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Demographic and Clinical Profiles of PCR Positive *Rickettsia felis* Infected Patients at A Tertiary Care Hospital in Bangladesh

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Abstract

Background: Rickettsial infections remain relatively unexplored, under recognized and under reported due to a lack of awareness and limited access to diagnosis. **Objective:** The purpose of the present study was to evaluate the demographic and clinical profile of PCR positive *Rickettsia felis* infected patients attended at a tertiary care hospital. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh from July 2013 to June 2014 for duration of one year. Patients with fever (102° to 104°F) more than 15 days of any age and gender, not responding to commonly used antibiotics and any other additional complaints and clinical features including headache, rash, lymphadenopathy or myalgia and eschars on the skin were included in this study. Patients with evident cause of fever like malaria diagnosed by blood smear or immunochromatography were excluded from the study. Blood was collected according to blood collection guidelines. PCR from blood was performed using standard protocol with specific primers to detect Rickettsia up to species level. **Results:** A total number of 50 respondents were recruited after fulfilling the inclusion and exclusion criteria. Among them 21(42%) cases were PCR positive. Among 21 PCR positive cases, the predominant age group was more than 15 to 30 years which was 11(52.4%), female patients were predominant than male patients which was 11(52.0%). All the cases with Rickettsial infection had fever 21(100.0%). Other associated features like headache was most commonly noticed in 10(47.6%) cases followed by body-ache, Cough and rash which was 7(33.3%) cases, 5(23.8%) cases and 1/21 (4.7%) cases respectively. **Conclusion:** This study demonstrated high prevalence of *Rickettsia felis* infection in patients in Bangladesh with unidentified febrile illness and suggests that this infection is endemic to the north-central area of this country. Females are more prone to get infected and most commonly noticed associated features was headache.

Keywords: Rickettsial Fever; *Rickettsia felis*; Rocky Mountain spotted fever; epidemic typhus; Zoonotic disease; PCR

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Introduction

Rickettsia has a universal distribution but the epidemiology of Rickettsial diseases and their health impacts differs according to geographical region[1]. *Rickettsia* comprises a group of microorganisms that phylogenetically occupy a position between bacteria and viruses[2]. They are a genus of non-motile, Gram-negative, non-spore forming, highly pleomorphic bacteria that can present as cocci (0.1 µm in diameter), rods (1 to 4 µm long) or thread-like (10 µ

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m long). Being obligate intracellular parasites, *Rickettsia* cannot live in artificial nutrient environments and grow either in tissue or embryo culture typically, chicken embryos are used[3]. The name *Rickettsia* honors Howard Taylor Ricketts for his brilliant experiments in 1909 Howard Taylor Ricketts first described small organisms that appeared to be associated with the disease Rocky Mountain spotted fever (RMSF)[3]. This was the first report of the organism *Rickettsia*. Rickettsiae are associated with arthropods, which may act as vectors, reservoirs and amplifiers in the life cycles of the bacteria. Ticks are the main vectors and reservoirs of SFG *rickettsiae*. The main arthropod reservoir and vector of *Rickettsia felis* is the cat flea. The expansion of *Rickettsia felis* hosts and potential vectors to include mites, lice and ticks further highlights the infancy of the field[4]. The animal hosts are dogs, cats, rodents, opossums, horses, sheep, goats and monkeys. The expansion of *Rickettsia felis* hosts and potential vectors to include mites, lice and ticks further highlights the infancy of the field. The animal hosts are dogs, cats, rodents, opossums, horses, sheep, goats and monkeys[5].

Man is an accidental host except for louse borne epidemic typhus. Transmission to humans occurs by infected arthropod vector or exposure to infected animal reservoir (Rodents, dogs and many more). Vector to human transmission occurs as vector defecate while feeding like flea feeding reflex so that feces contaminate pruritic bite wounds seen with typhus fever group or primarily by bite, where regurgitation of infected saliva occurs during feeding. They are not transmissible directly from person to person except by blood transfusion or organ transplantation. The influencing factors for transmission of *Rickettsial* diseases are overcrowded situations like prison, refugee camps and during wars etc. along with very poor hygienic conditions[3]. Increased travel may have played a role in spreading these flea-associated pathogens in recent decades as travelers and their accompanying[4]. The risk of developing *Rickettsia felis* infection was 1.6 times higher during the rainy season than during the dry season[6]. In Africa, from 2007 to 2008, the *rickettsial* seroprevalence among febrile patients was 28.0% to 58.0%. In the same period, *Rickettsia felis* infections was identified in 8.0% of febrile cases in Tanzania and 6.0% in rural Senegal[3]. The epidemiology and clinical picture of this emerging infection in the rest of Africa is largely unknown[6]. In India, during 2005 to 2009, rickettsial sero-positivity was 33.3% cases [7]. Rickettsial infections detected were scrub typhus 48.2% followed by spotted fever group 27.5% and typhus group 6.8% cases[7].

Rickettsia survival depends on entry, growth and replication within the eukaryotic host cells, typically endothelial cells. The Rickettsial diseases develop after infection through skin and incubation period ranges from 3 to 14 days[3]. From the portal of entry in the skin, rickettsiae spread via the bloodstream to infect the endothelium and sometimes the vascular smooth muscle cells. *Rickettsia species* enter their target cells, multiply by binary fission in the cytosol, and damage heavily parasitized cells directly, causing hyperplasia of the endothelial cells and thrombus formation, which leads to obstruction of blood flow and escape of red blood cells into the surrounding tissue. Papules develop when inflammatory cells migrate into the tissue. Necrosis in the center of the papule causes the typical clinical sign of rickettsial infection, the eschar. The pathologic effects of rickettsial diseases originate from the multifocal areas of endothelial injury and vasculitis with loss of intravascular fluid into tissue spaces (edema), resultant low blood volume, reduced perfusion of the organs and impaired function of the tissues with damaged blood vessels like encephalitis, pneumonitis, and hemorrhagic rash[8]. Early signs and symptoms of rickettsial infections are nonspecific and mimic viral illnesses, making diagnosis more difficult[2].

Rickettsia felis, which belongs to the spotted fever group of rickettsiae, causes febrile illness in humans. The main vector of this bacterium is the cat flea (Ctenocephalides felis). *Rickettsia felis* infection in humans worldwide has been increasingly described, especially in the Americas, Europe, Africa, and eastern Asia[16]. *Rickettsia felis* infection is common among febrile patients (≈15%) in tropical Africa[6] and among apparently healthy persons in eastern coastal provinces of China[17]. However, little is known about prevalence of *Rickettsia felis* infection of humans in southern Asia, although 3 serologically diagnosed cases in Sri Lanka have been described[18] and *Rickettsia felis* has been detected in rodent fleas in Afghanistan[19].

Clinical manifestations of human *Rickettsia felis* infection include fever (more than 38°C), fatigue, headache, maculopapular rash and eschar[9]. Due to shared symptoms with other rickettsial and viral infections, it is thought that many human cases are currently misdiagnosed[4]. *Rickettsia felis* infection may be confused with infection due to other rickettsial agents like *Rickettsia typhi* and some members of the

SFG, as well as other infectious diseases like dengue, malaria, brucellosis, leptospirosis or even other clinical conditions like kawasaki disease. The presence of a cutaneous eschar at the bite site is possible, although it may be infrequent. Respiratory and digestive symptoms, including cough, pulmonary edema, pneumonia, nausea, vomiting and diarrhea have been reported. Neurological signs have also been documented. Although *Rickettsia felis* infection in most cases has been observed as a mild to moderate illness, no deaths attributed to *Rickettsia felis* infection are reported in the literature[9]. To the best of our knowledge, there is no study on *Rickettsia* except very few serology based study in Bangladesh. Considering the above mentioned facts this study was designed to evaluate the demographic and clinical profile of PCR positive *Rickettsia felis* infected patients attended at a tertiary care hospital in Bangladesh.

Methodology

Study Settings and Population: This cross-sectional study was conducted in the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh. This study was carried out from July 2013 to June 2014 for duration of one year. All suspected patients of Rickettsial fever attending at outpatient and inpatient department of medicine and pediatrics unit of Mymensingh Medical College Hospital, Mymensingh were selected as a study population. Patients with fever (102-104°F) more than 15 days of any age and gender, not responding to commonly used antibiotics and any other additional complaints and clinical features including headache, rash, lymphadenopathy or myalgia and eschars on the skin were included in this study. Patients with evident cause of fever like malaria diagnosed by blood smear or immunochromatography were excluded from the study.

Study Procedure: Informed written consent was taken from all patients or their legal guardians before specimen collection. A set of questionnaire was used for each of the cases. All the relevant information like history, clinical and laboratory findings and data were systematically recorded in a pre-designed data sheet.

Laboratory Procedures: For specimen collection and storage, venous blood was collected aseptically following universal safety precaution. After wearing a sterile disposable glove, the puncture area was washed with iodine and 70.0% alcohol. Then with a sterile disposable syringe, 4 ml of blood was collected. From the blood sample, it was transferred in a tube with EDTA for PCR and immediately refrigerated at -20°C for molecular study.

Polymerase Chain Reaction: Nested PCR from blood was designed and performed by using standard protocol for the detection of the target gene of 17kDa antigen gene (17kDa protein antigen). Three major steps of PCR was performed which included DNA extraction from blood samples, DNA amplification in thermal cycler and visualization/documentation under UV light.

Table 1: Primers used for PCR of *Rickettsia-specific* 17 kDa anitigen gene

Primers	Primer sequence	Product size (bp)
Sp1	5'- GCT CTT GCA ACT TCT ATG TT -3'	434 bp
Sp2	5'- CAT TGT TCG TCA GGT TGG GG -3'	
Sp3	5'- CAT TAC TTG GTT CTC AAT TCG GT -3'	232 bp
Sp4	5'- GTT TTA TTA GGT GTT ACG TAA CC -3'	

Interpretation: Samples were scored as positive when a PCR product of 434 bp in case of 17 kDa antigen gene could be detected in first round PCR. In nested PCR, samples were scored as positive when a PCR product of 230 bp in case of 17 kDa was detected.

Observations of Polymerase Chain Reaction:

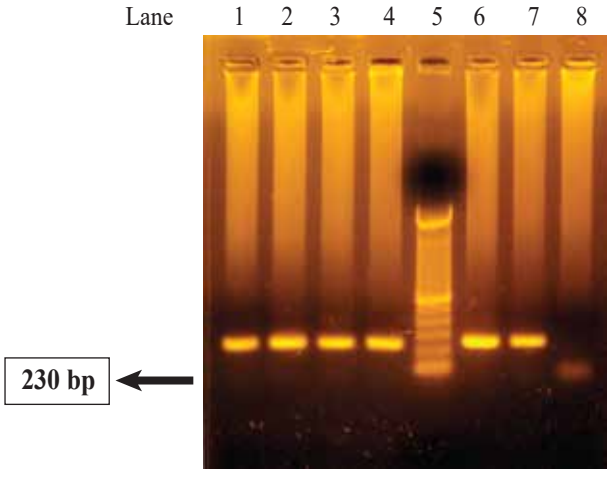

Figure I: Nested PCR product of 17kDa antigen gene (230 bp)

Nested PCR was done to detect the 17kDa antigen gene for detection of *Rickettsia*. Lane 1-4 and 6-7 showing bands of the amplified product of 230 bp regions and is indicated by the arrow. Lane 5 shows

the 100 bp ladder and 230 bp region indicates 17 kDa antigen gene. Lane 8 shows negative result (Figure I).

Observations of DNA Sequencing: PCR products from 7 samples were randomly selected and sent to the Department of Hygiene, Sapporo Medical University, Sapporo, Japan for sequence analysis. All nucleotide sequences from the 17-kDa antigen gene (230-bp) were identical to that of reference strain *Rickettsia felis* URRWXCa12 (GenBank accession no. CP000053).

Statistical Analysis: Statistical analyses were performed with SPSS software, versions 27.0 (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp.). Continuous data that were normally distributed were summarized in terms of the mean, standard deviation, median, minimum, maximum and number of observations. Categorical or discrete data were summarized in terms of frequency counts and percentages.

Ethical Consideration: Approval of the research protocol and ethical permission were obtained from the Ethics Review Committee of Mymensingh Medical College, Mymensingh, Bangladesh [ERC/MMC/2013]. All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

A total number of 50 respondents were recruited after fulfilling the inclusion and exclusion criteria. Among them 21(42%) cases were PCR positive (Figure II).

Among 21 PCR positive cases, the predominant age group was >15-30 years which was 11 (52.4%) followed by age group >30-45 years, 8-15 years and >45-80 years which was 5 (23.8%), 3(14.3%) and 2(9.5%) respectively (Table 2).

Among 21 PCR positive cases, female patients were predominant than male patients which was 11(52%) and 10(48%) respectively. The male female ratio was 1:1.1 (Table 3).

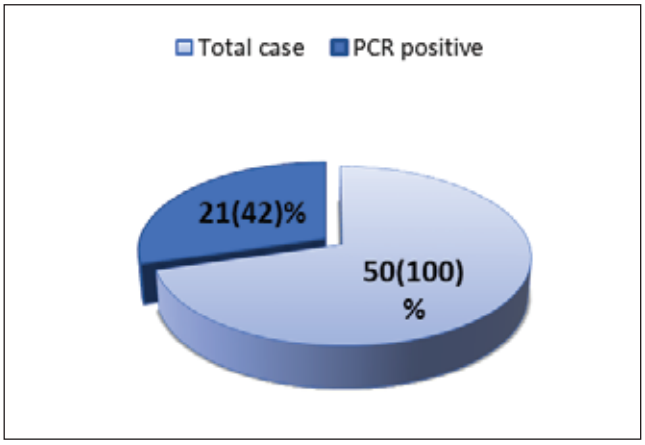

Figure II: Pie chart showing distribution of PCR positive cases

Table 2: Distribution and Percentage of PCR-positive *Rickettsia cases* according to Age Groups (n=21)

Age in years	Frequency	Percent
8 to 15	3	14.3
15 to 30	11	52.4
30 to 45	5	23.8
45 to 80	2	9.5
Total	**21**	**100.0**

Table 3: Distribution and Percentage of PCR positive *Rickettsia cases* according to Gender (n=21)

Gender	Frequency	Percent
Male	10	48.0
Female	11	52.0
Total	**21**	**100.0**

Out of 21 PCR positive cases majority were found in housewives 07(33%) followed by the students 05(23.8%), farmer 04(19%), service 03(14.3%) and day laborer 02/21 (9.5%) (Table 4).

Table 4: Distribution and percentage of PCR positive *Rickettsia cases* according to Occupation (n=21)

Occupation	Frequency	Percent
Farmer	4	19.0
Housewife	7	33.0
Student	5	23.8
Service	3	14.3
Day Laborer	2	9.5
Total	**21**	**100.0**

All the cases with Rickettsial infection had fever 21 (100%). Other associated features like headache was most commonly noticed in 10 (47.6%) cases followed by body ache, Cough and rash which was 7 (33.3%), 05 (23.8%) and 01/21 (4.7 %) cases respectively (Table 5).

Table 5: Distribution and Percentage of PCR positive *Rickettsia cases* according to Occupation (n=21)

Clinical features	Frequency	Percent
Fever	21	100.0
Fever with Headache	10	47.6
Fever with Body ache	7	33.3
Fever with Cough	5	23.8
Fever with Rash	1	4.7

Discussion

In this Study, Nested PCR selective for the Rickettsia specific 17-kDa antigen gene was used to screen for rickettsial diseases according to the method described previously. During Observations of PCR products from 7 random samples for DNA Sequence analysis, we sent them to the Department of Hygiene, Sapporo Medical University, Sapporo, Japan. All nucleotide sequences from the 17-kDa antigen gene (230-bp) were identical to that of reference strain of *Rickettsia felis* URRWXCa12 (GenBank accession no. CP000053).

The study has been conducted to evaluate the demographic and clinical profile of PCR positive Rickettsial fever. In this study 21(42%) cases were positive by PCR for *Rickettsia felis*. A previous study in Mymensingh[3] reported 69(46%) of 150 human cases were positive by PCR for *Rickettsia felis*, which is almost similar to the present study. In India, 58.6% of the clinically suspected cases of spotted fever rickettsioses were confirmed by nested PCR[10]. From Kenya[11], 3.7% cases were positive by PCR and in Senegal[12], the prevalence of flea-borne spotted fever was 4.4% cases. *Rickettsia felis* infections were identified in 8.0% of febrile cases in Tanzania[13] and 6.0% in rural Senegal[13]. The prevalence of *Rickettsia felis* infection in different countries differs considerably in terms of arthropod vectors, geographic distribution, and virulence.

In the present study, most of the positive cases of study population were in age group 15 to 30 years 52.4% and 30 to 45 years 23.8%. In Malaysia, 20 to 40 years and 40 to 60 years of age groups were more affected[14]. In India, maximum number of rickettsial infections (79.3%) was in age group of 11 to 60 years[7]. Our study corresponds with the studies mentioned.

Among 21 positive cases 11 (52.0%) were female and 10 (48.0%) were male. The male female ratio in our study was found to be 1:1.1. Similar study from Senegal reported the male female ratio of 1:1.1[6]. In Malaysia, another study reported a male to female ratio of 2:1 in 2000[14]. These findings reflect that females are more likely to get infected. Reasons for this difference are not established still now.

Out of 21 PCR positive cases majority were found in housewives 7(33.0%) followed by the students 5(23.8%), farmer 4(19.0%), service 3(14.3%) and day laborer 2/21 (9.5%). All the cases with Rickettsial infection had fever 21(100.0%). Other associated features like headache was most commonly noticed in 10(47.6%) cases followed by body ache, cough and rash which was 7(33.3%) cases, 5(23.8%) cases and 01/21(4.7%) cases respectively Rash was present only in 4.7% cases in the present study. In a previous study in Mymensingh, rash was present in 2.9% cases that was almost similar to this study. From Spain, Oteo et al[15] reported that after flea bites, rickettsial patients may present without a rash. From Asia in Thai-Myanmar border, among 7 febrile patients 4 were without any rash or eschar[16]. Seasonal activities of the vectors, reservoirs and the human behavior determine the epidemiology of Rickettsial diseases. Further study should be done in large scale throughout the country to find out the prevalence of Rickettsial infection in Bangladesh.

Conclusion

This study demonstrated *Rickettsia felis* infection in patients in Bangladesh with unidentified febrile illness. The high prevalence of *Rickettsia felis* infection suggests that this infection is endemic to the north-central area of this country and might be associated with contact between humans of low socioeconomic status and the large number of stray cats and dogs. Females are more likely to get infected. All the cases with Rickettsial infection had fever and most commonly noticed associated features was headache. The presence of *Rickettsia felis* is represents a high potential risk for public health. For confirmation of spread of this infectious disease, further study should be done in large scale throughout the country to find out the prevalence of *Rickettsia felis* infection in Bangladesh.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

Financial Disclosure

The author(s) received no specific funding for this work.

Authors' contributions
Rajib Ahmed, Muhammad Akram Hossain, Shyamal Kumar Paul- conceived and designed the study, analyzed the data, interpreted the results; Tarana Jahan, Rumana Hasan Sharmi- wrote up the draft manuscript. Rajib Ahmed, Tarana Jahan, Tanzila Rawnuck - contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Habiba Begum, Farzana Boby- involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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