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Evidence for a high prevalence of spotted fever group rickettsial infections in diverse ecologic zones of Inner Mongolia

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SUMMARY

A 3-year study of spotted fever group rickettsial ecology in Inner Mongolia revealed that nearly half of the human population tested had antibodies to *Rickettsia sibirica* detected by complement fixation test. Infected persons, ticks and a high proportion of seropositive livestock and wild rodents were found in all five vegetation zones (desert, steppe, forest, forest-grassland and grassland).

INTRODUCTION

North Asian tick typhus is a tick-borne infection caused by *Rickettsia sibirica*, a spotted fever group (SFG) rickettsia. The rickettsiae are maintained in nature by transovarian transmission in the tick host and transient horizontal transmission involving various mammalian hosts [1]. Human infections occur when persons are bitten by infected ticks. First described by scientists in the USSR in 1935, North Asian tick typhus is recognized in Russia, Kazakhstan, Girgizistan, Mongolia and China [2]. Although *R. sibirica* has been recovered from ticks in a particular location, such as Pakistan, human infections may not be recognized and diagnosed [3]. *Rickettsia sibirica* has been isolated from humans and ticks in Inner Mongolia, Xinjiang and Heilongjiang, but few cases are diagnosed by medical personnel [4–8]. In order to evaluate the intensity and geographic distribution of SFG rickettsial infection in Inner Mongolia, a study of humans, domestic animals, wild rodents and ticks was conducted in all five vegetational zones of the province during the period 1988 through 1990.

METHODS

Study design

Twelve counties were selected representing each of five dominant vegetation types and were divided into three investigation levels (Table 1, Fig. 1). These counties are representative of Inner Mongolia Autonomous Region, a political

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Table 1. *Study design of SFG rickettsial ecology and epidemiology in Inner Mongolia*

County	Vegetation type	Rainfall*	Frost-free days	Study level†
Azuo	Desert	120	130–170	1
Damao	Steppe	126	120	1
Siziwang	Steppe	310	99–120	2
Zhalute	Grassland	388	102–140	1
Kailu	Grassland	343	156	2
Huolinguole	Grassland	354	80	3
Balinyuo	Forest-grassland	358	124	1
Keshiketeng	Forest-grassland	386	72–120	2
Zhalantun	Forest-grassland	455	126	3
Ezuo	Forest	450	60–80	1
Yakeshi	Forest	373	60–90	2
Elunchun	Forest	500	100	3

* Average annual rainfall (mm).

† Study level 1: complete collection of human blood for rickettsial isolation and SFG serosurvey, animal blood for serosurvey, tick seasonal variation, haemolymph survey for rickettsiae, rickettsial isolation and climatologic data; Study level 2: collection of fewer samples and omission of tick season variation investigation; Study level 3: serosurveys of healthy humans and animals.

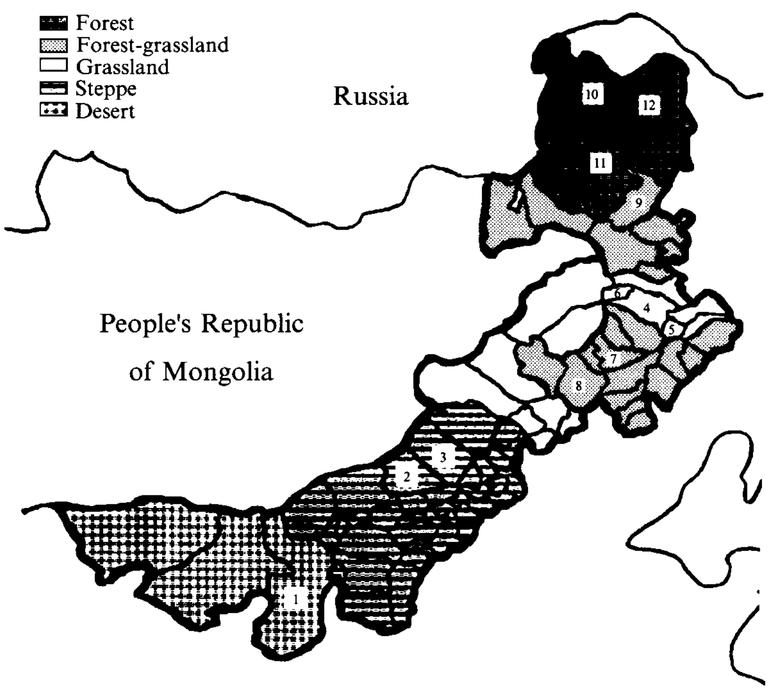

Fig. 1. Map of Inner Mongolia demonstrating the five vegetational zones and the 12 investigated counties: (1) Azuo; (2) Damao; (3) Siziwang; (4) Zhalute; (5) Kailu; (6) Huolinguole; (7) Balinyuo; (8) Keshiketeng; (9) Zhalantun; (10) Ezuo; (11) Yakeshi; (12) Elunchun.

area equivalent to a province containing a recognized minority population. Inner Mongolia occupies a plateau in northern China bordering Mongolia and Russia. The mean temperature in January is −20 °C to −10 °C and in July 17 °C to 20 °C.

Serologic assay

Sera were collected from 50–100 healthy persons in each county during each year from 1988 through 1990. In each level 1 and 2 study county, sera were also collected at slaughterhouses in winter from 30–50 sheep, cattle, goats and camels. Sera were collected from 30 wild rodents in level 1 counties. Sera were assayed for antibodies to SFG rickettsiae by the micro-complement fixation (CF) assay [9] with *R. sibirica* (strain 246) antigen and positive control serum (1:128) purchased from the Chinese National Institute of Microbiology and Epidemiology (Changping, PRC). Sera reactive at a titre of 8 or greater were considered positive.

Rickettsial isolation

Blood collected from 49 persons with a history of recent tick bite and aliquots of triturated pools of 30–50 ticks of a particular area and species were inoculated intraperitoneally into guinea-pigs. Guinea-pig sera were collected before inoculation and 28 days after inoculation to test for seroconversion.

Haemolymph test

A drop of haemolymph from an amputated tick leg was placed on a glass slide, dried, heat-fixed, stained by the Giemsa method and examined microscopically for rickettsiae.

Seasonal variation of ticks

At 10-day intervals, ticks were collected by handpicking from five goats or, in the desert area only, camels and counted until no ticks were found on two consecutive occasions.

RESULTS

Evidence of human infection

Human infections were documented in 9 persons among 49 subjects with history of recent tick bite. Although guinea-pigs inoculated with the blood of persons from each vegetation area (steppe, 3 persons; desert, 3 persons; forest, forest-grassland and grassland, 1 person each) developed anti-*R. sibirica* CF antibodies ($\geqslant 256$), none of these rickettsial strains were established by continuous passage in the laboratory.

Sera from 1261 (48·86%) of 2581 healthy subjects contained antibodies to *R. sibirica*. There was statistically significant variation in the prevalence of seropositivity and geometric mean titres from year to year in different vegetation zones (Table 2), and in the same vegetation zone in different counties (data not shown).

Evidence of SFG rickettsial infections of animals

A high proportion of sera of domestic animals and wild rodents contained antibodies to *R. sibirica* indicating that SFG rickettsiae are widely distributed in the ecosystems of Inner Mongolia (Fig. 2). Overall, antibodies to SFG rickettsiae

Table 2. *Prevalence of antibodies to SFG rickettsiae among persons in Inner Mongolia, 1988–90*

Vegetation zone	No*	1988	1989	1990	Total
Desert	438	23† (34)‡	78 (84)	39 (33)	43 (51)
Steppe	532	20 (12)	66 (31)	42 (13)	39 (19)
Grassland	398	66 (30)	75 (36)	39 (17)	58 (28)
Forest-grassland	766	49 (28)	56 (13)	47 (52)	51 (25)
Forest	447	67 (23)	58 (12)	51 (57)	55 (22)

* No, number of subjects tested.
† Percentage of sera with CF titre ⩾ 8.
‡ Geometric mean titer of positive sera.

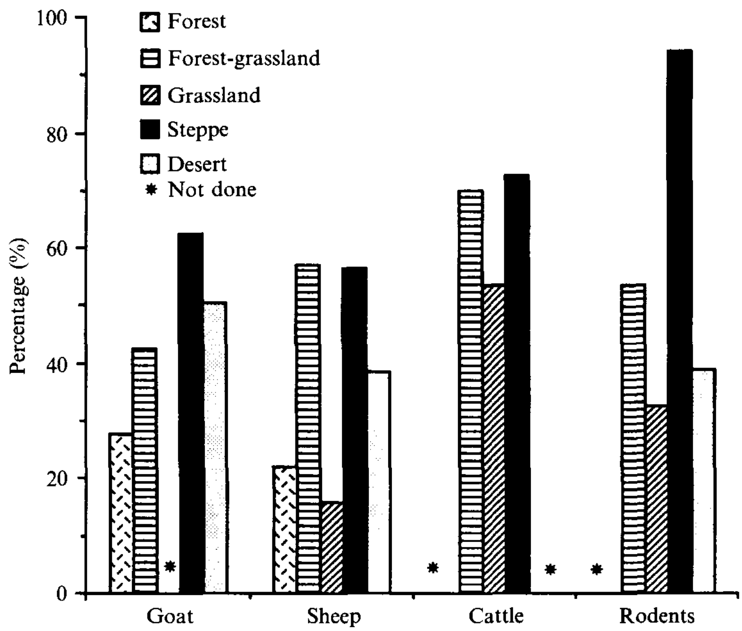

Fig. 2. Prevalence of antibodies to goat, sheep, cattle and rodents in the five vegetational zones of Inner Mongolia.

were detected in 198 (47 %) of 425 goats (GMT, 40), 218 (68 %) of 322 cattle (GMT, 40), and 312 (47 %) of 664 sheep (GMT, 34). Antibodies were present in 37 (32 %) of 116 camels (GMT, 25) in the desert area. Antibodies to SFG rickettsiae were present in the sera of 69 (53 %) of 129 *Citellus undulatus* rodents in the forest-grassland areas, 38 (33 %) of 116 *C. undulatus* in the grasslands zones, 48 (94 %) of 51 *Meriones unguiculatus* in the steppe areas, and 14 (39 %) of 36 *M. unguiculatus* in the desert area.

Evidence of R. sibirica *in ticks*

Guinea-pigs inoculated with 18 (23 %) of 77 pools of *Dermacentor nuttallii* and 7 (24 %) of 29 pools of *Hyalomma asiaticum* developed antibodies to *R. sibirica*. With 30–50 ticks per pool, these results indicate that at least 0·5–1 % of these ticks contain SFG rickettsiae capable of stimulating an antibody response in guinea-

Table 3. *Comparison of detected prevalence of rickettsiae in ticks by haemolymph test and guinea-pig inoculation*

Vegetation zone	No*	Guinea-pig inoculation†	No*	Haemolymph test‡
Desert	817	7/29 (24)	494	3
Steppe	571	7/20 (35)	219	12
Grassland	530	4/13 (31)	267	4
Forest-grassland	830	4/19 (21)	1192	11
Forest	420	3/15 (20)	ND§	ND

* No, number of ticks collected and examined.
† Proportion of tick pools stimulating seroconversion to *R. sibirica* (%).
‡ Percentage of ticks containing stained rickettsia-like organisms.
§ ND, no data available.

pigs, presumably because they are pathogenic. The haemolymph test detected rickettsia-like organisms in 3–12% of ticks in different vegetation zones and different years (Table 3). *Hyalomma asiaticum* was the prevalent tick in the desert area, and *D. nuttallii* was the principal tick in the forest, forest-grassland, grassland and steppe regions.

Peak of tick activity was observed in Balinyuo County (forest-grassland) in early April, Zhalute County (grassland) in late April and early May, in Damao county (steppe) in late March to mid-April, and Azuo County (desert) in late April and early May. All of the peaks were directly related to a rise in relative humidity.

DISCUSSION

Rickettsia sibirica was shown to be a prevalent organism throughout Inner Mongolia, where humans and domestic animals are highly exposed to this tick-borne rickettsia. Other studies of humans in Inner Mongolia have shown a lower prevalence of antibodies to *R. sibirica* than the 49% observed in this study by CF test although the assays are not all the same, e.g. 11% by CF in Abagnar in 1958 [10] and 26·1% by IFA in Zhalute, Balinyuo and Donwu counties in 1988 [11]. Farm workers in the Mongolian People's Republic also had lower seroprevalence rates reported in 1984, ranging from 2·6–10·6% in different locations [2]. Because antibodies are detected by the CF test during convalescence from SFG rickettsial infection but generally do not persist indefinitely, it would appear that a high proportion of the population of all vegetation zones of Inner Mongolia has been infected with *R. sibirica* or other SFG rickettsiae in the not so remote past. Further evidence for an intense exposure to SFG rickettsiae-infected ticks is the consistently high seroprevalence of antibodies in cattle, sheep, goats and camels. Rickettsiae were detected also in substantial portions of *D. nuttallii* and *H. asiaticum* ticks. Transovarian transmission is the most important factor in the maintenance of *R. sibirica* in nature. However, horizontal transmission from larval or nymphal ticks to mammals such as *Citellus* and *Meriones*, which develop rickettsemia and serve as the source of infection to other feeding larval or nymphal ticks, may also be an important factor in maintenance and periodic increases of rickettsiae in the ecosystem.

Russian scientists have studied SFG rickettsial ecology carefully since the 1930s [2]. The ecology of the Primorye region, which has been investigated extensively, is quite different from that of Inner Mongolia. However, the steppes of Russia and Mongolia have some similarities to the SFG rickettsial ecology of Inner Mongolia, including the dominant tick vector, *D. nuttallii*.

This work contributes to the knowledge of SFG rickettsioses in the desert area of Asia. *Hyalomma asiaticum* is the dominant tick host of SFG rickettsiae in the Inner Mongolian desert. High prevalences of antibodies to SFG rickettsiae in humans, goats, sheep, camels and *Meriones* rodents in desert foci indicate that this environment is capable of supporting the hosts of these rickettsiae. Whether the SFG rickettsiae in the desert areas include *R. sibirica* or other species in addition to the novel species described by Yu and colleagues [12] remains to be determined. Whether the unnamed *Rickettsia* sp. isolated from *H. asiaticum* ticks in Inner Mongolia causes human infection and illness also is unknown at this time.

The enigma of why North Asian tick typhus is diagnosed clinically so infrequently in Inner Mongolia in the face of such a high prevalence of infection remains an important, unsolved problem. Spotted fever group rickettsioses are generally relatively mild in young patients [13–15]. With such a high prevalence, it is possible that most persons are infected at an early age and are diagnosed as having a viral exanthem such as rubella, measles or enteroviral infection. These self-limited illnesses might differ little from a mild attack of *R. sibirica* infection. This emphasises the need to examine the patient's entire skin surface including scalp and pubis for an eschar or maculopapular rash. Because some patients with SFG rickettsioses lack the eschar or rash, it behoves the physician to keep rickettsial infection in the differential diagnosis of all febrile patients exposed to ticks during the season of tick activity. Even the mildly ill patient's course is ameliorated by a short course of tetracycline.

Ultimately, it should be determined whether the human infections following tick bite and the high seroprevalence are caused by *R. sibirica*, the *H. asiaticum* SFG rickettsia or other *Rickettsia* species. Although isolation and identification of the rickettsiae from patients is the gold standard, this goal has been achieved by contemporary standards for only two human isolates of *R. sibirica* from Russia and three human isolates from China [8, 16–18]. An alternative approach that could elucidate the identity of the SFG rickettsial pathogen(s) of humans in Inner Mongolia would be development of a monoclonal antibody-based, epitope-blocking assay for species-specific antibodies to *R. sibirica* and for the *Rickettsia* species recovered from *H. asiaticum* as has been established for *R. rickettsii* [19]. The geographic limits of known rickettsioses such as *R. sibirica* and the full list of human SFG rickettsioses might then be more completely evaluated.

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