

Detection of Crimean-Congo hemorrhagic fever and theileriosis in livestock, Madhya Pradesh, Central India

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Abstract

Tick borne diseases impinge veterinary and public health, worldwide causing threat to nutritional security, mortality and huge economic losses. Crimean-Congo haemorrhagic fever (CCHF) and theileriosis are the two important tick-borne diseases in humans and animals respectively, risk the public health due to mortality and pose major threat to animal husbandry and livestock industries. Sero-epidemiological and genetic analysis are the key aspects in determining the prevalence of CCHF virus (CCHFV) circulation and molecular prevalence of theileriosis in region wise, which are overlooked in the India, where the country known for largest cattle population, globally. Thus, the present study aims to evaluate the prevalence of CCHFV in animals and genetic diversity of the prevailing *Theileria annulata* in Jabalpur, Madhya Pradesh. Systematically, livestock population was screened for presence of CCHFV antibodies and *T. annulata* through commercial IgG ELISA kits and PCR, respectively. Overall, 16% of samples (n=63/393) tested were positive for CCHFV-specific IgG antibodies and 15.9% (n=21/132) animals were positive for *T. annulata* infections, respectively. It indicates that, CCHFV is circulating in this region in an unnoticed hidden manner. It recommends that, screening of human cases showing hemorrhagic manifestations in health care setup and active surveillance of ticks as a preparedness to combat future outbreaks. Moreover, designing region wise preventive strategic plan may curb these emerging as well as economic tick-borne diseases.

Rapid communication

Title: Detection of Crimean-Congo hemorrhagic fever and theileriosis in livestock, Madhya Pradesh, Central India

Short title: CCHF & theileriosis in livestock, Central India

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Summary

Tick borne diseases impinge veterinary and public health, worldwide causing threat to nutritional security, mortality and huge economic losses. Crimean-Congo haemorrhagic fever (CCHF) and theileriosis are the two important tick borne diseases in humans and animals respectively, risk the public health due to mortality and pose major threat to animal husbandry and livestock's industries. Sero-epidemiological and genetic analysis are the key aspects in determining the prevalence of CCHF virus (CCHFV) circulation and molecular prevalence of theileriosis, which are overlooked in the India, where the country known for largest cattle population, globally. Thus, the present study aims to evaluate the prevalence of CCHFV in animals and genetic diversity of the prevailing *Theileria annulata* in Jabalpur, Madhya Pradesh. Systematically, livestock population was screened for presence of CCHFV antibodies and *T. annulata* through commercial IgG ELISA kits and PCR, respectively. Overall, 16% of samples (n=63/393) tested were positive for CCHFV-specific IgG antibodies and 15.9% (n=21/132) animals were positive for *T. annulata* infections, respectively. It indicates that, CCHFV is circulating in this region in an unnoticed hidden manner. So, screening of human cases showing hemorrhagic manifestations in health care setup and active surveillance of tick is need of the hour to tackle public health outbreaks and to design preventive strategic measures to control these emerging as well as economic tick borne diseases.

Key words : Crimean-Congo haemorrhagic fever virus, *Hyalomma anatolicum*, Livestock, Sero-survey, Theileriosis

Introduction

As per World Health Organization (WHO) estimates, 17% all infectious diseases are owing to vectors, results in more than 700000 deaths annually (). In recent days, many public health emergency situation have been noticed globally, due to upsurge in emerging infectious diseases of zoonotic origin, and most of them are transmitted by ticks and mosquitoes (Al-Abri et al., 2017). The Ixodid tick, *Hyalomma spp* is one of the widely distributed tick species in Asian countries, acts as a potential vector for CCHF infection in humans and tropical bovine theileriosis in animals, respectively (Manjunathachar et al., 2019). Crimean-Congo hemorrhagic fever (CCHF) is a trans-boundary disease, caused by CCHFV belonging to the family *Nairoviridae* (Spengler and Bente, 2017). The CCHF infection is endemic in many regions, such as Africa, Eastern and Southern Europe, and Central Asia where numerous wild and domestic animals serve as asymptomatic reservoirs and play important role in natural circulation of virus (Ergonul, 2006; Mourya et al., 2015; Spengler 2016; Mourya et al., 2019, Al-Abri et al., 2017). The virus is maintained in nature in Ixodid ticks mostly of genus *Hyalomma* by trans-ovarial and trans-stadial transmission (Malik et al., 2020). Considering its havoc on public health and wide geographic distributions, WHO has enlisted CCHF as the top priority disease and urged research and development actions (Mehand et al., 2018).

In recent years, the virus appears to be extending its horizon across the countries and many human cases are reported due to globalization (Mourya et al 2012; Al-Abri et al., 2017). In India, after a first confirmed case of CCHF, sporadic cases and outbreaks were reported since 2011, from different districts of Gujarat State. Similarly, in Rajasthan state after reporting animal positivity in the year 2010, several CCHF outbreaks, constant engagement and positivity rate of CCHF in ticks was recorded (Yadav et al, 2016; Mourya et al 2012, 2019). Nevertheless, the asymptomatic animals could provide virus for tick-borne transmission to susceptible humans, thus play an important role in maintenance and epidemiology of the disease. ICMR- National Institute of Virology, Pune have been reported 81 CCHF positive human cases in from 2011 to

December 2018 in India, depicts the need of active surveillance (Mourya et al., 2019). Recently, health authorities issued a high alert situation in Gujarat and Rajasthan states of India upon encountering of cases (35-50% mortality), urges the need for proper diagnosis and preventive measures (IDSP, 2019).

The apicomplexan protozoan parasites such as *Theileria* or *Babesia* spp. are also transmitted by Ixodid ticks and can infect domestic, wild animals and humans (Chen et al., 2014; Gabrielli et al., 2014). *Theileria annulata* causes lymphoproliferative disease in cattle and responsible for high mortality and morbidity, thus leads to food insecurity and heavy economic losses. In India, the estimated losses accounted due to theileriosis is US 1295 million dollars annually (Narladkar, 2018). Few scientific shreds of evidence show that some *Theileria* spp. are causes severe life-threatening infection in humans (Chen et al., 2014; Gabrielli et al., 2014). Considering the world largest population of cattle and its importance in national economy, food security, control and prevention of tick borne diseases is paramount important (Dept. Animal Husbandry & Dairying, Govt. of India, 2019). Although evidence of CCHFV and theileriosis in livestock were reported in India, constant surveillance and monitor the presence and regional distribution of pathogens is much needed to define potential risk zones (Yadav et al, 2016; Mourya et al 2012, 2019; Narladkar, 2018). Considering the ecological niche of study area, where rich in bio-diversity, abundance of competent vector species, constant wild-domestic-human interface, and the Madhya Pradesh is bordered by three outbreak states (Gujarat, Rajasthan and Uttar Pradesh), study was designed to evaluate the prevalence of CCHFV in animals and genetic diversity of the *Theileria annulata* in Jabalpur, Madhya Pradesh.

Materials and Methods:

A cross-sectional study was conducted in the Jabalpur region (23.1815° N, 79.9864° E), Madhya Pradesh between March, 2018- June, 2019 to investigate the prevalence of CCHF and theileriosis in livestock's and its associated risk factors (Fig.1). To cover urban and rural/tribal areas, five villages were selected purposely from peripheral villages of study region.. Simple random sampling method was applied to select the animals from the herd. The sample size was estimated based on the assumption of 10% sero-positivity rate, the level of confidence as 95 percent and a absolute error of 5 %. A minimum desired sample size was 140, so the desired sample bovine and goat/sheep was 140 each. Overall, a total of 155 bovine and 238 goats' blood samples were collected from the jugular veins following biosafety measures. The pre-designed structured interview schedule was used to collect information on animal husbandry activities and risk factors. Sera were separated and subjected to two commercial ELISA kits (1 for bovine -Catalogue No.CCHFCTIGG-48, and 1 for goats -Catalogue No. CCHFSGIGG-48) (Zydus Diagnostics, Ahmedabad, India). The manufacturer's instructions were followed for testing samples and based on the ratio >1.5, the positivity was determined for both the kits. The DNA was isolated from the CCHF IgG negative bovine blood samples using DNA Sure Mini Kit (Nucleo-pore, GENETIX, India) as per the manufacturer's protocol. The isolated DNA samples were subjected to PCR amplification targeting the cytochrome b (312 bp segment) gene of the *Theileria annulata* (Bilgic et al., 2010). The PCR products were sequenced analyzed and submitted to GenBank. The necessary ethical permission for the conduct of this study was obtained from the Institutional Animal Ethics Committee (IAEC) and Institutional Ethics Committee (NIRTH/IEC/3756/2018).

Results:

The results of this survey showed that 18 (11.6%) bovines and 45 (18.9%) goats were having antibodies specific for CCHFV. Out of 132 CCHF negative bovine samples subjected for PCR amplification targeting the 312 bp product of cytochrome b gene of *T. annulata*, 21 samples showed positivity. The overall PCR based prevalence rate of bovine tropical theileriosis is 15.9 %. Phylogenetic analysis revealed that Jabalpur *T. annulata* isolate (GenBank accession no. MN893432 to MN893434) share 100% nucleotide sequences identity with Rewa, Madhya Pradesh (MG787986.1), Mhow, Madhya Pradesh (MG787985.1), Mathura, Uttar Pradesh (MG787984.1), Hisar, Haryana (MG787983.1), and Bareilly, Uttar Pradesh (MG787980.1) isolates (Fig.2). Physical examination of animals revealed that, animals are severely infested with *Hyalomma* spp.ticks. Further, farmers covered under survey were scored and analysis revealed that, they are having lower knowledge on zoonotic diseases and tick-borne diseases (Table.1). The education level of farmers is directly correlated with the knowledge of animal husbandry activities. Interestingly, majority of the farmers reported

that, the general practice of tick control is hand pick the ticks /groom the animal and crush compared to use of chemical acaricides.

Discussion:

Livestock play an important integral role in food, nutritional security and improvement of the Indian economy (<http://dadf.gov.in/sites/default/files/Annual%20Report.pdf>). The majority of rural/tribal people are dependent on the livestock sector for their livelihood, employment, health, and nutritional security. However, tick borne diseases and lack of timely preventive and control measures, jeopardize the public health and animal husbandry sectors. Globally, tick and tick-borne diseases (TTBDs) are prevalent in 80% of the cattle population and the reported economic impact in terms of production losses is 21.38–28.76 billion US dollars annually (Manjunathachar et al., 2014; Narladkar, 2018). Madhya Pradesh is a tribal dominant state adjoining with three CCHF outbreak states (Gujarat, Rajasthan and Uttar Pradesh), rich in bio-diversity and humid subtropical climate favors higher tick activity. The population is mainly involved in agricultural, animal husbandry and forest-related activities. Since, the CCHF is an incidental disease, the constant interaction between wild, domestic animals and humans, increase the chances of spillover of pathogens. Studies are reported that the outbreak of CCHF is directly correlated with an increase in invertebrate host and ticks population above the maintenance level (Spengler et al., 2017). The present study clearly shows the higher prevalence of CCHF IgG antibodies in animals compare to earlier study reported by Mourya et al. (2015) of 4.7% (7/150) in bovines and 7.5% (3/40) sheep/goat. This finding suggested that, CCHFV is circulating in an unnoticed, amplified manner in Jabalpur, Madhya Pradesh, Central India, which is also endemic for malaria and other arbo-viral hemorrhagic diseases (Singh et al., 2014). Besides, the CCHF signs and symptoms in the initial stage of illness are very similar with arbo-viral hemorrhagic infections and create a void in differential diagnosis, clinically. Thus, it is obvious that, clinicians may not be able to recognize it, and this may pose a threat to public health as a nosocomial infection (Yadav et al., 2016).

Among the ruminant species studied, the rate of CCHF prevalence is more in goats compare to cattle. The similar type of finding was observed in Nepal where 38.2% CCHFV antibodies were recorded in goats and no cattle sample was found positive (Wangchuk et al., 2016). India having the second largest goat population in the world and largest exporter of goat/sheep (18,425 MT in 2018-19) meat to the United Arab Emirates, Saudi Arabia, Qatar, Kuwait and Oman countries (<http://apeda.gov.in/apedawebsite/SubHead.-Products/Sheep.Goat.Meat.htm>). The high positivity in goats pose risk to the slaughter house workers and animal handlers and act as a vehicle for diseases transmission due to increased trading of small ruminants in the absence of cattle slaughter in India. Trade in live animals, export of meat and meat products serve as a mobile pool for diseases like CCHF and pose threat to national economy. Hence, it necessitates the importance of improved time to time surveillance against emerging viral pathogens.

Amongst number of hemo-protozoan parasitic infections in cattle, theileriosis accounts huge economic losses due to high mortality, reduction in milk yield, emaciation as well as high treatment cost (Narladkar, 2018). Further, impeding the problem is due to non-availability of data on the prevalence rate and lack of implementation of control and prevention policies tackle the disease. Present study revealed the high prevalence rate of 15.9 % for *T. annulata* infections in cattle by utilizing cytochrome b gene based PCR. The present data based on cytochrome b gene, highly sensitive PCR target will shed light on the occurrence of *T. annulata* infection and devising control strategies to detect and eliminate the infection in tribal dominated regions of Jabalpur, Madhya Pradesh. Further, phylogenetic analysis revealed that, Jabalpur isolates are 100% nucleotide similarities with North-Indian isolates. Currently, piroplasmosis is mainly encounter in domestic animals. However, several zoonotic piroplasmosis cases are reported in humans globally, warrants the need of active investigations in India, owing to transmission dynamics in vector tick species, mutations in parasites, lack of epidemiological data and involvement of wild animals in the ecosystem (Gabreilli et al., 2014). It is noted that, the examined animals are non-descript and cross breeds and they possess high rate of tick infestations. Based on the geographical evidences, it is owing due to grazing of animals in the buffer zones of forest region where, the amplifying host like small vertebrates (hares, hedgehogs) harbor high rate of immature ticks (Brown, 1997). Further, lack of insecticide usage in these regions may help in

maintenance of enzootic-tick-vertebrate cycle in field condition. Hand picking and crushing of the ticks, pose a serious risk to the farmers and treating ill patients in resource poor health facilities at village level pose a risk to the medical staff. So, detection of CCHFV antibodies in domestic animals in the study, shed light on the possible hotspots for the localization of CCHFV foci, evidences for virus circulation and highlights the potential risk for human infection. This is the high time to elucidate the factors involved in increasing the prevalence rate over a while and /or studies on any circulation of low pathogenic strains in this region. Although our findings provide sufficient serologic and molecular proof for the occurrence of CCHFV and theileriosis infections respectively, among domestic ruminants in Jabalpur region, because there is no vaccine currently available against CCHF disease, however suffers due to lack of risk group's immune status against CCHF infection.

Conclusion:

The study highlights the high prevalence of CCHFV in domestic animals in Jabalpur, Madhya Pradesh and the humans are at risk. Though, the region has encountered many arbo-viral outbreaks in humans, but to avoid CCHF outbreaks in near future, it is recommended to enhance public health awareness for livestock owners, slaughter house workers to follow strict hygienic measures when handling livestock or its products. Further, increasing diagnostic infrastructure and awareness among physicians/health care workers to screen arbo-viral patients showing compatible clinical presentations with those of CCHFV. In addition, management of hyalomma ticks under the threshold by using an integrated management system and education in this tribal dominated region may help in early intervention and preparedness between animal-human interfaces.

Conflict of Interest

There is no conflict of interest among the authors.

Acknowledgments

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Data Availability

The authors confirm that, the original data used for analyses can be obtained from the author after approval by the Indian Council of Medical Research, New Delhi

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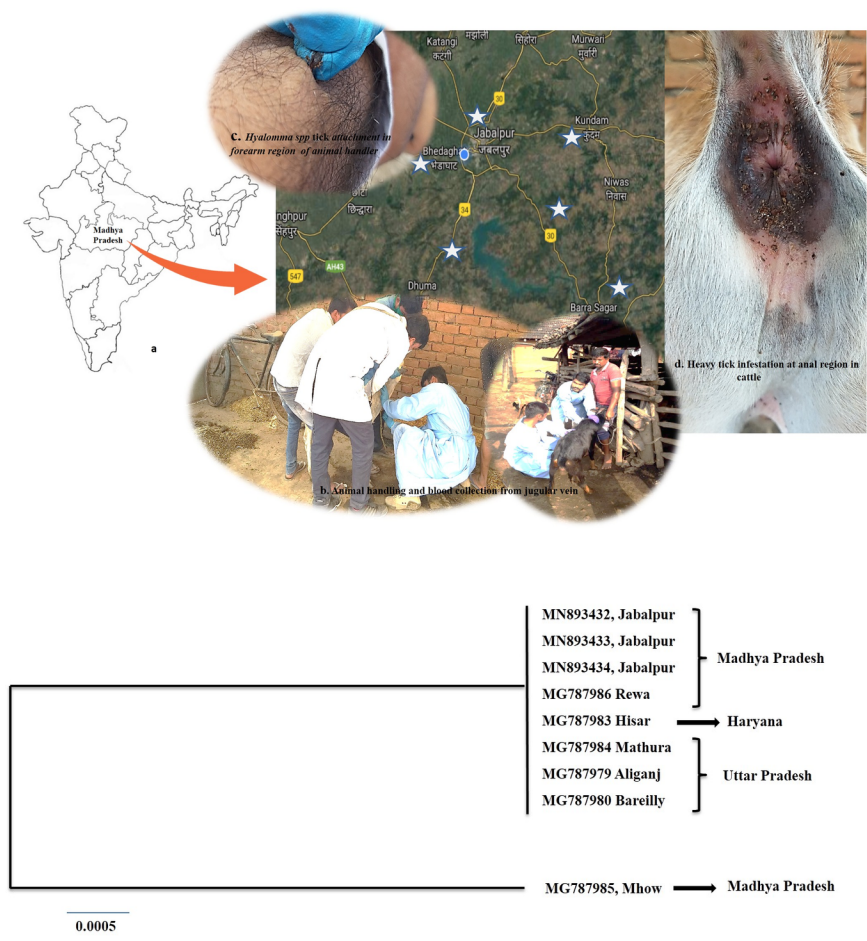
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