Findings of Antibodies to *Mycoplasma suis* on Swine Farms in Serbia

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ABSTRACT

**Background:** Hemoplasmas are eperythrocytic procaryotes, including *Mycoplasma* species which were recently transferred from the genera *Eperythrozoon* and *Haemobartonella*, based on their similarity of the 16S rRNA sequences, and newly identified hemotropic mycoplasmas. Eperythrozoonosis of pigs is caused by the haemotropic bacterium *M. suis*, and the disease has a worldwide distribution. The disease manifests as a severe and often fatal acute febrile icteroanemia, mainly in piglets, pregnant sows before parturition and fattening pigs exposed to stress. The aim of this study was to determine the seroprevalence of IgG to *M. suis* in swine populations in Serbia, using a Western Blot test with the recombinant protein MSG1.

**Materials, Methods & Results:** Four farms were chosen to represent the main swine-producing geographic regions of Serbia, including South Bačka District (farms 1 and 2), North Bačka District (farm 3) and Braničevo District (farm 4). A total of forty-six clinically healthy pigs, age 8-20 weeks, were included in the study. Blood samples from pigs randomly selected from the four farms were collected by jugular vein puncture into serum vacutainer tubes with clot activator. After clotting at room temperature for 1 h, blood samples were centrifuged at 1500 g, for 15 min at room temperature. Sera were carefully harvested and stored at -20°C until assayed. The sera samples were tested by the Western Blot test with recombinant protein MSG1 (p40). Production of recombinant protein MSG1 (p40), dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting were performed as previously described by Hoelzl. Specific IgG antibodies to MSG1 of *M. suis* were identified in 20 of the 46 samples tested, giving a total seroprevalence of 43.47%. The *M. suis*-specific antibody response was detected in pigs from all tested farms, within farm seroprevalences of 54.54% on farm 1; 27.27% on farm 2; 36.36% on farm 3 and 53.84% on farm 4.

**Discussion:** Reports on the prevalence of swine infected with *Mycoplasma suis* in other countries are rare, but have been communicated for USA, Brazil, Japan, Portugal, China and Germany. Previously published values of the prevalence of the pigs infected with haemoplasmas in Serbia, determined through a microscopic examination of the peripheral blood smear according to Giemsa was 39% and with Acridine orange was 47%. These prevalences are similar to the overall prevalence found in this study (43.47%). In this present study, the prevalence of *M. suis* in the farm pigs studied in Serbia (43.47%) was higher than the serological evidence of the infection reported in pigs in the USA, Brazil and Japan, but lower than the prevalence in sows in Portugal and China. In this present study, the prevalence of *M. suis* on the studied pig farms in Serbia (43.47%) was more similar to results obtained in Germany, where *M. suis* infections were detected in 79 out of 196 pig farms (40.3%) by employing a quantitative real-time LightCycler PCR. Also, *M. suis* was detected in 36 out of 359 wild German boars (10.03%) with similar methodology. Our seroprevalence of anti-*M. suis* IgG in farmed pigs is probably higher than that in wild boar because of the conditions of intensive breeding on pig farms, but perhaps this may also be partly due to the different geographic locality, and/or to different analytical methods. This overall determined seroprevalence of 43.47% from a small sample within a relatively small area suggests a significantly higher presence of infection on pig farms in Serbia, and hence, significantly more economic losses in pig production than could be expected based on the worldwide reports of *M. suis* prevalence.

**Keywords:** *Mycoplasma suis*, *Eperythrozoon suis*, swine, seroprevalence, Serbia.
INTRODUCTION

In recent years, the economic impact of erythrozoonosis on commercial pigs has been increasing [2,9]. Due to the latent nature and the non-specific clinical symptoms of the disease, it seems likely that the prevalence of *Mycoplasma suis* in swine populations may be considerably greater than the number of clinical cases might lead one to suppose [8]. Swine erythrozoonosis is also a zoonotic disease and it is important in public health [12,16].

Diagnosis of *M. suis* infection among acutely diseased pigs in most diagnostic laboratories relies on microscopic examination of blood smears stained by Giemsa or Acridine-orange, or a PCR test for detection *M. suis* genome. Also, for the serodiagnosis of *M. suis* infection, three methods are currently used: Complement fixation assay, Enzyme-linked immunosorbent assay test and Indirect hemagglutination [4,5,18]. In addition, the recombinant expression of two proteins of *M. suis*, MSG1 (p40) and HspA1 (p70), was recently reported in *Escherichia coli*, which provides new possibilities in the serodiagnostic of swine erythrozoonosis [5].

The first microscopic detection of *M. suis* in peripheral blood smears in the swine populations on farm pigs in Serbia was conducted by Lako [7]. The same year, identification of *M. suis* using PCR was confirmed by Potkonjak [11]. To date, however, serological examination of pigs in Serbia for their exposure to erythrozoonosis has not been conducted. Therefore, the aim of this study was to determine the seroprevalence of IgG to MSG1 of *Mycoplasma suis* in farm pigs in Serbia, using a Western Blot test.

MATERIALS AND METHODS

Experimental design

Four farms were chosen to represent the main swine-producing geographic regions of Serbia, including South Bačka District (farms 1 and 2), North Bačka District (farm 3) and Braničevo District (farm 4). A total of forty-six clinically healthy pigs, age 8-20 weeks, were included in the study.

Preparation of samples

Blood samples from pigs randomly selected from the four farms were collected by jugular vein puncture into serum vacutainer tubes with clot activator. After clotting at room temperature for 1 h, blood samples were centrifuged at 1500 g, for 15 min at room temperature. Sera were carefully harvested and stored at -20°C until assayed.

Western Blot assay

The sera samples were tested by the Western Blot test with recombinant protein MSG1 (p40). Production of recombinant protein MSG1 (p40), dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting were performed as previously described [4].

RESULTS

Specific IgG antibodies to MSG1 of *Mycoplasma suis* were identified in 20 of the 46 samples tested, giving a total seroprevalence of 43.47% (Figure 1). The *M. suis*-specific antibody response was detected in pigs from all tested farms, within farm seroprevalences of 54.54% on farm 1; 27.27% on farm 2; 36.36% on farm 3 and 53.84% on farm 4 (Figure 2).

DISCUSSION

Reports on the prevalence of swine infected with *Mycoplasma suis* in other countries are rare, but have been communicated for USA, Brazil, Japan, Portugal, China and Germany. Sisk [14] reported that 24% of sows had suspect titres to *M. suis* by the indirect haemagglutination in South Georgia counties (USA). Guimaraes [1] reported 22 of the sows (18.2%) they tested were positive by PCR, while 40 (33.1%) were positive by Southern blot; only one piglet and one boar were positive in Brazil. Real-time PCR was used to examine 120 swine blood specimens from clinically healthy pigs in eleven farms in Japan, and only six (5.0%) were positive for *M. suis*, 18 (15.0%) were positive for *M. parvum*, and three (2.5%) were mixed infection by both hemoplasma species [15].

Perestrelo-Vieira [10] showed, for the first time in Portugal, the existence of *M. suis* sub-clinical infections mainly in sows and boars in intensive breeding herds. Among 200 pigs examined by ELISA (44 sows, 30 boars and 126 piglets), 59% of sows, 50% of boars and 0.8% of piglets were *M. suis* positive [10]. Infections in animals in China have been recognized since 1995, and the number of cases has been increasing rapidly. For example, >600,000 pigs infected with *M. suis* were reported in 2003. The prevalence of the disease in pigs and humans has reached an alarming level in China [6]. Yuan et al. shows that 86% (148/172) of swine and 49% (32/65) of humans had positive PCR assay results.
for *M. suis* infection in Shanghai [17]. Wu [16] found the highest prevalence on pig farms in China was more than 90% and also that farms with higher infection rates occurred in pig-raising areas during epidemic seasons.

Previously published values of the prevalence of the pigs infected with haemoplasmas in Serbia, determined through a microscopic examination of the peripheral blood smear according to Giemsa was 39% and with Acridine orange was 47% [7]. These prevalences are similar to the overall prevalence found in this study. However, microscopic examination of the peripheral blood smear can produce false-positive results because of confusion of *M. suis* with Howell-Jolly or Heinz bodies, background debris and staining artifacts. Also, false-negative microscopic detection may result from low bacterial loads or inapparent infections with bacteria being absent from blood [5]. Therefore, it difficult to directly compare overall *M. suis* prevalences in different studies when these data have been obtained by different methods.

In this present study, the prevalence of *M. suis* in the farm pigs studied in Serbia (43.47%) was higher than the serological evidence of the infection reported in pigs in the USA [14], Brazil [1] and Japan [15], but lower than the prevalence in sows in Portugal [10] and China [6,16,17]. In this present study, the prevalence of *M. suis* on the studied pig farms in Serbia (43.47%) was more similar to results published by Ritzmann [13] in Germany, where *M. suis* infections were detected in 79 out of 196 pig farms (40.3%) by employing a quantitative real-time LightCycler PCR. Hoelzle [3] detected *M. suis* in 36 out of 359 wild German boars (10.03%) using a quantitative real-time LightCycler PCR, which was the first detection of *M. suis* in wild boars. Our seroprevalence of anti-*M. suis* IgG in farmed pigs is probably higher than that in wild boar because of the conditions of intensive breeding on pig farms, but perhaps this may also be partly due to the different geographic locality, and/or to different analytical methods.

Figure 1. Results of Western blot test for IgG antibodies to rMSG1 of *Mycoplasma suis*.
CONCLUSION

In summary, a Western blot test with recombinant protein MSG1 was used for the first time for the seroepidemiological study of swine infections with M. suis in Serbia. The overall determined seroprevalence of 43.47% from a small sample within a relatively small area suggests a significantly higher presence of infection on the pig farms and hence significantly more economic losses in pig production than could be expected based on the worldwide reports of Mycoplasma suis prevalence. Further studies are necessary to confirm these findings.

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Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES


