Opis przypadku *Case report*

International trade – a potential source of brucellosis in pigs

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Summary

Brucellosis was confirmed in boars imported for breeding purposes to Poland from one of the EU countries. In serological investigations, in which the RBT, ELISA, CFT, SAT and 2-ME were used, positive reactions to brucellosis were found in 9 out of 23 animals. All the animals were slaughtered, and bacteriological examination was performed. Brucellae were isolated from the tissues of 7 boars. The characteristics of the strains isolated showed that they belonged to Brucella suis biovar 2, which is typical for Europe. The examination of animals for brucellosis at quarantine stations seems to be crucial for the protection of herds from Brucella infection.

Keywords: brucellosis, diagnosis, pigs

Brucellosis in pigs is an infectious and contagious disease caused by the bacteria *Brucella suis.* It is primarily a disease of pigs, but it can also occur in other domestic and wild animals and man. The species of *Brucella suis* consists of five biovars, but the infection in pigs is caused by *B. suis* biovars 1, 2 and 3. Porcine brucellosis is of widespread occurrence; however, the prevalence is low, with the exception of South America and South-East Asia, where the prevalence is higher. In Europe the disease is caused by *Brucella suis* biovar 2 (except for Croatia, where biovar 1 was observed), and the natural reservoir of this biovar, which is rarely pathogenic for humans (6, 18), are wild boars and hares (2, 3).

Common manifestations of brucellosis in pigs are abortions occurring at any time during gestation, temporary or permanent sterility, the birth of stillborn or weak piglets, orchitis and swelling of one or both testicles of infected boars, abscesses in subcutaneous tissues, kidneys and muscles. Boars and sows can become lame or even paralyzed because of severe arthritis (2, 4).

Porcine brucellosis is transmitted venereally. Infected boars infect sows during mating, and conversely, infected sows shed the bacterium in the discharges from their uterus, and boars can contract the disease during copulation. Sometimes, uninfected herds are contaminated by wild boars mating with domestic sows. The disease can also be transmitted by ingestion, by inhalation, via the conjunctiva or cutaneously.

As brucellosis in pigs does not always cause symptoms, or clinical signs can be very few while abortions can be caused by a number of other conditions, diagnosis must be performed by laboratory testing including serological tests and the culture and identification of *Brucella*. The risk of introducing brucellosis into a swine herd is related to wild animals and purchased infected pigs. Semen for artificial insemination should also be considered as a risk factor.

As regards the intra-Community trade in pigs, under Council Directive 64/432/EEC of 26 June 1964 on health problems affecting intra-community trade in bovine animals and swine, until 1997 pigs had to be certified as originating from brucellosis-free countries. Since porcine brucellosis was thought to have disappeared from UE countries and due to the technical development of pig husbandry, Directive 97/12/EEC of 17 March 1997 removed this requirement.

In Poland, last outbreaks of brucellosis in pigs were recorded in 1994 and 1999. At present, testing primarily involves boars used for insemination, and the main methods employed for diagnosing brucellosis are the rose bengal test (RBT) and ELISA. Additionally, according to the Instruction of Chief Veterinary Officer, serum agglutination test (SAT), 2 mercaptoethanol test (2-ME) and complement fixation test (CFT) can be used to explain doubtful results (10). If positive serological results are ascertained, pigs are slaughtered, and culture methods are used to isolate *Brucella*.

This paper reports a case of *Brucella suis* infection in boars imported to Poland.

Material and methods

Twenty three clinically healthy boars imported from an EU country for breeding purposes and brought to 2 quarantine stations were examined. First, blood samples were examined. The boars from the first station (21 animals) were examined twice with a 3-week interval, whereas the boars from the second station (2 animals) were examined only once. After a serological examination all boars were slaughtered, and material for bacteriological examination was taken. The material consisted of testicles, lymph nodes, liver and spleen.

Serological methods. ELISA, RBT, SAT, CFT, 2-ME were used for the serological examination. The diagnostic kit used in the ELISA had been developed at the National Veterinary Research Institute in Pulawy, and it was described previously (15). 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. Controls in the ELISA consisted of a strong positive swine serum (S++) with a high titer of anti-*Brucella* antibodies, a weak positive serum (S+) with a low titer of anti-*Brucella* antibodies and a negative one (N–). The results of the ELISA were read when the absorbance values (OD-optical density) of the weak positive controls were above 0.250. At this level the cut-offs between positive and negative results were settled. The RBT, SAT, CFT and 2-ME were performed according to official instructions and protocols (7-9, 12).

Bacteriological examination. Farrell's medium was used for the culture of specimens used for isolating *Brucellae*. The plates were incubated for 10 days at 37°C in an atmosphere with 5-10% CO_2 added. Those typical of *Brucella* colonies were stained by Gram's method, then affiliation for the genus *Brucella* was confirmed by agglutination with anti-*Brucella* standard serum and positive results in tests for catalase and oxidase. Further antigenic characteristic was performed by using monospecific anti-A and anti-M sera and conducting further tests: test for CO_2 requirement, production of H_2S and urease, growth on thionin and basic fuchsin, and lysis by phages (Tbilisi at its routine test dilution – RTD and $10^4 \times$ RTD) (1).

Results and discussion

Tab. 1 summarizes the results of the serological examination of sera from boars at both quarantine stations. Sera from 7 out of 21 boars from the first station were ultimately classified as positive. Six samples classified as positive reacted positively in all tests: RBT, SAT, CFT, 2-ME and ELISA. One of the samples was positive in CFT and ELISA but negative in RBT, SAT and 2-ME. The comparison of titers of antibodies in SAT, CFT and 2-ME shows that in most samples the level of antibodies detected in the second examination was markedly higher. As for the sera from boars from the second station, both were positive in all tests.

As seen in tab. 2, positive results of bacteriological examination were obtained in 7 cases. *Brucella* microorganisms were isolated from the tissues of 5 boars from station 1 and both boars from station 2. All isolates had the same characteristics: agglutination with anti-*Brucella* standard serum and monospecific anti-A serum, positive results of oxidase, catalase and urease tests, no CO_2 requirement for growth, no H_2S production, growth on thionin, no growth on basic fuchsin, and lysis by TB phages at a higher concentration ($10^4 \times$ RTD).These characteristics are typical of *B. suis* biovar 2 (1).

Wildlife (wild boars and hares) undoubtedly plays a key role in the epidemiology of *B. suis* (5, 13, 17). The presence of *B. suis* infection in wild boars and hares has been reported in many European countries. The potential risk

Tab. 1. The results of serological examination of boars for anti-*Brucella* antibodies

No. of animal	1st examination					2nd examination					Result
	RBT	SAT (IU/ml*)	CFT (icftu/ml**)	2-ME (titer)	ELISA	RBT	SAT (IU/ml)	CFT (icftu/ml)	2-ME (titer)	ELISA	
1	+	+ (574.5)	+ (320)	1/320	+	+	+ (410.5)	+ (640)	1/320	+	+
2	+	+ (164)	+ (160)	4/80	+	+	+ (256)	+ (744)	2/160	+	+
3	+	+ (492.5)	+ (848)	2/320	+	+	+ (656.5)	+ (1696)	4/320	+	+
4	+	61,5	– (8.4)	2/40	+	+	492.5	+ (320)	1/320	+	+
5	–	–	–	–	–	+	+ (287)	+ (40)	2/40	+	+
6	–	+ (61.5)	+ (106)	–	+	–	– (15.5)	+ (93)	–	+	+
7	+	+ (61.5)	+ (46.5)	1/10	+	+	+ (82)	+ (93)	4/20	+	+
8	–	– (13)	–	–	–	–	– (15.5)	–	–	–	–
9	–	– (18)	–	–	–	–	– (18)	–	–	–	–
10	–	–	–	–	–	–	– (15.5)	–	–	–	–
11	–	–	–	–	–	–	– (15.5)	–	–	–	–
12	–	– (15.5)	–	–	–	–	– (15.5)	–	–	–	–
13-21	–	–	–	–	–	–	–	–	–	–	–
22	+	+ (492.5)	+ (1696)	2/320	+						+
23	+	+ (205)	+ (186)	1/160	+						+

Explanations: * – international complement fixation test units per ml, ** – international units per ml; no 1-21 – boars from quarantine station no 1, no 22-23 – boars from quarantine station no 2

Tab. 2. The results of bacteriological examination of boars for brucellosis

No. of animal	Culture (+/–)	Characteristics of isolates											
		CO_2 requirement	Oxidase	Catalase	Urease	Agglutination in sera			H_2S	Growth on dyes		Lysis by phages Tb	
						Standard serum	A	M		Thionin	Basic fuchsin	RTD*	$10^4 \times$ RTD
1	+	–	+	+	+	+	+	–	–	+	–	–	+
2	+	–	+	+	+	+	+	–	–	+	–	–	+
3	+	–	+	+	+	+	+	–	–	+	–	–	+
6	+	–	+	+	+	+	+	–	–	+	–	–	+
7	+	–	+	+	+	+	+	–	–	+	–	–	+
22	+	–	+	+	+	+	+	–	–	+	–	–	+
23	+	–	+	+	+	+	+	–	–	+	–	–	+
4, 5, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21	–	–	–	–	–	–	–	–	–	–	–	–	–

Explanation: * – routine test dilution

of infection concerns primarily outdoor pig holdings, popular in some regions, which are situated close to infected environment.

The movement of animals from country to country in Europe has not been presented as a likely way to transfer porcine brucellosis in the literature yet. The case presented above was probably caused by latent infection as the boars had been examined serologically before the transfer with negative results and reacted positively after the transfer. It is very important to use a set of different methods to analyze dubious samples. One should always bear in mind the possibility of cross reactions caused in pigs primarily by *Yersinia enterocolitica O:9,* which are sometimes difficult to differentiate with specific *Brucella* reactions (11, 14, 19). No serological method is fully reliable in diagnosing brucellosis in individual pigs (2). Another essential factor is the need to examine animals twice, with a 3-4 week interval. An earlier study (16) showed how it may be possible to distinguish infected pigs from those in which false positive serological reactions (FPSR) are observed. It recommended the inclusion of such parameters as the percentage of positive results in the RBT, ELISA, SAT and CFT, the titers of antibodies in the SAT and the absorbance values of positive samples in the ELISA, the presence of antibodies which are not inactivated by 2-mercaptoethanol, and the permanent character of anti-*Brucella* antibodies (long-lasting serological reply). These suggestions were followed in this case.

Bacteriological examinations showed that the causative agent of the infection was *B. suis* biovar 2, and it is not surprising since, as already mentioned, in Europe brucellosis in pigs is caused by this biovar.

In the present case the imported boars did not arrive at their points of destination, i.e. insemination stations. They were kept at quarantine stations, where they were examined and diagnosed with brucellosis. The examination of animals for brucellosis at quarantine stations seems to be crucial for the protection of herds from *Brucella* infection.

References

1. *Alton G. G., Jones L. M., Angus R. D., Verger J. M.*: Techniques for the Brucellosis Laboratory. INRA, Paris, France 1988.
2. *Anon.*: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, France 2009.
3. *Anon.*: Porcine brucellosis (Brucella suis). Scientific opinion of the Panel on Animal Health and Welfare (Question No EFSA-Q-2008-665). 2009, 1144, 1-112.
4. *Anon.*: The Merck Veterinary Manual. A Handbook of Diagnosis, Therapy, and Disease Prevention and control for the Veterinarian, Merck&Co., Inc., Rahway, USA 1991.
5. *Garin Bastuji B., Hars J., Thiebaud M., Artois M.*: Brucellosis of domestic pigs. Reemergence of Brucella suis biovar 2 in France. Epidémiol. Santé Anim. 2000, 38, 1-5.
6. *Garin Bastuji B., Vaillant., Albert D., Tourrand B., Danjean M. P., Lagier A., Rispal P., Bnequet B., Maurin M., De Valk H., Mailles A.*: Is brucellosis due the biovar 2 of Brucella suis an emerging zoonosis in France? Two case reports in wild boar and hare hunters, [in:] Proc. Internat. Soc. Chemotherapy Disease Management Meeting, 1st International Meeting on Treatment of Human Brucellosis, 7-10 November 2006, Ioannina, Greece.
7. Instruction no 26/2003 of the Chief Veterinary Officer, Warsaw 2003.
8. Instruction no 27/2003 of the Chief Veterinary Officer, Warsaw 2003.
9. Instruction no 28/2003 of the Chief Veterinary Officer, Warsaw 2003.
10. Instruction no GIWz401/Bru-28/2006 of the Chief Veterinary Officer, Warsaw 2006.
11. *Jungersen G., Soerensen V., Giese S. B., Stack J. A., Riber U.*: Differentiation between serological responses to Brucella suis and Yersinia enterocolitica O:9 after natural or experimental infection in pigs. Epidemiol. Infect. 2006, 134, 347-357.
12. *Królak M., Stryszak A.*: Standardowa technika odczynu z 2-merkaptoetanolem (OME) w rozpoznawaniu brucelozy zwierząt. Instytut Weterynarii, Puławy, Polska 1979.
13. *Melzer F., Lohse R., Nieper H., Liebert M., Sachse K.*: A serological study on brucellosis in wild boars in Germany. Eur. J. Wildl. Res. 2006, 53, 153-157.
14. *Singh D. K., Warayan K. G.*: Serological study of swine yersiniosis in a farm. Indian J. Anim. Sci. 1991, 5, 506-508.
15. *Szulowski K., Pilaszek J., Truszczyński M.*: Zestaw ELISA do badania surowic świń w kierunku brucelozy. Medycyna Wet. 1996, 52, 513-515.
16. *Szulowski K., Iwaniak W., Pilaszek J.*: Porcine brucellosis in Poland: problems accompanying serological surveys conducted in 1995-2000. Bull. Vet. Inst. Pulawy 2001, 45, 153-161.
17. *Szulowski K., Iwaniak W., Pilaszek J., Murat J.*: Wild boars and hares as reservoirs of Brucella suis biovar 2 in Poland. Brucellosis 2008 Internat. Res. Conf. (including the 61th Brucellosis Res. Conf.), London 10-13 September 2008, 137 p.
18. *Teyssou R., Morvan J., Leleu J. P., Roumegou P., Goullin B., Carteron B.*: About a case of human brucellosis due to Brucella-Suis Biovar-2. Med. Mal. Infect. 1989, 19, 160-161.
19. *Wrathall A. E., Broughton E. S., Gill K. P. W., Goldsmith G. P.*: Serological reactions to Brucella species in British pigs. Vet. Rec. 1993, 132, 449-451.

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