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Full Length Research Paper

A survey of ectoparasites from wild rodents and *Anourosorex squamipes* in Sichuan Province, South-west China

Lei Wei[1, 2], Xinwei Wang[1], Chengmin Wang[1] and Hongxuan He[1]*

[1]National Research Center for Wildlife Born Diseases, Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, P. R. China. [2]Faculty of Animal Science, Suzhou Vocational Technology College, Anhui, Suzhou, 234000, China.

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This study was to investigate the populations and species of ectoparasites from *Apodemas agrariu*s (Rodentia: Muridae; Pallas, 1771), *Rattus norvegicus* (Rodentia: Muridae; Berkenhout, 1769), *Rattus nitidus* (Rodentia: Muridae; Hodgson, 1845), *Rattus fulavipectus* (Rodentia: Muridae; Gray,1847), *Mus musculus* (Rodentia: Muridae; Linnaeus, 1758), *Micromys minutus* (Rodentia: Muridae; Pallas, 1771) and *Anourosorex squamipes*, (Insectivora: Soricidae; Milne-Edwards,1872) which have been identified as the main wild reservoir of disease, trapped from six districts of Sichuan province, southwest China, with the objectives of determining the prevalence of ectoparasites and identifying the potential risk factors associated with human health. Parasitological examination was performed by optical microscopy. Out of 282 *A. squamipes* and 175 rodents species examined, 70.5% of *A. squamipes* and 66.4% of rodents species were found to be infested with more ectoparasites (average: 68.45%). A total of 56 species of parasites, including 34 species of chigger mite, 14 species of mesostigmatid (gamasid) mite, six species of flea and two species of sucking louse were examined. The ectoparasites identified in *A. squamipes* were chigger mite (60.0%), mesostigmatid (gamasid) mite (22.2%), flea (13.3%) and sucking louse (4.4%). In rodents, chigger mite (60.7%), mesostigmatid (gamasid) mite (25.0%), flea (10.7%) and sucking louse (3.6%) were identified also. Both in rodents and *A. squamipes*, significant variation ($p < 0.05$) in ectoparasite infestation was observed in relation to body weight between females and males in all ectoparasites and chigger mites. Most species of ectoparasite were relatively uncommon, but a few were abundant. Within this ectoparasite complex, 16 species have previously been reported to be vectors of human disease agents. These mammals would appear therefore to be a natural reservoir for plague bacilli and epidemic haemorrhagic fever (Korean haemorrhagic fever) viruses. The results suggest that parasite viability studies are needed in order to assess the potential risk for human health.

Key words: *Anourosorex squamipes,* China, ectoparasites, wild rodents.

INTRODUCTION

Understanding the richness of ectoparasite species provides valuable insights into the ecological roles they play in the regulation of their host populations and communities (Poulin and Rohde, 1997; Poulin, 1998; Stanko et al., 2002; Krasnov et al., 2004). Many ectoparasites are vectors of bacteria, viruses, cestodes, nematodes and other disease agents, and mammals with higher parasite loads and species richness may harbour more such vectors. *Apodemas agrarius*, *Rattus norvegicus*, *Rattus nitidus*, *Rattus flavipectus*, *Mus musculus*, *Micromys minutus* and *Anourosorex squamipes* are widespread in Sichuan province, and are well known as a reservoir of plague in southwest China (Tian, 1998; Zhang et al., 2002; Wang et al., 2007). Some ectoparasites of rodent species and *A. squamipes*, notably fleas, can infest humans and may lead to the development of

*Corresponding author. E-mail: hehx@ioz.ac.cn.

Table 1. Species and numbers of all trapped small mammals in six districts, Sichuan province.

	A. squamipes		***A. agrarius***		***R. norvegicus***		***R. tanezumi***		***R. nitidus***		***M. minutus***		***M. musculus***	
Sites	m	f	m	f	m	f	m	f	m	f	m	f	m	f
Dujingyan	66	53	6	4					2	3				
Pengzhou	32	28	3	2	13	7	3	2	3	2				
Shifang	10	7			3	2	2	3	3	2				
Mianzhu	50	41	21	14							3	2		
Anxian	3	2	25	20			3	2					3	2
Beichuan			5	5	3	2								
Total	161	121	50	45	19	11	8	8	8	7	3	2	3	1

Note: m, males; f, females.

dermatitis. Moreover, the capacity of fleas to act as vectors for several microparasites was recognized, and has important implications for human health. For example, fleas are known to transmit the etiologic agent of bubonic plague, *Yersinia pestis*, from rodents and *A. squamipes* to humans (Rust et al., 1971; Antolin et al., 2002; Gage and Kosoy, 2005). In addition, fleas have been implicated in the transmission of the main etiologic agent of catscratch zoonosis (Comer et al., 2001; Chomel et al., 2006) and several trypanosomatids (Zanatta-Coutinho and Marcos-Linardi, 2007). Rodents and *A. squamipes* are well known as a reservoir of plague and also a natural reservoir of other disease agents, such as epidemic haemorrhagic fever (EHF = Korean haemorrhagic fever) viruses (Su et al., 1989). This study will aid in assessment of the distribution patterns of ectoparasite, and is an important tool in developing control strategies and in evaluation of ectoparasite-borne disease risk in humans.

This is the first study of prevalence of ectoparasites was investigated on *A. squamipes* and rodents at Dujiangyan, Pengzhou, Shifang, Mianzhu Beichuan and Anxian, Sichuan province after Wenchuan earthquake. No studies had focus on infestation with ecto-parasites on small mammals in this area under natural condition. The objective of this study was to determine by the prevalence of ectoparasites on these animals and to provide theoretical data for preventing the occurrence of zoonotic diseases.

MATERIALS AND METHODS

Study area

The study was conducted on Dujiangyan, Pengzhou, Shifang, Mianzhu Beichuan and Anxian (103° 40`-104° 27`N, 31° 03`-31°41`E; elevation 790 m) Prefecture, Sichuan province, southwest China. The six districts were located on area of earthquake and the climate of field investigations is moist and rainy. *A. agrarius*, *R. norvegicus*, *R. nitidus*, *R. lavipectus*, *M. musculus*, *M. minutus* and *A. squamipes* were trapped from June to November, 2008 in farmland, scrubland and forest with cage-traps (10 cm × 10 cm × 20 cm) baited with carrot and sunflower seeds. The cage-traps were examined and re-baited every morning. Trapped small mammals were removed from traps shortly after dawn and were put into white cloth bags in the field and brought to the laboratory for the collection of parasites.

Sampling collection

Before laboratory examination, all trapped small mammals were identified according to their morphological features (Huang et al., 1995). The gender and body weight of rodents and *A. squamipes* were recorded and statisticed, respectively. Species and numbers of all trapped small mammals were in Table 1. In the laboratory, ectoparasites were collected from each trapped small mammal. Each mammal was put on a large, square, white plate and all ectoparasites were collected from the body surface; those scattered on the white cloth bag or leaving the body were also collected with the help of a magnifier (Deng et al., 1993; Li et al., 1997; Jin, 1999; Xie and Zeng, 2000). Ectoparasites were divided into four groups: chigger mites; mesostigmatid (gamasid) mites; fleas and sucking lice.

Laboratory examinations

Ectoparasites were collected and identified using difference methods. Ticks, lice and fleas were collected and put into containers and preserved in 70% alcohol as described in Urquhart *et al*. (1996). Skin scrapings for mites were processed according to the method described by Cole (1986). The collected samples were examined by stereomicroscope and identification was performed according to the identification key given by Okello-onen et al. (1999) for tick and Wall and Shearer (1997) and Urquhart et al.

(1996) for lice, fleas and mites. The identification was confirmed at National Research Center for Wildlife borne Diseases, Chinese Academy of Sciences. We quieted the ectoparasites that were too damaged to identify.

Data management and analysis

Non-parametric Mann–Whitney *U*-tests were used to test differences in the abundance of ectoparasites between female and male hosts. Spearman correlation analysis was used to analyze the relationship between ectoparasites and the body parameters of hosts. The constituent ratio (C), prevalence (P) and average ectoparasite abundance (A) were calculated using the following formulae:

$$C = \frac{N_I}{N} \cdot 100\% \ ; \ P = \frac{H_I}{H} \cdot 100\% \quad A = \frac{N_I}{H} \cdot 100\%$$

where N*i* represents the number of individual ectoparasites in group *i* (total ectoparasites, chigger mites, mesostigmatid mites, fleas or sucking lice) and N represents the total number of ectoparasites. H represents the total number of mouse hosts and H_i represents the number of individual mouse hosts parasitized by group *i*.

RESULTS

Ectoparasite prevalence

A total of 282 *A. squamipes* and 175 rodents species, 195 females and 262 males, were captured (Table 1). A total of 3622 individual ectoparasites were collected and 55 species identified were divided into four groups (Table 2). A large proportion (average: 68.45%) of *A. squamipes* and rodents species were infested, giving an average ectoparasite abundance of 8.51 (Table 3). Chigger mites were the most numerous among the four ectoparasite groups, followed by sucking lice, mesostigmatid mites, fleas and sucking lice infestations. In this investigation, 27 and 33 species of chigger mite were observed on *A. squamipes* and rodents, respectively; 10 and 14 species of mesostigmatid mites were observed on *A. squamipes* and rodents respectively; 6 species of fleas and 2 species of sucking lice were observed on *A. squamipes* and rodents, respectively (Table 2). Most dominants were similar in *A. squamipes* and rodents species except *Frontopsylla spadix* in *A. squamipes* (Table 4).

Prevalence of ectoparasites by gender and body weight

There was a high proportion in male than female in terms of the abundance of chigger mites on *A. squamipes* and rodents species. The results show significant differences between male and female of *A. squamipes* and rodents species in terms of the abundance of chigger mites (U = 24862, d.f. = 198, 280, P = 0.004). The species abundance of both ectoparasites and chigger mites differed significantly between male and female hosts (U = 24568, d.f. = 202, 264, P = 0.004 for all ectoparasites; U = 24248, d.f. = 202, 276, P = 0.001 for chigger mites); the species richness of all ectoparasites and chigger mites on male hosts was, respectively, 1.46 and 1.65 times that on female hosts. However, there were no significant differences between male and female hosts that were discovered in mesostigmatid abundance, sucking louse abundance or flea abundance.

There was a high proportion in heavy body than thin body in terms of the abundance and richness of all ectoparasites and chigger mites on *A. squamipes* and rodents species (Table 5). Correlation analysis using 282 *A. squamipes* and 175 rodents species showed that the species abundance and richness of both all ectoparasites and chigger mites were positively correlated with the body weight of the host (r = 0.108, d.f. = 456, P = 0.030 for all ectoparasite abundance; r = 0.130, d.f. =456, P = 0.008 for chigger mite abundance; r = 0.098, d.f. =456, P = 0.046 for the species richness of all ectoparasites; r = 0.114, d.f. = 456, P = 0.022 for the species richness of chigger mites). But there were no significant differences in mesostigmatid mites' abundance, sucking louse abundance or flea abundance between host body parameters and mesostigmatid mites, sucking lice or fleas.

DISCUSSION

Some ectoparasites found in this investigation have already been reported and high prevalence was recorded on rat in Yunnan province (He et al., 1997). The results were consistent with previous studies. But it is the first that these ectoparasites were found on *A. squamipes*. The problem of ectoparasites in rodents and *A. squamipes* of this study area should to be very important as they are widely distributed in southwest China. In this investigation, 33 species of chigger mite were observed on rodents and *A. squamipes* (Table 2). Chigger mites which are related to many human diseases represented the most numerous of the ectoparasite groups collected. *Leptotrombidium scutellare*, one of the two main vector chigger mites transmitting *Orientia tsutsugamushi* to humans to cause *tsutsugamushi* disease in China (Li et al., 1999), is the most important ectoparasites observed on rodents and *A. squamipes*, accounting for 38.03% and 36.66% of all chigger mites, respectively (Table 4). *Leptotrombidium deliense*, the second most important ectoparasite and another principal vector of tsutsugamushi disease in China, was also observed on rodents and *A. squamipes* (6 individuals). These species have also recently been shown to be vectors of EHF virus (Song, 2000; Wu et al., 2004). *Laelaps echidninus*, the most numerous gamasid mite, of which 110 individuals were collected in this study, is a potential reservoir of *Y. pestis*, *Coxiella burnetii*, *O. tsutsugamushi* and *Leptospira interrogans* (Wang and Wang, 1962; Wang et al., 1996) (Table 2). Reportedly, *L. echidninus* can cause popular urticaria (Zhao, 2002). *Hirstionyssus sunci*, the third most numerous mesostigmatid mites, of which 49 individuals were collected, can cause human dermatitis (Deng et al., 1993). Fleas can cause skin damage and anaphylaxis by biting humans and sucking blood, and can also transmit zoonotic pathogens such as those that cause endemic typhus and tularaemia. Six species of flea were observed on rodents and *A. squamipes*. *Neopsylla specialis*, which has been described as the dominant flea species on rat in Yunnan province (He et al., 1997), was the most numerous of the fleas observed in this investigation, accounting for 14 individuals and 11 individuals or 38.89% and 35.48% of all fleas collected from *A. squamipes* and Rodents, followed by *Ctenophthalmus quadratus*, *Stenischia humilis*, *F. spadix*, *Leptopsylla*

Table 2. Ectoparasite species, by group, collected from rodents and *Anourosorex squamipes* in Sichuan Province, China in 2008.

Anourosorex squamipes		**Rodents**	
Species of parasites	**Individuals**	**Species of parasites**	**Individuals**
Chigger mites		**Chigger mites**	
Gahrliepia latiscutata	3	*Gahrliepia latiscutata*	4
Gahrliepia linguipelta	10	*Gahrliepia linguipelta*	8
Gahrliepia megascuta	17	*Gahrliepia megascuta*	14
Gahrliepia yunnanensis	90	*Gahrliepia yunnanensis*	72
Helenicula simena	35	*Gahrliepia radiopunctata*	2
Herpetacarus hastoclavus	112	*Helenicula simena*	30
Leptotrombidium apodevrieri	38	*Herpetacarus hastoclavus*	100
Leptotrombidium bambicola	4	*Leptotrombidium apodevrieri*	20
Leptotrombidium biji	6	*Leptotrombidium bambicola*	3
Leptotrombidium bishanense	28	*Leptotrombidium biji*	4
Leptotrombidium deliense	4	*Leptotrombidium bishanense*	22
Leptotrombidium densipunctatum	36	*Leptotrombidium deliense*†	2
Leptotrombidium dianchi	5	*Leptotrombidium densipunctatum*	24
Leptotrombidium eothenomydis	6	*Leptotrombidium dianchi*	4
Leptotrombidium gongshanense	8	*Leptotrombidium eothenomydis*	5
Leptotrombidium jinmai	10	*Leptotrombidium gongshanense*	6
Leptotrombidium kaohuense†	3	*Leptotrombidium jinmai*	8
Leptotrombidium longimedium	63	*Leptotrombidium kaohuense*†	4
Leptotrombidium robustisetum	12	*Leptotrombidium longimedium*	40
Leptotrombidium rusticum	200	*Leptotrombidium robustisetum*	12
Leptotrombidium scutellare†	500	*Leptotrombidium rusticum*	180
Leptotrombidium shuqui	15	*Leptotrombidium scutellare*†	480
Leptotrombidium sinicum	110	*Leptotrombidium shuqui*	10
Leptotrombidium xiaguanense	10	*Leptotrombidium sinicum*	100
Leptotrombidium xiaowei	7	*Leptotrombidium xiaguanense*	7
Leptotrombidium yongshengense	5	*Leptotrombidium xiaowei*	11
Leptotrombidium yui†	27	*Leptotrombidium yongshengense*	9
		Leptotrombidium yui†	33
Mesostigmatid mites		*Trombiculindus bambusoides*	20
Hirstionyssus sunci†	29	*Trombiculindus chilie*	3
Hypoaspis pavlovskii	1	*Trombiculindus yunnanus*	1
Laelaps chini	3	*Walchia ewingi*	32
Laelaps echidninus†	60	*Walchia koi*	2
Laelaps guizhouensis	6		
Laelaps nuttalli	47	**Mesostigmatid mites**	
Laelaps paucisetosa	3	*Lasioseius trifurcipilus*	1
Laelaps turkestanicus†	6	*Proctolaelaps pygmaeus*	13
Ornithonyssus bacoti†	1	*Gymdolaelaps weishanensis*	1
Proctolaelaps pygmaeus	7	*Haemogamasus oliviformis*	2
		Hirstionyssus sunci†	20
Fleas		*Hypoaspis pavlovskii*	1
Ctenophthalmus quadratus†	7	*Laelaps chini*	2
Neopsylla specialis specialis†	14	*Laelaps echidninus*†	50

Table 2. Contd.

Stenischia humilis†	6	**Fleas**	
Frontopsylla spadix†	4	*Ctenophthalmus quadratus*†	6
Leptopsylla segnis†	3	*Neopsylla specialis specialis*†	11
Paradoxopsyllus custodis†	2	*Stenischia humilis*†	5
		Frontopsylla spadix†	3
Sucking lice		*Leptopsylla segnis*†	3
Hoplopleura affinis	226	*Paradoxopsyllus custodis*†	3
Polyplax serrata	240		
Laelaps guizhouensis	5	**Sucking lice**	
Laelaps nuttalli	30	*Hoplopleura affinis*	200
Laelaps paucisetosa	2	*Polyplax serrata*	219
Laelaps turkestanicus†	5		
Ornithonyssus bacoti†	1		
Proctolaelaps pygmaeus	6		

†This ectoparasite is a vector of human disease.

Table 3. Constituent ratio, prevalence and average abundance of ectoparasites on rodents and *Anourosorex squamipes* in Sichuan Province, China, 2008.

Groups	Number of individuals	Number of species	Constituent ratio (C)	Prevalence (P)	Average abundance (A)
All ectoparasites	3890	55	100	68.45	8.51
Chigger mites	2626	34	67.51	41	5.75
Sucking lice	885	2	22.75	33	1.94
Mesostigmatid mites	302	14	7.76	18	0.66
Fleas	67	6	1.72	8	0.15

Table 4. Dominant species (representing >10% of its group) in the four groups of ectoparasite on rodents and *A. squamipes*.

	A. squamipes		**Rodents**	
Species	**Individuals**	**Percentage of group**	**Individuals**	**Percentage of group**
Chigger mites				
Leptotrombidium scutellare	500	36.66	480	38.03
Leptotrombidium rusticu	200	14.66	180	14.26
Sucking lice				
Polyplax serrata	240	51.50	219	52.26
Hoplopleura affinis	226	48.49	200	47.73
Mesostigmatid mites				
Laelaps echidninus	60	36.81	50	35.97
Laelaps nuttalli	47	28.83	30	21.58
Hirstionyssus sunci	29	17.79	20	14.38
Fleas				
Neopsylla specialis	14	38.89	11	35.48
Ctenophthalmus quadratus	7	19.44	6	19.35
Stenischia humilis	6	16.67	5	16.13
Frontopsylla spadix	4	11.11		

Table 5. Infestations of ectoparasites on *A. squamipes* and Rodents species by body weight.

	A. squamipes		Rodents species	
	Heavy body (n=189) (%)	Thin body (n=93) (%)	Heavy body (n=112) (%)	Thin body (n=63) (%)
All ectoparasites	62.37	38.56	60.23	36.75
Chigger mites	45.62	21.24	46.36	19.52
Mesostigmatid mites	9.34	6.86	7.35	5.23
Fleas	7.13	4.25	8.12	5.32
Sucking lice	10.24	8.26	19.89	7.35

segnis and *Paradoxopsyllus custodies*, all of which had been previously reported to be naturally infected with *Y. pestis* (Table 2). *N. specialis specialis* is a vector of plague bacilli and the most important vector in the sylvatic plague focus in southwest China (Zhang et al., 1996; He et al., 1997). This study found that the abundance of chigger mites and total ectoparasite on *A. squamipes* and rodents was significant differences between male and female (Table 5). Correlation analysis using 282 *A. squamipes* and 175 rodents species showed that ectoparasite abundance, chigger mite abundance, and the species richness of both all ectoparasites and chigger mites were positively correlated with the body weight of the host. The species richness of both all ectoparasites and chigger mites differed significantly between male and female hosts; the species richness of all ectoparasites and chigger mites on male hosts was, respectively, 1.46 and 1.65 times that on female hosts. On the contrary, no correlations were observed between host body parameters and mesostigmatid mites, sucking lice or fleas.

This is the first study to prevalence of ectoparasites on *A. squamipes* and rodents after Wenchuan earthquake. No previous study has examined such a large ectoparasites population sample in this geographical zone. Our results showed that many of the ectoparasites found on *A. squamipes* and rodents were able to transmission zoonotic pathogens (Table 2). Prior to this work, little information was available on the ectoparasites of *A. squamipes* and rodents in Sichuan Province, although most zoonotic diseases, such as plague, *Tsutsugamushi* disease, endemic typhus, EHF and Leptospirosis, occur there. *A. squamipes* and rodents were known to be a natural reservoir of plague and EHF, it remains to be verified whether it serves as a reservoir for other infections. Ectoparasites on small field mammals have been less researched than those on house mice, more studies on the ectoparasites of small, wild mammals such as *A. squamipes* and rodents are needed.

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