Detection of serum antibodies to *Brucella* in Russian aquatic mammals

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ABSTRACT. A serologic survey of *Brucella* infection was performed in Caspian seals (*Pusa caspica*, n=71), Baikal seals (*P. sibirica*, n=7), ringed seals (*P. hispida hispida*, n=6), and beluga whales (*Delphinapterus leucas*, n=4) inhabiting Russian waters, by enzyme-linked immunosorbent assay (ELISA) using *Brucella abortus* and *B. canis* as antigens. The sera of 4 Caspian seals (4%) tested positive for *B. abortus*. The same sera samples demonstrated weaker yet detectable affinity for *B. canis* antigens. Several discrete bands against *B. abortus* and *B. canis* antigens were detected on Western blot analysis of the ELISA-positive seal sera; the bands against *B. canis* were weaker than those against *B. abortus*. The sera of 3 beluga whales (75%) were positive for *B. abortus* antigens but showed no binding to *B. canis* antigens in the ELISA. The positive whale sera showed a strong band appearance only against *B. abortus* antigens in the Western blot analysis. Many detected bands were discrete, while some of them had a smeared appearance. The present results indicate that *Brucella* infection occurred in Caspian seals and beluga whales inhabiting Russian waters, and that the *Brucella* strains infecting the seals and the whales were antigenetically distinct.

KEY WORDS: antibody, Baikal seal, beluga whale, *Brucella*, Caspian seal

*Brucella* are gram-negative intracellular pathogenic bacteria infecting many species of mammals and causing reproductive disorders including abortion. In terrestrial mammals, besides 6 classical *Brucella* species (*Brucella melitensis*, *B. abortus*, *B. ovis*, *B. canis*, *B. suis* and *B. neotomae* [6]), 3 new species of *Brucella*, i.e., *B. microti* [39], *B. inopinata* [40] and *B. papionis* [42] have been reported. In marine mammals, 2 novel species, *B. ceti*, which is preferentially associated with cetaceans, and *B. pinnipediais*, which is preferentially associated with pinnipeds, have been identified so far [13]. Relatively little is known about the pathogenicity of *Brucella* infection in marine mammals. Some pathological changes in reproductive organs and nervous system were suggested as those induced by *B. ceti* infection [16, 19, 32, 36]. The abortion-inducing potential of *B. ceti* has been shown in captive and stranded dolphins and porpoises [11, 20, 25]. Apart from a report of placentitis in *Brucella*-infected northern fur seals (*Callorhinus ursinus*) [8], there is little evidence to suggest that *B. pinnipediais* causes brucellosis or abortion.

*Brucella* infection in marine mammals has been reported to occur widely in various species inhabiting the European and North American sea [16, 19]. Although several species of marine mammals populate the coast of Russia and its inner lakes, little is known about their *Brucella* infection. Caspian seal (*Pusa caspica*) and Baikal seal (*P. sibirica*) are endemic species restricted exclusively to the Caspian Sea and the Lake Baikal, respectively [15, 26]. Caspian seal is registered as an endangered species in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List (2017-3 version). Ringed seal (*P. hispida*) is a species of the circumpolar Arctic coasts with a broad geographic distribution and is classified into 5 distinct subspecies [17]. The subspecies *P. hispida hispida* has the largest population widely distributed in the Arctic Ocean. These 3 seal species belong to the same genus *Pusa*, as determined by the similarity in skull morphology and genetics [3, 38]. Beluga whale (*Delphinapterus leucas*), a member of the family Monodontidae, is supremely adapted to life in cold waters and inhabits the Arctic and sub-Arctic [30]. To obtain detailed knowledge of pervasiveness and character of *Brucella* infection in marine mammals inhabiting Russian waters, we conducted a serologic survey of Caspian seals, Baikal seals, ringed seals, and beluga whales found in this area.

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**MATERIALS AND METHODS**

**Samples**

We have conducted the Russian-Japanese Joint Research Program for Biological and Environmental Studies with the permission from the Russian Federation and the local governments. This study was performed in accordance with the guidelines of the Animal Ethic Committee of the University of Tokyo. Sera samples from Caspian seals were obtained on the Pearl Island (Fig. 1; 45° 03’ N, 48° 18’ E) located in the north-west area of the Caspian Sea, between November 5–11, 1993; August 15–16, 1997; September 12–16, 1998; and September 24–October 8, 2000. Sera samples from 7 Baikal seals in the Lake Baikal (Fig. 1; 53° 46’–52’ N, 108° 23’–31’ E) were collected on May 21 and 22, 1998. The sera of six ringed seals were obtained at Dickson in the southern part of the Kara Sea from May 2 to 22, 2002 (Fig. 1; 73° 30’ N, 80° 31’ E). The ages of Caspian seals and ringed seals were estimated by counting the growth layers in both the dentine and cementum of the canine [4]. The 3 species of seals were all subjected to gross pathological observation. Sera from 4 beluga whales were collected at Anadyr Firth on July 20, 2001 (Fig. 1; 64° 83’ N, 176° 72’ E). The life stage of the beluga whales was estimated based on their body length [30].

**Enzyme-linked immunosorbent assay**

Anti-Brucella serum antibody was detected in the enzyme-linked immunosorbent assay (ELISA) according to the protocol described previously [1]. Briefly, commercially available inactivated *B. abortus* strain 125 (Kaketsuken Co., Kumamoto, Japan) and *B. canis* strain QE-13B (Kitasato Institute Co., Tokyo, Japan) were solubilized and absorbed to the inner surface of each well (50 µg/50 µl/well) of a 96-well microtiter plate. The sera diluted at 1:100 were used as the primary antibody, and horseradish peroxidase-conjugated Protein A/G (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) diluted at 1:5,000 was used for detection. The absorbance at 405 nm was measured and the arithmetic mean of triplicate absorbance data points with the standard deviation (SD) was calculated. Serum samples showing the absorbance value higher than 0.2 against *B. abortus*, were regarded as positive, based on the values of serum samples from captive animals [1].

**Western blot analysis**

Western blot analysis was performed according to the method described previously [36]. Proteins of the solubilized *B. abortus* strain 125 and *B. canis* strain QE-13B were respectively separated on a 10% polyacrylamide gel by SDS polyacrylamide gel electrophoresis (20 µg/lane) and blotted onto a polyvinylidene difluoride membrane (Millipore Co., Billerica, MA, U.S.A.). Serum samples diluted to 1:100 were used as the first antibodies, and then horseradish peroxidase-conjugated Protein-A/G (Thermo Fisher Scientific Inc.) diluted to 1:5,000 was used for detection.

**RESULTS**

**Anti-Brucella antibodies assessed by ELISA**

All serum samples were examined for antibodies against *B. abortus* and *B. canis* antigens by ELISA. Some samples from Caspian seals and beluga whales showed a value higher than 0.2 only for *B. abortus* antigens (Table 1). The sera of 4 among the examined 71 Caspian seals showed positive absorbance for *B. abortus* antigens (OD at 405 nm: 0.22–0.26) (Table 1). The absorbance for *B. canis* antigens in the same samples ranged from 0.12–0.19, which was relatively higher than that of other sera samples, though lower than 0.2 threshold. The age of the Caspian seals, estimated by counting the canine layers, ranged widely from less than 1 to 32 years. The 4 ELISA-positive Caspian seals were estimated to be adults at 11.5–31.5 years of age, based on...
The sera of 3 out of the 4 examined Beluga whales also demonstrated positive absorbance against \textit{B. abortus} antigens (Table 1).

Based on the body length, 2 of the examined whales appeared to be adults (395 and 350 cm) and the other 2 were juveniles (318 and 302 cm) [30]. One of the ELISA-positive whales was an adult and 2 were juveniles. The serum sample of the juvenile female whale showed high absorbance (OD at 405 nm=0.72), and that of the other 2 demonstrated lower absorbance (OD at 405 nm=0.26 and 0.27). No serum sample from the 4 examined whales reacted with \textit{B. canis} antigens (OD at 405 nm=0.04–0.06).

Western blot analysis for antibody specificity against \textit{B. abortus} and \textit{B. canis}

Since ELISA showed differences in antibody reactivity to \textit{B. abortus} and \textit{B. canis} in the antibody-positive serum samples between Caspian seals and beluga whales, the antigen specificity was further examined by Western blot using 2 available Caspian seal serum samples and all 4 beluga whale serum samples. The 2 Caspian seal sera (Serum ID: 98C46 and 00C17) previously showed the lowest absorbance in ELISA (OD at 405 nm=0.22) out of the 4 positive serum samples. In Western blot analysis, they displayed similar band patterns: some weak yet sharp bands were formed as a response to both antigens of \textit{B. abortus} and \textit{B. canis} (Fig. 2a). The Western blot band patterns of the 3 ELISA-positive serum samples from beluga whales (Serum ID: No. 1, No. 10 and No. 11) were similar to each other. Many bands, including the smeary bands, were detected in \textit{B. abortus} Western blots while none were observed in case of \textit{B. canis} (Fig. 2b). Western blot was also applied to the negative seal sera showing the absorbance close to the significance threshold of 0.2 (OD at 405 nm: 0.15–0.16) and 1 negative beluga whale serum (OD at 405 nm: 0.13), but no bands were observed (data not shown).

**DISCUSSION**

The present serologic study demonstrated that \textit{Brucella} infection occurred in Caspian seal and beluga whale populations inhabiting Russian waters. The presence of anti-\textit{Brucella} antibodies in Caspian seals is consistent with the findings in the previous

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**Table 1.** Prevalence of serum antibodies against \textit{B. abortus} in Caspian seals, Baikal seals, ringed seals and beluga whales

| Species                  | Year | Male | Female | Total (%) |
|--------------------------|------|------|--------|-----------|
| Caspian seal (\textit{Pusa caspica}) | 1993 | 0/9  | 0/3    | 0/12      |
|                          | 1997 | 1/4  | 0/3    | 1/7 (14)  |
|                          | 1998 | 0/3  | 1/12   | 1/15 (7)  |
|                          | 2000 | 2/14 | 0/23   | 2/37 (5)  |
|                          | Total| 3/30 | 1/41   | 4/71 (6)  |

| Baikal seal (\textit{Pusa sibirica}) | 1998 | 0/6 | 0/1 | 0/7 |
| Ringed seal (\textit{Pusa hispida hispida}) | 2002 | 0/3 | 0/3 | 0/6 |
| Beluga whale (\textit{Delphinapterus leucas}) | 2001 | 1/1 | 2/3 | 3/4 (75) |

a) Absorbance greater than 0.2 at 405 nm in ELISA is regarded as positive.
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Recent global warming is bringing out an environmental change affecting animal distribution, especially the melting of ice in the Arctic sea. It may cause a critical ecological influence on the animals in the Arctic sea and the connecting North Asian waters including Anadyr Firth. We must not only continue the serologic survey of the inhabiting mammals but also accelerate the isolation and characterization of Brucella from them. In addition, pathological studies including reproductive disorders are warranted, although the evidence of abnormality of reproduction in seals and whales were not observed in the present study. These studies would contribute to the conservation of wild animals and their ecosystems.

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REFERENCES

1. Abe, E., Ohishi, K., Ishinazaka, T., Fujii, K. and Maruyama, T. 2017. Serologic evidence of Brucella infection in pinnipeds along the coast of Hokkaido, the northernmost main island of Japan. Microbiol. Immunol. 61: 114–122. [Medline] [CrossRef]

2. Al Dahouk, S., Köhler, S., Occhialini, A., Jiménez de Bagüés, M. P., Hammerl, J. A., Eisenberg, T., Vergnaud, G., Cloeckaert, A., Zygmunt, M. S., Whatmore, A. M., Melzer, F., Drees, K. P., Foster, J. T., Wattam, A. R. and Scholz, H. C. 2017. Brucella spp. of amphibians comprise genomically diverse motile strains competent for replication in macrophages and survival in mammalian hosts. Sci. Rep. 7: 44420. [Medline] [CrossRef]

3. Amano, M., Koyama, Y., Petrov, E. A., Hayano, A. and Miyazaki, N. 2000. Morphometric comparison of skulls of the subgenus Pusa. pp. 315–323. In: Lake Baikal (Minoura, K. ed.), Elsevier Press, Amsterdam.

4. Amano, M., Miyazaki, N. and Petrov, E. A. 2000. Age determination and growth of Baikal seals (Pusa sibirica). pp. 449–462. In: Advances in Ecological Research, (Rossiter, A. and Kawabata, H., eds.), Vol. 31: Ancient Lakes: Biodiversity, Ecology and Evolution. Academic Press, London.

5. Avalos-Téllez, R., Ramirez-Pfeiffer, C., Hernández-Castro, R., Aparicio, E., Sánchez-Dominguez, C., Zavala-Norzagaray, A., Arellano-Reynoso, B., Suárez-Guemes, J., Aguira, A. I. and Aurelios-Gamboa, D. 2014. Infection of California sea lions (Zalophus californianus) with terrestrial Brucella spp. Vet. J. 202: 198–200. [Medline] [CrossRef]

6. Corbel, M. J. and Banai, M. B. 2005. Genus Brucella. pp. 370–386. In: Bergey’s Manual of Systematic Bacteriology. Vol. 2. (Brenner, D. J., Kreig, N. R. and Staley, J. T., eds.), Springer Press, New York.

7. Di Guardo, G., Marruchella, G., Agrimi, U. and Kennedy, S. 2005. Morbillivirus infections in aquatic mammals: a brief overview. J. Vet. Med. A Physiol. Pathol. Clin. Med. 52: 88–93. [Medline] [CrossRef]

8. Duncan, C. G., Tiller, R., Mathis, D., Stoddard, R., Kersh, G. J., Dickerson, B. and Gelatt, T. 2014. Brucella placentitis and seroprevalence in northern fur seals (Callorhinus ursinus) of the Pribilof Islands, Alaska. J. Vet. Diagn. Invest. 26: 507–512. [Medline] [CrossRef]

9. Durymanova, A. A., Dimov, S. K., Kuznetsova, V. N., Khuras’kin, L. N., Zolotykh, S. I. and Shestopalov, A. M. 2004. Antibodies to Brucella in caspian seals. Zh. Mikrobiol. Epidemiol. Immunobiol. 6: 99–101 (in Russian). [Medline]

10. Eisenberg, T., Rille, K., Schauerte, N., Geiger, C., Blom, J. and Scholz, H. C. 2017. Isolation of a novel ‘atypical’ Brucella strain from a spotted ribbontail ray (Taeniura lyra). Antonie van Leeuwenhoek. 110: 221–234. [Medline] [CrossRef]

11. Ewalt, D. R., Payeur, J. B., Martin, B. M., Cummings, D. R. and Miller, W. G. 1994. Characteristics of a Brucella species from a bottleneck dolphin (Tursiops truncatus). J. Vet. Diagn. Invest. 6: 448–452. [Medline] [CrossRef]

12. Forbes, L. B., Nielsen, O., Measures, L. and Ewalt, D. R. 2000. Brucellosis in ringed seals and harp seals from Canada. J. Wildl. Dis. 36: 596–598. [Medline] [CrossRef]

13. Foster, G., Osterman, B. S., Godfroid, J., Jacques, I. and Cloeckaert, A. 2007. Brucella ceti sp. nov. and Brucella pinnipedialis sp. nov. for Brucella strains with cetaceans and seals as their preferred hosts. J. Exp. Vet. Sci. 57: 2688–2693. [Medline] [CrossRef]

14. Garner, M. M., Lambourn, D. M., Jeffries, S. J., Hall, P. B., Ryan, J. C., Ewalt, D. R., Polzin, L. M. and Cheville, N. F. 1997. Evidence of Brucella infection in Paraparadoxes lungworms in a Pacific harbor seal (Phoca vitulina richardsoni). J. Vet. Diagn. Invest. 9: 298–303. [Medline] [CrossRef]

15. Goodman, S. J. 2017. Caspian seal. pp. 164–166. In: Encyclopedia of Marine Mammals, 3rd ed. (Wursig, B., Thewissen, J. G. M. and Kovacs, M. A. eds.), Academic Press, San Diego.

16. Guzmán-Verri, C., González-Barrientos, R., Hernández-Mora, G., Morales, J. A., Baquero-Calvo, E., Chaves-Olarte, E. and Moreno, E. 2012. Brucella ceti and brucellosis in cetaceans. Front. Cell. Infect. Microbiol. 2: 3. [Medline] [CrossRef]

17. Hammill, M. O. 2017. Ringed seal. pp. 822–824. In: Encyclopedia of Marine Mammals. 3rd ed. (Wursig, B., Thewissen, J. G. M. and Kovacs, M. A. eds.), Academic Press, San Diego.

18. Helmke, K. C., Garner, M. N., Ryan, J. and Bradway, D. 2018. Clinopathologic features of infection with novel Brucella organisms in captive waxy tree frogs (Phyllomedusa sauvagii) and Colorado river toads (Incilius alvarius). J. Zoo Wildl. Med. 49: 153–161. [Medline] [CrossRef]

19. Hernández-Mora, G., Palacios-Alfaro, J. D. and González-Barrientos, R. 2013. Wildlife reservoirs of brucellosis: Brucella in aquatic environments. Rev. Off. Int. Epizoot. 32: 89–103. [Medline] [CrossRef]

20. Jauniaux, T. P., Brenez, C., Fretin, D., Godfroid, J., Haelters, J., Jacques, I., Kerkhoff, F., Mast, J., Sarlet, M. and Caignol, F. L. 2010. Brucella ceti infection in harbor porpoise (Phocoena phocoena). Emerg. Infect. Dis. 16: 1966–1968. [Medline] [CrossRef]

21. Kimura, M., Koyama, Y., Sugizaki, S., Park, E. S., Imaoka, K. and Morikawa, S. 2017. Isolation of Brucella inopinata-like bacteria from White’s and Denny’s tree frogs. Vector Borne Zoonotic Dis. 17: 297–302. [Medline] [CrossRef]

22. Lambourn, D. M., Garner, M., Ewalt, D., Raverty, S., Sidor, I., Jeffries, S. J., Ryan, J. and Jaydos, J. K. 2013. Brucella pinnipedialis infections in Pacific harbor seals (Phoca vitulina richardsoni) from Washington State, USA. J. Wildl. Dis. 49: 802–815. [Medline] [CrossRef]

23. Larsen, A. K., Godfroid, J. and Nymo, I. H. 2016. Entry and elimination of marine mammal pathogens from aquatic environments. Acta Vet. Scand. 58: 9. [Medline] [CrossRef]

24. Miller, W. G., Adams, L. G., Ficht, T. A., Cheville, N. F., Payeur, J. P., Harley, D. R., House, C. and Ridgway, S. H. 1999. Brucella-induced abortions and infection in bottlenose dolphins (Tursiops truncatus). J. Zoo Wildl. Med. 30: 100–110. [Medline] [CrossRef]

25. Miyazaki, N. 2017. Baikal seal, pp. 57–58. In: Encyclopedia of Marine Mammals, 3rd ed. (Wursig, B., Thewissen, J. G. M. and Kovacs, M. A. eds.), Academic Press, San Diego.

26. Nymo, I. H., Tryland, M., Frie, A. K., Haug, T., Foster, G., Redven, R. and Godfroid, J. 2013. Age-dependent prevalence of anti-Brucella antibodies in hooded seals Cystophora cristata. Dis. Aquat. Organ. 106: 187–196. [Medline] [CrossRef]

27. Nymo, I. H., Tryland, M., Remen, R., Beckman, K., Larsen, A. K., Redven, B., Beckman, K., Larsen, A. K., Tryland, M., Quakenbush, L. and Godfroid, J. 2018. Brucella antibodies in Alaskan true seals and eared seals –two different stories. Front. Vet. Sci. 5: 8. [Medline] [CrossRef]

28. O’Corry-Crowe. 2017. Beluga whale. pp. 93–96. In: Encyclopedia of Marine Mammals, 3rd ed. (Wursig, B., Thewissen, J. G. M. and Kovacs, M. A. eds.), Academic Press, San Diego.

doi: 10.1292/jvms.18-0330
31. Ohishi, K., Ninomiya, A., Kida, H., Park, C. H., Maruyama, T., Arai, T., Katsumata, E., Tobayama, T., Boltunov, A. N., Khuraskin, L. S. and Miyazaki, N. 2002. Serological evidence of transmission of human influenza A and B viruses to Caspian seals (Phoca caspica). Microbiol. Immunol. 46: 639–644. [Medline] [CrossRef]

32. Ohishi, K., Zenitani, R., Bando, T., Goto, Y., Uchida, K., Maruyama, T., Yamamoto, S., Miyazaki, N. and Fujise, Y. 2003. Pathological and serological evidence of Brucella-infection in baleen whales (Mysticeti) in the western North Pacific. Comp. Immunol. Microbiol. Infect. Dis. 26: 125–136. [Medline] [CrossRef]

33. Ohishi, K., Kishida, N., Ninomiya, A., Kida, H., Takada, Y., Miyazaki, N., Boltunov, A. N. and Maruyama, T. 2004. Antibodies to human-related H3 influenza A virus in Baikal seals (Phoca sibirica) and ringed seals (Phoca hispida) in Russia. Microbiol. Immunol. 48: 905–909. [Medline] [CrossRef]

34. Ohishi, K., Takishita, K., Kawato, M., Zenitani, R., Bando, T., Fujise, Y., Goto, Y., Yamamoto, S. and Maruyama, T. 2004. Molecular evidence of new variant Brucella in North Pacific common minke whales. Microbes Infect. 6: 1199–1204. [Medline] [CrossRef]

35. Ohishi, K., Takishita, K., Kawato, M., Zenitani, R., Bando, T., Fujise, Y., Goto, Y., Yamamoto, S. and Maruyama, T. 2005. Chimeric structure of omp2 of Brucella from Pacific common minke whales (Balaenoptera acutorostrata). Microbiol. Immunol. 49: 789–793. [Medline] [CrossRef]

36. Ohishi, K., Bando, T., Abe, E., Kawai, Y., Fujise, Y. and Maruyama, T. 2016. Long-term and large-scale epidemiology of Brucella infection in baleen whales and sperm whales in the western North Pacific and Antarctic Oceans. J. Vet. Med. Sci. 78: 1457–1464. [Medline] [CrossRef]

37. Ross, H. M., Jahans, K. L., MacMillan, A. P., Reid, R. J., Thompson, P. M. and Foster, G. 1996. Brucella species infection in North Sea seal and cetacean populations. Vet. Rec. 138: 647–648. [Medline] [CrossRef]

38. Sasaki, H., Numachi, K. and Grachev, M. A. 2003. The origin and genetic relationships of the Baikal seal, Phoca sibirica, by restriction analysis of mitochondrial DNA. Zool. Sci. 20: 1417–1422. [Medline] [CrossRef]

39. Scholz, H. C., Hubalek, Z., Sedlácek, I., Vergnaud, G., Tomas, H., Al Dahouk, S., Melzer, F., Kämpfer, P., Neubauer, H., Cloeckaert, A., Maquart, M., Zygmunt, M. S., Whatmore, A. M., Falsen, E., Bahn, P., Göllner, C., Pfeffer, M., Huber, B., Busse, H. J. and Nöckler, K. 2008. Brucella microti sp. nov., isolated from the common vole Microtus arvalis. Int. J. Syst. Evol. Microbiol. 58: 375–382. [Medline] [CrossRef]

40. Scholz, H. C., Nöckler, K., Göllner, C., Bahn, P., Vergnaud, G., Tomas, H., Al Dahouk, S., Kämpfer, P., Cloeckaert, A., Maquart, M., Zygmunt, M. S., Whatmore, A. M., Pfeffer, M., Huber, B., Busse, H. J. and De, B. K. 2010. Brucella inopinata sp. nov., isolated from a breast implant infection. Int. J. Syst. Evol. Microbiol. 60: 801–808. [Medline] [CrossRef]

41. Tryland, M., Sørensen, K. K. and Godfroid, J. 2005. Prevalence of Brucella pinnipediae in healthy hooded seals (Cystophora cristata) from the North Atlantic Ocean and ringed seals (Phoca hispida) from Svalbard. Vet. Microbiol. 105: 103–111. [Medline] [CrossRef]

42. Whatmore, A. M., Davison, N., Cloeckaert, A., Al Dahouk, S., Zygmunt, M. S., Brew, S. D., Perrett, L. L., Koylass, M. S., Vergnaud, G., Quance, C., Scholz, H. C., Dick, E. J. Jr., Hubbard, G. and Schlabritz-Loutsevitch, N. E. 2014. Brucella papionis sp. nov., isolated from baboons (Papio spp.). Int. J. Syst. Evol. Microbiol. 64: 4120–4128. [Medline] [CrossRef]

43. Zygmunt, M. S., Blasco, J. M., Letesson, J. J., Cloeckaert, A. and Moriyón, I. 2009. DNA polymorphism analysis of Brucella lipopolysaccharide genes reveals marked differences in O-polysaccharide biosynthetic genes between smooth and rough Brucella species and novel species-specific markers. BMC Microbiol. 9: 92. [Medline] [CrossRef]