EPIDEMIOLOGICAL STUDIES ON HOST ANIMALS OF SCRUB TYPHUS OF THE AUTUMN-WINTER TYPE IN SHANDONG PROVINCE, CHINA

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Abstract. In order to elucidate the host animals of scrub typhus in Shandong Province, epidemiological studies on host rodents of the autumn-winter type scrub typhus were carried out from 1995 to 2002 at four localities in the Shandong Province. Based upon ecological observations of the composition, seasonal fluctuation of animal hosts, isolation of *Orientia tsutsugamushi*, detection and identification of serotypes of antibodies to *O. tsutsugamushi* were conducted. Two thousand eight hundred and eighty-four rodents and insectivores were captured, including 2,055 *Apodemus agrarius* (71.26%), 408 *Cricetulus triton* (14.15%), 64 *C. barabensis* (2.22%), 12 *Crocidura suaveolens* (0.42%), 313 *Rattus* (R.) *norvegicus* (10.85%), 32 *Mus* (M.) *musculus* (1.11%). *A. agrarius* was predominant in the field and the seasonal fluctuation was correlated significantly to that of scrub typhus ($r=0.810$, $p<0.005$). *R. norvegicus* was predominant indoors. The average capture rate per year in the field was 12.76% from 1995 to 1997. Of the total 2,884 rodents and insectivores captured out- and in-doors, 527 were living rodents (including 335 *A. agrarius*, 119 *C. triton*, 6 *C. barabensis*, 2 *C. suaveolens*, 63 *R. norvegicus* and 2 *M. musculus*, and 15,467 chigger mites were collected from them. Two hundred and fifty-three of 335 *A. agrarius* were parasitized by chiggers, showing 75.52% (253/335) of the infestation rate and 17.53 of the chigger index; 106 *C. triton* were parasitized by chiggers, showing 89.08% (106/119) infestation rate and 75.93 of the chigger index. The average antibody positive rate of rodents was 14.78%. The seasonal change of the antibody positive rate was higher during December-February (the second year), and varied from 20% to 28%, but the level of antibodies remained relatively low (5.26-16.67%) during March-November. The results of serotyping with 47 antibody-positive sera were as followings: 39 sera were Gilliam types, 7 sera were Karp types, 1 serum was Kato type. Twelve strains of *O. tsutsugamushi* were isolated from *A. agrarius* (8 strains), *C. triton* (3 strains) and *R. norvegicus* (1 strain), out of the isolated 12 strains, 10 were Gilliam strains, 2 were Karp strains. *A. agrarius* and *R. norvegicus* were the main host animals in out- and indoors respectively.

INTRODUCTION

Investigations over the past decade have reported a maximum of 194 clinical cases of scrub typhus per year (Cheng *et al*,1988; Wei *et al*,1989; Wang *et al*,1995; Liu *et al*, 1999), representing a small fraction of the total Shandong population. These reports represent only those infections with *Orientia tsutsugamushi* that caused serious illness resulting in hospitalization, and probably omit many clinical cases of scrub typhus that were undiagnosed because of lack of reagents for specific serological tests, low physician awareness of clinical presentation, and common self medication outside the official medical system. From these partial reports, we can know that scrub typhus is prevalent in the Shandong Province. Although the vector mite and clinical epidemiology of scrub typhus were investigated previously by Cheng *et al* (1988); Wei *et al* (1989); Feng *et al* (1989); Wang *et al* (1995) and Liu *et al* (1999), in Mongyin County, Feixian County and eastern Jinan, but the host animals of *O. tsutsugamushi* in those areas were not investigated systemically. In order to obtain more detailed information, from 1995 to 2002, we carried out an investigation on animal hosts of scrub typhus in this area.

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MATERIALS AND METHODS

Surveyed areas, capture of rodents

Feixian County, Junan County, eastern Jinan and Zouping County, where more people are seriously infected with scrub typhus were chosen as the locations of the investigation. From 1995 to 2002, rodents and insectivores were collected monthly, with traps set in the fields and in houses. The composition and the capture rate of each rodent or insectivore species were calculated, and the seasonal fluctuation of rodents was observed. Living rodents were killed and their sera were collected for the detection of *O. tsutsugamushi*. Antibodies to *O. tsutsugamushi* in the sera of rodents were detected and serotyped by IFA. During the endemic season, pools of three to five rodents of the same species were dissected for livers, spleens and kidneys, which were homogenized in physiological saline to make a 10% homogenate, then injected directly into the peritoneal cavities of three laboratory mice (0.5 ml/mouse) to isolate *O. tsutsugamushi*.

Collection of vector mites

Chigger mites were collected from captured rodents, the species composition and seasonal fluctuations of each species of chigger mite in relation to scrub typhus were observed.

Technique for isolation of *O. tsutsugamushi*

After having been inoculated with the homogenates of rodent internal organs, passage mice received injections of cyclophosphamide (CY), at a dosage of 0.25 mg/g body weight, on 0, 5 and 10 days for its immuno-suppressant effect. Inoculated mice were observed daily and the surviving mice were autopsied after 12~14 days. Smears from peritoneal fluid, liver, spleen and kidney were made and Giemsa stained to reveal *O. tsutsugamushi*. Serum antibody to *O. tsutsugamushi* was tested by IFA. All negative specimens were blindly passaged three to four times in mice for further confirmation.

Detection of antibodies to *O. tsutsugamushi* and identification of serotypes

All sera from rodents and passage mice were kept in a -20℃ freezer until use. The method for IFA and serotyping was that of Guo *et al* (1994).

Detection of LD_{50}, cross-immunity with standard Karp strain of the isolated *O. tsutsugamushi*

The LD_{50} of isolated strains was detected, and cross-immunity with standard Karp strain was observed as normal (Cheng *et al*,1988).

RESULTS

Composition and fluctuation of hosts

Two thousand eight hundred and eighty-four rodents and insectivores were captured in the field during the survey, including 2,055 *Apodemus agrarius* (71.26%), 408 *Cricetulus triton* (14.15%), 64 *C. barabensis* (2.22%), 12 *Crocidura suaveolens* (0.42%), 313 were *Rattus norvegicus* (10.85%), 32 were *Mus musculus* (1.11%). *A. agrarius* was predominant in the field. The population fluctuation curves of rodents in the field during three years were very similar, and the total population fluctuation curve of *A. agrarius* had two peaks (Fig 1): The former was in July (the capture rate was 13.50%), while the latter in October (14.56%). The population fluctuation curve of *C. triton* had only one peak, it was in July (4.67%). *R. norvegicus* was predominant in houses.

Correlation between incidence of scrub typhus and capture rate of rodents

The capture rates of rodents and incidence rates of scrub typhus were all showed in Table 2

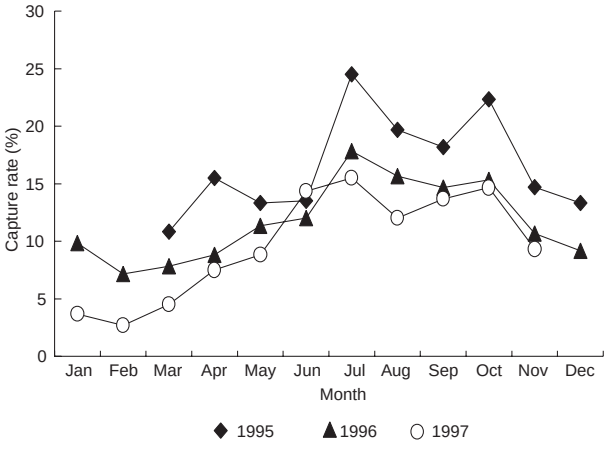

Fig 1–The seasonal fluctuation of rodent capture rate in Fei county from 1995 to 1997.

Table 1
The correlation analysis between the capture rate of rodents and incidence rate of scrub typhus.

Year	Month	Capture rate (%) of *A. agrarius*	Capture rate(%) of *C. triton*	Incidence rate (%)
1995	September	12.17	5.50	0.56
	October	16.83	5.30	87.08
	November	11.00	2.50	21.35
1996	September	12.20	2.50	2.25
	October	13.70	1.70	28.65
	November	10.40	0.30	18.54
1997	September	11.50	0.63	1.69
	October	13.17	1.17	32.58
	November	9.00	0.33	6.18

(patients with the disease occurred from September to November, thus only the incidence rates of the three months could be provided yearly). The coefficient correlation between the capture rates of *A. agrarius* and the incidence rates was 0.810 ($p<0.005$), showing statistical significance; but that between the capture rates of *C. triton* and the incidence rates was 0.414 ($p>0.05$), showing no statistical significance.

Chigger index of host

A total of 15,467 chigger mites, consisting of 6 species from two genera, were collected from 527 living rodents (including 335 *A. agrarius*, 119 *C. triton*, 6 *C. barabensis*, 2 *C. suaveolens*, 63 *R. norvegicus*, 2 *M. musculus)* captured in the foci of scrub typhus, including 4,649 *W. pacifica*, 3,834 *L. scutellare*, 2,833 *L. linhuaikongense*, 1,649 *L. palpale*, 466 *L. taishanicum*. The hosts of *L. scutellare*, *L. linhuaikongense*, *L. palpale* and *L. taishanicum* were extensive, but *W. pacifica* could only be found on *C. triton*. *C. triton* was parasitized by five species of chigger mites, and carried more mites, the parasitized rate was 89.08%, the chigger index was 75.93, *A. agrarius* was parasitized by four species, the parasitized rate was 75.52%, and the chigger index was 17.53. The seasonal fluctuation of the total chigger mite index was related positively to that of the total capture rate of rodents ($r=0.811$, $p<0.001$).

Detection and serotyping of antibodies to *O. tsutsugamushi* in sera of rodents

Six hundred and fifteen sera of various ro-

Table 2
Detection of antibodies to *O. tsutsugamushi* in the sera of rodents in Shandong Province.

Species	Total no.	Positive no.	Positive rate (%)
A. agrarius	260	39	15.00
C. triton	89	10	11.24
R. norvegicus	236	34	14.41
M .musculus	27	3	11.11
C. suaveolens	3	0	0
Total	615	86	13.98

Table 3
Seasonal fluctuation of antibodies to *O. tsutsugamushi* in the sera of rodents in Fei Xian county.

Year	Month	Total no.	Positive no.	Positive rate (%)
1995	May	10	1	10.00
	Jun	23	3	13.04
	Jul	24	4	16.67
	Aug	45	7	15.56
	Sep	15	2	13.33
	Oct	68	9	13.24
	Nov	28	3	10.71
	Dec	25	7	28.00
1996	Jan	13	3	23.08
	Feb	30	6	20.00
	Mar	18	1	5.56
	Apr	19	1	5.26
	Total	318	47	14.78

Table 4
Isolation and serotyping of *O. tsutsugamushi* from the rodents in Shandong Province.

Species of rodents	Pools inoculated	Strains isolated	Serotyping		
			Gilliam	Karp	Kato
A. agrarius	26	8	7	1	0
C. triton	9	3	2	1	0
R. norvegicus	8	1	1	0	0
Total	43	12	10	2	0

dents were collected in the Shandong Province, including 260 sera of *A. agrarius*, 89 sera of *C. triton*, 3 sera of *C. suaveolens*, 236 sera of *R. norvegicus*, and 27 sera of *M. musculus*. Eighty-six of the sera had antibodies to *O. tsutsugamushi*, the positive rate was 13.98% (Table 3). By serotyping with 47 antibody-positive sera collected in Feixian County, the results were: 39 sera belonged to Gilliam type (31 sera from *A. agrarius*, 6 sera from *C. triton*, 2 sera from *R. norvegicus*), 7 sera to Karp type (5 sera from *A. agrarius*, 2 sera from *C. triton*), and one serum to Kato (from *C. triton*).

Fluctuation of antibodies to *O. tsutsugamushi*

From May 1995 to April 1996, we observed monthly the seasonal fluctuation of antibodies to *O. tsutsugamushi* in the sera of rodents in Feixian County (Table 4). During December-Feburary (the second year), the antibody level was higher, varying from 20% to 28%. During March-November, the level of antibodies remained relatively low (5.26%~16.67%).

Isolation of *O. tsutsugamushi* from rodents (Table 5)

Eight strains of *O. tsutsugamushi* were isolated from 26 pools of internal organs of *A. agrarius*; three strains were isolated from nine pools of *C. triton*; one strain was isolated from eight pools of *R. norvegicus*. The results of serotype identification of the isolated strains were as followings: two strains isolated from *A. agrarius* and *C. triton* belonged to the Karp type and the other seven strains belonged to the Gilliam type. The LD_{50} of the two strains was determined: the one isolated from *A. agrarius* was $10^{-1.875}$, the other isolated from *C. triton* was $10^{-1.375}$. Both strains had cross-immunity with the standard Karp strain.

DISCUSSION

In our investigative focus, scrub typhus was mainly epidemic during September- November, with the peak in October (Yang *et al*, 1997; Liu *et al*, 1999), and we proved that *L. scutellare* was the main transmitting vector of *O. tsutsugamushi* in the area (Liu *et al*, 1997) . When cases of scrub typhus were abundant, the numbers of this mite infesting the bodies of *A. agrarius*, *C. triton*, *C. suaveolens* and *R. norvegicus* were abundant. Studies of *A. agrarius* revealed that this rodent was predominant in the field and the seasonal fluctuation correlated significantly with that of scrub typhus ($r=0.810$, $p<0.005$). We isolated *O. tsutsugamushi* from *A. agrarius*, and antibodies to *O. tsutsugamushi* were also detected in this species, thus we can conclude that this species is the most important host of *O. tsutsugamushi* in this focus. *C. triton* occupied a certain ratio in the epidemic season, and naturally infected with *O. tsutsugamushi*. it could play an important role in transmission of *O. tsutsugamushi*; *R. norvegicus* was predominant in households, and was infected with *O. tsutsugamushi*. It was the main host of *O. tsutsugamushi* in households.

A previous study proved that only the Gilliam type of *O. tsutsugamushi* existed in the sera of *A. agrarius* from Shandong (Wang, 1992) . In the present study, the existance of Karp and Kato types of *O. tsutsugamushi* in *A. agrarius* and *C. triton* was first reported by us.

The seasonal fluctuation of antibodies to *O. tsutsugamushi* revealed that after the epidemic season of scrub typhus, the positive rate of antibodies increased rapidly, but before the epidemic season, the positive rate was relatively low. This

result was similar to that obtained from the sera of rodents in Korea (Ree *et al*, 1991).

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