

parasites can easily be missed or barely noticeable so that their identification can be quite difficult. In such instances, videodermoscopy might lead to the diagnosis and should be considered as a useful diagnostic aid. Image storage and sharing can also facilitate collaboration with experts and can enable timely recognition of unusual parasitic disorders imported from different geographic areas or tropical countries.

The cost of the equipment varies according to resolution quality, magnification capability, and image storage facility; costs range from 500 (for simple systems) to 10,000 (for sophisticated systems) euros. The expense is greatly outweighed by the advantages of avoiding the high cost of managing outbreaks of epidemic parasitoses resulting from misdiagnosis, treatment failures, and incomplete posttreatment monitoring (10).

Videodermoscopy is a noninvasive way to diagnose some pruritic disorders while avoiding unnecessary, uncomfortable, and sometimes expensive investigations and treatments. Physicians without access to such equipment should consider promptly referring patients to the nearest available videodermoscopy service for effective management.

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DOI: <http://dx.doi.org/10.3201/eid2006.130767>

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Distinguishing Nontuberculous Mycobacteria from Multidrug-Resistant *Mycobacterium tuberculosis*, China

To the Editor: Mycobacteria are commonly characterized by positive acid-fast staining. Most mycobacterial species belong to the nontuberculous mycobacteria (NTM), excluding species in the *Mycobacterium tuberculosis* complex and *M. leprae*. Both *M. tuberculosis* and NTM can induce pulmonary infection with similar symptoms and pulmonary radiographic findings (1). These similarities have led to difficulty in distinguishing these infections clinically.

As in many developing countries, the acid-fast stain is the only bacteriologic basis for diagnosing tuberculosis (TB) in primary health care institutions in China, where facilities are limited for *M. tuberculosis* culture, strain identification, and drug resistance detection. Thus, NTM is easily misdiagnosed as *M. tuberculosis*, and multidrug-resistant (MDR) TB is unable to be accurately identified. Patients with misdiagnosed TB usually are treated with the standard anti-TB regimens recommended by the Chinese government (i.e., 2HRZE/4HR [2 months of isoniazid (INH), rifampin (RIF), pyrazinmid, and ethambutol, followed by 4 months of INH and RIF 1 time daily] and 2H₃R₃Z₃E₃/4H₃R₃ [2 months of INH, RIF, pyrazinamide, and ethambutol followed by 4 months of INH and RIF 3 times weekly]) (2), which often results in treatment failures. Misdiagnosis is a key hurdle for effective prevention and treatment of TB (3–5). To evaluate the effect of misdiagnosis on TB prevention, we determined the proportion of patients with MDR TB and NTM infection in primary health care institutions in Zhejiang Province, China. Our

findings would be useful for improving TB prevention and treatment.

During 2011–2012, sputum samples from 13,882 patients suspected of having TB in 8 counties in Zhejiang Province were used to culture mycobacteria. Each sample was seeded onto 2 pieces of Löwenstein-Jensen medium. A total of 1,410 samples grew mycobacteria confirmed as acid-fast bacilli by using Ziehl-Neelsen staining. The 1,410 samples were further identified by using the Mycobacteria Identification Array Kit (CapitalBio, Beijing, China). The kit contains 17 types of bacilli-specific 16S rRNA probes (i.e., *M. tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. gilvum*, *M. xenopi*, *M. smegmatis*, *M. aurum*, *M. terrae*, *M. goodii*, *M. chelonae/abscessus*, *M. phlei*, *M. scrofulaceum*, *M. fortuitum*, *M. szulgai*, *M. ulcerans*, *M. marinum*, and *M. kansasii*). With this method, *M. tuberculosis* and NTM can be distinguished, and the species of NTM can be identified (6–8). Of 1,410 positive strains, we identified 1,332 (94.5%) as *M. tuberculosis* and 78 (5.5%) as NTM. NTM strains were further identified as follows: *M. intracellulare*, 39 isolates; *M. chelonae/abscessus*, 12 isolates; *M. kansasii*, 13 isolates; *M. avium*, 3 isolates; *M. fortuitum*, 4 isolates; and *M. scrofulaceum* and *M. szulgai*, 1 isolate each. For 5 isolates, strain could not be classified.

We detected drug resistance of 1,332 *M. tuberculosis* strains using a

Tuberculosis Drug Resistance Detection Array Kit (CapitalBio) (9). The mutant points for RIF resistance were identified as follows: *rpoB*/C531G, C531T, CG531AC, A526C, A526G, A526T, C526A, C526G, C526T, T533C, A516G, A516T, G516T, T511C, T511G, C513A, A513T, and C522T (Table). Moreover, the kit contained 5 mutant points for INH resistance, including *katG* (G315A, G315C, G315T, and C315) and *inhA* (C-15T) (Table). Of 1,332 *M. tuberculosis* strains, we identified 1,115 (83.7%) RIF/INH-sensitive strains, 88 (6.6%) MDR TB strains, 83 (6.2%) INH-resistant strains, and 47 (3.5%) RIF-resistant strains. Of the 1,410 positive strains, 88 (6.2%) were MDR *M. tuberculosis* strains.

The epidemiology of TB in Zhejiang Province reflects the situation in China and some developing countries (10). The clinical diagnosis and treatment of >80% TB cases in China is performed mainly by primary health care institutions. However, almost 80% of these medical institutions do not have the capability to culture *M. tuberculosis*, detect drug resistance, and identify strains (7). Of 1,410 strains obtained from the patients in 8 counties of Zhejiang Province, 218 (15.5%) were MDR TB, INH resistant, and RIF resistant. These affected patients could not be effectively treated with the national standard regimens. Specifically, 88 patients with MDR TB would be at risk for extensively drug-resistant TB, and 83

patients with INH-resistant TB and 47 with RIF-resistant TB would be at risk for MDR TB. In addition, we identified 78 (5.5%) NTM strains. With the acid-fast stain, these illnesses would be misidentified as TB and, in most instances, also would be reported as treatment failures. Clearly, accurate diagnosis provided by the technologies used in this study for distinguishing NTM and *M. tuberculosis*, *Mycobacterium* strain identification, and drug-resistance detection would increase the cure rate and effectively prevent TB epidemics.

For INH resistance, *katG315* was a main mutant point of the *M. tuberculosis* strain; 140 (81.4%) of the 172 INH-resistant mutations were related to *katG315*. For RIF resistance, *rpoB531* was a main mutant point; 84 (60.0%) of 140 RIF-resistant mutations were associated with *rpoB531*. Therefore, in future studies, more attention should be paid to the molecular epidemiology of *katG315* and *rpoB531*.

In conclusion, using the techniques for *M. tuberculosis* culture, *Mycobacterium* strain identification, and drug-resistance detection is necessary. It should be urgently pursued for accurate TB diagnosis in primary health care institutions in China to improve the prevention, treatment, and control of TB.

This study was funded by the National Scientific and Technological Major Project of China (grant no. 2009ZX10004-901, 2011ZX10004-901),

Table. Gene mutations of 214 drug-resistant tubercle bacilli, Zhejiang Province, China, 2011–2012

Drug	Mutant points	Mutant times	
		Mutant times for single site, no. (%)*	Total no. mutant times of sites related to drug resistance
Isoniazid	<i>inhA</i> -15 (C→T)	32 (18.6)	172†
	<i>katG</i> 315 (G→C), (G→A)	140 (81.4)	
Rifampin	<i>rpoB</i> 511 (T→C)	10 (7.1)	140‡
	<i>rpoB</i> 513 (A→C)	2 (1.4)	
	<i>rpoB</i> 516 (A→T), (A→G), (G→T)	19 (13.6)	
	<i>rpoB</i> 526 (A→G), (A→T), (C→G), (C→T)	21 (15)	
	<i>rpoB</i> 531 (C→G), (C→T)	84 (60.0)	
	<i>rpoB</i> 533 (T→C)	4 (2.9)	

*No. mutant times for single site/total no. mutant times of sites related to drug resistance.

†A strain simultaneously had *katG*315 (G→C) and *inhA*-15 (C→T).

‡Five strains had the double mutation of *rpoB*.

State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, and the National Natural Science Foundation of China (grant no.81070285).

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DOI: <http://dx.doi.org/10.3201/eid2006.130700>

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Schmallenberg Virus Circulation in High Mountain Ecosystem, Spain

To the Editor: Schmallenberg virus (SBV) is an emerging vector-borne virus mainly associated with *Culicoides* spp. midges (1,2). Factors affecting the density and distribution of vectors may help determine the prevalence of SBV infection in particular areas. Altitude could be one limiting factor for virus transmission; however, little information is available regarding SBV in high-altitude regions.

During December 29, 2012–February 21, 2013, morphologic anomalies were identified in 4 stillborn calves from different farms in northeastern Spain, and infection with SBV was suspected. The cases were clustered in the Ripollès and Garrotxa regions of Catalonia and appeared in beef cattle herds that spent the grazing season (May–November) in the alpine meadows (>2,000 m altitude) of the National Game Reserve of Freser-Setcases in the Eastern Pyrenees Mountains. The calves had severe arthrogryposis, ankylosis of several joints, abnormal curvature of the vertebral column, and severe muscle atrophy. Malformations of the central nervous system included bilateral hydrocephalus, cerebellar hypoplasia, and micromyelia, characterized by the presence of few neurons in the ventral horns and moderate to severe bilateral reduction of white matter in the ventral and lateral funiculi.

SBV infection was confirmed by real-time reverse transcription qualitative PCR (RT-qPCR) (1,3) or serologic testing in 3 of the 4 calves and all 4 of the mothers (Table). Serum samples were tested by using a commercial indirect ELISA (ID.vet; Innovative Diagnostics, Montpellier, France) and a virus neutralization test using the BH80/11–4 isolate (provided by the Friedrich-Loeffler-Institut, Isle of Riems, Germany) (4). Consistent results were obtained from both of these techniques, and the proportions of calves positive by ELISA and RT-qPCR were similar to those found in previous studies (5).

The neurologic and musculoskeletal lesions found in the calves indicated that fetal infection probably occurred at 5–6 months' gestation (6). Gestation started in mid-April to mid-May; therefore, maternal infection most probably occurred in late summer 2012 (September–October), when cows were grazing in the alpine meadows.

We then performed a serologic study in domestic and sympatric wild ruminants from the National Game