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Serosurvey of Malsoor virus among *Rousettus leschenaulti* bat & human population residing nearby Robber's cave, Mahabaleshwar, Maharashtra, India

Sir,

Malsoor virus, a novel *Phlebovirus*, was isolated for the first time from the *Rousettus leschenaulti* bats from Maharashtra, India, by National Institute of Virology, Pune, during 2010[1] . It is genetically closely related with severe fever with thrombocytopenia syndrome (SFTS) virus and heartland virus[1-4]; both these viruses are known to cause severe human diseases.

The virus was isolated from Robber's cave located at Mahabaleshwar, Satara district, Maharashtra[1]. The village Malsoor after which the virus was named is located 10 km away from Robber's Cave. The caves are occasionally visited by villagers. These caves harbour large colonies of fruit bats *R. leschenaulti* (approximately 23,000) and insectivorous microchiropteran bat *Miniopterus schreibersii* (approximately 4200). Besides these species, Rufous horseshoe bats (*Rhinolophus rouxii*) have also been spotted in small numbers[5].

Among the genus *Phlebovirus*, in the *Bunyaviridae* family, 12 viruses have been linked to disease in humans including Rift Valley fever, heartland virus and SFTS virus. Recently, a novel *Phlebovirus* was isolated from Turkey named Adana virus against which seropositivity (0.7%) was recorded among human population[6]. Similar results were observed in Tunisia for Punique virus, where human samples (0.4%) were positive for anti-Punique IgG antibodies[7].

During the earlier study in 2010[8], of the 69 bats collected from Robber's cave, 19 bats were found to be positive for Malsoor virus by nested reverse transcriptase polymerase chain reaction (nRT-PCR) and sequencing. Malsoor virus is genetically closely related to SFTS (S, M and L segments sequences have revealed that these viruses have maximum homology at nucleotide level 64.0%>60.16%> 59.13%> 50.23% for L>M>N> NSs segments) which causes disease in human. Looking at the disease-causing potential of *Phlebovirus*[9,10], a preliminary investigation was conducted during 2014-2015 among human population residing adjacent to Robber's caves to determine the seroprevalence of Malsoor virus if any. This study was important due to lack of data about this novel virus and its disease-causing potential in human. Prior permission and approval of Institutional Human Ethical Committee and Animal Ethical Committee were obtained to initiate this study. During 2014-2015, blood samples (3-5 ml) of 174 participants were collected from three villages within a radius of <5 km from Robber's cave (Malusar wadi, Malusar village and Chikhali). Tapola village was included as a control village which was 22 km away from the caves. Details of the human population investigated are mentioned in the Table. Among all the participants, only three persons had a history of epistaxis. Besides these, three persons had a history of fever. No other relevant major illnesses were reported.

Indigenous anti-Malsoor IgG ELISA assays for screening of bat and human serum samples were developed to determine the presence of Malsoor virus among bat and human population. ELISA plates (Nunc Maxisob plates, Thermofisher Scientific, USA) were coated with Gamma-irradiated Malsoor virus antigen[11] (Row A to D) and *Vero CCL-81* cell (ATCC, USA) control antigen (Row E to H) in carbonate buffer (*p*H 9.2, 0.025 M) overnight at 4°C. Subsequently, wells were blocked [1 h incubation with wash buffer containing 2% bovine serum albumin (BSA)]. One hundred microlitres of diluted sample (1:100 in 1% BSA in 1× phosphate-buffered saline containing 0.1% Tween) was added to duplicate wells of Malsoor virus and control antigen-coated wells and incubated at 37°C for one hour. One hundred microlitres of anti-human IgG horseradish peroxidase (HRP)

Table. Characteristics of populations investigated for evidence of Malsoor virus infection, Western Ghats, Maharashtra State, India

Name of village	Population	Distance from the caves (km)	Nature of contact of the villagers	Samples collected	Relevant clinical information
Malusar Wadi	150	<1	Go around the caves for wood picking regularly.	40	1 adult (65 yr) and two children (11 and 14 yr) had a history of bleeding through the nose during last three months. Two people with low-grade fever.
Malusar village	120	2.5	Visit the cave regularly every 2-4 wk for worshipping the idol placed close to the entrance of the caves, though rarely enter the caves.	17	One person with history of low-grade fever during last one month.
Chikhali	260	5	Visit the caves for idol worshipping every 4-8 wk.	42	-
Tapola (control village)	750	22	Rare or no contact with the concerned caves.	75	-
Total	1280			174	

antibodies (Thermofisher Scientific) (1:10,000) were added and further incubated one hour at 37°C. After each incubation, the ELISA plate was washed four times with wash buffer. One hundred microlitres of (3,3',5,5'-Tetramethylbenzidine (TMB) substrate was added and incubated for 10 min. The reaction was stopped by 1N H_2SO_4 and absorbance was measured at 450 nm. As positive human serum sample against Malsoor virus was not available, triplicates of substrate control and negative control (normal human serum) were used for optimization of ELISA. The cut-off for ELISA was calculated using sufficient numbers (50-100) of negative samples from the population. The cut-off was calculated as mean OD of the negative control plus 3 standard deviations. For anti-Malsoor bat IgG ELISA, anti-bat IgG HRP conjugate (1:8000) was used. Bat sample positive for Malsoor virus by nRT-PCR was used as positive control for optimization of anti-Malsoor Bat IgG ELISA.

R. leschenaulti bats were captured using appropriate tools. Further, necropsies of bats were carried out in field laboratories under appropriate biosafety conditions as described earlier[1]. Serum samples were used for screening of IgG antibodies.

Of the 51 *Rousettus* bat blood samples (2 ml each) collected during the current study, serum samples of 47 bats were tested for IgG antibodies depending on the availability of serum. Twenty five bat serum samples were found to be positive for anti-Malsoor IgG antibodies (57%). Of the 174 human blood samples tested for Malsoor virus-specific IgG antibodies, none was found positive. Samples of three febrile illness cases were also tested for Malsoor and Dengue virus by nRT-PCR[12] and found to be negative for both[8].

To assess future threats posed by zoonotic viruses of bats, there is a need for proactive surveillance among bat populations from different areas. This was a pilot study for detecting Malsoor virus IgG antibodies among human and bat population in a specific area nearby to Robber's cave. The presence of Malsoor virus and anti-Malsoor virus IgG antibodies in bat population in Mahabaleshwar and major distribution of *Rousettus* bats in different States of India suggest possible risk to humans. Indigenously developed serological tests for detecting Malsoor virus antibodies could be used to determine the prevalence of this virus among bats and human population from different parts of the country.

Though all human samples collected from Malusar wadi, Malusar village and Chikhali villages were found to be negative for Malsoor virus-specific IgG antibodies, there could be antibodies present in the samples but remained undetected by ELISA. Therefore, the negative results do not rule out the possibility of occasional spill over to humans.

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***Conflicts of Interest*:** None.

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