SHORT COMMUNICATION

Wildlife Plague Surveillance Near the China–Kazakhstan Border: 2012–2015

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Plague is endemic in Xinjiang Uygur Autonomous Region (XUAR), which is the largest province in China and is bordered by five Central Asian countries (Sun et al., 2012). Eleven plague epidemics occurred in humans in XUAR between 1953 and 1992. Forty-one persons were infected when hunting marmots, or through flea bites. Twenty-three of them died involving 41 people who were bitten by fleas from marmots they had captured (Zhang, 1992). Although no cases of human plague have been reported since 1993, many cases have been reported in wildlife (Zhang et al., 2008).

In 2003, four wild strains of Yersinia pestis were isolated from dead marmots (Marmota baibacina) in north-west XUAR near Altaw Mountain, Wenquan County, a region bordering the Republic of Kazakhstan (Pulati et al., 2004). This region was then listed as a plague focus for the first time. Serological monitoring and bacteriological monitoring conducted between 2004 and 2011 have shown that the plague seropositivity rates in the long-tailed ground squirrel (Spermophilus undulatus) and shepherd dogs were 0.72% (16 of 2218 tested animals) and 3.91% (12 of 307 tested animals), respectively. In the same interval, nine wild strains of Y. pestis were isolated from dead marmots, and the area around Biezhentao Mountain, which is partially located in Wenquan County, was designated as a new plague focus in 2009 (Pulati et al., 2010, 2012). This study reports surveillance data obtained between 2012 and 2015, including serological assays in Marmota baibacina marmots, S. undulatus ground squirrels, and sheepdogs (all from the same geographical area), bacterial isolation and genotypic identification.

This study was approved by the Animal Ethics Committee of Shihezi University (Approval No. AECSU2013-17). A total of 1191 serum samples from 477 marmots, 100 shepherd dogs and 614 long-tailed ground squirrels were collected at eight sites (shown in Fig. 1) in the vicinity of Altaw Mountain and Biezhentao Mountain at an altitude of 2100–3000 m. The marmots and long-tailed ground squirrels are valuable sentinel animals for plague serosurveillance in endemic disease foci, although their infections are usually asymptomatic (Vet. Microbiol., 172, 2014 and 339).
squirrels were captured as previously described (Guo et al., 2015). Dog owners agreed to take blood samples in shepherd dogs. The samples were screened with a passive haemagglutination assay to detect immunoglobulin (Ig)G antibodies against the F1 antigen of Y. Pestis, using a standard protocol of the Diagnostic Kit produced in Jilin Baird medical immunology Products Co. Ltd. In addition, a total of 101 dead marmots and 56 samples of resident Oropsylla silantiewi fleas were obtained during collections conducted each year from June to August. Samples included liver, spleen and bone marrow, which were streaked onto Yersinia nutrient agar medium and incubated at 28°C for 24 h. Any suspected colonies that grew were transferred to duplicate plates, cultured again and further tested by phage lysis for detection of Y. pestis. Isolation of bacteria from fleas was carried out as previously described (He et al., 2011; Wang et al., 2011). All positive colonies were screened for the presence of the caf1 and pla genes and then genotyped by multiple-locus variable-number tandem repeat analysis (MLVA) using 14 loci (Norkina et al., 1994; Zhang et al., 2009). To check the accuracy of MLVA profiles, samples were sequenced, and the repeat number of 14 loci was evaluated with BioNumerics version 4.0 software (Applied Maths, Kortrijk, Belgium).

The detailed results of the serological survey are shown in Table 1. The passive haemagglutination assay confirmed that ten of the 477 marmots, ten of the 100 shepherd dogs and six of the 614 long-tailed ground squirrels sera were positive to the Y. pestis F1 antigen. None of the negative control samples were positive for F1-specific antigen used in this assay. Seventeen suspected wild strains of Y. pestis were isolated at the eight sampling sites (Table 1 and Fig. 1), and evaluated by phage lysis tests, caf1, pla gene detection and MLVA typing. Of these, 16 wild strains were isolated from dead marmots and one was isolated from O. silantiewi fleas infesting a dead marmot. The isolates

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**Table 1.** Serological and bacterial findings in the surveillance area near the China–Kazakhstan border

| Year  | Positive No./No. of Serological detection | Bacterial detection | Origin |
|-------|------------------------------------------|---------------------|--------|
|       | Marmota baibacina | Shepherd | Spermophilus undulatus | Strain No. & position |                  |
| 2012  | 3/116                                        | 5/20                | 2/151 | 1 (Mengke valley, MKG) | Bone marrow (11 natural-dead M. baibacina) |
|       |                                               |                     |       | 1 (Sukesheyi valley, SKSYG) |                  |
| 2013  | 3/152                                        | 1/20                | 0/151 | 1 (Dalede valley, TLDG) | Liver (30 natural-dead M. baibacina) |
| 2014  | 3/162                                        | 2/30                | 3/178 | 1 (Dalede valley, TLDG) | Bone marrow (30 natural-dead M. baibacina) |
|       |                                               |                     |       | 1 (Juiledeshayi valley, JLDYSYG) |                  |
| 2015  | 1/47                                         | 2/30                | 1/134 | 4 (Kuketama valley, KKTWG) | 1 strain from Oropsylla silantiewi fleas infesting freshly natural-dead M. Baibacina, the other originated from livers (9 natural-dead M. baibacina) |
|       |                                               |                     |       | 3 (Mohate Valley, MHTG) | Liver (6 natural-dead M. baibacina) |
|       |                                               |                     |       | 3 (Awute valley, AWTG) | Spleen (7 natural-dead M. baibacina) |
|       |                                               |                     |       | 2 (Sulubiezhen valley, SLBZG) | Spleen (8 natural-dead M. baibacina) |
shared the same MLVA type (MT86: 2-2-2-4-6-8-7-5-2-7-3-3-2-5). Phylogenetic analysis showed that MT86 was an ancestral bacterial genotype of MT39, MT40 and MT41 which were all isolated from the Pamirs Plateau plague focus (Fig. 2) (Zhang et al., 2009). The sequences were deposited in GenBank with the accession numbers KT923491, KT923492 and KU645287–KU645300).

To date, 15 natural plague foci have been identified in China, covering an area of more than 1.4 million km² (Zhang et al., 2015). Of these, the M. baibacina–S. undulatus focus of the Tianshan Mountains, designated as the B focus of China, is mainly located north of Tianshan Mountains (Zhang et al., 2009). The region of Altaw Mountain and Biezhentao Mountain is included in the B focus. This comprehensive serological and pathogen study of M. baibacina marmots, S. undulatus ground squirrels and shepherd dogs showed that wildlife plague is very active in the region of Altaw Mountain and Biezhentao Mountain.

Marmots are the main reservoir of Y. pestis in Xinjiang, and it is clear that plague can be transmitted when hunting marmots. In June through August, the peak occurrence of plague epidemics coincides with the peak of the marmot population in XUAR. During those months, livestock returns to summer pastures, where they share the habitat with marmots, long-tailed ground squirrels, shepherd dogs and herdsmen. Plague can circulate in this unique ecological system (Zhang, 1992; Zhang et al., 2009). Oropsylla silantiewi is a dominant flea species infesting M. baibacina and can represent 89.87% of the ectoparasitic flea population (Zhang et al., 2012). As a vector for Y. pestis, O. silantiewi can feed on multiple hosts which enhances the risk of a plague outbreak (Li et al., 1997). Seventeen isolates were obtained from dead marmots and O. silantiewi. Although there were no official reports of human plague infection, the surveillance data convinced us that it is necessary for the local residents to avoid capturing, skinning dead marmots and against flea bites. Additionally, S. undulatus, with a range in China (Heilongjiang, XUAR), Kazakhstan, Mongolia, and the Russian Federation, is also a reservoir of Y. pestis. Yersinia pestis was isolated from 3.22% (66/2051) of S. undulatus from the Borohoro mountain area, which is in the M. baibacina–S. undulatus plague focus (Zhang and Sheng, 1991; ZipcodeZoo, 2004). In the present study, our 4-year surveillance results found an S. undulatus seropositivity rate of 0.98% (6/614). Although Y. pestis was not isolated from S. undulatus in our eight surveillance regions, S. undulatus should not be overlooked.

Serological and bacterial findings throughout the 4-year surveillance period showed that plague in marmots and the long-tailed ground squirrels is active in the region of Altaw Mountain and Biezhentao Mountain. Of the eight sites where Y. pestis was isolated, six proved to be new plague locations, which expands the plague area. Seventeen isolates shared the same MLVA type (MT86: 2-2-2-4-6-8-7-5-2-7-3-3-2-5), which was seen as an ancestral MT when compared to MT39, MT40 and MT41. Finally, shepherd dogs have an epidemiological role as a sentinel animal of Y. pestis infection and plague surveillance. Although there have been no official reports of human plague in XUAR.
since 1993, it is clear that plague is maintained by long-tailed ground squirrels and marmots and that their parasites should be of great concern in the Altay and Biezhentao Mountain region.

*Marmota baibacina* marmots and *S. undulatus* ground squirrels have the same or similar habitat on both sides of the China–Kazakhstan border. However, to the best of our knowledge, there is very limited data available on *M. baibacina* marmots and *S. undulatus* ground squirrels in this region, nor is their role as hosts or reservoirs of plague in the Alma-Ata region, east of Kazakhstan known (Wang and Sun, 1999; ZipcodeZoo, 2004; Wilson and Reeder, 2005; Lowell et al., 2007). Herein we suggest that Kazakhstan should strengthen plague surveillance from wildlife, particularly in the area bordering China.

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