would conclude that it might increase spread of bacterial drug resistance. Prompt recognition of carbapenem-resistant *Salmonella* spp. and initiation of appropriate infection control measures are essential to avoid spread of these organisms.

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References

1. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, *bla*$_{(NDM-1)}$, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. http://dx.doi.org/10.1128/AAC.00774-09
2. Nordmann P, Poirel L, Walsh TR, Livermore DM. The emerging NDM carbapenemases. Trends Microbiol. 2011;19:588–95. http://dx.doi.org/10.1016/j.tim.2011.09.005
3. Cabanes F, Lemant J, Picot S, Simac C, Cousty J, Jalin L, et al. Emergence of *Klebsiella pneumoniae* and *Salmonella* metallo-beta-lactamase (NDM-1) producers on Reunion Island. J Clin Microbiol. 2012;50:3812. http://dx.doi.org/10.1128/JCM.01029-12
4. Savard P, Gopinath R, Zhu W, Kitchel B, Rasheed JK, Tekle T, et al. First NDM-positive *Salmonella* sp. strain identified in the United States. Antimicrob Agents Chemother. 2011;55:5957–8. http://dx.doi.org/10.1128/AAC.05719-11
5. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 22nd informational supplement. CLSI document M100–S22. Wayne (PA): The Institute; 2012.
6. Wang M, Tran JH, Jacoby GA, Zhang Y, Wang F, Hooper DC. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. Antimicrob Agents Chemother. 2003;47:2242–8. http://dx.doi.org/10.1128/AAC.47.7.2242-2248.2003
7. Johnson TJ, Lang KS. IncA/C plasmids: an emerging threat to human and animal health? Mob Genet Elements. 2012;2:55–8. http://dx.doi.org/10.4161/mge.19626
8. Su LH, Wu TL, Chiu CH. Development of carbapenem resistance during therapy for non-typhoid *Salmonella* infection. Clin Microbiol Infect. 2012;18:E91–4. http://dx.doi.org/10.1111/j.1469-0691.2012.03767.x
9. Cui S, Li J, Sun Z, Hu C, Jin S, Guo Y, et al. Ciprofloxacin-resistant *Salmonella enterica* serotype Typhimurium, China. Emerg Infect Dis. 2008;14:493–5. http://dx.doi.org/10.3201/eid1403.070857
10. Sjölund-Karlsson M, Joyce K, Blickenstaff K, Ball T, Haro J, Medalla FM, et al. Antimicrobial susceptibility to azithromycin among *Salmonella enterica* isolates from the United States. Antimicrob Agents Chemother. 2011;55:3985–9. http://dx.doi.org/10.1128/AAC.00590-11

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Contagious Caprine Pleuropneumonia in Endangered Tibetan Antelope, China, 2012

To the Editor: Contagious caprine pleuropneumonia is a severe respiratory disease of goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), a member of the *M. mycoides* cluster (*1*). Mccp infection is associated with a 60% mortality rate and 90% illness rate, and the disease can cause substantial losses of livestock (*1*,*2*). We report a 2012 outbreak of contagious caprine pleuropneumonia in endangered Tibetan antelope (*Pantholops hodgsonii*) in China.

In 2000, the International Union of Conservation of Nature first listed the Tibetan antelope as an endangered species (*3*), and in 2004, the number of these antelope was estimated at 150,000 (*4*). Most Tibetan antelope live on China's Qinghai–Tibet Plateau at an altitude of 3,700–5,500 m (*3*).

During September–December 2012, ≈2,400 endangered Tibetan antelope were found dead in the Naqu area of Tibet; the dead animals represented 16% of the 15,000 Tibetan antelope thought to live in the area. Necropsy was performed on 13 of the antelope at sites within the Shenzha, Shuanghu, and Nima localities of the Naqu area (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/20/1/13-0067-Techapp1.pdf). Gross pathologic lesions were localized exclusively to the lung, where severe pleuropneumonia with partial hepatization was observed (Figure, panel A). The lungs of some affected antelope displayed a thickening of the interlobular septa, pleuritis, and an accumulation of straw-colored pleural fluid. The pleural exudate solidified to form a gelatinous covering on the lung (Figure, panel B).

Samples of lung tissue from 5 of the antelope were selected for histologic examination. Four of the samples showed fibrinous pneumonia with serofibrinous fluid and an inflammatory cell infiltrate consisting mainly of lymphocytes in the alveoli (Figure, panel C) and bronchioles (Figure, panel D). One sample showed pulmonary edema with a protein-rich fluid effusion in alveoli.

Lung tissue from each of the 13 antelope was minced and inoculated into modified Hayflick broth, which has been used extensively to isolate *Mycoplasma* spp. from animals. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 (*5*). The medium was examined daily by comparing inoculated broth with an uninoculated control broth. Moderate turbidity, a color change from pink to yellow, and an appreciable swirl of the culture when rotated were used as indicators of

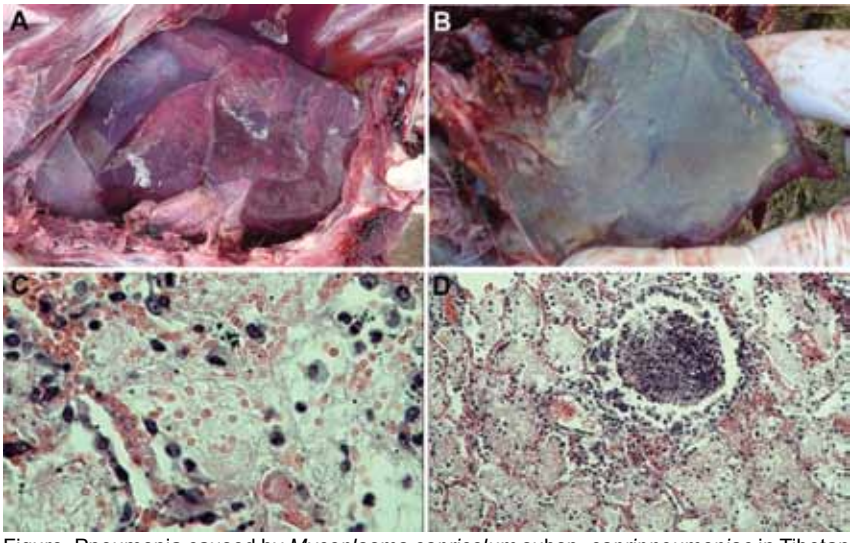

Figure. Pneumonia caused by *Mycoplasma capricolum* subsp. *capripneumoniae* in Tibetan antelope *(Pantholops hodgsonii*), Tibet, 2012. A) Lung of a caprine pleuropneumonia–infected Tibetan antelope (sample SZM2) showing lung hepatization. B) Lung of a caprine pleuropneumonia–infected Tibetan antelope (sample SH3) showing fibrin deposition. C and D) Fibrinous pneumonia with serofibrinous fluid and an inflammatory cell infiltrate, consisting of mainly lymphocytes, in the alveoli (panel C, sample SZM2, hematoxylin and eosin stain; original magnification ×400) and bronchioles (panel D, sample SH3, hematoxylin and eosin stain; original magnification ×100). Refer to online Technical Appendix Table 1 (wwwnc.cdc.gov/EID/article/19/12/13-0067-Techapp1.pdf) for details of the lung samples used to generate images for this figure.

mycoplasma growth. After 2–3 passages in culture, 11 of 13 samples showed growth of mycoplasma. The presence of mycoplasma-like particles in the 11 growth-positive cultures was confirmed by electron microscopy (online Technical Appendix Figure 1). Collectively, these observations implicated mycoplasma as the cause of disease in the affected antelope.

We next screened lung samples from each of the 13 Tibetan antelope by PCR for evidence of *M. mycoides* cluster and *M. bovis*. Eleven samples were positive for Mccp, but no other types of mycoplasma were detected (online Technical Appendix Tables 1, 2). We conducted PCR as described (*6*) on the *arc*D gene of Mccp. In brief, we conducted 35 cycles of 30 s at 94°C, 15 s at 47°C, and 15 s at 72°C. Of note, lung sample SH7, which showed pulmonary edema, was negative for mycoplasma by PCR and culture. Lung samples from the 13 dead Tibetan antelope were also tested for an additional 16 potential pathogens (online Technical Appendix Tables 1, 2) by PCR or reverse transcription PCR. No pathogens other than Mccp were detected.

To assess the relationship of the Mccp strain isolated from infected Tibetan antelope with previously isolated Mccp strains and the closely related *M. capricolum* subsp. *capricolum* (Mcc), we analyzed a 562-bp segment of the H2 gene of Mccp, which was used to distinguish the Mccp and Mcc as reported by Lorenzon et al. (*7*), isolated from an infected Tibetan antelope in Shuanghu county (sample SH3). The partial H2 sequence (GenBank accession no. KC441725) had higher sequence identity with Mccp isolates (99.3%–99.7%) than with Mcc isolates (90.2%–91.2% (online Technical Appendix Figure 2). This phylogenetic analysis demonstrated that the Mccp isolated from infected Tibetan antelope belongs to the same clade as Mccp strains previously isolated in Africa and Asia.

The changing habitat of endangered Tibetan antelope may lead to increased exposure to Mccp, which can cause devastating outbreaks, such as the one reported here. Goats and sheep are herded on grasslands at an altitude of 4,300–5,000 m, the same area where Tibetan antelope reside. Goats are a reservoir for Mccp, and Mccp has been isolated from sheep in mixed herds with goats (*8*). Rail lines traverse the rangelands in this region, limiting the normal migration patterns of the Tibetan antelope population. Interaction among goats, sheep, and Tibetan antelope in this region, combined with the effect of human infringement on their rangeland, may increase the risk for disease emergence and transmission.

Our results show that contagious caprine pleuropneumonia may pose a substantial threat to the survival of endangered Tibetan antelope. Surveillance for Mccp infection among Tibetan antelope populations and domestic and wild goat and sheep populations that have close contact with the Tibetan antelope should be considered.

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References

1. Nicholas R, Churchward C. Contagious caprine pleuropneumonia: new aspects of an old disease. Transbound Emerg Dis. 2012;59:189–96. http://dx.doi.org/10.1111/j.1865-1682.2011.01262.x
2. Fischer A, Shapiro B, Muriuki C, Heller M, Schnee C, Bongcam-Rudloff E, et al. The origin of the '*Mycoplasma mycoides* cluster' coincides with domestication of ruminants. PLoS ONE. 2012;7:e36150. http://dx.doi.org/10.1371/journal.pone.0036150
3. International Union for Conservation of Nature and Natural Resources. *Pantholops hodgsonii*. In: IUCN red list of threatened species [cited 2013 Oct 15. http://www.iucnredlist.org/details/15967/0.
4. Zhinong X, Lei W. Tracking down Tibetan antelopes. Beijing: Foreign Languages Press; 2004. p. 28.
5. Eshetu L, Yigezu L, Asfaw Y. A study on contagious caprine pleuropneumonia (CCPP) in goats at an export oriented abattoir, Debrezeit, Ethiopia. Trop Anim Health Prod. 2007;39:427–32. http://dx.doi.org/10.1007/s11250-007-9041-1
6. Woubit S, Lorenzon S, Peyraud A, Manso-Silvan L, Thiaucourt F. A specific PCR for the identification of *Mycoplasma capricolum* subsp. *capripneumoniae*, the causative agent of contagious caprine pleuropneumonia (CCPP). Vet Microbiol. 2004;104:125–32. http://dx.doi.org/10.1016/j.vetmic.2004.08.006
7. Lorenzon S, Wesonga H, Ygesu L, Tekleghiorgis T, Maikano Y, Angaya M, et al. Genetic evolution of *Mycoplasma capricolum* subsp. *capripneumoniae* strains and molecular epidemiology of contagious caprine pleuropneumonia by sequencing of locus H2. Vet Microbiol. 2002;85:111–23. http://dx.doi.org/10.1016/S0378-1135(01)00509-0
8. Bölske G, Mattsson JG, Bascuñana CR, Bergström K, Wesonga H, Johansson KE. Diagnosis of contagious caprine pleuropneumonia by detection and identification of *Mycoplasma capricolum* subsp. *capripneumoniae* by PCR and restriction enzyme analysis. J Clin Microbiol. 1996;34:785–91.

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Unexpected *Brucella suis* Biovar 2 Infection in a Dairy Cow, Belgium

To the Editor: Belgium was declared free of bovine brucellosis by the European Union in 2003 (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2003:156:0074:0078:EN:PDF). To maintain this status, the Federal Agency for the Safety of the Food Chain implemented a monitoring program, approved by the European Union, that consists of random serologic surveys and mandatory reporting of spontaneous abortion. This reporting enabled the detection of 2 outbreaks of bovine brucellosis in cattle caused by *Brucella abortus* biovar 3, in 2010 and 2012, but the origin of these outbreaks has not been identified.

As part of an epidemiologic survey conducted to prevent the spread of the infection, ELISA testing (Brucellosis Antibody Test Kit; Idexx, Hoofddorp, the Netherlands) was performed on bulk milk samples from 9,013 dairy farms in the country; 75 farms had positive test results and were classified as reactor farms. All cows in milk production on these farms were serologically tested, first by using slow agglutination test with the addition of EDTA to the antigen, and then, if results were positive, by a commercial ELISA. If results of the ELISA were positive, a confirmatory internal ELISA was performed at the national reference laboratory. A total of 41 seropositive cows from 27 farms were identified. All confirmed seropositive cows were slaughtered for bacteriologic investigation; all had negative test results for *B. abortus*.

On March 23, 2012, bulk milk sample testing for a farm in the province of Namur showed positive results. Testing performed in January 2011 on milk collected from the same farm had yielded negative results. The 150 cattle (including 55 dairy cows) on this farm were further serologically tested. One nonpregnant dairy cow had positive test results by slow agglutination test and ELISAs and was slaughtered on April 23, 2012. The cow was >4 years old, born in the farm, last calved in March 2011, and showed no clinical sign of brucellosis.

Bacteriologic examination was conducted on spleen, uterus, lymph nodes, and udder tissue samples; *Brucella* spp. were cultured from the spleen and uterus. Bacterial colonies grew on *Brucella* agar supplemented with 5% horse serum in the presence of basic thionine and safranine O; CO_2 was not required for growth, and H_2S was not produced. The isolates showed catalase, oxidase, and urease activity, a biochemical profile typical of *B. suis* biovar 2; identity was confirmed by real-time PCR on DNA extracted directly from the uterus (*1*).

A stamping out with compensation policy was implemented for this farm by the Federal Agency for the Safety of the Food Chain, according to European Union regulations, and subsequent epidemiologic investigations were performed. The farm owner is not a hunter. The culture-positive cow originated from a group of 10

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Contagious Caprine Pleuropneumonia in Endangered Tibetan Antelope, China

Technical Appendix

Technical Appendix Table 1. Histopathologic observations and pathogen testing of dead Tibetan antelopes evaluated during an outbreak of contagious caprine pleuropneumonia, China, 2012*

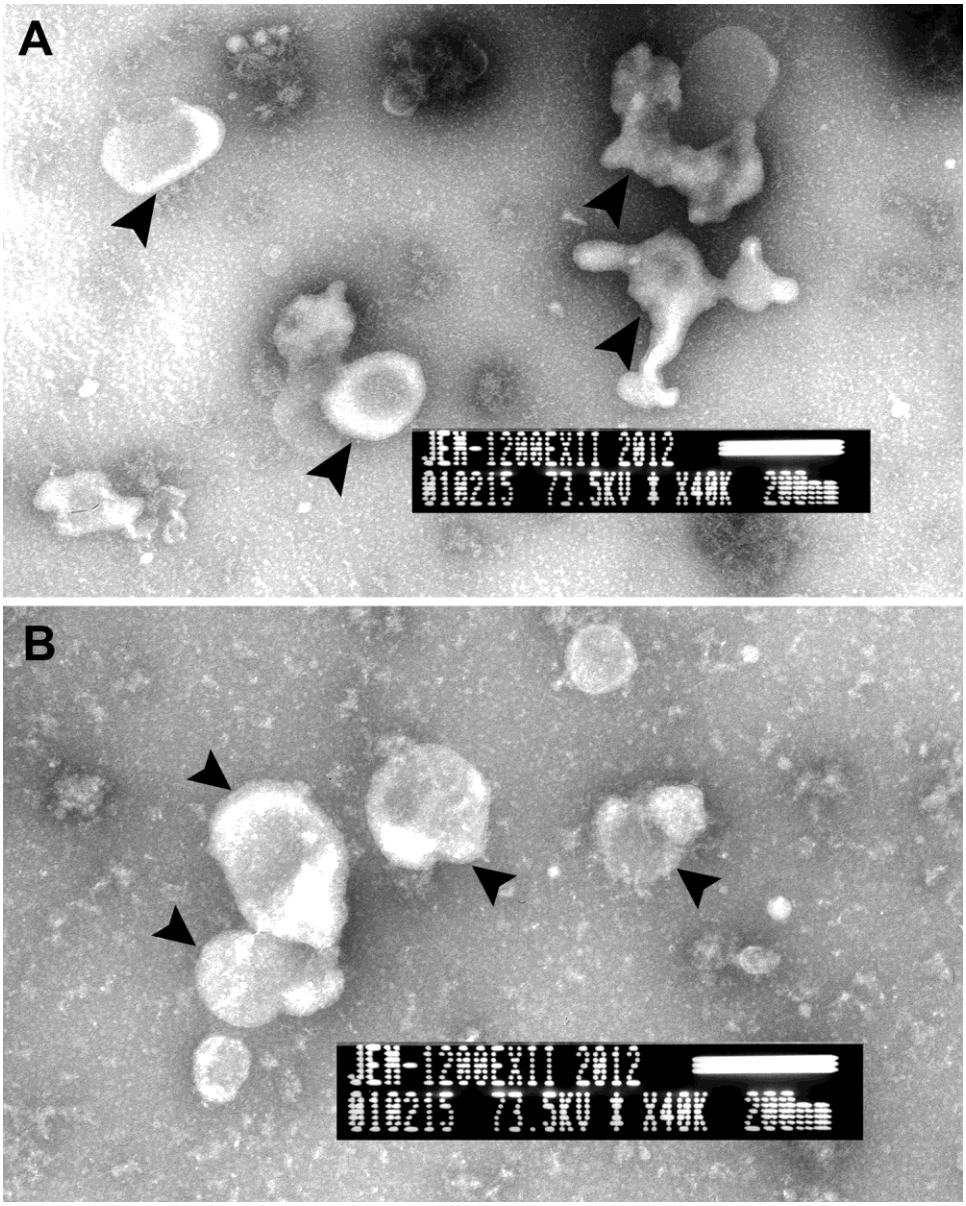
						PCR or reverse transcription PCR detection	
Sample	Locality*	GPS position	Sex, age, y	Histopathologic changes	Mccp isolated	Mccp	Additional pathogens†
SZM1	Shenzha	N30°54.777′, E08°21.170′	F, 5	Fibrinous pneumonia	+	+	–
SZM2	Shenzha	N30°54.776′, E08°21.167′	F, 4	Fibrinous pneumonia	+	+	–
SH1	Shuanghu	Not determined	F, 5	Not done	+	+	–
SH2	Shuanghu	N31°58.591′, E087°27.822′	M, 6	Not done	+	+	–
SH3	Shuanghu	N31°58.169′, E087°28.435′	M, 7	Fibrinous pneumonia	+	+	–
SH4	Shuanghu	N32°00.173′, E087°29.028′	M, 3	Fibrinous pneumonia	+	+	–
SH5	Shuanghu	N31°58.583′, E087°27.825′	F, 4	Not done	+	+	–
SH6	Shuanghu	N31°58.583′, E087°27.825′	F, 7	Not done	+	+	–
SH7	Shuanghu	Not determined	F, 9	Serous pneumonia	–	–	–
NM2	Nima	N31°58.256′, E087°22.439′	F, 3	Not done	+	+	–
NM3	Nima	Not determined	F, 6	Not done	+	+	–
NM4	Nima	N31°58.256′, E087°22.439′	F, 5	Not done	+	+	–
NM5	Nima	N31°57.492′, E087°22 164′	F, 4	Not done	–	–	–

*GPS, global position system; Mccp, *Mycoplasma capricolum* subsp. *Capripneumoniae*.

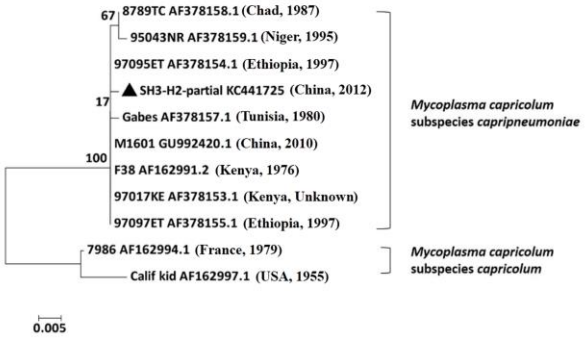
†Sixteen additional pathogens were tested by PCR or reverse transcription PCR: bluetongue virus, maedi-visna virus, goat arthritis encephalitis virus, foot and mouth disease virus, rinderpest virus, bovine parainfluenza virus 3, *Coxiella burnetii*, *Clostridium welchii*, *Pasteurella* spp., *Mycoplasma bovis*, *Mycoplasma leachii*, *Mycoplasma mycoides* subsp. *mycodies* large colony type, *Mycoplasma mycoides* subsp. *capri*, *Mycoplasma capricolum* subsp. *capricolum*, *Mycoplasma ovipneumoniae*, and *Mycoplasma mycoides* subsp. *mycoides* small colony type.

Technical Appendix Table 2. Primer pairs used for the detection of pathogens

Pathogen	Primers (5′→3′)
Bluetongue virus	BTV-251-F: TCGCTGCCATGCTATCCG
	BTV-251-R: CGTACGATGCGAATGCAG
Coxiella burnetii	IS111F1–485-F: TACTGGGTGTTGATATTGC
	IS111F1–485-R: CCGTTTCATCCGCGGTG
Maedi-visna virus	MVV-87-F: GAGGGATCAAGGATAAAAATGG
	MVV-87-R: GGTATCGYTGCAGYAACAT
Goat arthritis encephalitis virus	CAEV-296-F: CAAGCAGCAGGAGGAGAAGCTG
	CAEV-296-R:TCCTACCCCCATAATTTGATCCAC
Clostridium welchii	CPA-402-F: GTTGATAGCGCAGGACATGTTAAG
	CPA-402-R: CATGTAGTCATCTGTTCCAGCATC
Pasteurella spp.	C70/B37–1505-F: AGAGTTTGATYMTGGC
	C70/B37–1505-R: TACGGYTACCTTGTTACGA
Foot and mouth disease virus	FMD-ARS4-F: ACCAACCTCCTTGATGTGGCT
	FMD-NK61-R: GACATGTCCTCCTGCATCTG
Rinderpest virus	RPV-237-P1: ACAAACCNAGGATTGCTGAAATGAT
	RPV-237-P2: CTGAAYTTGTTCTGAAYTGAGTTCT
Bovine parainfluenza virus 3	RspV1/ MR2–739-F: GATCAGGAACTCTTAAAGGC
	RspV1/ MR2–739-R: TTTTCCCGACCCCTTCTAT
Mycoplasma bovis	Vsp-F: TGCTATTCATTTCTTTGTAGTATTTTATGT
	Vsp-R: TTTATTTCCTTTACCAATTACATATATTCG
M. leachii	7500bp1L: GTTGGTTTTGGATCAACTGG
	3480bp-R: TCTGATTTAGTTGGATTGAGTTCA
M. mycoides subsp. *mycodies* large colony type and *M.mycoides* subsp. *capri*	MMC2-L:CAATCCAGATCATAAAAAACCT
	MMC1-R: CTCCTCATATTCCCCTAGAA
M. capricolum subsp. capricolum	MCCPL1-L: AGACCCAAATAAGCCATCCA
	MCCPL1-R: CTTTCACCGCTTGTTGAATG
M. mycoides subsp. *mycoides* small colony type	SC3NEST1-L: ACAAAAAGAAGATATGGTGTTGG
	SC3NEST1-R: ATCAGGTTTATCCATTGGTTGG
M. capricolum subspecies *capripneumoniae*	Mccp-spe-F: ATCATTTTTAATCCCTTCAAG
	Mccp-spe-R: TACTATGAGTAATTATAATATATGCAA
M. ovipneumoniae	LMFI: TGAACGGAATATGTTAGCTT
	LMRI: GACTTCATCCTGCACTCTGT
H2 gene partial of *M. capricolum subspecies capripneumoniae*	m-h2a:CGGGGATCCGGTATTGTTGTTGGAAGT
	m-h2b:CGGGTCGACGCTCCATCAAACATAGAT



Technical Appendix Figure 1. Negative staining electron micrograph of *Mycoplasma capricolum* subsp. *capripneumoniae* particles. A) Electron micrograph of lung tissue of an infected Tibetan antelope SH3. Magnification ×40,000. B) Electron micrograph of culture supernatant after inoculation with tissue from the lungs of affected Tibetan antelope SH3. Magnification ×40,000. Arrowheads indicate pleomorphic mycoplasma-like particles appearing as short rods and spirals with diameters ranging from 100 to 300 nm. Scale bars indicate 200 nm. Refer to Technical Appendix Table 1 for details of the lung samples referred to in this Figure.



Technical Appendix Figure 2. Phylogenetic tree of *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) and *M. capricolum* subspecies *capricolum* (Mcc) strains was generated by using partial H2 gene nucleotide sequences. Sequences are identified by strain name and GenBank accession number. The tree was created by using MEGA5 software (http://megasoftware.net/) according to the neighbor-joining method. Bootstrapping with 1,000 replicates was performed to determine the percentage reliability for each internal node. Horizontal branch lengths are proportional to genetic distances. The black triangle indicates the Mccp field isolate from an infected Tibetan antelope (sample SH3 in online Technical Appendix Table 1) in the Naqu area, China. Scale bar indicates nucleotide substitutions per site.