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Seroprevalence and risk factors of *Mycoplasma suis* infection in pig farms in central China

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ABSTRACT

Mycoplasma suis, the causative agent of porcine infectious anemia, causes large economic losses to the swine industry worldwide. A questionnaire-based survey was conducted in 69 pig farms in Hubei Province, China, from November 2011 to August 2013 to ascertain the prevalence and associated risk factors of *M. suis*. Four thousand and four blood samples from pigs of all the age groups were tested for *M. suis* antibodies using the established rMSG1–ELISA assay. Among these 4004 samples, 1615 blood samples from multiparous sows were examined to identify the association between seroprevalence and different seasons. Information on risk factors collected from farmers or attending veterinarians was recorded on a pre-designed questionnaire. The overall test seroprevalence of *M. suis* infection at the animal level was 31.9% (1277/4004; 95% CI: 30.5%, 33.4%), whereas at the farm level, this value was 95.65% (66/69; 95% CI: 87.8%, 99.1%). The seroprevalence of *M. suis* was higher in replacement gilts (40.6%; 95% CI: 35.1%, 46.3%), multiparous sows (48.2%; 95% CI: 45.8%, 50.7%) and boars (44.4%; 95% CI: 34.5%, 54.8%), as compared to piglets (13.0%; 95% CI: 9.4%, 17.3%), weaned-piglets (10.8%; 95% CI: 8.9%, 13.0%), and growing-finishing pigs (25.0%; 95% CI: 22.0%, 28.3%). In terms of seasons, the prevalence of *M. suis* in pigs was significantly higher in summer (65.3%; 95% CI: 61.0%, 69.5%) and autumn (65.0%; 95% CI: 59.0%, 70.6%) compared to spring (30.1%; 95% CI: 26.0%, 34.4%) and winter (36.4%; 95% CI: 31.4%, 41.5%). Farm-level risk factors were identified by multivariable logistic regression analysis. The associated factors retained in the final multivariable logistic regression model were drug treatment, presence of mosquitoes and flies, and frequency of disinfection. Drug treatment (OR = 0.24; 95% CI: 0.07, 0.88; P = 0.031) and frequency of disinfection (OR = 0.23; 95% CI: 0.06, 0.90; P = 0.035) were protective factors, and the presence of mosquitoes and flies (OR = 5.994; 95% CI: 1.56, 23.00; P = 0.009) was a risk factor for *M. suis* infection on farms. The results of the present study provide the first insight into the impact of associated determinants on *M. suis* infection in central China.

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1. Introduction

Mycoplasma suis, previously classified as the rickettsial agent *Eperythrozoon suis*, is the causative agent of eperythrozoonosis in domestic pigs (Neimark and Kocan, 1997; Neimark et al., 2001). This agent is a small erythrocytic parasite that adheres to or invades red blood cells (RBCs) (Groebel et al., 2009; Hoelzle, 2008). The attachment of *M. suis* to erythrocytes alters cell permeability and osmotic fragility, causing cell deformation and even the eryptosis (Heinritzi and Plank, 1992; Felder et al., 2011). It can cause acute hemolytic anemia or chronic infections characterized by anemia, decreased weight gain in feeder pigs and decreased reproductive efficiency in sows (Heinritzi, 1989; Büttner et al., 1995; Messick, 2004; Yuan et al., 2007).

M. suis can be transmitted experimentally by subcutaneous, intravenous, intraperitoneal and oral inoculation (Hoelzle, 2008). In addition, natural infections can be most commonly traced back to simple mechanical transmission by contaminated needles and surgical instruments (Henry, 1979). Transplacental transmissions of *M. suis*, bovine hemoplasmas and *Eperythrozoon* spp. in humans are also evident (Henderson et al., 1997; Yang et al., 2000; Hornok et al., 2011). Arthropods, such as mosquitoes, flies, lice and ticks, have been reported as transmission vectors for *Eperythrozoon* species (Prullage et al., 1993; Hofmann-Lehmann et al., 2004; Hornok et al., 2011; Song et al., 2013). Furthermore, recent studies have reported that humans in close contact with *M. suis*-infected pigs can also be infected with these organisms (Yuan et al., 2009), suggesting the possibility of *M. suis* as a potential zoonotic pathogen.

Several diagnostic techniques for *M. suis* have been established, including molecular and serological diagnostic methods with different specificity and sensitivity (Hoelzle et al., 2007a,b; Guimaraes et al., 2011; Liu et al., 2012; Zhang et al., 2012). Among these, ELISA using recombinant MSG1 protein is the most widely used method. MSG1 protein is located on the surface of *M. suis* and is involved in the adhesion of the organism to erythrocytes (Hoelzle et al., 2007c). Experimental evidence suggests that rMSG1 can induce a strong humoral and cellular immunity against *M. suis*. Because of the high sensitivity and specificity of rMSG1, this protein is being used in the routine diagnosis and large-scale epidemiological investigations of *M. suis* (Hoelzle et al., 2007a,b,c; Hoelzle et al., 2009).

M. suis has been reported worldwide and causes serious economic losses to the swine industry (Dipeolu et al., 1982; Hoelzle, 2007; Ritzmann et al., 2009; Zhou et al., 2009). *M. suis* infections in weaned pigs, slaughter pigs, and wild boars have been reported (Gresham et al., 1994; Henderson et al., 1997; Hoelzle et al., 2010). Several studies have been conducted to establish the prevalence of *M. suis* infection in pigs in different countries. The reported prevalences range from 30% (Wu et al., 2006) to 86% (Yuan et al., 2009) in China, 18.2% in Brazil (Guimaraes et al., 2007), and 80% in Switzerland (Guimaraes et al., 2011). Despite the apparent importance of *M. suis* to the pig industry worldwide, few published studies have performed a systematic analysis to determine the risk factors associated with porcine eperythrozoonosis. The identification of risk factors is essential for the development of cost-effective and efficient control programs. China is the largest pork producer of the world, being responsible for 50% of the world's pork production. Hubei Province, which is located in central China, is the fifth-largest pork producer in China (Zhang et al., 2007; Tao et al., 2011). However, the epidemiology of *M. suis* in pig farms in central China has yet to be ascertained.

Therefore, an established rMSG1–ELISA assay was applied to determine the seroprevalence of *M. suis* in pig farms in Hubei, China. The various risk factors influencing the prevalence of *M. suis* were also identified.

2. Materials and methods

2.1. Study areas

Hubei Province is located in central China. The province has a land area of 1.859 million km^2 and a human population of 60 million. The study area extends from latitude 29°05′ N to 33°20′ N and from longitude 108°21′ E to 116°07′ E. A subtropical monsoon climate provides both abundant sunshine and rainfall (800–1600 mm). The average temperature ranges between 15 °C and 17 °C, with a high temperature of 40 °C. According to the classification criterion of seasons in the Hubei area, the whole year was divided into four seasons: (a) spring (March 22 to May 27), (b) summer (May 27 to September 29), (c) autumn (September 29 to November 27), and (d) winter (November 27 to March 22). The topography of Hubei Province is varied and complicated, with towering mountains, basins of different sizes, undulating plateaus and hills, and flat and fertile plains. Therefore, the sampled pig farms in this study were located in different topographical areas. The study site selection was based on the distribution of pig farms in Hubei Province (Fig. 1). The western part of Hubei Province is Shiyan and Enshi. The topography of these two cities is defined by rugged terrain. Due to the poor road networks and limited resources, pig farms are scarce in this region. Therefore, the selected sampling sites did not cover the western part of Hubei Province.

2.2. Study design and sampling

Assuming a default 50% seroprevalence value, a 95% confidence level and a 10% absolute error, at least 97 animals from each age group were calculated as being necessary for inclusion in this study (Thrusfield, 2005). In accordance with the survey progress, we performed multistage sampling trimonthly during November 2011 to August 2013 in eight cities. For each sampling, we selected one or two farms randomly in each city according to its breeding density. The farms that were selected included pigs with all age stages. In addition, the farms that had already been selected before were not included in the next sampling stage. Then, 4% of pigs were selected on each farm. Within each farm, the ratio for sampled piglets (≤21 days), weaned-piglets (22–70 days), growing-finishing pigs (≥71 days), replacement gilts, multiparous sows, and boars was 2:6:5:2:10:1 according to the age structure of the herds. According to the sampling procedure, a total of 4004 blood samples from 69 pig farms located in eight cities of Hubei Province were collected.