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Brucella melitensis in Asian Badgers, Northwestern China

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We isolated *Brucella melitensis* biovar 3 from the spleen of an Asian badger (*Meles leucurus*) in Nilka County, northwestern China. Our investigation showed that this isolate had a common multilocus variable-number tandem-repeat analysis 16 genotype, similar to bacterial isolates from local aborted sheep fetuses.

[1]These authors contributed equally to this article.

Brucellosis can be transmitted between domestic animals and wildlife (*1*). *Brucella melitensis* has been isolated from wildlife, such as chamois (*Rupicapra rupicapra*) (*2*), Alpine ibex (*Capra ibex*) (*3*), and Iberian wild goat (*Capra pyrenaica*) (*4*). Badgers are major predators in forests and consume a broad spectrum of food items, including small terrestrial vertebrates and their cadavers (*5*), which might result in contact with pathogens from tissues of these vertebrates. We report an Asian badger (*Meles leucurus*) in China naturally infected with *B. melitensis* biotype 3.

This study was approved by the Animal Ethics Committee of Shihezi University (approval no. AEC-SU2017–04). In 2017, a total of 7 illegally hunted and dying badgers in Nilka County, northwestern China, were confiscated by the local government.

We identified the animals as Asian badgers by using a PCR targeting the 16S rDNA gene (GenBank accession no. MH155253). We collected different organs or tissues, including heart, liver, spleen, lung, kidney, small intestine, large intestine, and blood, from all badgers. We separated serum from blood samples by centrifugation at 1,000 × *g* for 15 min and tested serum by using the rose bengal test (RBT) and serum agglutination test (SAT) (*6*). To detect *Brucella* antigens, we used immunohistochemical staining of liver and spleen tissue sections by pipetting mouse anti–*Brucella melitensis* IgG diluted 1:100 in 30% bovine serum albumin/phosphate-buffered saline onto each section. For comparison, we collected samples from 14 aborted sheep fetuses from Nilka County.

We extracted genomic DNA from all samples by using a commercial kit (Blood and Cell and Tissue Kit; BioTeke, http://www.bioteke.com). We used the partial *omp*22 gene (238 bp) encoding 22-kD outer membrane protein to identify the *Brucella* genus and the *IS*711 gene to identify *Brucella* species. We used PCRs that have been described (*7*). We used *Brucella* reference strains (*B. melitensis* 16M and *B. abortus* 2308) as positive controls and double-distilled water as a negative control.

We homogenized spleen samples of badgers and the 14 aborted sheep fetuses and inoculated these homogenates onto individual *Brucella* agar plates, which we then incubated at 37°C in an atmosphere of 5% CO_2 for 5 days. We tested putative *Brucella* colonies using by H_2S production, dye inhibition, agglutination by monospecific serum, and sensitivity to bacteriophages (Appendix Table, https://wwwnc.cdc.gov/EID/article/26/4/19-0833-App1.pdf). We analyzed colonies by using a multilocus variable-number tandem-repeat analysis (MLVA) typing assay (*8*).

Only serum from badger no. 2 was positive for smooth *Brucella* antigen by RBT and SAT; the specific

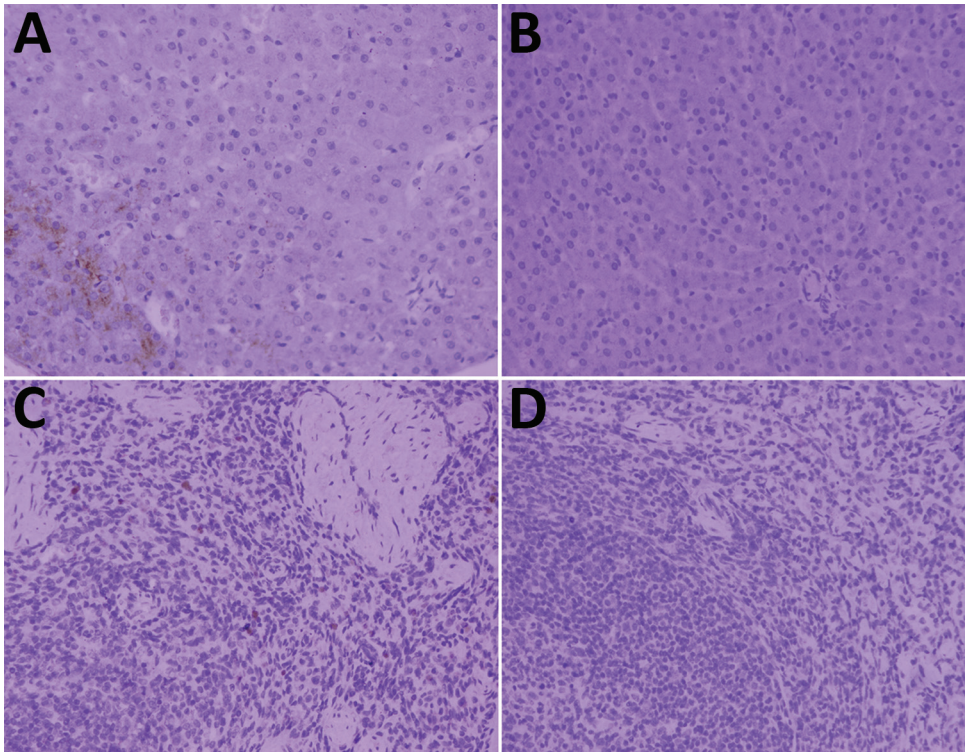

Figure. *Brucella* antigen in liver and spleen from Asian badger infected with *Brucella melitensis*, northwestern China. A) *Brucella* antigen in liver of Asian badger no. 2; B) liver of an uninfected Asian badger; C) *Brucella* antigen in spleen of Asian badger no. 2; D) spleen of badger without *Brucella* antigen. Diaminobenzidine staining; original magnification ×400.

antibody titer was 1:160 (≈125 IU/mL). We successfully amplified 2 genetic markers (regions of the *omp*22 and *IS*711 genes) from blood, heart, liver, spleen, lung, kidney, small intestine, and large intestine from badger no. 2 but not from samples of other badgers. In addition, we isolated *B. melitensis* biotype 3 from badger no. 2 and 5 aborted sheep fetuses according to phenotypic identification (Appendix Table). MLVA-16 typing indicated that the isolates from badger no. 2 and aborted sheep fetuses had a common MLVA-16 type (1-5-3-13-2-2-3-2-4-40-8-8-4-3-7-7). In addition, immunohistochemical staining with a brown chromogen (diaminobenzidine) identified *Brucella* antigens in liver and spleen of badger no. 2 (Figure).

B. melitensis is isolated mainly from goats and sheep, in which it causes fetal abortion (*1*). The Asian badger is a semihibernating, burrowing animal species that has not been reported to harbor this pathogen. In a previous study, Li and Hu reported that 0.30% (12/4,015) of sheep in Nilka County, China, were serologically positive for smooth *Brucella* antigen by RBT and 9.75% (145/1,485) were serologically positive for smooth *Brucella* antigen by SAT (*9*). The habitats of Asian badgers and the grazing areas of sheep and goats partially overlap, which can be most likely explained by observations of shepherds that Asian badgers eat aborted fetuses or their placentas during lambing season in winter. In this study, *B. melitensis* biovar 3 isolates, designated as XJ1802 and XJ1804 strains, were found in aborted sheep fetuses and an Asian badger. MLVA-16 typing indicated that they shared a common MLVA-16 type (Appendix Figure). This finding suggests that the Asian badger is a *Brucella* spillover host that becomes infected from sheep that act as a reservoir host.

Another study reported that coyotes were infected probably through ingestion of aborted fetuses and placentas in enzootic brucellosis areas (*10*). In our study, we detected *Brucella* DNA from blood, heart, liver, spleen, lung, kidney, small intestine, and large bowel of badger no. 2 and identified *B. melitensis* biovar 3 from spleen tissue. This finding suggests that pathologic changes in multiple organs or tissues caused by *B. melitensis* might occur.

In the future, it will be essential to evaluate the clinical status of Asian badgers naturally infected with *B. melitensis*. In addition, more extensive surveillance is necessary to expand our knowledge on the epidemiologic interface between wildlife and domestic animals in the context of *Brucella* infections.

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Multicenter Study of Azole-Resistant *Aspergillus fumigatus* Clinical Isolates, Taiwan[1]

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[1]Results from this study were presented in part at the 30th International Congress of Chemotherapy and Infection, November 24–27, 2017, Taipei, Taiwan.

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In a multicenter study, we determined a prevalence rate of 4% for azole-resistant *Aspergillus fumigatus* in Taiwan. Resistance emerged mainly from the environment (TR_{34}/L98H, TR_{34}/L98H/S297T/F495I, and TR_{46}/Y121F/T289A mutations) but occasionally during azole treatment. A high mortality rate observed for azole-resistant aspergillosis necessitates diagnostic stewardship in healthcare and antifungal stewardship in the environment.

Worldwide emergence of azole-resistant *Aspergillus fumigatus* since the late 2000s threatens human health (*1*). Azole resistance in *A. fumigatus* might develop during patient therapy with medical azoles or through exposure to azole fungicides in the environment; environmental exposure predominantly involves TR_{34}/L98H and TR_{46}/Y121F/T289A mutations in *cyp51A* (*1*).

Taiwan is an island country in eastern Asia that is geographically separated from mainland Eurasia and has a long history of azole fungicide use. To delineate the influence of clinical and environmental use of azoles on resistance, we conducted a multicenter study that investigated 375 *A. fumigatus* sensu stricto isolates collected during August 2011–March 2018 from 297 patients at 11 hospitals in Taiwan (Appendix Table 1, Figure 1, https://wwwnc.cdc.gov/EID/article/26/4/19-0840-App1.pdf).

We confirmed the presence of azole resistance by using the Clinical Laboratory Standard Institute method (Appendix Table 1) (*2*). Isolates resistant to ≥1 medical azoles (itraconazole, voriconazole, posaconazole, and isavuconazole) were defined as azole-resistant *A. fumigatus* and examined for resistance mechanisms, microsatellite-based phylogenetic relatedness, and growth rates following previously described methods (*3*,*4*).

Overall, 19 isolates from 12 patients were azole-resistant *A. fumigatus*. These isolates had resistance rates of 4.0%/patient and 5.1%/isolate analyses (Appendix Tables 2, 3). Ten (83.3%) patients harbored azole-resistant *A. fumigatus* that had environmental mutations, including TR_{34}/L98H (5 isolates, 5 patients), TR_{34}/L98H/S297T/F495I (7 isolates, 4 patients), and TR_{46}/Y121F/T289A (1 isolate) mutations. This observation

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Appendix

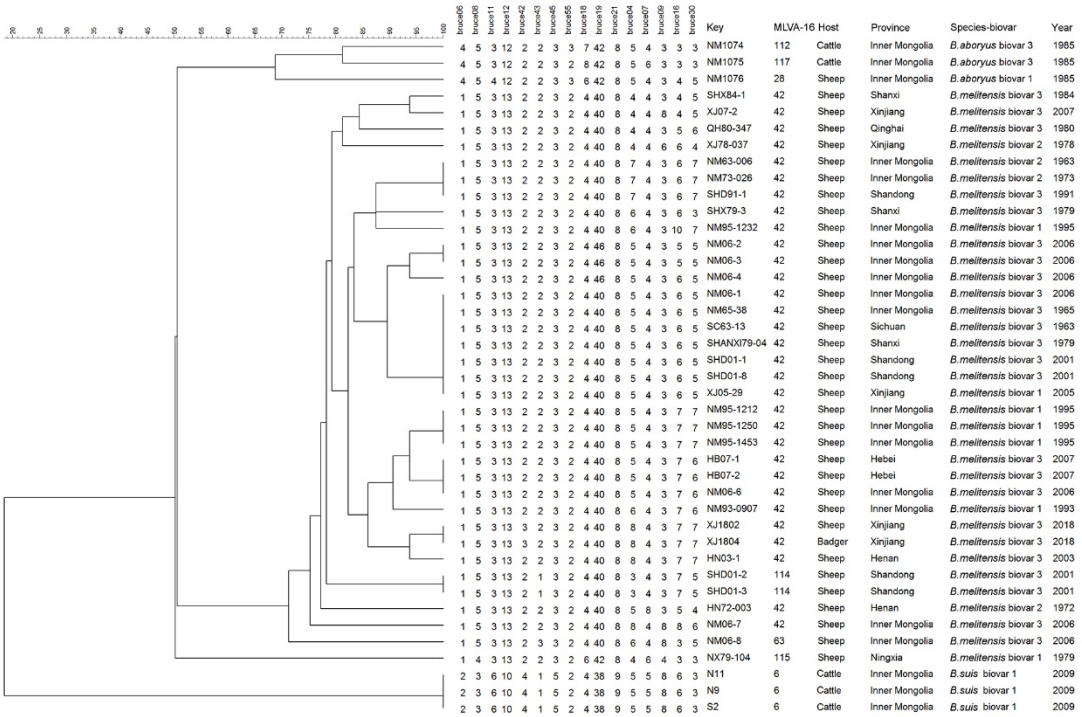
Appendix Table. Characteristics of *Brucella* isolates from Asian badger and other sources, northwestern China*

Characteristic	*Brucella* isolate in spleen of Asian badger	*Brucella* isolates in aborted sheep fetuses (n = 5)	International reference strains		
			16M	544A	1330s
CO_2 requirement	–	–	–	–	–
H_2S produced†	–	–	+	++	+++
Dye inhibition‡					
Thionin	+	+	+	–	+
Basic fuchsin	+	+	+	+	–
Lysis by bacteriophage at RTD					
Tb RTD	–	–	–	+	–
Tb 10^4 RTD	–	–	–	+	+
Iz	–	–	–	–	–
BK2	+	+	+	+	+
Agglutination of monospecific serum					
A	+	+	–	+	+
M	+	+	+	–	–
R	–	–	–	–	–
Species	*B. melitensis*	*B. melitensis*	*B. melitensis*	*B. abortus*	*B. suis*
Biotype	3	3	1	1	1

*RTD, routine test dilution; –, negative; +, positive.

†+, weakly positive; ++, positive; +++, strongly positive.

‡Dye concentration in *Brucella* agar medium is 20 μg/mL (1:50,000 dilution).



Appendix Figure. Dendrogram based on the MLVA-16 genotyping assay showing relationship between *Brucella* isolates from an Asian badger and aborted sheep fetuses and other bacteria, northwestern China. Shown are serial numbers for *Brucella* isolates; MLVA-16 genotype and host from which the bacteria was isolated; province; Species-biovar (biotyping result corresponding to each isolate); and year of isolation. Scale bar indicates percent relatedness. MLVA, multiple locus variable number tandem repeat analysis typing assay