

# *WELCOME*



# **Molecular characterization of Orientia tsutsugamushi causing scrub typhus among febrile patients in the north-central Bangladesh**

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# **Molecular characterization of Orientia tsutsugamushi causing scrub typhus among febrile patients in the north-central Bangladesh**

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# BACKGROUND

- ✓ Scrub typhus is an acute febrile illness, caused by *Orientia tsutsugamushi*.
- ✓ *Orientia tsutsugamushi* is a gram negative obligate intracellular bacterium transmitted by mites.
- ✓ Endemic to Asia pacific region.
- ✓ Neglected but serious public health problem.
- ✓ Threatens one billion people globally
- ✓ Causes illness in one million people each year.
-

# Cont.

- ✓ Scrub typhus restricted to *Tsutsugamushi triangle* which includes most of south and southeast Asian countries.
- ✓ Outside Asia scrub typhus also found in South America and in southern Chili.
- ✓ Seroprevalence is 40% in general population in these region.
- ✓ 20% of all febrile hospital admission and mortality is 0-70%.

# Cont.

- ✓ Enormous **genetic diversity** is a characteristic of *O.tsutsugamushi*.
- ✓ **Multiple genotypes** has been emerged on the sequence variation of **56 kDa** antigen.
- ✓ **Karp, Kato, Gilliam, Kawasaki and Kuroki** are well known genotypes.
- ✓ **New genotypes** are continuously being identified, worldwide

# Cont.

- ✓ Bangladesh is located is within the *Tsutsugamushi* triangle.
- ✓ Seroprevalence was described as 23.7%.
- ✓ There are very few information available regarding the genotypes or genetic traits.
- ✓ Clarifying genotypes and genetic diversity is essential for rapid diagnosis and development of vaccines.

# GENERAL OBJECTIVE

- ✓ To detect *O.tsutsugamushi* causing **scrub typhus** with determination of their **genotypes** and **genetic** characteristics among the febrile patients of suspected **Rickettsial illness**, in north-central region of Bangladesh (Mymensigh).

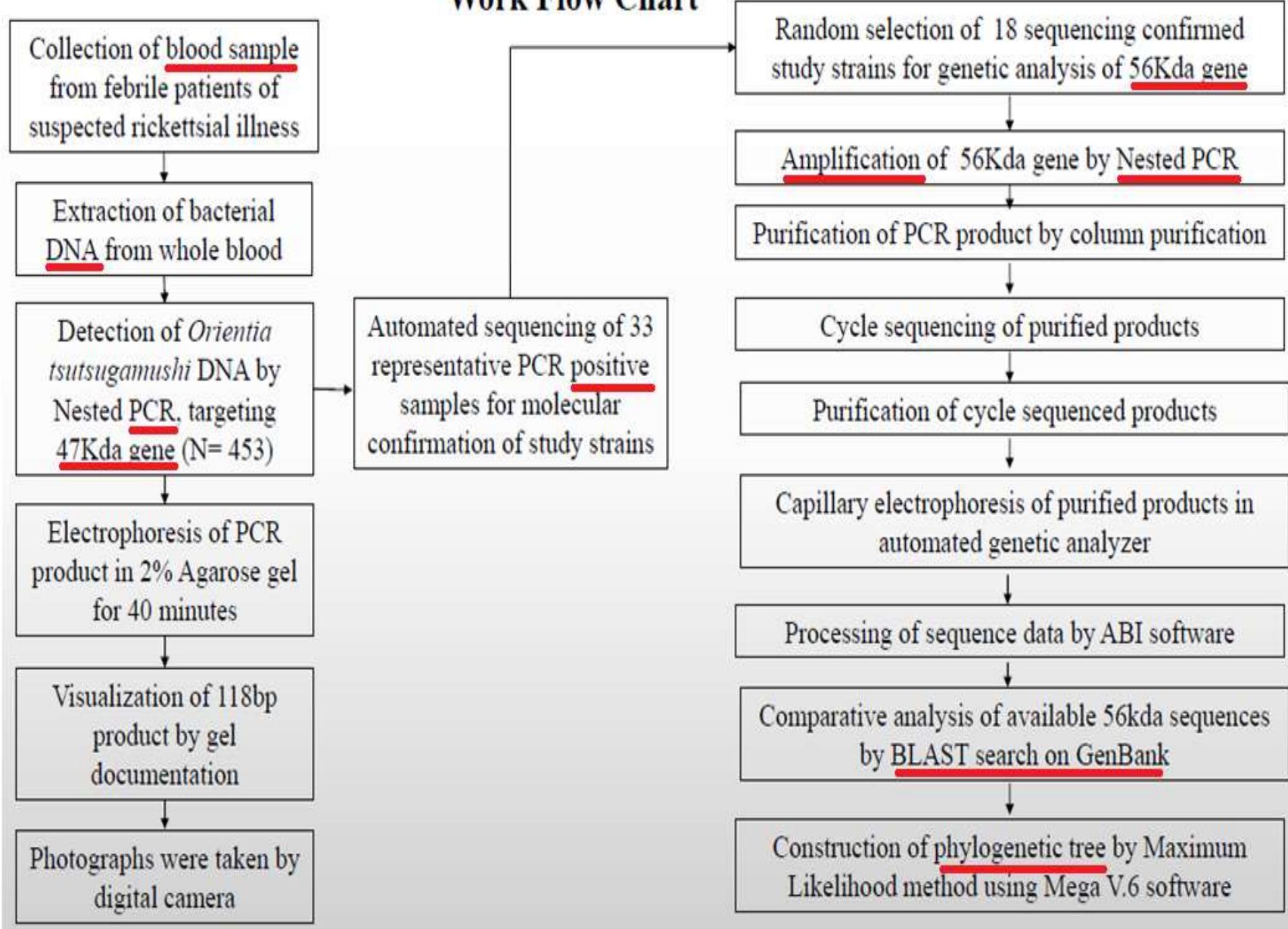
# MATERIALS AND METHODS

- ✓ Type of study: Cross-sectional type.
- ✓ Place of study: Department of Medicine and Microbiology, Mymensingh Medical College(MMC), Bangladesh.
- ✓ Study period: March 2018 to December 2018

## Cont.

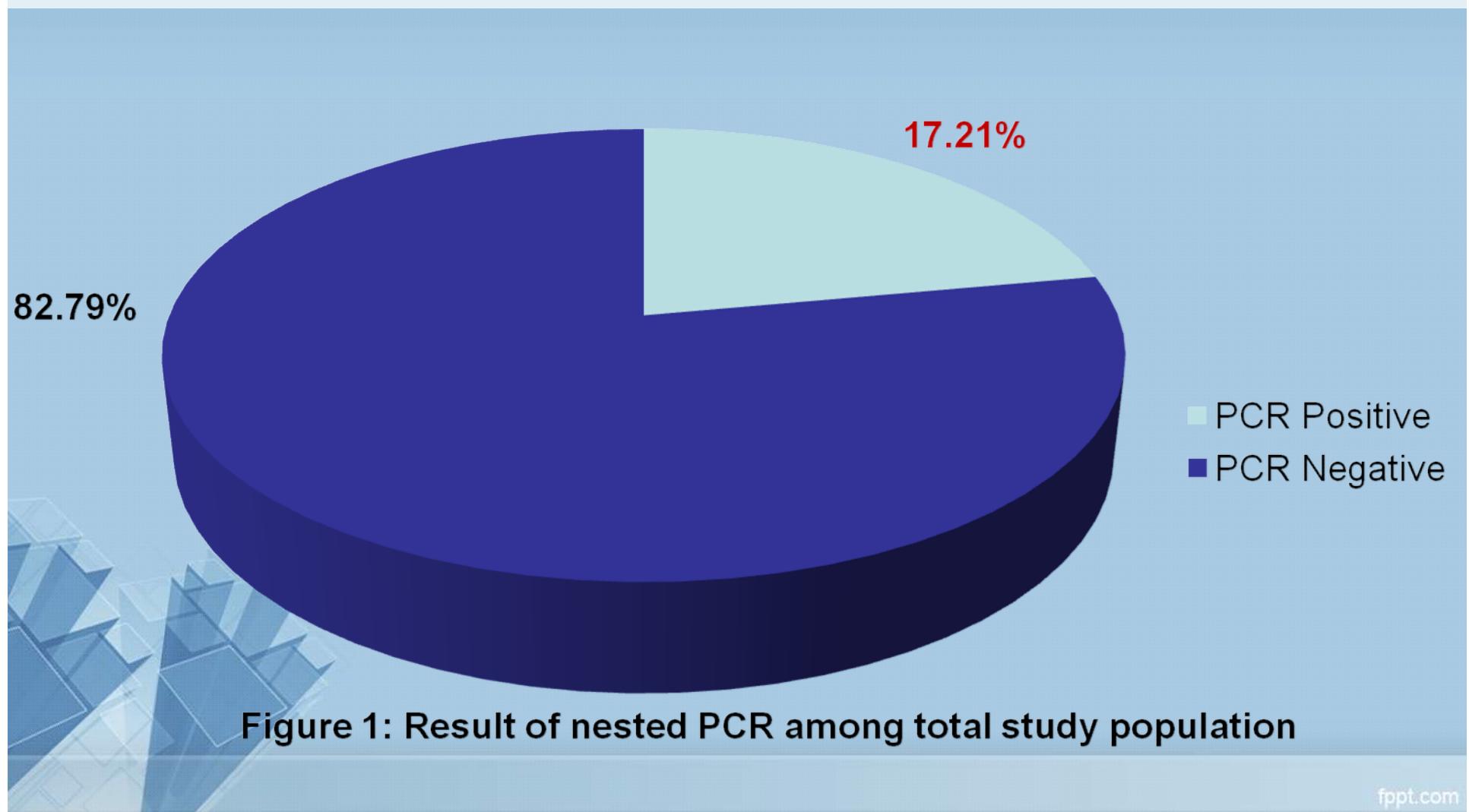
- ✓ Study population : A total of 453 Febrile patients of suspected Rickettsia illness.
- ✓ Cases were selected irrespective of age and sex with specified inclusion criteria from out patient and in patient Department of Medicine and Pediatrics, Mymensingh medical college hospital(MMCH).

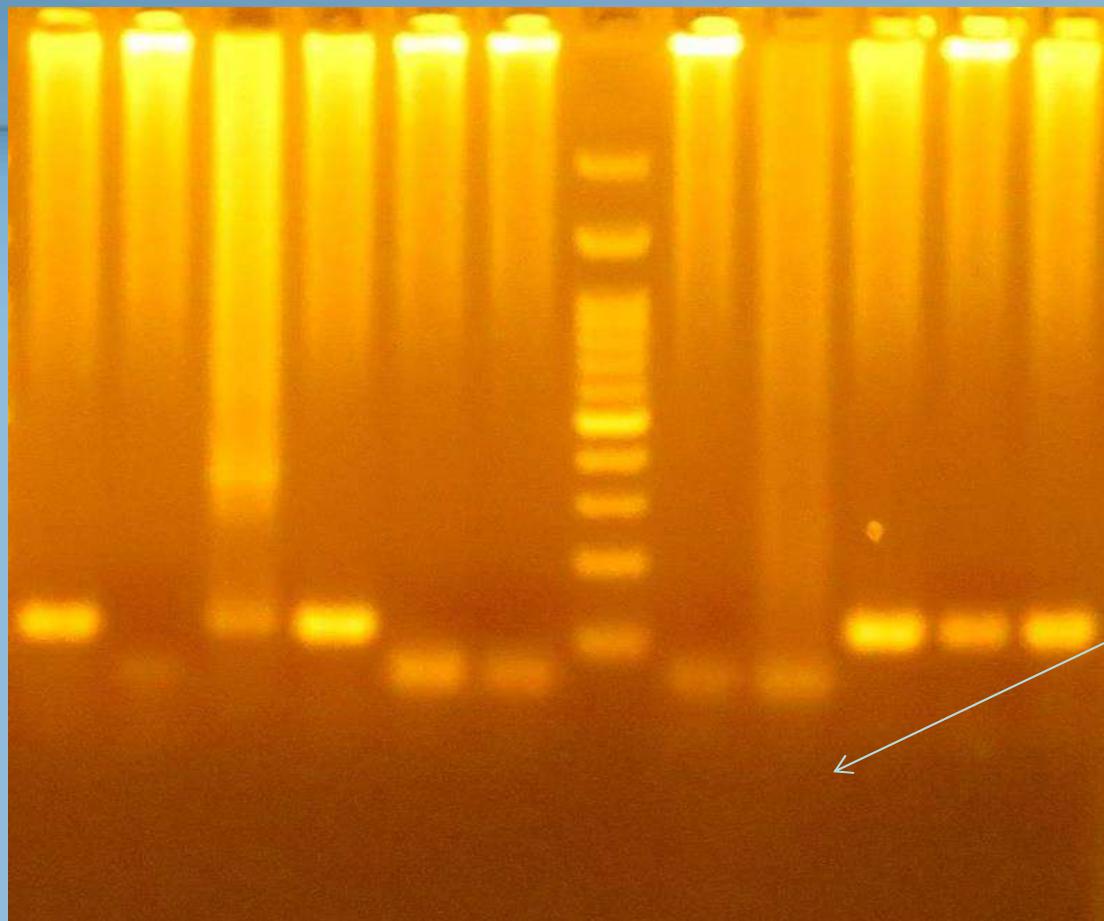
## Work Flow Chart



# RESULTS

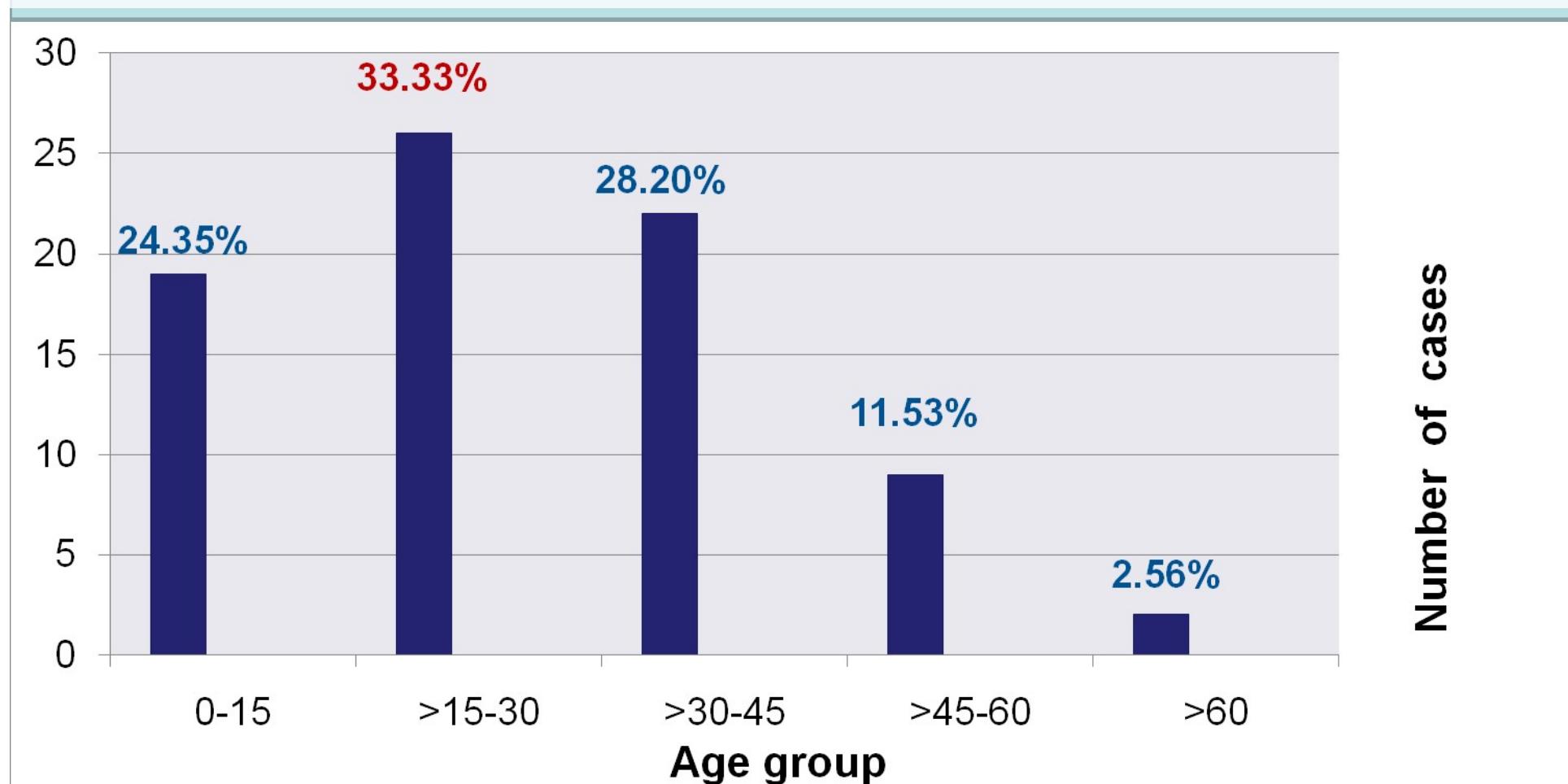
**Pie diagram showing the Result of Nested PCR among the total study population (N=453). Out of 453 samples, total 78(17.21%) were positive by Nested PCR for *O.tsutsugamushi* and remaining 375 (82.79%) were negative.**





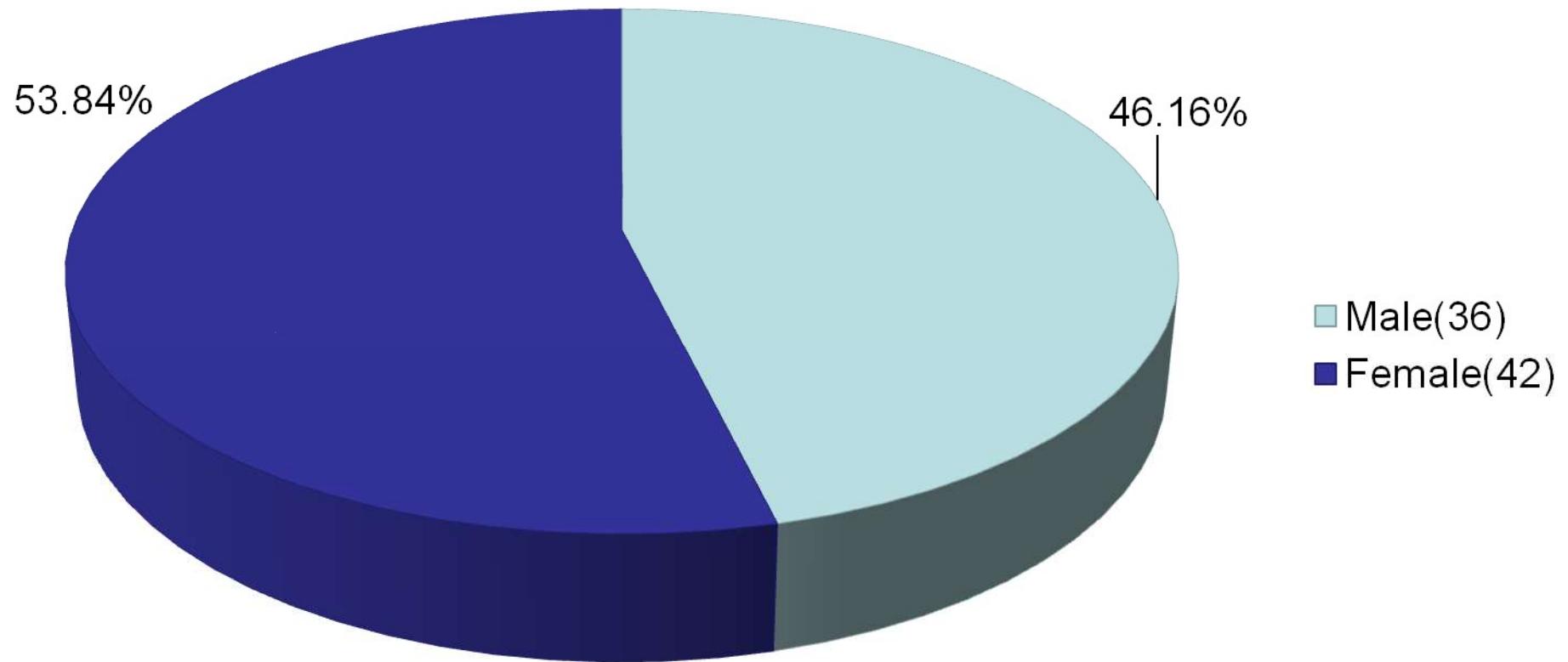
**Photograph: Visualization of 118 bp product of Nested PCR targeting 47Kda antigen gene on 2% Agarose gel**

**Bar diagram Showing distribution of PCR Positive cases according to age group (n=78). Among the 78 PCR positive cases, 26(33.33%) were in age group >15-30 years, followed by 22(28.20%) were in age group >30-45 years. Age group 0-15 years accounted for 19(24.35%) cases also.**



**Figure-2: Distribution of PCR positive cases according to age group**

**Pie diagram showing the Sex distribution of PCR positive cases (n=78). Out of 78 PCR positive cases, 42(53.84%) were female and 36(46.16%) were male. Positive cases were more in female group.**



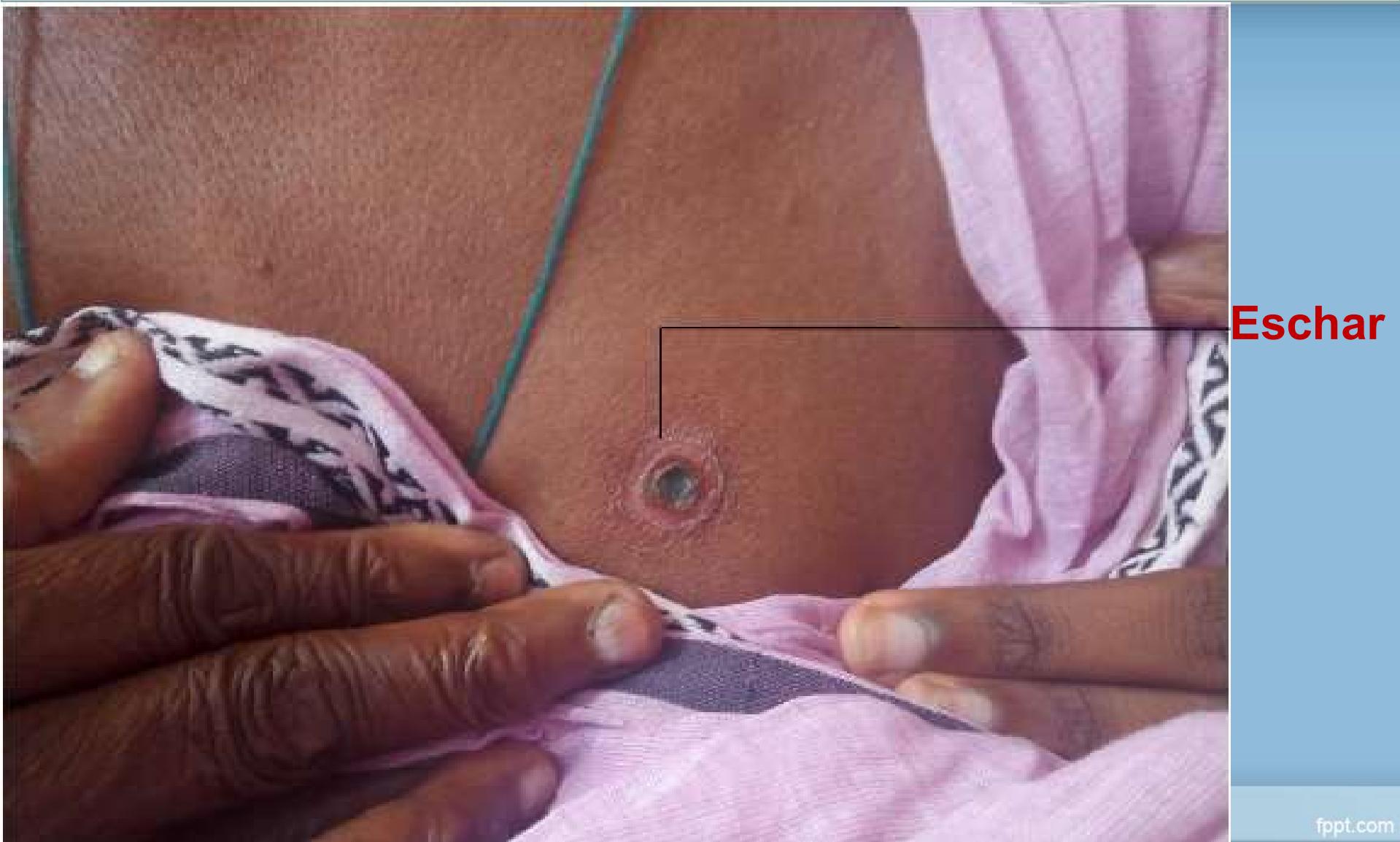
**Table-1: Distribution of PCR positive cases according to occupation (n= 78)**

<b>Occupation</b>	<b>No. of cases</b>	<b>Percentage (%)</b>
<b>Student</b>	<b>24</b>	<b>30.76</b>
<b>Housewife</b>	<b>19</b>	<b>24.35</b>
<b>Farmer</b>	<b>17</b>	<b>21.79</b>
<b>Day laborer</b>	<b>8</b>	<b>10.25</b>
<b>Unemployed</b>	<b>8</b>	<b>10.25</b>
<b>Teacher</b>	<b>2</b>	<b>2.56</b>
<b>Total</b>	<b>78</b>	<b>100</b>

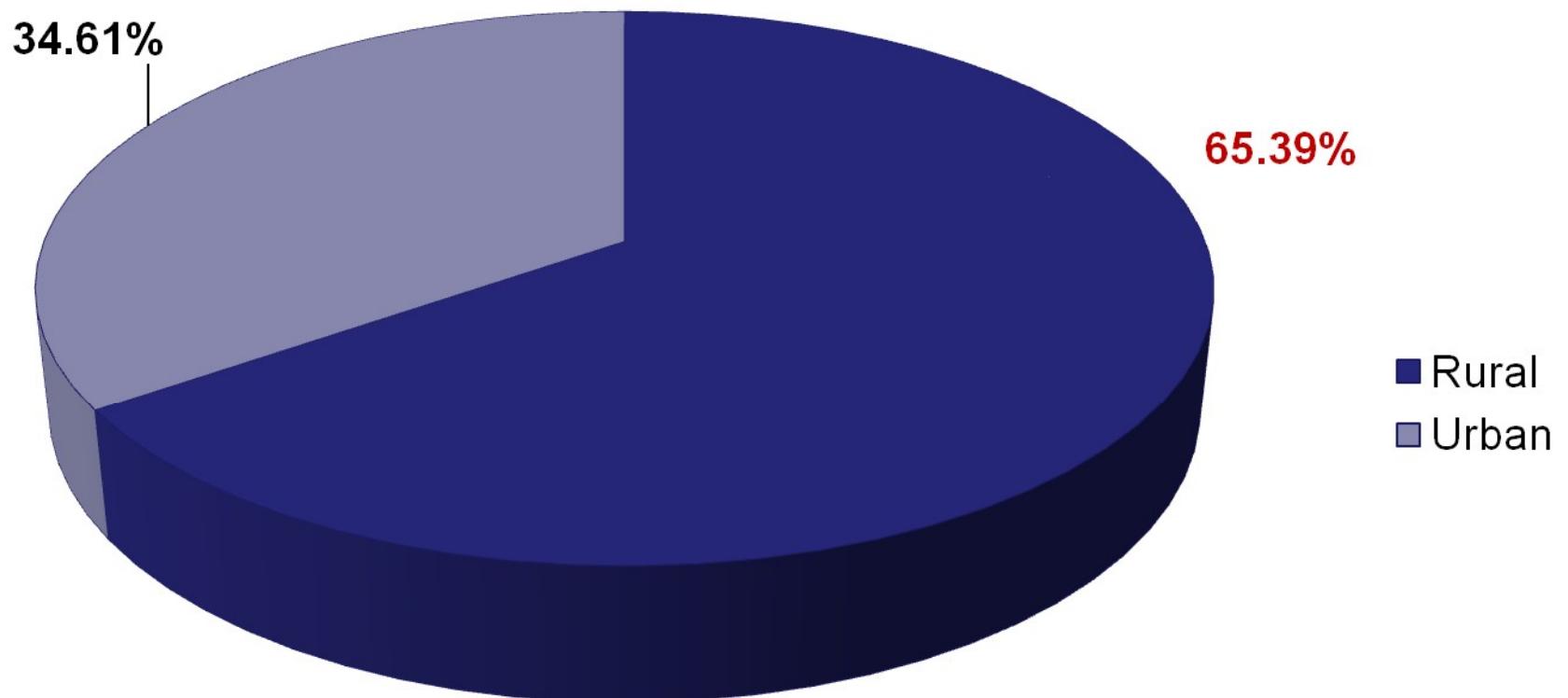
**Table-2: Clinical features of Scrub typhus among PCR positive cases (n=78)**

Clinical features	No. of cases	Percentage (%)
Fever	78	100
Myalgia	48	61.53
Headache	44	56.41
Cough	44	56.41
Eschar	14	17.94
Vomiting	14	17.94
Respiratory distress	12	15.38
Anemia	9	11.53
Skin Rash	8	10.25
Oliguria	8	10.25
Jaundice	7	8.97
Neck rigidity	4	5.12

## Photograph of typical Eschar of scrub typhus

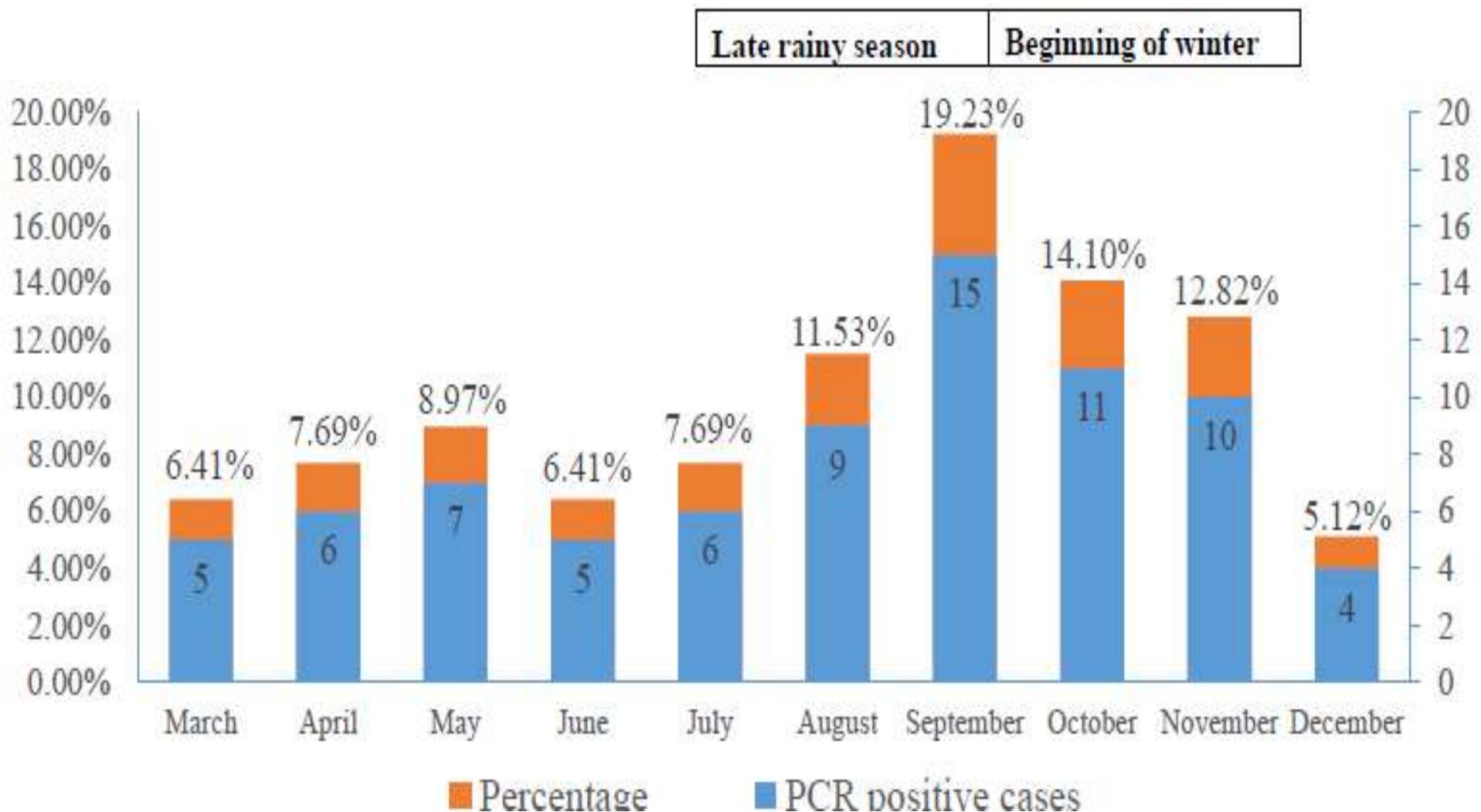


Pie diagram showing the distribution of PCR positive cases according to locality(n=78). Out of 78 PCR positive cases, 51(65.39%) were from rural areas and 27(34.61%) were from urban areas. Majority of the PCR positive cases were from rural areas.



**Figure-4 : Distribution of PCR positive cases according to locality**

Bar diagram showing month wise distribution of PCR positive cases(n=78). Number of PCR positive cases, began rise from August(9;11.53%) and reached to its maximum during the month of September(15;19.23%).The case number remained high during the month of October(11;14.10%) and November(10;12.82%) also. December accounted for the least number (4;5.12%) of PCR positive cases.

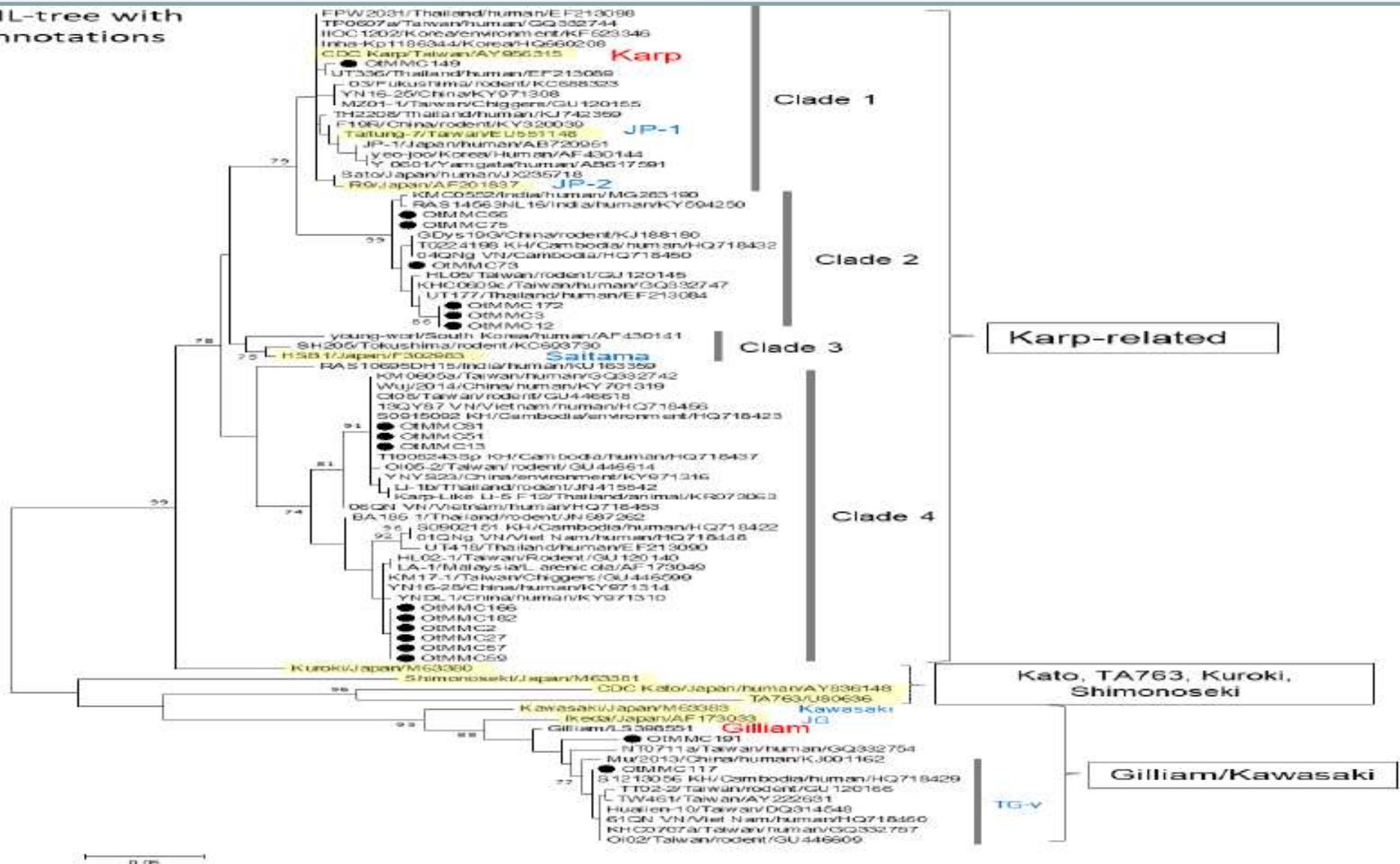


## Results of Nucleotide Sequencing

- ✓ Through nucleotide sequencing and analysis of determined sequences on **GenBank** by **BLAST** search, all the 33 (**100%**) representative PCR positive study strains were genetically confirmed to be *O. tsutsugamushi*.

**Figure-6: Phylogenetic tree of *O. tsutsugamushi* strains based on 56Kda gene sequences**

ML-tree with annotations



**Table-4: percentage of nucleotide sequence identities among representative *O. tsutsugamushi* study strains**

Ot MMC	149	166	172	182	191	66	75	2	3	12	13	27	51	57	59	73	81	117
149																		
166	93%																	
172	93%	94%																
182	93%	99%	94%															
191	76%	76%	75%	77%														
66	95%	90%	94%	90%	72%													
75	95%	90%	94%	90%	72%	100%												
2	93%	99%	94%	100%	75%	90%	90%											
3	93%	94%	99%	95%	76%	94%	94%	95%										
12	93%	94%	99%	95%	76%	94%	94%	95%	100%									
13	94%	91%	89%	91%	73%	91%	91%	91%	89%	89%								
27	93%	99%	94%	99%	75%	90%	90%	99%	95%	99%	91%							
51	94%	91%	89%	91%	73%	91%	91%	91%	89%	91%	100%	91%						
57	93%	99%	94%	100%	75%	90%	90%	100%	95%	100%	91%	99%	91%					
59	93%	99%	94%	100%	75%	90%	90%	100%	95%	100%	91%	99%	91%	100%				
73	92%	94%	98%	94%	74%	96%	96%	94%	98%	94%	88%	94%	88%	94%	94%	94%		
81	94%	91%	89%	91%	73%	91%	91%	91%	88%	91%	100%	91%	100%	91%	90%	88%		
117	79%	81%	81%	81%	90%	78%	78%	81%	81%	81%	79%	80%	79%	80%	80%	80%	78%	

**Observation:** Representative Study strains showed 72%-100% sequence identity among each other

**Table-5:nucleotide sequence identities between the study strains and prototype strains**

Prototype Strains	Study Strains (OtMMC)	Range of sequence identities between prototype and study strains (%)
Karp prototype strain (CDC Karp/Taiwan/AY956315)	Karp related Study strains	88 to 94 %
Gilliam prototype strain (Gilliam / LS 398551)	Gilliam Study strains	88 to 94 %

# DISCUSSION

- ✓ In the present study in north-central Bangladesh the genetically defined detection rate of *O. tsutsugamushi* was **17.2%**.
- ✓ In one previous study in south-east Bangladesh(Chittagong) detection rate was 1`0.9%

# Cont.

- ✓ Present series is **consistent** with study done in eastern **Taiwan**(16%), northern **Thailand**(21%) and **north-east India**(16%).
- ✓ So, **north-central Bangladesh** is also considered to be an **endemic area** of scrub typhus in south Asia.
- ✓ **Karp genotypes** found in present study is consistent with the above mentioned studies.

# Conclusion

- ✓ The present study documented a very **high**(17.21%) detection rate of *O.tsutsugamushi* by Nested PCR.
- ✓ Phylogenetic analysis demonstrated the presence of **Karp** related and **Gilliam** genotype of *O.tsutsugamushi*, with **Karp** being the predominant one.

Cont.

- ✓ A significant diversity was found among the study strains of *O.tsutsugamushi*.
- ✓ Further epidemiological study at national level is needed to ascertain the prevalence and characteristic of this pathogen for the control scrub typhus.

**Thank  
you**



