Clinical characteristics of *Scrub Typhus* in Gedu and Mongar (Bhutan)

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Abstract

Scrub typhus caused by Orientia tsutsugamushi is an acute, mite-borne, febrile illness that occurs in the Asia-Pacific region. Bhutan falls under scrub typhus endemic region but diseases was not under the scan of physician although lot of undifferentiated fever was reported across the country. The surveillance on undifferentiated fever in Gedu hospital has confirmed the presence of scrub typhus. The confirmation was base on pair samples antibody titer by IFA from five patients although all most all samples from other patients also had moderately high antibody titer against scrub typhus. The clinical characteristics of scrub typhus cases (confirmed and probable) are also in concordance with findings of studies conducted on clinical characteristics of confirmed scrub typhus cases by other endemic countries.

1. Background

Scrub typhus is a rickettsial disease caused by Orientia tsutsugamushi, which is transmitted to humans through infected chigger mites. Scrub typhus is widely distributed and endemic in Southeast Asia and the Pacific Rim covering 13,000,000 km2 area (Figure 1). The scrub typhus is transmitted by the Leptotrombidium deliense mite. The reservoir hosts are rodents mainly including Rattus losea, R. flavipectus, and Apodemus agrarius. Transmission of the etiological agent to the rodent host or the human incidental host occurs during feeding of the parasitic larval or "chigger" stage of mites primarily of the genus Leptotrombidium. Vertical or transovarial transmission appears to be essential to the maintenance of the infection in nature; thus, the mite serves as both the vector and the reservoir. The vectors can be found in a variety of ecological conditions, from the mountainous regions of northern India to the tropical climates of the Malay Peninsula and

Indonesia. To date, there have been 120 antigenically distinct strains reported, including the initially characterized prototypic strains Karp, Gilliam, and Kato.

Rickettsia is transmitted through the bite of an infected mite to human, it begins to proliferate at the bite site and a characteristic skin lesion, known as an eschar, is formed. The pathogen then spreads systemically via the hematogenous and lymphogenous routes. Infected people develop various systemic symptoms and reactions including fever, cutaneous rash, lympadenopathy, elevations of C-reacting protein (CRP) and liver enzymes. Human cases caused by scrub typhus have common clinical features including fever, cutaneous rash, eschar and local lympadenopathy and the associated complications were typically severe. Doxycline is the drug of choice but even chloramphenicol also dramatically and effectively eliminate mortality among cases recognized early enough for initiation of treatment. Thus, the effort to produce an effective vaccine was not given priority primarily because of the existence of effective, rapid-acting antibiotic treatments. However, the need for greater emphasis on prevention by an effective vaccine has been prompted by increasing evidence that suggests the presence of antibiotic refractility and the difficulty in diagnosing a disease that mimics several other febrile illnesses.

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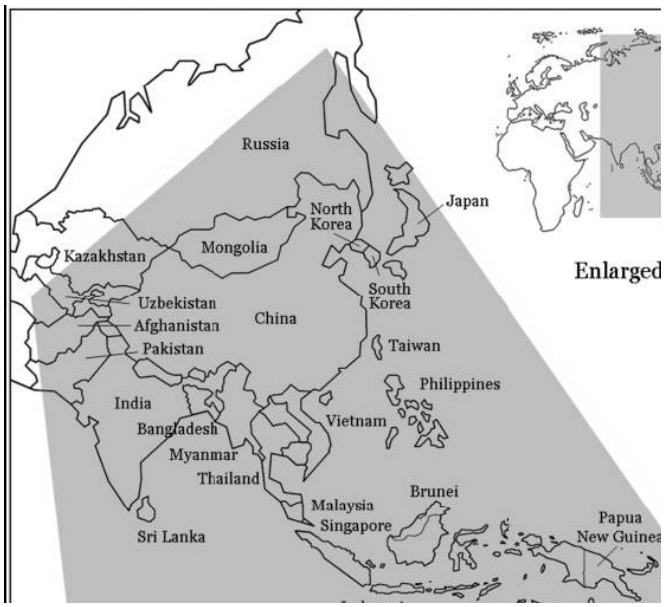


Figure 1: Map of the area where scrub typhus is endemic.

Bhutan falls under endemic belt (Figure 1) but the evidence of presence of scrub typhus was not established until recently after Public Health Laboratory has kept surveillance in Gedu hospital since March 2009 following number of PUO reported in 2008.

2. Methods

2.1 The study site

PHL has kept surveillance from March 2009 in Gedu hospital following number of PUO reported and referred to JDWNRH in 2008. The Mongar cases were coincidental.

2.2 Criteria for case selections

Any patients who visits hospital presenting acute undifferentiated fever and clinical manifestations indicative of scrub typhus. Acute and convalescent blood samples were obtained from few patients but only single sample was obtained from most patients. All samples were sent to Wellcome Rust-Mahosot Hospital- Oxford University – Mahosot Hospital – Tropical Medicine Research Collaboration (Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR) for laboratory diagnosis.

2.3 Confirmatory diagnosis

For each suspected case, the serum was assayed by IFA for detecting the IgM and IgG antibodies against *O. tsutsugamushi* and *Rickettsia typhi* antigens. A confirmed scrub typhus case was defined as (1) IgG titer ≥ 4-fold increase in paired serum specimens; and (2) IgM titer ≥ 1:80 or IgG titer ≥ 1:400 in a single serum sample.

2.4 Data collection

Data were collected in sample collection form sent by PHL to Gedu hospital although it was not designed to capture clinical details. However, form sent to Mongar had provision to capture clinical details. Detail information of patients from Gedu hospital was taken out from investigation report of CPO, VCDP. The data included for analysis was (1) demography: age, sex, (2) epidemiologic data: symptom onset date, residence place, (3) clinical signs and symptoms: fever, eschar, rash, lymphadenopathy, and others; (4) routine hematological

examination: white blood cell (WBC) counts, platelet (PLT) counts, red blood cell (RBC), hemoglobin (HGB) and hematocrit (HCT).

3. Results

3.1 Confirmed cases of scrub typhus

PHL had sent 33 samples in total to reference lab from which five patients had paired samples. Based on IFA examination result, 23 patient samples had high titer of IgM and IgG antibody against scrub typhus and 12 samples against murine typhus. In addition, 6 patients had primary dengue infection (Table 2). Since all positive samples had high titer against both IgM ang IgG, the results were inconclusive to confirm whether it is an acute infection or history of past infection. However, from paired samples results from 5 patients; 1 patient was confirmed to have suffered from primary dengue, scrub and murine typhus infection, 1 patients from acute infection against both scrub and murine typhus and 3 patients from acute murine typhus infection (Table 1). The interpretation was based on fourfold or greater rise of antibody between paired serum specimens. The serotypes of all IFA-positive samples were not done in this study.

Table 1: IFA results for paired samples

Case	Age/Sex	1st Sample				2nd Samples				Interpretation
		Scrub antibody (End titer)		Murine antibody (End titer)		Scrub antibody (End titer)		Murine antibody (End titer)		
		IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	
1	7/F	800	800	3200	1600	800	400	800	800	Acute murine typhus infection
2	23/F	400	400	3200	1600	400	400	>3200	>3200	Acute murine typhus infection
3	7/F	<400	1600	800	1600	3200	800	>3200	>3200	Acute scrub and murine infection
4	7/F	<400	400	<400	800	3200	800	>3200	3200	Acute scrub and murine infection
5	7/F	400	400	800	800	800	800	>3200	>3200	Acute murine typhus infection

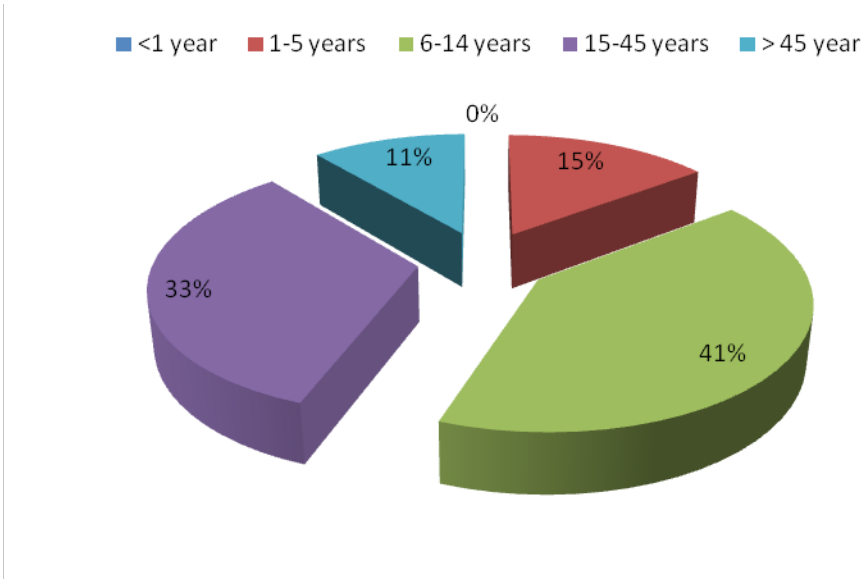
Table 2: IFA results for 23 patients

Demography			Dengue ELISA Results		Murine typhus				Scrub typhus				Interpretation
					1:3200		End point		1:3200		End point		
Case #	Age	sex	NS1 results	Infection status	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	
1	6	F	Negative	**Primary Dengue**	pos	pos	3200	3200	pos	pos	>3200	>3200	Prob acute scrub typhus inf
2	1.4	F	Negative	Negative	neg	neg	800	400	pos	pos	>3200	>3200	Prob acute scrub typhus inf
3	3.3	F	Negative	**Primary Dengue**	neg	neg	<400	<400	pos	pos	3200	3200	Prob acute scrub typhus inf
4	36	M	Negative	Negative	neg	neg	1600	800	pos	pos	3200	3200	Prob acute scrub typhus inf
5	40	F	Negative	Negative	neg	neg	<400	800	pos	pos	>3200	>3200	Prob acute scrub typhus inf
6	3.3	M	Negative	Negative	neg	neg	800	<400	pos	neg	>3200	3200	Prob acute scrub typhus inf
7	6	M	Negative	Negative	neg	neg	800	400	pos	pos	>3200	>3200	Prob acute scrub typhus inf
8	6	M	Negative	Negative	neg	neg	800	<400	neg	pos	800	>3200	Prob acute scrub typhus inf
9	4	M	Negative	**Primary Dengue**	pos	neg	>3200	800	pos	pos	>3200	>3200	Prob acute scrub typhus inf
10	33	F	Negative	Negative	neg	neg	<400	<400	pos	pos	>3200	3200	Prob acute scrub typhus inf
11	28	F	Negative	Negative	neg	neg	<400	<400	neg	pos	800	3200	Prob acute scrub typhus inf
12	11	M	Negative	Negative	neg	neg	neg	800	pos	pos	3200	>3200	Prob acute scrub typhus inf
13	17	F	Negative	Negative	neg	neg	<400	<400	neg	pos	800	3200	Prob acute scrub typhus inf
14	7	F	Negative	Negative	neg	neg	<400	<400	neg	neg	800	1600	Prob acute scrub typhus inf
15	33	F	Negative	Negative	neg	neg	<400	<400	neg	neg	800	1600	Prob acute scrub typhus inf
16	57	F	Negative	Negative	neg	neg	<400	400	neg	pos	800	>3200	Prob acute scrub typhus inf
17	66	F	Negative	Negative	neg	neg	<400	<400	neg	pos	<400	>3200	Prob past scrub typhus inf
18	10	M	Negative	Negative	neg	neg	<400	<400	neg	neg	<400	800	Prob past scrub typhus inf
19	14	F	**Positive**	**Primary Dengue**	neg	neg	<400	<400	neg	neg	400	800	Prob acute scrub typhus inf
20	50	M	Negative	**Primary Dengue**	neg	neg	<400	<400	neg	pos	800	>3200	Prob acute scrub typhus inf
21	18	M	Negative	Negative	neg	neg	<400	<400	neg	neg	<400	<400	No evidence of acute typhus inf
22	11	M	Negative	Negative	neg	neg	<400	<400	neg	neg	<400	<400	No evidence of acute typhus inf
23	29	F	Negative	Negative	neg	neg	<400	<400	pos	pos	>3200	>3200	Prob acute scrub typhus inf

3.2 Sex and age distributions of cases

Of 28 cases, 57% (16/28) were female patients and 43% (12/28) were male, respectively. Most cases were among aged group 6-14 years (41%), followed by 15-45(33%) (Figure: 2). The youngest and oldest cases were 1.4 and 66 years respectively.

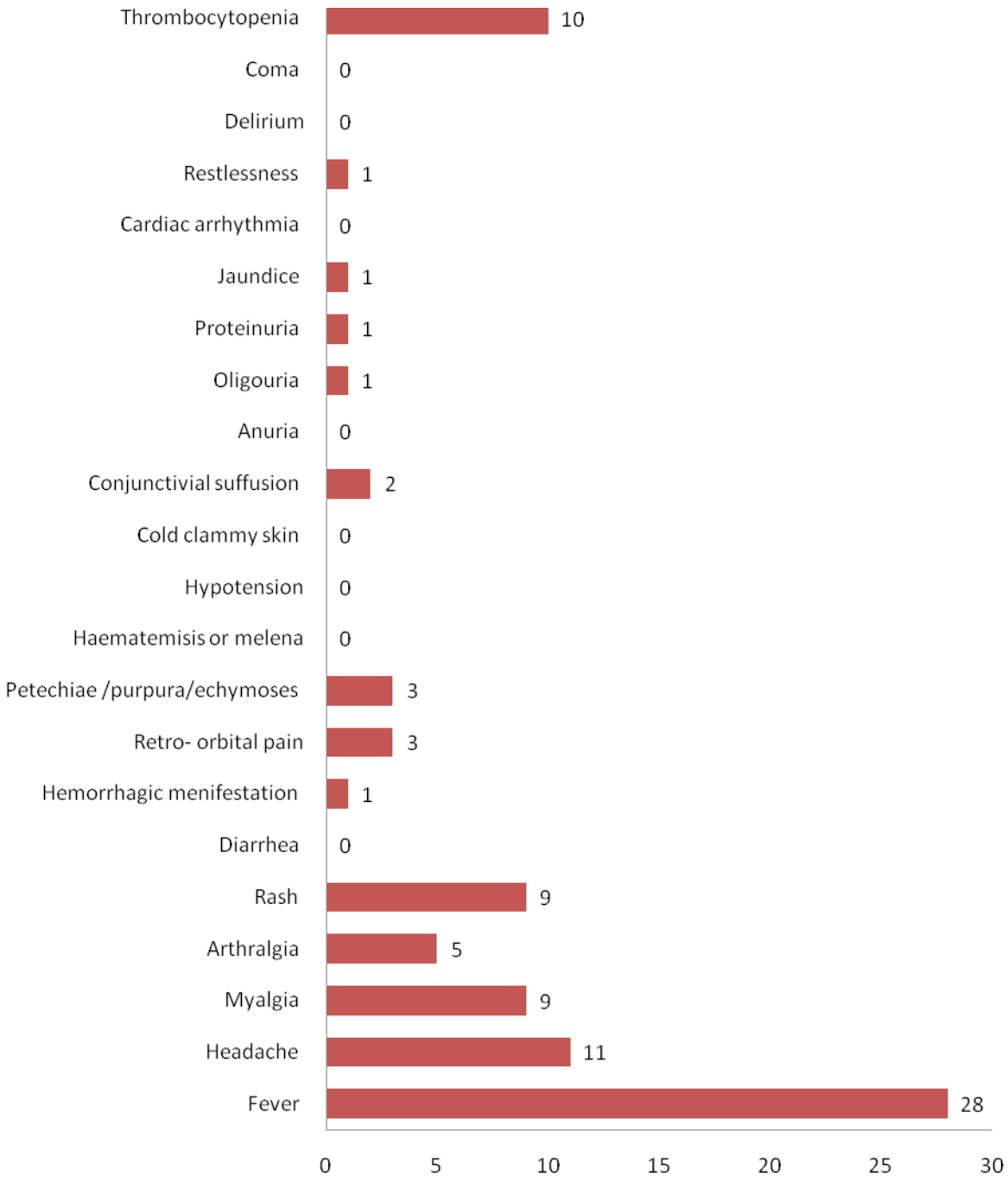
Figure: 2 Age distribution



3.3 Clinical symptoms

Figure 3 summarize the signs and symptoms of all 28 cases presented during OPD visit and at the time of admission to hospitals. The cases manifested with the common clinical signs and symptoms of scrub typhus: fever (100.0% of cases), headache (39.0%), myalgia/arthalgia (45%), rash (32%), eschar (21%), retro-orbital pain (10%), petechiae/purpura/echymoses (10%), regional lympadenopathy (10%) and thrombocytopenia (40.0%) were observed respectively. Hepatosplenomegaly (7%) were also reported.

Figure 3: Clinical signs and symptoms



3.4 Location of eschars

Of 28 probable scrub typhus cases, 21% (6/28) exhibited eschar; 5 with one eschar and 1 with multiple eschar. Eschars were found in axilla (4/6), upper arm (1/6) and below breast (1/6).

3.5 Routine hematological examinations

Hematological examinations of the case (n=17) was done at the time of OPD and admission. Both normal and abnormal (e.g. increased or decreased counts) hematological parameters were recorded. Normal results were observed in 82.3% (WBC counts), 35.2% (PLT counts), 88.2% (RBC counts), 64.7% (HGB), and 47.0% (HCT) of cases, respectively, while 17.6%, 64.7%, 11.7%, 35.2%, and 52.9% of cases showed increased or decreased counts for each of these categories, respectively.

4. Discussion

Laboratory results provide evidence that patients from Gedu and Mongar had scrub and murine typhus infection (Table 2). The results of pair samples collected from 5 patients during acute and convalescent has confirmed the acute stage of scrub and murine typhus infection (Table 1). However, rickettsial serology results from other samples suggest that the moderately high titres apparently against *R. typhi* (causing murine typhus) were due to scrub typhus infection because murine typhus (epidemic typhus) is usually more severe and it cause neurological symptoms including meningoencephalitis and delirium, and coma can occur in 50% of cases which is not seen among the patients from Gedu and Mongar. Moreover, secondary infections by *R. typhi* can result in myocarditis, bronchopneumonia or gangrene. Since background immunity against rickettsial diseases in the country is unknown and there are inadequate data on the longevity of IgM and IgG against *O. tsutsugamushi* and no data on the longevity of IgM and IgG against *R. typhi* after infection (Blacksell *et al.* 2007). It is therefore possible that the high titre against *O. tsutsugamushi* is indicative of recent infections or acute disease in line with 2 acute scrub typhus confirmed cases. However, to define true acute disease, quantification of antibodies against rickettsial diseases in well people would help to establish appropriate cutoffs for IFAs among Bhutanese population.

In this surveillance, occurrence periods for scrub typhus cases was not documented however, all cases have been recorded during the months of June - July (summer). However, it will be too

early to comment on seasonal variation at the moment. Studies in China and Korea found that the seasonal variations of scrub typhus increase in October and peak in November. This pattern was, however, different from those reported in Japan. In Japan, a bimodal pattern of occurrence of cases – one in spring and another in autumn-winter was reported. Because humans are infected through bites of the larva of the chigger mites, seasonal variations in scrub typhus infections may be in part due to seasonal fluctuations of the larval chigger mites as well as their rodent hosts. In Japan, the bimodal pattern of occurrence of cases was reported to relate to population dynamics of the two different species of chigger mites.

With limited number of cases, age and sex distribution among the cases holds no significance for the current scrub typhus disease study. However, data shows that most affected age group is 6-14 years (41%) and higher prevalent among females (57%) which could be due to occupation related although occupation of cases have not be recorded (Figure 2).

Based on data collected from patients about clinical symptoms of scrub typhus, common reported signs and symptoms presented by patients are high persistent fever, headache, rashes, mylagia, arthalgia, retro-orbital pain and thrombocytopenia (Figure 3). Some cases are associated with regional lymphadenopathy and hepatomegaly. Besides above signs and symptoms, electrocardiogram abnormity, hepatosplenomegaly, and some severe complications such as toxic myocarditis, alimentary tract hemorrhage, pleural fluid, or abdominal dropsy were reported in studies conducted in China and Japan.

Eschar formation is one of the common phenomenon in scrub typhus and t*he distribution of eschar on body surface might be associated with dressing styles and personal hygiene, as the two factors affect how and where chiggers entered and stayed on the body surface.* Common site for echar formation among population in the country will be known once proper surveillance and study is in place. *Irons et al (1947) reported that 45% of confirmed eschars in US army personnel were detected on the feet and legs. Perineum, inguinal area, and axilla were also the preferentially eschar-manifested areas. Kim et al (2007) reported that among the 162 adult scrub typhus cases in southwestern area of Korea, most cases had eschars on the front of the body. Eschars were primarily detected in*

males within 30 cm below the umbilicus. Yet a different pattern was seen in females – the most prevalent area in females was the front chest above the umbilicus.

In addition, PLT count (64.7%) and HCT (*52.9%)* abnormalities is mostly commonly seen among the patients (*64.7%)*, while WBC and other CBC counts are not common abnormalities (WBC- *17.6%, RBC- 11.7%, and HCB - 35.2%). It is very evident that because of abnormal PLT count and HCT level observed in most patients, clinicians mis-diagnose case as dengue fever due to low awareness of the presence of scrub typhus in the country. In scrub typhus, HCT is not a common abnormal blood parameter seen but higher HCT abnormal rate seen among the scrub typhus could be due to primary dengue infection, dual infection seen in 6 patients. Dual infection phenomenon is not uncommon among diseases especially in different disease endemic places.* Like dengue fever and DHF, scrub typhus can be clinically confirmed base on CBC results without waiting for serology or other confirmatory test. In scrub typhus cases, high transaminase (ALT) level (2 folds), thrombocytopenia and leucoytosis is common and positive predictive value is 85%. The positive predictive value further increases if eschar is observed in patient.

Scrub typhus caused by *O. tsutsugamushi has three strains Gilliam, Karp, and Kato. Like dengue strains, secondary infection is usually severe in form if the second time infection is caused by different strain. The reason is not known at the moment but it could be probably due to immune-pathogenesis. Strains typing need to done to know the presence of strains in the country and its epidemiology.*

The drug of choice for scrub typhus is doxycyline and chloramphenicol is also found effective. Both antibiotic drugs are bacteriostatic and its mode of action is inhibits protein synthesis by preventing attachment of aminoacyl transfer RNA to its acceptor site on the ribosome (30S and 50S subunit ribosome). The drug is very effective if administer during early time of infection because drugs can stop multiplication of bacteria and prevent dissemination to major organs. Ampicillin and amoxicillin are found ineffective for scrub typhus in Gedu hospital because they are effective only in gram negative bacteria while *O. tsutsugamushi* is gram positive bacteria and intercellular organism.

The limitation of current surveillance is lack of adequate data collection, lab tests and samples size. More studies will be required to understand the clinical presentations, geographic distribution, seasonality, animal reservoir(s), vector and different subspecies that transmit these rickettsial diseases in the country.

5. Conclusion

This surveillance establishes that scrub typhus and other rickettsial diseases are not uncommon in Bhutan and should be considered as emerging new diseases of high public health importance and should be kept in the differential diagnosis of acute febrile illness. *The clinical characterizations of scrub typhus cases from two places (Gedu and Mongar) also fall in line with clinical characterization studies conducted in China and other endemic countries. These findings will provide valuable information in assisting physicians in the diagnosis, treatment, and prevention of the disease.*

References

1. *Kelly et.al, Scrub Typhus: The geographic distribution of phenotypic and genotypic variations of Orientia tsutsugamushi, CID 2009:48 (Suppl3).*
2. *Yun-Xi Liu et.al, Clinical characteristic of the autumn-winter type scrub typhus cases in south of Shandong province, northern China, BMC Infectious Diseases 2009, 9:82.*
3. *Varghese et.al, Scrub typhus among hospitalized patients with febrile illness in South India: magnitude and clinical predictors, British Infectious Society 2005.*
4. *Senthayanon et.al, Association of high Orientia tsutsugamushi DNA loads with disease of greater severity in adults with Scrub typhus, Journal of Clincial Microbiology, Feb 2009.*
5. Interim Report to the Ministry of Health, Royal Government of Bhutan on a series of 38 sera from patients with fever, Wellcome Trust-Mahosot Hospital-Oxford University-Mahosot Hospital-Tropical Medicine Research Collaboration, Microbiology Laboratory, Mahosot Hospital,Vientiane, Lao PDR, V6.10.2009.