



Chigger Mite (Acari: Trombiculidae) Survey of Rodents in Shandong Province, Northern China

Xiao-Dan Huang^{1,†}, Peng Cheng^{1,†}, Yu-Qiang Zhao¹, Wen-Juan Li², Jiu-Xu Zhao³, Hong-Mei Liu¹, Jing-Xuan Kou^{1,*}, Mao-Qing Gong^{1,*}

¹Shandong Academy of Medicine Sciences, Shandong Institute of Parasitic Diseases, Jining, Shandong Province 272033, People's Republic of China; ²Institute of Forensic Medicine and Laboratory Medicine, Jining Medical University, Jining, Shandong Province 272067, People's Republic of China; ³Jining Health School, Jining, Shandong Province 272031, People's Republic of China

Abstract: Chigger mites are parasites of rodents and other vertebrates, invertebrates, and other arthropods, and are the only vectors of scrub typhus, in addition to other zoonoses. Therefore, investigating their distribution, diversity, and seasonal abundance is important for public health. Rodent surveillance was conducted at 6 districts in Shandong Province, northern China (114-112°E, 34-38°N), from January to December 2011. Overall, 225/286 (78.7%) rodents captured were infested with chigger mites. A total of 451 chigger mites were identified as belonging to 5 most commonly collected species and 3 genera in 1 family. *Leptotrombidium scutellare* and *Leptotrombidium intermedia* were the most commonly collected chigger mites. *L. scutellare* (66.2%, 36.7%, and 49.0%) was the most frequently collected chigger mite from *Apodemus agrarius*, *Rattus norvegicus*, and *Microtus fortis*, respectively, whereas *L. intermedia* (61.5% and 63.2%) was the most frequently collected chigger mite from *Cricetulus triton* and *Mus musculus*, respectively. This study demonstrated a relatively high prevalence of chigger mites that varied seasonally in Shandong Province, China.

Key words: *Leptotrombidium scutellare*, *Leptotrombidium intermedia*, chigger mite, species, rodent, Shandong Province, China

INTRODUCTION

Chigger mites belonging to the families Leeuwenhoekidae and Trombiculidae (suborder Actinotidida, order Trombidiformes, subclass Acari, and class Arachnida) are ectoparasitic mites, which can lead to skin disorders, e.g., trombidiosis (trombiculiasis). Moreover, chigger mites are commonly regarded as vectors of scrub typhus, a disease caused by the intracellular pathogen *Orientia tsutsugamushi* [1-5]. Worldwide, a total of 3,000 chigger mite species that parasitize mammals (especially rodents), birds, reptiles, amphibians, invertebrates, and other arthropods have been recorded, with >420 species reported in China [6-8].

O. tsutsugamushi, a gram-negative bacterium, is transmitted transovarially to larval chigger mites that infest and transmit the bacterium to various hosts, including humans [9,10]. Scrub typhus has been reported in southern China (south of

the Yangtze River) for decades, with the main epidemic season occurring during the summer months. Scrub typhus was not previously reported in northern China (north of the Yangtze River), including Shandong Province, until 1986, but is now considered to be an emerging infectious disease in this area, with seasonal epidemics occurring during the autumn and winter seasons [11,12]. The annual incidence of scrub typhus increased from 0.23 to 0.64 per 100,000 people from 2006 to 2012 in Shandong Province, China [13]. Thus, studies of rodent populations and their associated ectoparasites provide information on the seasonal and geographic distributions of chigger mites. This study reports on the species composition, species diversity, and seasonal distributions of chigger mites in Shandong Province, China.

MATERIALS AND METHODS

Ectoparasite collection and identification

Rodents were collected from 6 districts (Binzhou, Liaocheng, Heze, Jining, Linyi, and Qingdao) in Shandong Province, north China (114-112°E, 34-38°N) from January to December 2011 (Fig. 1). Rodent live capture traps (7.5×9×23 cm; HC Hareware Products Co., Ltd, Tianjin, China) were set

•Received 17 January 2017, revised 8 September 2017, accepted 11 October 2017.

*Corresponding authors (gmg2005@163.com; koujx123@163.com)

†Xiao-Dan Huang and Peng Cheng contributed equally to this paper.

© 2017, Korean Society for Parasitology and Tropical Medicine

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

at 17:00-18:30 hr and retrieved early the following morning (07:30-08:30 hr), and were set in both indoor (every 15 m² at rodent haunts) and outdoor habitats bordering rivers, canals, roads, bridges, burial sites, and residential areas. Traps with rodents were placed in white cloth bags labelled with the time, date and location, and then placed in a styrofoam cooler and transported to our laboratory where they were euthanized in accordance with an approved animal use protocol and then identified to species based on their general external morphological features [14,15].

Chigger mites were collected from rodent hosts using currettes and lancets, and then placed in individually labelled vials containing 70% ethanol [16]. All mites were rinsed with distilled water to remove the ethanol and then mounted on glass slides with Hoyer's medium, and later identified at 400× microscope (Olympus CX41, Hamburg, Germany) [15,17]. Voucher chigger mites and representative rodents were deposited at the Medical Entomology Department, Shandong Institute of Parasitic Diseases, China.



Fig. 1. Six small mammal and chigger mite collection sites in Shandong Province, northern China.

General statistical analysis of chigger mites

The proportion (*P*) of each chigger mite species, the chigger mite infestation rate (*I_r*), and the mean number of chigger mites per rodent host (*MA*) were calculated [18,19], where $P = \frac{N_i}{N} \times 100\%$; $I_r = \frac{H_p}{H_T} \times 100\%$; and $MA = \frac{M}{H_T} \times 100\%$ (*N_i*=the number of each specific mite species, *N*=the total number chiggers from all mite species, *H_p*=the number of infested rodents, *H_T*=the total number of rodents, and *M*=the mean number of each species of mites. The above indices were calculated using SPSS, version 16.0 (SPSS, Chicago, Illinois, USA).

RESULTS

Chigger mite species

A total of 225/286 (78.7%) rodents collected from the 6 survey locations were infested with chigger mites. A total of 451 chigger mites were identified as belonging to the 5 most commonly collected species and 3 genera 1 family. *Leptotrombidium scutellare* and *Leptotrombidium intermedia* were the 2

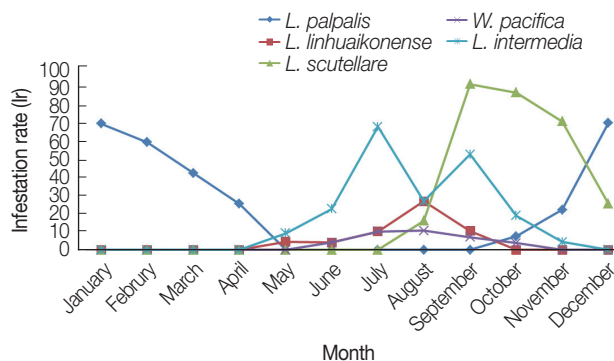


Fig. 2. Seasonal variations of the infestation rate (*I_r*) in Shandong Province, northern China.

Table 1. No. of rodents collected, no. (%) infested with the 5 most commonly collected chigger mites, and the mean no. of chigger mites per rodent host

Rodent Species	No. Rodent collected	No. (%) Rodent infested	<i>L. scutellare</i>		<i>L. palpalis</i>		<i>L. linhuaikonense</i>		<i>W. pacifica</i>		<i>L. intermedia</i>		Total	Chigger mite index ^a
			No. (%) collected	Chigger mite index ^a	No. (%) collected	Chigger mite index ^a	No. (%) collected	Chigger mite index ^a	No. (%) collected	Chigger mite index ^a	No. (%) collected	Chigger mite index ^a		
<i>A. agrarius</i>	167	136 (81.4)	190 (66.2)	1.40	53 (18.5)	0.39	14 (4.9)	0.10	10 (3.5)	0.07	20 (7.0)	0.15	287	2.11
<i>R. norvegicus</i>	36	22 (61.1)	11 (36.7)	0.50	9 (30.0)	0.41	0 (0.0)	0.00	0 (0.0)	0.00	10 (33.3)	0.45	30	1.36
<i>C. triton</i>	15	12 (80.0)	0 (0.0)	0.00	0 (0.0)	0.00	0 (0.0)	0.00	5 (38.5)	0.42	8 (61.5)	0.67	13	1.08
<i>M. musculus</i>	7	7 (100.0)	7 (36.8)	1.00	0 (0.0)	0.00	0 (0.0)	0.00	0 (0.0)	0.00	12 (63.2)	1.71	19	2.71
<i>M. fortis</i>	61	48 (78.7)	50 (49.0)	1.04	21 (20.6)	0.44	4 (3.9)	0.08	0 (0.0)	0.00	27 (26.5)	0.56	102	2.13
Total	286	225 (78.7)	258 (57.2)	1.15	83 (18.4)	0.37	18 (0.0)	0.08	15 (0.0)	0.07	77 (17.1)	0.34	451	2.00

^aChigger mite index = Mean no. of the 5 most commonly chigger mites collected by species per rodent.

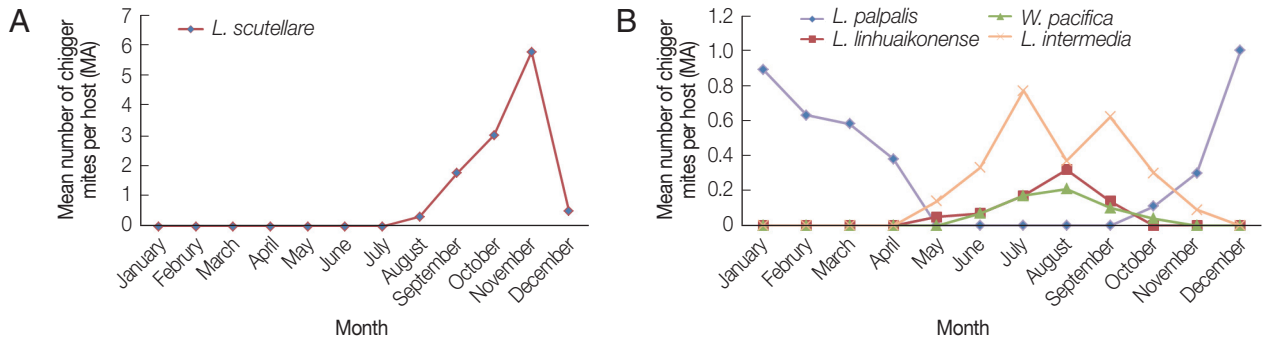


Fig. 3. (A) Seasonal variations of the mean number of *Leptotrombidium scutellare* per rodent host (MA) in Shandong Province, northern China. (B) Seasonal variations of the mean number of *Leptotrombidium palpalis*, *Leptotrombidium linhuaikonense*, *Walchia pacifica*, and *Leptotrombidium intermedia* per rodent host (MA) in Shandong Province, northern China.

most commonly collected chigger mite species.

L. scutellare (66.2%, 36.7%, and 49.0%) was the most frequently collected chigger mite from *Apodemus agrarius*, *Rattus norvegicus*, and *Microtus fortis*, respectively, while *L. intermedia* (61.5% and 63.2%) was the most frequently collected from *Cricetulus triton* and *Mus musculus*, respectively (Table 1).

Infestation rate of chigger mites (Ir)

L. palpalis was collected from January to April and from October to December 2011 (Fig. 2). The percent of rodents infested decreased from high in December (68.8%) to low in May-September and then increased from October (7.4%) to highs in December (68.8%) and January (68.4%) the following year. *Leptotrombidium linhuaikonense* was collected from May to September, with the highest infestation indices occurring from July to September (10.0-26.3%). *Walchia pacifica* was collected from June to October with infestation indices increasing slowly and peaking in August (10.5%). *L. scutellare* was collected from August to December with infestation indices of 89.7%, 85.2%, and 69.6% for September, October, and November, respectively, and was the most frequently collected mite species during the autumn season. *L. intermedia* mites were collected from May to November with infestation indices ranging from 26.3% to 66.7%, and were more frequently collected from July to September.

Mean no. of chigger mites per rodent host (MA)

L. scutellare was the most commonly collected chigger mite with mean numbers increasing from July (0.32), reaching 1.76, 3.00, and 5.74 in September, October, and November, respectively (Fig. 3A). The reported cases of scrub typhus in September, October, and November were 13, 357, and 70, re-

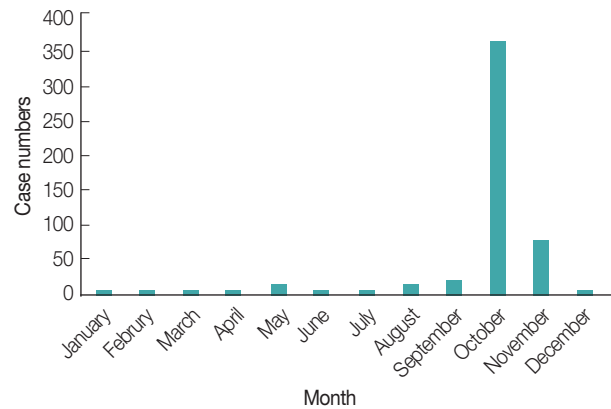


Fig. 4. Seasonal distribution of scrub typhus cases in Shandong Province, China.

spectively (Fig. 4). Thus, the seasonal distribution of scrub typhus corresponded with the seasonal fluctuations in the prevalence of *L. scutellare*. The MA for *L. palpalis* decreased gradually from January (0.89) to April (0.38) and increased again from October (0.11) to December (1.00) (Fig. 3B). The MA for *L. linhuaikonense* increased from May (0.05) to August (0.32), while the MA for *W. pacifica* increased gradually from June (0.07) to August (0.21), and then decreased in September (0.10) and October (0.04). The MA for *L. intermedia* increased gradually from May (0.14) to July (0.77), and then decreased gradually from October (0.30) to November (0.09).

DISCUSSION

Scrub typhus, transmitted by larval chigger mites, is endemic in the Asia-Pacific region [20-23]. A total of 10,860 chigger mites collected from small mammals in South Korea were identified as only 8 species belonging to 4 genera [4], and a

survey in Japan identified a total of 16,369 individual chigger mites belonging to only 10 species and 3 genera [3]. In contrast, the species diversity of chigger mites in China is much higher than the diversity cited in reports on chigger mites in other Asian countries, with >400 chigger mite species that vary among the different provinces. For example, 18, 12, 15, and 41 chigger mite species were collected from small mammals (*A. agrarius*, *R. norvegicus*, *M. musculus*, *C. triton*, *Rattus flavipectus*, and *Apodemus speciosus*) from Liaoning, Jilin, Heilongjiang, and Hubei Provinces, respectively [24]. To date, at least 17 chigger mite species, belonging to 6 genera and 3 families have been identified from Shandong Province [25,26]. The present investigation resulted in the identification of the 5 most commonly collected chigger mite species belonging to 3 genera in 1 family. *L. scutellare* was the predominant chigger mite species collected, and *A. agrarius* was the primary host for all the chigger species, a finding consistent with previous studies [24-26]. Our study showed a low species dominance, which may imply low thresholds for specific host preferences, and may increase their probability of encountering humans, as well as their transmission of scrub typhus among various hosts [27,28]. Chigger mite species composition has a comparatively high species richness and diversity, which may be attributed to the ecological situation in Shandong Province [29-31], which has a temperate and monsoonal climate suitable for various rodent hosts and chigger mite survival.

Seasonal differences in the relative abundance of chigger mites showed that *L. palpalis* infestations occurred mainly during autumn and winter, and that it was the dominant species collected during the winter. *L. linhuaianense*, *W. pacifica*, and *L. intermedia* were collected more frequently during the summer and were the dominant species collected during that period. *L. scutellare* appeared in the autumn and early winter and was the dominant species collected from September to November and corresponded to higher numbers of scrub typhus in humans, with the majority of patients reporting to health clinics beginning in late August, numbers peaking in October, and then declining to low numbers in December. Shandong Province is an economically developed province in China, with new rural reconstruction and urbanization, and invasion of rodent habitats, there is increased opportunity for mites to come into contact with the humans.

This study has provided key scientific data that identifies potential disease risks for the development of preventive and potential control measures against scrub typhus in Shandong

Province. However, future studies should aim to increase the rodent host sample size and diversity and investigate in greater detail the overall geographical distribution of chigger mites in Shandong Province.

ACKNOWLEDGMENTS

We are grateful to staff members Huai-Wei Wang and Hai-Fang Wang for their contributions in previous field collection and investigations and participating student Qi-Qi Shi at Shandong Institute of Parasitic Diseases. This project was financed by grants from the National Natural Science Foundation of China (grant nos. 81672059 and 81471985), Natural Science Foundation of Shandong province (grant nos. ZR2015YL032, ZR2014YL031, and ZR2015YL023), the Scientific Research Foundation of Jining Medical University (grant no. JY2015BS26), and the Innovation Project of Shandong Academy of Medical Sciences.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Varma RN. Prevalence of *Leptotrombidium deliense*, the scrub typhus vector, in the eastern Himalayas. *Nature* 1969; 222: 984-985.
2. Asanuma K, Kitaoka M, Shimizu F, Kano R. *Leptotrombidium scutellare* as a vector of scrub typhus at the endemic area of the foothills of Mt. Fuji, Japan. *J Hyg Epidemiol Microbiol Immunol* 1974; 18: 172-184.
3. Iwasa M, Kasuya S, Noda N, Hioki A, Ito A, Ohtomo H. Trombiculid mites (Acari: Trombiculidae) and *Rickettsia tsutsugamushi* isolated from wild rodents in a new endemic area of Japan. *J Med Entomol* 1990; 27: 501-508.
4. Lee IY, Kim HC, Lee YS, Seo JH, Lim JW, Yong TS, Klein TA, Lee WJ. Geographical distribution and relative abundance of vectors of scrub typhus in the Republic of Korea. *Korean J Parasitol* 2009; 47: 381-386.
5. Yu J, Deng XZ, Yang ZQ, Yao PP, Zhu HP, Xiong HR, Li CL, Zhang Y. Study on the transmission of Hantaan virus and *Orientia tsutsugamushi* by naturally dual infected *Leptotrombidium scutellare* through biting. *Zhonghua Yu Fang Yi Xue Za Zhi* 2010; 44: 324-328 (in Chinese).
6. Daniel M, Stekolnikov AA. Chigger mites (Acari: Trombiculidae) new to the fauna of Cuba, with the description of two new species. *Folia Parasitol (Praha)* 2003; 50: 143-150.

7. Daniel M, Stekolnikov AA. Chigger mites (Acari: Trombiculidae) from Makalu region in Nepal Himalaya, with a description of three new species. *J Med Entomol* 2009; 46: 753-765.
8. Loan HK, Cuong NV, Takhampunya R, Klangthong K, Osikowicz L, Kiet BT, Campbell J, Bryant J, Promstaporn S, Kosoy M, Hoang NV, Morand S, Chaval Y, Hien VB, Carrique-Mas J. *Bartonella* species and trombiculid mites of rats from the Mekong Delta of Vietnam. *Vector Borne Zoonotic Dis* 2015; 15: 40-47.
9. Jensenius M, Fournier PE, Raoult D. Rickettsioses and the international traveler. *Clin Infect Dis* 2004; 39: 1493-1499.
10. Cho NH, Kim HR, Lee JH, Kim SY, Kim J, Cha S, Kim SY, Darby AC, Fuxelius HH, Yin J, Kim JH, Kim J, Lee SJ, Koh YS, Jang WJ, Park KH, Andersson SG, Choi MS, Kim IS. The *Orientia tsutsugamushi* genome reveals massive proliferation of conjugative type IV secretion system and host-cell interaction genes. *Proc Natl Acad Sci U S A* 2007; 104: 7981-7986.
11. Yang LP, Zhao ZT, Liu YX, Feng YQ, Wang XJ, Li Z. Genotype identification and sequence analysis of *Orientia tsutsugamushi* isolated from Shandong area. *Zhonghua Liu Xing Bing Xue Za Zhi* 2006; 27: 1061-1064 (in Chinese).
12. Zhang M, Zhao ZT, Wang XJ, Li Z, Ding L, Ding SJ. Scrub typhus: surveillance, clinical profile and diagnostic issues in Shandong, China. *Am J Trop Med Hyg* 2012; 87: 1099-1104.
13. Wu YC, Qian Q, Magalhaes RJ, Han ZH, Haque U, Weppelmann TA, Hu WB, Liu YX, Sun YS, Zhang WY, Li SL. Rapid increase in scrub typhus incidence in Mainland China, 2006-2014. *Am J Trop Med Hyg* 2016; 94: 532-536.
14. Deng GE, Wang DQ, Gu YM, Meng YC. Economic insect fauna of China. Fasc 40, Acari, Dermansysoidea. Science Press. Beijing, China. 1993, pp 62-320.
15. Huang WJ, Chen YX, Wu D, Zhou DH. Rodents of China. Fudan University Press. Shanghai, China. 1995, pp 1-308.
16. Durden LA, Ellis BA, Banks CW, Crowe JD, Oliver JH Jr. Ectoparasites of gray squirrels in two different habitats and screening of selected ectoparasites for *Bartonellae*. *J Parasitol* 2004; 90: 485-489.
17. Li JC, Wang DQ, Chen XB. Trombiculid mites of China. Guangdong Science and Technology Press. Guangzhou, China. 1997, pp 97-438.
18. Bush AO, Lafferty KD, Lotz JM, Shostak AW. Parasitology meets ecology on its own terms: Margolis et al. revisited. *J Parasitol* 1997; 83: 575-583.
19. Men XY, Guo XG, Dong WG, Niu AQ, Qian TJ, Wu D. Ectoparasites of Chevrièr's field mouse, *Apodemus chevrièri*, in a focus of plague in southwest China. *Med Vet Entomol* 2007; 21: 297-300.
20. Kweon SS, Choi JS, Lim HS, Kim JR, Kim KY, Ryu SY, Yoo HS, Park O. Rapid increase of scrub typhus, South Korea, 2001-2006. *Emerg Infect Dis* 2009; 15: 1127-1129.
21. Wu YC, Qian Q, Magalhaes RJ, Han ZH, Haque U, Weppelmann TA, Wang Y, Liu YX, Li XL, Sun HL, Sun YS, Clements AC, Li SL, Zhang WY. Spatiotemporal dynamics of scrub typhus transmission in mainland China, 2006-2014. *PLoS Negl Trop Dis* 2016; 10: e0004875.
22. Lee HW, Cho PY, Moon SU, Na BK, Kang YJ, Sohn Y, Youn SK, Hong Y, Kim TS. Current situation of scrub typhus in South Korea from 2001-2013. *Parasit Vectors* 2015; 8: 238.
23. Takahashi M, Misumi H, Urakami H, Nakajima S, Furui S, Yamamoto S, Furuya Y, Misumi M, Matsumoto I. Mite vectors (Acari: Trombiculidae) of scrub typhus in a new endemic area in northern Kyoto, Japan. *J Med Entomol* 2004; 41: 107-114.
24. Liu Y, Zhao Z, Yang Z, Zhang J, Xu J, Wu Q, Peng Z, Miao Z. Epidemiological studies on host animals of scrub typhus of the autumn-winter type in Shandong Province, China. *Southeast Asian J Trop Med Public Health* 2003; 34: 826-830.
25. Xue J, Zhou GZ, Liu YX. The faunal study of chigger mites in Shandong Province. *Zhongguo Meijie Shengwuxue Ji Kongzhi Za Zhi* 2004; 15: 452-454 (in Chinese).
26. Yang Z, Liu Y, Yu X, Wu Q, Xing R. Investigation on natural foci of autumn-winter type tsutsugamushi disease in Shandong Province. *Zhonghua Liu Xing Bing Xue Za Zhi* 2000; 21: 283-286 (in Chinese).
27. Poulin R, Krasnov BR, Shenbrot GI, Mouillot DK, Hokhlova IS. Evolution of host specificity in fleas: is it directional and irreversible. *Int J Parasitol* 2006; 36: 185-191.
28. Zhang L, Zhao Z, Bi Z, Kou Z, Zhang M, Yang L, Zheng L. Risk factors associated with severe scrub typhus in Shandong, northern China. *Int J Infect Dis* 2014; 29: 203-207.
29. Krasnov BR, Shenbrot GI, Khokhlova IS, Degen AA. Flea species richness and parameters of host body, host geography and host 'milieu'. *J Animal Ecol* 2004; 73: 1121-1128.
30. Yang LP, Liu J, Wang XJ, Ma W, Jia CX, Jiang BF. Effects of meteorological factors on scrub typhus in a temperate region of China. *Epidemiol Infect* 2014; 142: 2217-2226.
31. Ding L, Wang XJ, Li Z, Ding SJ, Zhang M, Zhao ZT. Epidemic characteristics and related factors of autumn-winter type scrub typhus in Shandong area, 2010. *Chin J Public Health* 2013; 29: 543-545 (in Chinese).

