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Survey of the anti-Brucella antibody status determined by ELISA testing in wild boars in Poland

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Summary

Sera of 4407 wild boars (Sus scrofa) shot by hunters in Poland in 2012 were tested by ELISA for anti-Brucella antibodies. Samples originated from 11 out of 16 voivodeships, and 1077 (24.44%) seroreagents were detected. The highest prevalence of wild boars with anti-Brucella antibodies was found in Opolskie (39.9%) and Wielkopolskie (37.29%) voivodeships. The lowest percentage of positive results was observed in Kujawsko-Pomorskie (13.74%) and Łódzkie (15.47%) voivodeships. The results for particular districts revealed significant differences in the prevalence of anti-Brucella antibodies, which varied between 0 and 100%. The results of the surveys show that wild boars constitute an enormous reservoir of Brucella suis biovar 2 microorganisms in Poland. The next stage of the study will see examinations of wild boars continued by bacteriological and molecular methods.

Keywords: wild boars, brucellosis, Brucella suis biovar 2, ELISA

Porcine brucellosis is an infectious disease caused by *Brucella suis* biovar 1, 2, or 3. The main clinical feature of *B. suis* infection is reproductive failure characterized by abortion, stillbirth, and infertility in sows, and by testicular lesions, asymmetry of testicles, and infertility in boars. In Europe, the most common agent of brucellosis in pigs is *B. suis* biovar 2, and wildlife (wild boars and hares) constitutes a source of infection for domestic pigs. Several outbreaks of *B. suis* biovar 2 have been confirmed in porcine outdoor rearing systems, even on fenced premises, and the source of infection has been traced to contacts with wild boars. Transmission from wild boars to pigs is believed to occur through the venereal route, but other routes are also possible (11).

What is characteristic of *B. suis* biovar 2 infection and what distinguishes it from infection caused by *B. suis* biovar 1 or 3, are miliary lesions, particularly in reproductive tissues, that often become purulent. In contrast to *B. suis* biovar 1 and 3, biovar 2 is considered as rarely pathogenic or non-pathogenic for humans (9).

Systematic brucellosis monitoring in wildlife does not exist, and the surveillance of the animal health status is strictly regulated for domestic animals only. However, there are several publications showing the occurrence of brucellosis in wild boars in Europe. In Croatia, Cvetnic et al. (3) reported the presence of anti-*Brucella* antibodies identified by ELISA in 13.6% of serum samples from wild boars. Garin-Bastuji et al. (5) reported that in different regions of France positive serological reactions to brucellosis in wild boars ranged from 20% to 35%. In the Czech Republic, the frequency of positive reactions to brucellosis was 15% (7) and in north-eastern Germany 22% (1).

The aim of the study was to establish the prevalence of anti-*Brucella* antibodies in wild boars from Poland by ELISA testing. According to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (9), ELISA is one of the prescribed tests for international trade purposes in pigs. Additionally, and in contrast to other serological methods used in the diagnosis of brucellosis, the test makes it possible to detect anti-*Brucella* antibodies even when the quality of serum is poor, which is very common when the material is obtained from wild animals.

Material and methods

ELISA test. The indirect ELISA kit developed at the National Veterinary Research Institute in Pulawy (12), and used since 1996 to examine swine sera for anti-*Brucella* antibodies, was applied to sera from wild boars. Briefly, the lipopolysaccharide (LPS) obtained from the strain

B. abortus S19 was used as the antigen, anti-swine immunoglobulins, conjugated with horseradish peroxidase, were used as the conjugate, and ABTS with H_2O_2 as the substrate. Control sera consisted of strong positive (S++) and weak positive (S+) sera prepared on the basis of sera obtained from naturally *Brucella*-infected pigs, and a negative serum (N–) prepared from sera from healthy animals. Criteria for the assessment of sera from wild boars were the same as for porcine sera. The results of the test were read when the absorbance values (OD – optical density) of the S+ serum exceeded 0.300. At this level, cut-offs between positive and negative results were settled. Sera with OD values, expressed as a percentage relative to S+, equal or higher than 100% were regarded as positive, and those with OD values lower than 100% as negative.

Sera. A total of 4407 sera of wild boars, hunted in 2012 and originating from 11 out of 16 voivodeships in Poland, were examined. Blood samples were taken from each animal from the thoracic cavity, heart, or pericardium into plastic tubes and allowed to clot. The sera were then separated by centrifugation and stored at –20°C until tested. The ELISA test was then performed as described previously (12). Briefly, the examined and control sera diluted 1 : 100 in PBST buffer (PBS containing 0.05% of Tween 20) were transferred in a quantity of 100 µl to the wells of microplates coated with LPS antigen and incubated for 90 min at room temperature. After incubation, the plates were washed three times with PBST by a fully-automated plate washer (Labsystems, Multiwash, Finland), followed by the addition of antibody conjugate against swine immunoglobulins with horseradish peroxidase (Dako, Denmark), diluted 1 : 5000 (100 µl/well). The plates were incubated for 60 min at room temperature and washed again three times, after which the substrate (ABTS, Sigma, USA) with hydrogen peroxide (100 µl/well) was added. The results were read by a plate reader (Labsystems, Multiscan, Finland) at a wavelength of 405 nm.

Results and discussion

Out of 4407 samples examined, 1077 (24.44%) reacted positively in the ELISA test for brucellosis. Figure 1 presents the distribution of OD values obtained by ELISA for serum samples. Among sera classified as negative, the highest number of samples exhibited OD values in the ranges of 20-30% (N = 2085) and 30-40% (N = 470) in relation to the OD of positive control serum S+. On the other hand, among sera classified as positive, the largest number of samples showed OD values in the ranges of 100-150% (N = 274) and 150--200% (N = 215). The highest OD value of a positive sample exceeded 800%.

Figure 2 presents the distribution of positive results of the ELISA test in particular voivodeships. The highest percentages of positive samples were observed in Opolskie (39.9%), Wielkopolskie (37.29%), and Śląskie (34.48%) voivodeships, whereas the lowest percentages were found in Kujawsko-Pomorskie (13.74%), Łódzkie (15.47%) and Warmińsko--Mazurskie (18.66%) voivodeships.

The distribution of results over districts was also analyzed. As shown in Figure 3, in some districts (N = 18) the percentage of positive results was very high, exceeding 60%. In Raciborski District, all samples originating from that district reacted positively (N = 20). On the other hand, in 35 districts, all wild boars examined were classified as negative.

The previous serological survey concerning the prevalence of brucellosis in wild boars in Poland was performed in 2000 (15). Out of 933 sera tested, 115 (12.3%) reacted positively in ELISA testing. Additionally, 266 meat juice samples were tested, and a similar percentage was obtained (13.7%). The results of the current investigations, carried out on sig-

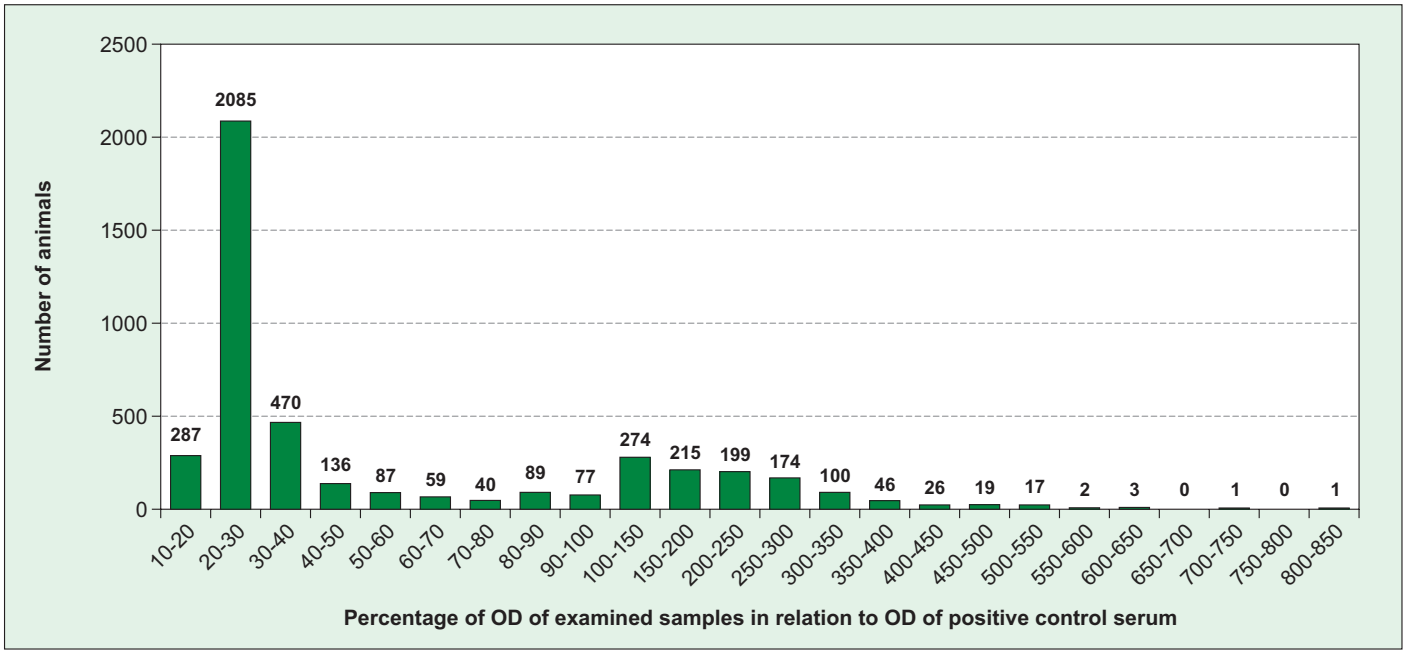

Fig. 1. Distribution of OD values obtained by the ELISA test in serum samples from wild boars

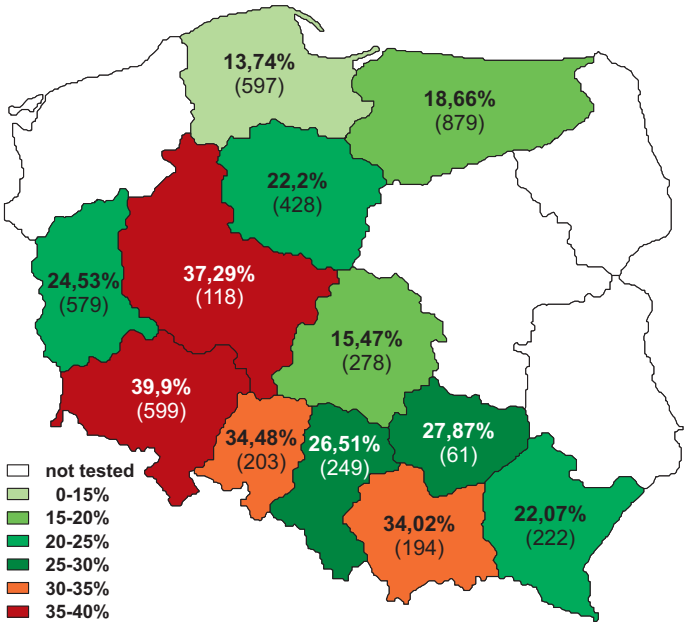

Fig. 2. The results of the examination of wild boars for anti-*Brucella* antibodies for particular voivodeships
Explanations: 4407 sera were examined; 1077 sera reacted positively. In brackets, the number of samples from the given voivodeship is shown.

nificantly more samples (N = 4407), confirm that wild boars in Poland, as in other European countries, constitute a very important reservoir of *Brucella* microorganisms, undoubtedly more so than hares. The prevalence, clearly higher than several years ago, is particularly high in the south-western part of the country, and lower in the north and centre. Bacteriological examinations on material from wild boars (usually lymph nodes) performed in Poland indicate that the causative agent of brucellosis and the presence of anti-*Brucella* antibodies in wild boars is always *B. suis* biovar 2 (14), and this phenomenon is typical of Europe (2, 6, 9, 11). Fortunately, this biovar is considered rarely pathogenic or non-pathogenic for humans, and only exceptionally has been described as the causative agent of human brucellosis (10, 16). However, its importance stems from the fact that *B. suis* biovar 2 can infect domestic pigs or even cattle (13). With regard to pigs, wild boars are potentially a dangerous source of infection, especially in those countries where porcine outdoor rearing systems are practiced (8, 11). The fact that this system is not popular in Poland may explain why, despite such a high level of *Brucella* infections in wild boars, brucellosis outbreaks in domestic pigs are very sporadic – the last one having been recorded in 1999 (data not published). On the other hand, our previous investigations concerning cattle revealed that *B. suis* biovar 2 influences the epidemiology and control of bovine brucellosis in Poland. In an examination of 176 cows slaughtered on account of a positive serological result for brucellosis, 5 cases exhibited *B. suis* biovar 2 organisms (13). The culture-positive animals originated from regions with large forests adjacent to open grassland, which allowed them free contact with wild animals (grazing on unfenced pastures). The results of examinations show that the prevalence of anti-*Brucella* antibodies in wild boars in this region exceeded 20% both in 2000 and in the present study. *B. suis* biovar 2 has also been recently isolated from cattle in Belgium (4).

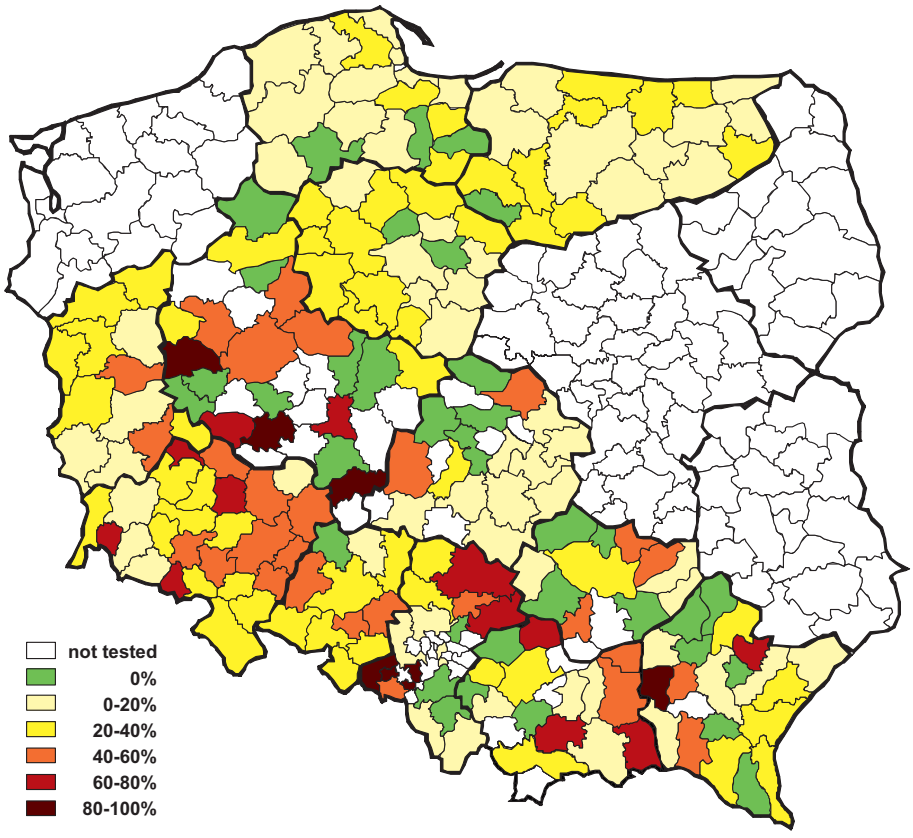

Fig. 3. The results of the examination of wild boars for anti-*Brucella* antibodies for particular districts

Assessing the prevalence of brucellosis in wild boars by serological methods (in this case ELISA), one should be aware that an unequivocal diagnosis of *B. suis* infections can be made exclusively by the isolation and identification of *Brucella*. It is possible that various cross-reacting bacteria, particularly *Y. enterocolitica* O:9, having the immunodominant epitope of O-polysaccharide and beingvery similar to that observed in *Brucella*, are responsible for some positive reactions that have been recorded in pigs (17, 18). The scale of this phenomenon in wild boars is totally unknown. Our next step, presently in progress, is to perform a bacteriological examination and PCR on material from wild boars in order to verify serological results.

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