.99%. These differences were statistically significant ($P<0.05$) (Table 1). However, there was no significant difference in *M. hyopneumoniae* seroprevalence between male (59.54%) and female (51.41%, $P>0.05$, Table 2) Tibetan pigs.

DISCUSSION

Although considerable efforts have been made to prevent *M. hyopneumoniae* infection and its harmful effects, significant economic losses have been caused to pig production worldwide (Thacker et al., 2006), however, no information regarding *M. hyopneumoniae* infection in Tibetan pigs was available. The present investigation revealed an overall 58.86% seroprevalence of *M. hyopneumoniae* infection in Tibetan pigs in Tibet, China, which is higher than the results of previous reports (Xing et al., 2009; Yao et al., 2010; He et al., 2011) in China of domestic pigs in low-altitude areas, and the average percentage of positive piglets is higher than in Europe (10.7%) (Villarreal et al., 2010). There are two main routes for *M. hyopneumoniae* to be introduced into a herd: direct transmission and airborne transmission

Table 2. Prevalence of antibodies to *Mycoplasma Hyopneumoniae* in Tibetan pigs by gender and area in Tibet, China determined using an ELISA Kit (IDEXX Co. Ltd., USA).

Biometric data	Gender			Total	Geographical location	
	Male	Female	Unknown		Mainling	Nyingchi
No. examined	173	142	108	423	195	228
No. positive	103	73	73	249	154	95
Prevalence (%)	59.54	51.41	67.59	58.86	78.97	41.67

(Batista et al., 2004). The most likely reason for the higher seroprevalence of *M. hyopneumoniae* in the herd of the examined Tibetan pigs is that all the Tibetan pigs in the present study were free-ranged in the field. *M. hyopneumoniae* can be transmitted between pigs by the egestas produced by sneezing and coughing and the pathogen may horizontally spread via direct contact from the infected pigs to the piglets (Morris et al., 1995) or from the sows to their offsprings vertically (Maes et al., 1996). In particular, pigs of all ages types are susceptible to *M. hyopneumoniae* infections (Maes et al., 1996) and the seroprevalence of *M. hyopneumoniae* infections is particularly high in pigs of mid-finishing to slaughter age (Sibila et al., 2009). Likewise, the growing Tibetan pigs had the second highest prevalence (72.99%) followed by fattening pigs (57.48%) in our research investigation, which are consistent with these reports. Previous studies have shown that *M. hyopneumoniae* could be transmitted horizontally between wild boars and domestic pigs, and wild boars have been found to be a potential reservoir of *M. hyopneumoniae* (Vengust et al., 2006; Marois et al., 2007). Similarly, Tibetan pigs in the present study are as susceptible to *M. hyopneumoniae* as wild boars and domestic pigs (Sibila et al., 2009). Therefore, further studies are needed to determine the transmission of *M. hyopneumoniae* between Tibetan pigs and domestic pigs because now, Tibetan pigs are raised in more places in addition to Tibet.

There are a number of ways to control *M. hyopneumoniae* infection in a herd, such as optimizing the management practices and housing conditions, and using medication and bacterins. Though these measures do not ensure the absence of *M. hyopneumoniae*, they can reduce the prevalence level in a herd and decrease the economic loss caused by *M. hyopneumoniae* infection (Maes et al., 2008). Tibetan pigs in the present study were raised by local Tibetans outdoors and free-ranging to seek for food. This kind of rough management practice may facilitate the spread of *M. hyopneumoniae* infection among Tibetan pigs, thus should be improved.

Conclusion

The present investigation revealed, for the first time, the high seroprevalence (58.86%) of *M. hyopneumoniae* infection in Tibetan pigs, in Tibet, China, and all ages types of Tibetan pigs are susceptible to *M. hyopneumoniae* infection. This raised animal health concerns. Effective strategies and measures should be taken to control *M. hyopneumoniae* infection in Tibetan pigs in this unique region of the world.

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