



Isolation of *Brucella suis* biovar 1 from a pig breeding farm in Brazil. Case Report

Isolamento de Brucella suis biovariedade 1 em granja de reprodutores suínos no Brasil. Relato de Caso

Rafael Massa^{1*}, Glaucenyra Cecília Pinheiro da Silva¹, Renata Ferreira dos Santos ^{*1}, Luis Ernesto Samartino², Luis Antonio Mathias¹

¹ Departamento de Medicina Veterinária Preventiva e Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV), Universidade Estadual Paulista (Unesp).

² Instituto Nacional de Tecnologia Agropecuária (INTA), Buenos Aires, Argentina

Abstract: The objective of this study was to confirm the occurrence of swine brucellosis by isolating *Brucella suis* from an outbreak that occurred in December 2012 in a Certified Pig Breeding Farm (Granja de Reprodutores Suínos Certificada, GRSC), in Guariba, São Paulo, Brazil. To this end, we collected the livers and spleens of seven stillborn piglets born to six different sows that had aborted. Furthermore, the stomach content from one piglet was also collected. The organs were prepared using sterile materials and seeded in Petri dishes containing *Brucella* agar with 5% rabbit serum medium. The routine tests confirmed that the *B. suis* biovar 1 strain was the causative agent. Therefore, the results showed that despite the reduced incidence of swine brucellosis caused by *Brucella suis* in the country, the causative agent of the disease is still present, able to reach herds with higher biosafety levels, threatening human and animal health.

Keywords: Isolation, Brucellosis, Swine.

Resumo: O objetivo deste trabalho foi confirmar a ocorrência de brucelose suína por meio do isolamento de *Brucella suis* de um foco ocorrido em dezembro de 2012 em uma Granja de Reprodutores Suínos Certificada (GRSC), localizada no município de Guariba, São Paulo, Brasil. Para isso, foram colhidos fígados e baços de sete leitões natimortos, filhos de seis matrizes diferentes, que apresentaram aborto. Além disso, foi colhido o conteúdo estomacal de um dos leitões. Os órgãos foram dilacerados, usando materiais estéreis, e semeados em placas de Petri com meio *Brucella* ágar com 5% de soro de coelho. A identificação foi confirmada por provas de rotina, e que levaram à conclusão de que se tratava de uma cepa de *B. suis* da biovariedade 1. Nesse sentido, observa-se que, apesar da redução da incidência de brucelose suína, por *Brucella suis*, no país, o agente etiológico da doença ainda está presente, atingindo até mesmo rebanhos com nível de biossegurança mais elevado e demonstrando ser um risco tanto à saúde humana quanto à saúde animal.

Palavras-chave: Isolamento, Brucelose, Suinocultura.

Autor para correspondência. E.Mail: * renatafdsantos@hotmail.com

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Introduction

Brucellosis is a subacute or chronic disease that can affect various animal species, especially domestic, caused by bacteria of the genus *Brucella* (XAVIER et al., 2010). *Brucella suis* is the only species of the genus *Brucella* that are known to cause systemic or generalized infection, causing reproductive problems in pigs (OLSEN et al., 2012).

In pigs, the main clinical signs include fever, abortion, endometritis, orchitis, loss of libido and infertility. Abortion can occur at any time during pregnancy, especially influenced by the exposure time to the agent of the disease. The pigs get infected by ingesting either contaminated water or food or by eating aborted fetuses and fetal membranes. Therefore, the disease characteristics suggest that the digestive tract is the most common entry gateway to the disease, and *B. suis* is also transmitted by venereal route (OLSEN et al., 2012).

Epidemiological information and the herd history are very important tools to assist in the diagnosis of swine brucellosis. This is because the most sensitive and specific method is isolation of the organism using culture techniques that are conducted using the products of abortions, vaginal swabs, testicular injury, abscesses, blood, and lymph nodes (ALTON, 1990). However, the conditions are not always adequate to perform this procedure.

An important tool to control *Brucella suis* is to form and maintain close herds, free of brucellosis. The creation of programs that allow identifying periodically, by diagnostic testing, positive cases of the disease can reduce the number of infected herds. Normative Instruction No. 19/2002 of the Ministry of Agriculture, Livestock and Supply (Ministério da Agricultura, Pecuária e Abastecimento, MAPA) establishes standards for certification of swine breeding farms. This Normative Instruction requires serologic testing for brucellosis to be performed every six months using the buffered acidified antigen test or other test approved by MAPA. The seropositive should be subjected to confirmatory tests, such as complement fixation or slow agglutination test carried out simultaneously with 2-mercaptoethanol. The breeding farm is considered free of infection if all tested animals are negative (BRASIL, 2002).

Case Report

In December 2012, an outbreak of porcine brucellosis in a Certified Pig Breeding Farm (GRSC) in Guariba, São Paulo, was reported to the official veterinary service.

Reproductive losses, such as miscarriages, stillbirths, mummified fetuses and weak piglets that are symptomatic of the disease had been observed. Samples of liver and spleen of seven stillborn piglets that were

born to six different sows were collected. In addition, the stomach contents of one piglet was collected.

All the material was sent to the laboratory. The organs were macerated using sterile materials and seeded in Petri dishes with *Brucella* agar with 5% rabbit serum medium (OIE, 2011). The cultures were incubated in bacteriological incubator at 37°C. Reading of the plates was conducted 24, 48 and 72 hours after cultivation for visualization of characteristic colonies. The replating was necessary to get colonies that were more isolated.

The isolates were submitted to microscopy with Gram staining, and those that presented morphology characteristic of *Brucella* spp. were subjected to an agglutination test with a known positive bovine serum.

Isolates were sent to the National Institute of Agricultural Technology (INTA), in Buenos Aires, Argentina, to identify and typify *Brucella* using the standard techniques described in the Manual of Diagnostics and Vaccines Test for Terrestrial Animals of OIE (2011). The typification was performed in growth media containing urea, thionine, and fuchsin. The production of hydrogen sulfide (H₂S) and agglutination with reagent blood swine sera were also tested.

The 15 initial samples processed in the laboratory showed intense microbial multiplication within a 48-hour period, of

which nine had colonies similar to smooth *Brucella*. Upon microscope examination, the isolates revealed the presence of Gram-negative coccobacilli. The three isolates with more characteristic morphology were positive to the agglutination test with known positive bovine serum.

The infection of animals by *Brucella* in the breeding farm was confirmed as Gram-negative coccobacilli by typification and hydrogen sulfide production. They multiplied rapidly in media containing urea and thionine but did not multiply in the presence of fuchsin. Furthermore, agglutination was observed in the presence of known positive swine serum while there was no agglutination in the presence of known negative pig serum. These results indicated a strain of *Brucella suis* biovar 1.

Discussion

Although brucellosis in pigs can also result from the infection with *B. abortus*, the *B. suis* biovar 1 is largely responsible for the disease in pigs (ROXO et al., 1996). As in the present study, Meirelles-Bartoli et al. (2012) also isolated *Brucella suis* biovar 1 in a farm in Jaboticabal, São Paulo, stressing that the etiologic agent is present in the region.

Although there are few reports in the literature on the isolation of *Brucella* spp. in pigs, some studies have shown serological responses to this etiologic agent. Braga et al. (2013) found a prevalence of 0.52% in animals of commercial herds in Piauí, Brazil,

whereas Rosa et al. (2012) reported a 3% prevalence in animals that were slaughtered in the same slaughterhouse but originated from 10 different properties in central São Paulo state; however, the titer obtained was considered low. Although these results show a low prevalence in the country, the damage caused during outbreaks in breeding properties can be very large (MEIRELLES-BARTOLLI et al., 2012). These authors observed prevalences of 93.7% and 27.4% in sows and finishing animals, respectively, during an outbreak in Jaboticabal, São Paulo. Furthermore, Leite et al. (2014) studied a pig herd in Mossoró, Rio Grande do Norte, and found a 17.5% prevalence in pigs using the complement fixation reaction test.

Conclusion

The isolation of *B. suis* biovar 1 in animals from a certified farm for breeding pigs shows that the causative agent is still widespread throughout the country and capable of overcoming the biosafety systems, threatening the pig production chain and the health of farm workers.

References

1. ALTON, G.G. *Brucella suis*. In: NIELSEN, K.; DUNCAN J.R. Animal Brucellosis. Boca Raton: CRC Press. Eds. Florida: CRC Press, 1990. p.411-422.
2. BRAGA, J.F.V.; TEIXEIRA, M.P.F.; FRANKLIN, F.L.A.A.; SOUZA, J.A.T.; SILVA, S.M.M.S.; GUEDES, R.M.C. Soroprevalência de pseudorraiva, peste suína clássica e brucelose em suínos do estado do Piauí. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, Belo Horizonte, v. 65, n. 5, p. 1321-1328, 2013.
3. BRASIL, 2002. Ministério da Agricultura. Instrução Normativa nº 19 de 15 de fev. de 2002. Normas para certificação de granjas de reprodutores suídeos. Diário Oficial da União, n. 41, 01 de março de 2002, Seção 1, 3--5
4. LEITE, A.I.; COELHO, W.A.C.; SILVA, G.C.P.; SANTOS, R.F.; MATHIAS, L.A.; DUTRA, I.S. Prevalência e fatores de risco para brucelose suína em Mossoró-RN. **Pesquisa Veterinária Brasileira**, Seropédica, v. 34, n.6, p. 537-541, 2014.
5. MEIRELLES-BARTOLLI, R.B.; MATHIAS, L.A.; SAMARTINO, L.E. Brucellosis due to *Brucella suis* in a swine herd associated with a human case in the State of São Paulo, Brazil. **Tropical Animal Health and Production**, Edinburgh, v. 44, n. 7, p.1575-1579, 2012.
6. OIE, 2011. Manual of diagnostic test and vaccines for terrestrial animals. CHAPTER 2.4.3 e 2.8.5. Disponível em: <<http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/>>. Acesso em: 30/10/2015
7. OLSEN, S.C.; GARIN-BASTUJI, B.; BLASCO, J.M.; NICOLA, A.M.; SAMARTINO, L. Brucellosis. In: ZIMMERMAN, J.J.; KARRIKER, L.; RAMIREZ, A.; SCHWARTZ, K.J.; STEVENSON, G.W. Diseases of swine. 10 ed. Iowa: Wiley-Blackwell, 2012. cap.51, p. 697-708.
8. ROSA, D.C.; GARCIA, K.C.O.D.; MEGID, J. Soropositividade para brucelose em suínos em abatedouros. **Pesquisa Veterinária Brasileira**, Seropédica, v. 32, n.7, p.623-626, 2012.
9. ROXO, E.; BERSANO, J.G.; PORTUGAL, M.A.S.C. *Brucella suis* em diferentes espécies de animais numa mesma propriedade rural. **Arquivos do Instituto Biológico**, São Paulo, v. 63, n.1, p.11-14, 1996.
10. XAVIER, M.N.; PAIXÃO, T.A.; DEN HARTIGH, A.B.; TSOLIS, R.M.; SANTOS, R. L. Pathogenesis of *Brucella* spp. **The Open Veterinary Science Journal**, Hilversum, v. 4, p. 109-118, 2010.

