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# Serosurvey of Crimean Congo Haemorrhagic fever (CCHF) among high risk group during 2011 and 2013 CCHF outbreak in Gujarat, India

KEYWORDS: Crimean-Congo haemorrhagic Fever (CCHF), seroprevalence, outbreak, Infectious disease, at risk population.


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**ABSTRACT** *Seroepidemiological study was conducted during the outbreak of Crimean-Congo hemorrhagic fever (CCHF) in village Kolat (January- February 2011) and village Karyana (July 2013) in Gujarat, India. Study was conducted to know the seroprevalence of CCHFV among at risk individuals which includes family contacts of CCHF case, household neighbourhood contacts, animal handlers and health care staff in the CCHF affected area. Detection of CCHF virus specific IgM and IgG antibodies were performed by using a commercial kit, Vector BEST Company. Sera from acute fever cases were screened for anti CCHFV IgM antibodies and for detection of CCHFV by Real Time RT-PCR. Anti-CCHFV IgG antibodies were not detected from village Kolat and 10 neighbouring villages of Kolat in January 2011 in Ahmedabad district. However, anti CCHFV IgG antibodies were detected in an animal handler from Ahemdabad. In year 2013, overall seroprevalence of 1.9% (5/261) was reported from Karyana village and 3.0% (1/33) from neighbouring Khambala village in Amreli District. This is the first serosurvey study in India performed in CCHF affected villages in Gujarat, which reported very low seropositivity of CCHF infection in the high-risk individual in the region, suggesting very low asymptomatic infection. The seroprevalence data in this study may be useful for public health to determine appropriate CCHFV interventions and preventive measures to be undertaken in the area where people are living in close proximity with the animals.*

**Introduction**

Crimean-Congo hemorrhagic fever (CCHF) is a severe acute febrile illness caused by the CCHF virus (CCHFV). This virus belongs to family *Bunyaviridae*, genus *Nairovirus*, and often causes severe viral haemorrhagic fever with overall case fatality of 9–50% (Chumakov MP, 2007). The disease is endemic in many countries and is a potentially fatal viral infection, found in parts of Africa, Eastern Europe and the Middle East (Hoogstraal H. 1979; Ergonul O. 2006). The virus is maintained in nature predominantly in *Ixodid* tick vectors. Among domestic animals, cattles, sheep, and goat play an important role in the natural cycle of the virus to cause human infections resulting in severe hemorrhagic fever (HF)(Hoogstraal H, 1979).

Presence of CCHFV was first confirmed in India during nosocomial outbreak in 2011 in tertiary care hospital in Ahemdabad district, Gujarat state, among human cases with hemorrhagic manifestations in the hospital staff (Mishra AC et al., 2011; Mourya DT et al., 2012). Transmission of CCHFV occurred through direct exposure to blood or other secretions. Instances of nosocomial transmission were also confirmed during 2010 (two cases), 2011 (eight cases) and 2012 (three cases) (Mourya DT et al., 2012; Yadav PD et al., 2014). In 2013, CCHFV etiology was detected in a family cluster (nine cases including two deaths) in Karyana village of Amreli District in Gujarat state (Yadav PD et al., 2014). Probable index case of the noscocomial outbreak and the only survived CCHF contact case detected during the outbreak were from Kolat village in Ahmedabad District (Mishra AC et al., 2011).

Serosurvey for CCHF antibodies had earlier been conducted among general population in various places in India. However, no confirmed case was detected in humans (Shanmugam J et al., 1976; Rodrigues FM et al., 1986). In the current study, we have conducted a seroepidemiological survey during the January 2011 and July 2013 CCHF outbreak among at-risk individuals in the CCHF affected area in rural part of Gujarat to know the seroprevalence of CCHFV.

**2. Material and Methods**

**2.1 Study area and serosurvey period**

Gujarat state in India is bordered by Rajasthan to the north, Maharashtra to the south, Madhya Pradesh to the east, and the Arabian Sea as well as the Pakistani province of Sindh on the west (Fig.1). In the rural economy of Gujarat, animal husbandry and dairying have played a vital role.

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

The rural area is classified as semi-arid to arid climatically and people are farmers and practice nomadic pastoralism, keeping cattles, goats, sheep in the house.

Serosurvey activity was conducted in January and February 2011 in the CCHF affected village Kolat and ten neighbouring villages (i.e Changodhar, Telav, Navapura, Moriya, Motidevi, Shela, Vaghjipura, Dholeshwar, Motipura, Sanatal ) within the radius of 10 km in Sanand Taluka, Ahmedabad District. Sera were also collected from Ahmedabad city in January 2011. Serosurvey activity was also conducted in CCHF affected village Karyana and neighbouring village Khambala (8 km North to village Karyana) from Amreli district in July 2013 (Fig. 1).

**2.2 Epidemiological characteristics and study population**
Information of housing, sanitary conditions around the houses, habits of individuals and their exposure to ticks was obtained through a predesigned questionnaire. Serosurvey was carried out in at risk study population in CCHF affected village Kolat (population 3978) and Karyana (population 2492). From CCHF affected village, study participants include family contacts of CCHFV case, immediate neighbourhood contacts living within 100 meter radius from CCHF confirmed house, health care staff involved in care of CCHF cases working in affected area. Other at risk participants were abattoir workers and animal handlers working in abattoirs run by Municipal Corporation in Ahmedabad, Gujarat. From neighbouring villages of CCHF affected village, study participants include acute fever cases and general population as control.

**2.3 Antibody testing**
Clinical samples were collected from the subjects after obtaining written consent. Approval was obtained from Institutional Human Ethics Committee, National Institute of Virology, Pune for the study. Sera collected from at risk study population in CCHF affected area was tested for anti CCHFV IgG antibodies. Sera collected from acute fever cases were tested for anti CCHFV IgM antibodies and detection of CCHF viral RNA by Real Time RT-PCR. Detection of CCHF virus specific IgM and IgG antibodies were performed by a commercial kit (Vector BEST Company, Vector Crimean-CHF IgG and IgM ELISA test kits; Vector-Best, Novosibirsk, Russia) as per the manufacturer's instructions. Sensitivity and specificity of standard panel of positive and negative samples is indicated as 100%. Sera from fever cases were also tested for Anti Dengue IgM antibodies using NIV ELISA kit.

**2.3.1 Interpretation of the results of both IgM and IgG ELISAs and CCHFV by Real Time RT-PCR**
The optical densities of the ELISA reactions are read at 450nm. The OD value of the negative control in the kit does not exceed 0.25, and that of positive control is not less than 1.0. The cut-off value is calculated as the average of optical densities of negative controls + 0.2. The samples are considered to be positive if OD of the samples is greater than or equal to the cut-off value

**3. Results**
A total of 73 sera from Kolat and sera from 84 fever case from neighbouring 10 villages [i.e Changodhar (19), Telav (20), Navapura (12), Moriya (9), Motidevi (9), Shela (5), Vaghjipura (4), Dholeshwar (4), Motipura (1), Sanatal (1)] were collected in January and February 2011. A total of 261 sera from Karyana and 33 sera from village Khambala were collected during July 2013. Majority (63.0%) of study subjects were female from village Kolat and Karyana (52.1%) of two different districts. Sera were collected from all age groups (Table 1). Sera were also collected from 23 health care workers from Karyana which includes 3 medical practitioners and 20 supporting health care staff from the affected area. Sera were also collected from 12 slaughter house workers and 16 animal handlers (all male) working in slaughter houses in Ahmadabad.

Anti CCHFV IgG, IgM antibodies and viral RNA were not detected from sera collected from Kolat and adjoining villages from year 2011. Sera collected from 16 animal handlers and 12 slaughter house workers, anti CCHFV IgG antibodies were detected in an animal handler from Ahmadabad. Anti CCHFV IgG antibodies were detected in 5 out of 261 sera collected from Karyana. Among these five subjects detected with anti CCHFV IgG antibodies, 3 subjects reported with recent history of fever which includes a 30 years female with history of direct contact with CCHF confirmed case and exposure of domestic animals. She was a 40 years female housewife and use to work in farm and a male farmer of 70 years who was involved in grazing activity. Sera collected from 23 fever cases were also negative for CCHF viral RNA and anti CCHFV IgM antibodies. Anti CCHFV IgG antibodies were detected in 35 years, female animal handler working in field form village Khambala (Table1).

Overall seroprevalence of 1.9% (5/261) was reported from Karyana village and 3.0% (1/33) from neighbouring Khambala village. Serpositivity among male was 2.4% in village Karyana and 5.9% in village Khambala. In village Karyana, seropositivity was observed in all age group (2-10, 11-20, 21-30, 31-40 and ≥ 61 years) except age group 41-60 years (Table 1). In village Karyana, seropositivity of 10.2 % was significant ($p<0.0001$) in population having animal contacts (5/49) as compared to those having no animal contacts (0/212). Among contact with CCHFV case, seropositivity of 15.4% (2/13) was significant ($p<0.01$) as compared to those who have no contact with CCHFV confirmed cases reported in Karyana village. Seropositivity of 20% (2/10) among persons with history of tick bite was significant ($p<0.002$) as compared with subjects reported with seropositivity of 1.2% (3/248) who have not reported history of tick bite (Table1). Sanitary conditions in CCHF affected village was poor as revealed in sanitary survey and people were very closely leaving in close proximity with animals and multiple cattle sheds around the houses particularly noted in houses of Bharwad community (Fig 2). Frequent livestock movements, seasonal migrations of animals in search of pasture were reported by study participants from Bharwad community. Their family members use to migrate with the cattle's to neighbouring districts and spend many days away from home.

**Discussion**
The present serosurvey was conducted to understand the seroprevalence of CCHF by detecting anti-CCHFV IgG antibodies among at risk population in CCHF affected village in year 2011 and 2013. Anti-CCHFV IgG antibodies were not detected from Kolat and ten neighbouring villages of Kolat in January 2011. Although people in the CCHF affected village Kolat were living in close proximity with domestic animals in which anti CCHFV IgG antibodies were detected (Mourya DT et al., 2012), but still no study participants detected with anti CCHFV IgG antibodies. In contrast, in July 2013, overall seroprevalence of CCHF was 1.9% from Karyana village and 3.0% from neighbouring Khambala village. Serosurvey study conducted in North-Easten Greece has reported seroprevalence of 3.1% persons by anti-CCHFV IgG antibodies (Papa A et al., 2011). Tokat and Sivas provinces in Turkey, showed higher seroprevalence up to 12.8% in high risk population (Gunes T

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

et al., 2009).

Anti-CCHF IgG antibodies were detected from an animal handler working in slaughter house in Ahemdabad. Exposure to blood and tissues of viremic animals during animal slaughter is a source of infection (Hoogstraal H et al, 1979; Ergonul O. et al., 2004). Source of infection of CCHF by exposure to blood and tissues of viremic animals during the slaughter is documented earlier (Hoogstraal H et al., 1979; Ergonul O., 2006). It has also been documented that low levels of viremia lasting for short duration was noted in domestic animals (Nalca A., 2007) and infection may not spread directly from animals to human being. In the present study, worker not involved in animal slaughtering activity was also found positive for anti-CCHFV IgG antibodies.

In the present study anti CCHFV IgG antibodies were detected in children and young adults in village Karyana. Other studies has reported that, CCHF seroprevalence in high risk population increased significantly with age, and highest proportion (23.5%) of seropositivity was reported among persons in age group 61-70 years of range. The important variables significantly associated with presence of antibody against CCHFV were history of tick bite or tick removal from animal, employment in animal husbandary or farming and age >40 years (Gunes T et al.,2009). In the present study, history of contact with the domestic animals, history suggestive of contact with CCHF case and tick bite was significantly associated with the seropositive subjects of CCHFV by detection of anti-CCHFV IgG antibodies. Though anti-CCHFV IgG antibodies were detected in three fever cases from Karyana village, sera collected from them were negative for anti-CCHFV IgM antibodies and CCHF qRT-PCR. This could be due to earlier exposure to CCHF of these cases.

Earlier, serosurvey done in year 2011 among animals (Mourya DT et al., 2012), about 17.6% (26/128) domestic animals from Kolat (Ahmedabad district) was positive for anti CCHF IgG. This shows that animals are infected in this region and human population in close contact with these animal population is at risk of getting CCHF infection from the infested vector ticks. This is also evident by the *H. anatolicum anatolicum* ticks positivity by PCR and viral isolation (Mourya DT et al., 2012). Though anti CCHFV IgG antibodies were not detected in any persons in Kolat village, first CCHF case from rural part of Gujarat was reported in January 2011 from Kolat in Ahemdabad district (Mishra AC et al., 2011; Mourya DT et al., 2012). However, death of consulting physician in February 2010 in Rajkot was confirmed with CCHF infection after retrospective analysis of sera tested in January 2011 for CCHF (Mourya DT et al., 2012). This confirms that infection foci of CCHFV were present in Gujarat from year 2010. Subsequent sporadic CCHF cases were reported from neighbouring districts of Ahemdabad from Gujarat highlighted CCHF as an important public health problem in the Gujarat (Yadav PD et al., 2014).

Similarly, anti-CCHF IgG antibodies were detected in 60.6% (20/33) domestic animals like cattles, goats and sheeps in the Karyana and Khambala village in Amreli District (Yadav PD et al., 2014). *Hyalomma* ticks were also positive for CCHF viral RNA from these villages (Yadav PD et al., 2014). A significant difference was observed in seropositivity of anti CCHFV IgG antibodies among domestic animals from Karyana and Kolat. This could be the reason of no detection of anti CCHFV IgG antibodies among study participants from village Kolat. High positivity of anti CCHFV IgG among domestic animals from Karyana, highlights how infection could have introduced by infestation of infected ticks to the domestic animals in these villages and affected rural population living in close proximity with domestic animals. It is also possible that during seasonal migrations of animals in search of pasture, animal handlers spend many days and nights outside, thus risking contact with infected animals and hungry ticks.

Animal movements could be one of the important reasons for spread of CCHF infection in the region which increases the risk of getting infection to people particularly the Bharwad community in Gujarat. The study in Greece showed climatic and environmental factors and infested livestock movements (legal and illegal) in a habitat suitable for ticks infestation might have played a role in the CCHF infection (Maltezou HC et al., 2007). CCHF being a zoonotic disease is associated with domestic animals. High risk population in this study was residing in close association of domestic animals. Particularly the Bharwad community which is mainly involved in animal handling is at risk of the CCHF infection as evident by clustering of CCHF cases in a family in village Karyana (Yadav PD et al., 2014). During the dry season people from Bharwad community use to migrate with the cattle's to neighbouring districts where water and pasture are abundant long after water resources have dried. Such migration patterns facilitate movement of potentially infected ticks across great distance with risk of exposure to tick borne diseases.

A serosurvey in three referral university hospitals in south eastern and central Iran, among health staff exposed to CCHF cases showed around 3.87% (5 of 129) positivity for anti CCHF IgG and none tested positive in unexposed group (Mardani M et al., 2007). In the present study no health staff showed anti CCHF IgG positivity. This may be due to safety precautions by health staffs using personnel protective equipment and majority health staff has not directly come into contact of confirmed CCHF cases.

Earlier very few studies were carried out in India to know the seroprevalence of CCHF in human population. Serosurvey carried out in India in 1973, informed about the detection of precipitating antibodies to CCHF virus in human sera from Trivandrum, Pondicherry and Shimoga district and its total absence in Delhi and Madras which would reflect the difference in exposure to tick bite (Shanmugam J et al., 1976). Study carried out in 1976 to determine prevalence of CHF-Congo virus in the western border districts in Jammu and Kashmir of India, no evidence of CHF-Congo virus activity was found by serosurvey among humans or domestic animals (Rodrigues FM et al., 1986).

This is the first serosurvey study in India performed in the CCHF affected villages in Gujarat, India. The study showed very low seropositivity of CCHF infection in the high-risk individual in the region suggesting very low asymptomatic infection. Study highlights the risk to people those are coming in contact with domestic animals. Confirmation of CCHF cases from other districts in Gujarat alarms the possible reporting of CCHF cases from other neighbouring state of Gujarat. The knowledge may be useful for public health to determine appropriate CCHFV interventions and preventive measures to be undertaken in the area where people are living in close proximity with the animals.

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**Declaration of Interest**

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

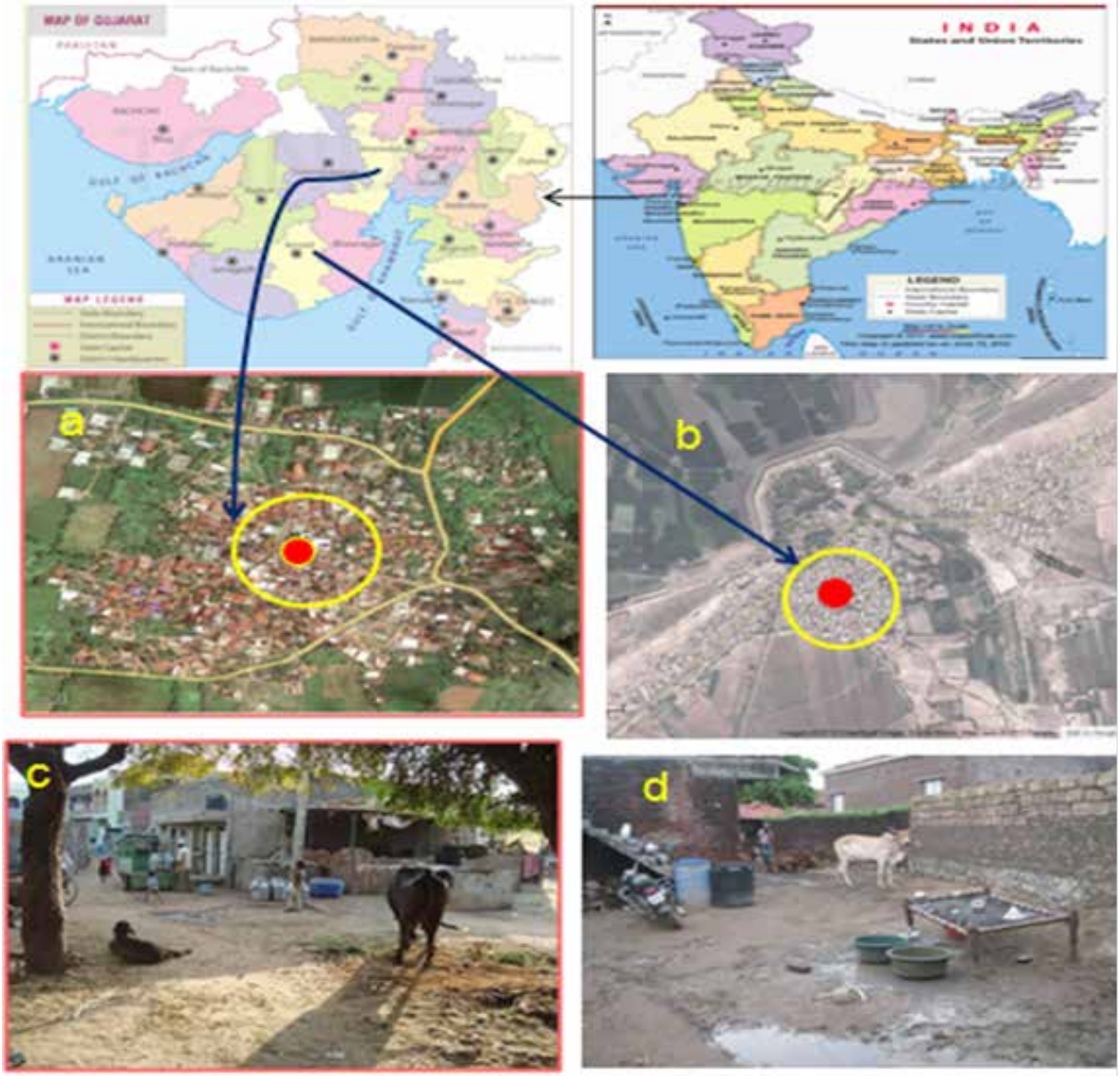


**Fig. 1. CCHF affected villages in Gujarat, India showing a) location (encircled in yellow colour) of CCHF case in Kolat village, b) Karyana village showing unhygienic conditions (cattle shed) around CCHF affected house in C) Kolat and d) Karyana village.**

**Table 1. Characteristics of study subjects showing seroprevalence of CCHFV in high risk population in CCHF affected area in Gujarat state.**

| Characteristics | Serosurvey in CCHF affected area with study population (n) and study period [month and year] | | | |
|---|---|---|---|---|
| | Kolat village (73) [Jan- Feb 2011] | Neighbouring ten villages# around Kolat ( 84) [Jan- Feb 2011] | Karyana village (261) [July 2013] | Khambala village (33) [July 2013] |
| Age (years) | | | | |
| Mean (± SD) | 28.9 (± 19.9) | 31.3 (± 21.1) | 28.7 (± 18.6) | 23.9 (± 15.1) |

Volume : 4 | Issue : 12 | Dec 2014 | ISSN - 2249-555X

| | | | | |
|---|---|---|---|---|
| Range | 2-75 | 2-97 | 2-78 | 2-51 |
| Gender, no.(%) | | | | |
| Male | 27 (37) | 28 (33.3) | 125 (47.9) | 16 (48.5) |
| Female | 46 (63) | 56 (66.7) | 136 (52.1) | 17 (51.5) |
| Total seroprevalence, no. positive (% ),[with 95% CI] | Nil | Nil | 5 ( 1.9)<br>[ 0.25 to 3.58] | 1 (3.0)<br>[ 0 to 8.88] |
| Seroprevalence by gender, no positive/no. tested (%), [with 95%CI) | | | | |
| Male | 0/27 (0) | 0/28 (0) | 3/125 (2.4) [0 to 5.1] | 0/16 (0) |
| Female | 0/46 (0) | 0/56(0) | 2/136 (1.5)<br>[ 0 to 3.5] | 1/17 (5.9)<br>[ 0 to 17.1] |
| Risk factors, no positive/exposed population | | | | |
| Age ≥20 | 0/45 (0) | 0/53 (0) | 3/165 (1.8) | 1/20 (5.0) |
| Contact with an domestic animals | 0/41 (0) | 0/21 (0) | 5/49 (10.2)* | 1/31 (3.0) |
| Contact with CCHFV patient | 0/16 (0) | Nil | 2/13 (15.4)* | 0/33 (0) |
| History of tick bite | Nil | 0/4 (0) | 2/10 (20)* | 0/33 |
| Seroprevalence by age group in years, no. positive/no. tested (%) | | | | |
| 2-10 | 0/6 (0) | 0/14 (0) | 1/46 (2.2) | 0/8 (0) |
| 11-20 | 0/22 (0) | 0/17 (0) | 1/50 (2) | 0/5 (0) |
| 21-30 | 0/16 (0) | 0/15 (0) | 1/68 (1.5) | 0/9 (0) |
| 31-40 | 0/14 (0) | 0/15 (0) | 1/29 (3.4) | 1/8 (12.5) |
| 41-50 | 0/8 (0) | 0/8 (0) | 0/31 (0) | 0/1 (0) |
| 51-60 | 0/5 (0) | 0/7 (0) | 0/22 (0) | 0/2 (0) |
| ≥61 | 0/2 (0) | 0/8 (0) | 1/15 (6.7) | Nil |

[#(Changodhar, Telav, Navapura, Moriya, Motidevi, Shela, Vaghjipura, Dholeshwar, Motipura, Sanatal); *Significant difference when compared with complementary category]

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