Serological evidence of *Brucella* species and *Leptospira interrogans* serovars in Greek swine herds

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Summary

In a serological survey in intensively managed swine herds in northern Greece (280 samples) and southern Greece (120 samples), 28.2% of samples were positive for *Leptospira interrogans* serovars and 3% for *Brucella* species. These pathogens of public health significance should be systematically investigated in Greece, a swine brucellosis-free country.

Keywords: swine, *Brucella*, *Leptospira interrogans*, serology, reproductive failure

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Intensive pig farming is common worldwide, allowing management of a large number of animals by a relatively small number of caretakers, and producing affordable meat of high nutritional value. The viability of this system depends greatly on the effectiveness of its management. Keeping large numbers of animals in small areas intensifies pig-to-pig and pig-to-environment interactions. Inferior management, facilities, or both may result in economic losses[1,2] or increased public health risks.[3–5] Financial losses result from animal mortality, cost of disease control, and cost of replacing sows with poor reproductive performance.[2,6] An estimated 88% of the total loss results from culling the breeding herd and production downtime.[2] Determining the seroprevalence of infectious agents of economic and public health significance may help determine management measures needed for minimizing health problems. This is especially true for agents associated with subclinical infections, which may be difficult to identify or isolate.[7] Thus, serologic testing is an economical and effective means of deciding on positive management changes in problematic herds, regardless of the specific agent. In some instances, serologic results may delineate risks to public health, for example, among those working with pigs or processing pork.[4,5] Two zoonotic agents that should be considered in herds where poor reproductive performance is a problem are *Brucella* species and *Leptospira interrogans* serovars.

The most frequently reported *Brucella* species infection in pigs is *Brucella suis*, a smooth *Brucella* species.[8] The rate of infection varies among pig herds, from farm to farm or by country,[9–11] by origin of tested pigs (wild or domesticated),[9,12] and by testing method used.[8,13] Agreement of serological tests with isolation of the organism varies among test methods.[12,14,15] The source of antigen used, for example a strain from bovine, porcine, or other species, must be known when some testing methods are used, for example, the rapid agglutination test.[13] The methods of preparing the antigen and the monoclonal or polyclonal conjugate used in serologic investigations of brucellosis are also important in the interpretation of results when some newer tests are used, for example, complement fixation and competitive ELISA.[7,14] All these factors should be taken into consideration when brucellosis is investigated and control measures are considered.

Swine brucellosis, caused by *Brucella suis,* has economic implications owing to the extensive investigations needed to enact control measures.[9] *Brucella* species may affect slaughterhouse workers, butchers, and consumers. Swine have been implicated in human cases of brucellosis in the United States and Australia.[4,5,16] The public health importance of brucellosis in food-producing animals, including pigs, has resulted in increased official vigilance, which has subsequently decreased the risk of infection and economic losses due to *Brucella* species, and simultaneously revealed a shift in the primary animal species infected, both in the European Union[9] and elsewhere.[4,16]

When poor reproductive performance in swine has been investigated in some countries, including Greece, less attention has been given to *Leptospira* serovars than to brucellosis. Infection with *Leptospira* organisms is maintained in the kidneys of carrier animals, including rodents. Organisms shed in urine contaminate the environment and infect other species, including man.[17–19] Inevitably, conditions of housing determine the numbers of mice and rats present and thus the number of pigs infected. Older pigs are more likely to be seropositive because of the persistence of

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antibodies and recurrent infection with various *Leptospira* serovars.[19–22] Because of the complex epidemiology of leptospirosis and the problems with interpretation of serologic results,[17,19] continuous, extensive investigations of leptospirosis in all farm animals are necessary to estimate losses accurately and enact appropriate prevention measures.

The objective of this study was to investigate the serological status of a sample of pig herds in Greece in regard to *Brucella* species and *Leptospira* serovars, which have not been investigated extensively in Greece. A competitive ELISA (cELISA) was selected to test for brucellosis, using as a conjugate a monoclonal antibody raised against *B melitensis* lipopolysaccharide (LPS).[23] The cELISA is not restricted to testing of a particular animal species[24] and is free of nonspecific reactions.[13] With a reported specificity of 99.5% and sensitivity of 78.5% for pig sera,[13] the cELISA is a reliable method for screening swine for *Brucella* antibodies in countries where control or eradication of brucellosis is a priority.[9] The microscopic agglutination test (MAT) was used to test for antibodies to *Leptospira* serovars. The MAT is more reliable when paired serum samples (acute and chronic) are tested,[19] but it is the most reliable method for investigating the prevalence of serovars. The MAT has a specificity of more than 97%, and somewhat low sensitivity (76% with convalescent serum).[25]

Materials and methods

Serum samples

Blood samples were collected from unvaccinated adult pigs in seven farrow-to-finish herds (40- to 70-sow herds) located in southern Greece, and in ten larger farrow-to-finish herds (100- to 200-sow herds) located more than 500 miles away, in northeastern Greece. In each herd, 25 to 40% of adults were sampled. Animals tested were selected by the owners on the basis of poor performance, defined as poor appetite, small litters, weak piglets, mummified fetuses, irregular estrus intervals, and low weaning weights. During the initial sampling, 120 samples were collected from the smaller herds and 280 from the larger herds. Serum samples were stored at -25°C for 1 to 6 months before testing.

Testing for *Brucella* species

The cELISA kit COMPELISA 400 for brucellosis diagnosis (Veterinary Laboratories Agency (VLA), New Haw, Addlestone, Surry, UK), detecting antibodies to smooth *Brucella* species, was used to test pigs for *Brucella* antibodies. The kit's manual[26] confirms its use in detection of *Brucella* antibodies in pig sera. The cELISA kit uses microplates pre-coated with *B melitensis* LPS, and the conjugate is a monoclonal anti-brucella antibody raised against *B melitensis* LPS, which competes with brucella-specific antibodies in the test serum. Tests were read at a wave length of 450 nm using a Microwell System ELISA reader (Organon Teknika, Netherlands). A sample with optical density equal to or less than 60% of the mean optical density of the conjugate control wells was considered positive.

Testing for *L interrogans* serovars

The MAT, internationally recognized as a reference test for *Leptospira* serovars,[19] was used to detect antibodies against *L interrogans* serovars. Each sample was first screened against six pools of 19 serovars used for serologic investigations of leptospirosis at the VLA (Table 1). Each pool was a mixture of equal quantities of 3- to 8-day live cultures of *Leptospira* serovars growing in liquid Ellinghausen and McCullough media (VLA, UK). Leptospira microorganisms were mixed with serum to a 1:25 dilution. Results were defined at the 1:25 dilution as follows: negative test, <30% agglutination; trace, 30 to 50% agglutination; and positive, >50% agglutination.[19] All trace and positive samples were further tested against each serovar in the pool at serial serum dilutions of 1:25 to 1:800 for each serovar tested.

Results

Seroprevalence to *Brucella* species

Weak positive reactions to the cELISA for smooth *Brucella* species were identified in samples from three herds in southern Greece (one, two, and six positive samples per herd) and in one herd in northern Greece (three positive samples), for a total of 12 positive reactions among the 400 samples tested (3%).

Seroprevalence to *L interrogans* serovars

Screening with pooled serovars identified trace or positive agglutination with one or more of the pools in 139 of the 400 samples (34.7%). Of these 139 samples, 62 (44.6%) showed trace agglutination and 77 (63.4%) were positive. No samples showed trace or positive agglutination to pools 4 or 6.

All samples that showed trace agglutination to an individual serovar at a dilution of 1:25 were negative to this serovar at a dilution of 1:50. At a dilution of 1:50, 113 samples (28.2%) showed trace or positive agglutination with one or more individual serovars. The most seroprevalent (trace or positive) serovars at the 1:50 dilution were Bratislava, 77 samples (68.2%); Australis, 26 samples (23.1%); Copenhageni, 23 samples (20.2%); and Autumnalis, 20 samples (18%). Thirty-nine percent of trace or positive samples were seropositive to more than one of the pool serovars. The prevalence of serovars at the 1:100 dilution was similar. However, trace and positive serovars at the 1:200 dilution were Bratislava, 19 samples; Australis, three samples; and Copenhageni, one sample. Only two small farms (southern Greece) had evidence of antibodies in serum diluted to 1:400: seven samples showed either trace (one sample) or positive agglutination. Six samples reacted with serovar Bratislava and one with serovar Copenhageni.

Table 1: *Leptospira interrogans* serovars represented in six pools used as antigens for a microscopic agglutination test in a study assessing the prevalence of *Leptospira* antibodies in 17 swine herds in Greece

Pool number					
1	2	3	4	5	6
Canicola Icterohaemorrhagiae Ballum Copenhageni	Pomona Grippotyphosa Tarassovi Mozdok	Australis Bratislava Autumnalis	Sejroe Hebdomadis Mini	Bataviae Zanoni Javanica	Hardjo (prajitno) Hardjo (bovis)

All farms had animals that were seropositive (positive or trace) to one or more serovars at a dilution of 1:50. The number of seroreactive animals at the 1:50 dilution ranged from five to 17 per herd. In one herd of 46 sows, 15 of 18 samples were seroreactive. Four of the six samples that were positive to serovar Bratislava at a dilution of 1:400 were from this herd.

None of the *Brucella*-positive pigs were seropositive to any of the *Leptospira* serovars.

Discussion

Zoonotic diseases are a public health problem worldwide. The prevalence of these diseases is related to the management practices of individual herds and the economic ability of a country to finance prevention or control programs.[9] The importance of herd management is apparent in the reported prevalence of pig brucellosis in the various pig herds within a country or a union.[9,11] In the case of swine brucellosis, even a small number of positive herds is important because of the public health implications of the infection. People at greatest risk of infection are those working with pigs or processing meat.[4,5]

In some countries where control programs for brucellosis in ruminants are successful, *Brucella suis*, the cause of pig brucellosis, has emerged as a source of human infection.[4,16] *Brucella suis* is reportedly absent in pigs in Greece.[9] This is the first report showing serologic evidence of Greek pig herds seropositive for brucellosis. The cELISA used in this investigation is reported as a method of high specificity (99.5%)[13] in detecting antibodies to smooth *Brucella* strains present in pig herds. Detection of even a small number of *Brucella*-seropositive pigs in this study suggests a need for an extensive microbiological investigation of the role of pigs in brucellosis.

Serologic cross reactions with other microorganisms, for example, *Yersinia enterocolitica* strain O:9, may cause false-positive reactions in some serological tests used to identify antibodies to smooth *Brucella* strains.[8,15] However, false-positive reactions are unlikely to occur with the cELISA, which uses as the competitive antibody a monoclonal antibody raised against *B melitensis* LPS, and which has reported specificity close to 100% (99.5%).[13]

In order to confirm the agent responsible for the immune response in the seropositive herds, attempts must be made to isolate the organism. If seroconversion was indeed caused by infection with *Brucella* species, the location of the positive farms is of great interest. These two groups of herds were physically separated by the bulk of the mainland, and were more than 500 miles apart. However, the location of all of these farms coincides with an area of high prevalence of *B melitensis* infection in small ruminants,[27] suggesting that *B melitensis* might have caused seroconversion in these herds. *Brucella melitensis* is of public health importance, but has not been reported as a cause of reproductive failure in pigs.[9] The very small number of reactors in both locations and the low antibody titers further support the possibility of infection with *B melitensis*. If these reactions were not false-positive, then the role of swine in the maintenance of brucellosis in Greece must be seriously considered.

Leptospirosis is another important zoonotic infection of pigs. Nevertheless, the presence in all herds of reactors to *Leptospira* serovars indicates that this microorganism is widely spread among Greek pigs. The prevalence of reactors is higher than that reported from other countries, where prevalence ranges from 10.3 to 22.2% when the same testing method is used.[20,22,28] This indicates a need for further investigation of leptospirosis as a cause of reproductive failure among Greek pigs. Among the serovars reported in other countries, serovar Pomona is the most important in pigs.[17,28–31] It has been suggested that swine are important maintenance hosts for serovars Pomona, Tarassovi, and Bratislava.[17] Studies in Northern Ireland and Australia report higher seroprevalence of serovars Bratislava, Australis, and Autumnalis among pigs showing signs of reproductive failure.[32–36] Anecdotal evidence from occasional serologic investigations in Greece suggested the possible role of serovar Bratislava in cases of poor reproductive performance in Greek pig herds, which has been confirmed with this survey. However, this is the first time serovars Copenhageni and Autumnalis have been reported in pigs in Greece, perhaps due to lack of investigation. Serovar Copenhageni, the third most seroprevalent serovar in this study, is rarely reported in intensively managed pigs worldwide. One such report associated this serovar with jaundice in piglets and adults.[37] Others report serovars Hardjo and Icterohaemorrhagiae as causes of reproductive failure in pigs.[20–22,29,33] The variety of *Leptospira* serovars reported as the most prevalent or pathogenic among pigs across the world is influenced by the complex epidemiology of leptospirosis, management practices in the farming of pigs, and serovars selected for investigation. Conditions on the farm, distance between farms, and animal movement between farms are apparently very important factors in the spread of different serovars.[38] Thus, the epidemiological importance of *Leptospira* for pig herds needs continuous and extensive investigation if effective control programs are to be implemented. In Greece, where little is known about the true prevalence of *Leptospira interrogans* serovars in pigs and their role in cases of reproductive failure, an extensive epidemiological investigation is needed to confidently determine the importance of leptospirosis in poor pig herd performance.

Implications

- Identification of a small number of pigs seropositive for *Brucella* species in two areas of Greece suggests a need for further investigation and microbiological identification of the organism among pigs with poor reproductive performance.
- As Greece is believed to be free of *B suis,* it is possible that the serological test used in these pigs identified a different *Brucella* species, for example, *B melitensis*, which exists in Greece among other animal species.
- The role of *Brucella* species other than *B suis* in cases of pig reproductive failure, or in the role of the pig as a maintenance host, should be systematically investigated in Greece.
- *Leptospira interrogans* serovars Copenhageni, Australis, and Autumnalis accounted for almost 50% of the serovars possibly involved in poor pig reproductive performance in the Greek pig herds investigated.
- Leptospirosis vaccines recommended for improving reproductive performance in Greek pig herds do not include serovars Copenhageni, Australis, and Autumnalis, and would be ineffective in herds where these serovars are prevalent.

- This report of a large number of *Leptospira interrogans* serovars in Greek pig herds suggests that leptospirosis should be seriously considered as a cause of reproductive failure and be systematically investigated by microbiological isolation and serological testing.

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