Perspective

Re-assessing the biosafety level requirement & defining surveillance need for
Kyasanur forest disease virus: Changed paradigm

Kyasanur forest disease (KFD) is a notifiable zoonotic infection associated with significant mortality in humans and monkeys. The KFD virus (KFDV) has expanded its geographical boundaries from a few districts of Karnataka to adjoining border districts of Maharashtra, Goa, Tamil Nadu and Kerala. High mortality is reported among the non-human primates (NHPs) *Macaca radiata* and *Semnopithecus entellus*, previously known as *Presbytis entellus*. The deaths in NHPs provide an alert to local people including health authorities about the beginning of epizootic and likely outbreak in humans. A wide range of tick vector species mainly *Haemaphysalis* and several mammalian hosts are involved in the maintenance cycle of KFDV. This perspective deals with some of the gaps and difficulties expressed by various public health workers and clinicians in their understanding of laboratory diagnosis as well as management of KFD during national consultation organized by the National Centre for Disease Control, Delhi, and Health and Family Welfare Department, Government of Karnataka in August 2019.

Some of the laboratory-related key questions were as follows; what is the appropriate transmission period of KFD? Whether surveillance should be done in humans or tick vector or both; whether surveillance should be done year-round or only during high transmission season? How to improve the timeline of sample collection and laboratory diagnosis? Can KFD suspected human samples be tested in biosafety level 2 (BSL-2) laboratories?

Season for high transmission of KFD

Role of human–wildlife interface resulting in spread of KFD is apparent. Studies carried out in the limited five affected districts of Karnataka State revealed high transmission season of KFDV from January to May. However, with increased surveillance in various States where this disease has been recognized, the peak transmission season in humans has been observed from October to June. Influence of social practices associated with cashew nut season in Goa as well as changing climate that supports increase of tick population affect occurrence of cases beyond defined seasons.

Emphasis on the human surveillance of KFD

Evergreen and semi-evergreen forests that harbour *Haemaphysalis* ticks are abundantly present in India. With the increased awareness, large number of suspected cases from naïve areas were screened for KFD prevalence underscroing the high probability of KFDV detection in new geographical areas. Tick surveillance is challenging because there is no standard method to detect virus in enzootic or epizootic phase. Therefore, a random collection of ticks neither gives any clue about the vector densities nor is the processing of tick pools ideal for virus detection. Serological surveys have already shown the presence of anti-KFDV antibodies in humans and animals in many of the areas where the presence of the virus has not been demonstrated. The parameter for assessing endemicity of KFD for majority areas is largely governed by the laboratory confirmed human cases recorded every year. The epicurve of outbreaks of this disease gives information about the beginning of human cases, and therefore, those areas should be kept under active surveillance. To understand the actual disease burden of KFD, active surveillance of human population seems to be the appropriate choice.

Effective timeframe for laboratory diagnosis of KFD

The earlier studies carried out by the Indian Council of Medical Research (ICMR)-National Institute of
Virology, Pune, have clearly shown that the reverse transcription polymerase chain reaction (RT-PCR) is the most preferred method for laboratory diagnosis during <4 days of post-onset days of the disease. The clinical specimens >4 to <18 days can be tested using either PCR and IgM-ELISA or both the assays. However, beyond 18 days, IgM and IgG provide an accurate diagnosis of KFD cases.

**Time to re-assess the biosafety level for handing human samples of KFD**

Laboratory-associated infections (LAIs) and fieldwork-associated KFD infections were reported earlier as most of the infectious work was carried out in biosafety level-2 (BSL-2) laboratories. The only available serological method used for diagnosis was complement fixation test for KFD diagnosis. This test required purified mouse brain-derived KFD antigen in high quantum. The limited understanding and awareness about biosafety were the main contributing factors to LAIs. Field-acquired infections can be attributed to limited/reduced/lack of appropriate personal protective equipment (PPE) usage and other biosafety precautions while performing the necropsy of infected monkeys and through infected tick bites. This led to the controversial hypothesis of human-to-human transmission and the suspected aerosol route of KFD infection. Apparently, there are no reports of human-to-human transmission of KFD during different outbreaks occurred in recent years. Initially, the KFDV was classified in category A98.2 by the International Classification of Diseases-10 (ICD-10). In 1974, the Centers for Disease Control and Prevention (CDC), USA, classified the concept of BSL (level 1-4) based on the susceptibility of the infecting organism (level 1-4) based on the principal characteristics and the route of transmission of the microorganisms. The CDC considers KFDV as infectious pathogen to be handled in BSL-4 laboratory.

The available data on the morbidity evidence of this disease are limited; studies report that the long-term sequelae of KFD infection are rare. The low case fatality rate (3-4%) in human and extension of geographical niche by the virus are the differing characteristics of pathogen from risk group 4 pathogen. Limited availability of BSL-3 laboratories necessitates the re-assessment of the risk group and biosafety level of KFDV.

The scope of risk assessment covers the potential harm caused by the pathogen to individual and the environment during the procedures or experimental activities. The procedures involved in laboratory diagnosis possess acceptable risk provided if laboratory worker follows standard personal protective equipment (PPE) in the BSL-2 laboratory setting and standard practices (Table). These procedures comprise pre-analytical, analytical and post-analytical phases such as sample collection, transportation to diagnostic laboratories, processing and disposal of the biological waste. While handling human samples likelihood of laboratory infection via inhalation exposure is 1.57, via percutaneous exposure is 2.6, via direct contact exposure 1.08, ingestion exposure 0.48, consequence of disease to human host is 1.35, proper biosafety practises, no risk to community is 98.65 (values derived from authors unpublished data). The likelihood is calculated based on analysis biosafety risk (R), likelihood of infection by the agent (Li), likelihood of exposure through an infectious route (Le), consequences of disease assuming infection (Cd): $R = F (Li, Le, Cd)$. The likelihood of exposure is assessed based upon the research procedures and required biosafety measures in-place and likelihood of infection and the consequences of disease are assessed for the risk for humans during laboratory procedures. Frontline diagnostic assays for KFDV are enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (RT-PCR). These assays tend to generate aerosols; however, the volume of the clinical sample is very small and gets diluted during the processing and testing. In the case of ELISA, the samples are inactivated at 56°C for 30 min and then used for further processing. In RT-PCR assays, the samples are treated with inactivating agents during the extraction of viral RNA. The sources of risk are identified during the procedures and mitigated by standard practices, safety equipment and facility requirements of BSL-2 laboratories.

**Risk mitigation**

With limited BSL-3 facilities in the country, it is recommended to handle KFDV suspected human samples in BSL-2 laboratories that follow a uniform standard operating procedure to minimize risk. The procedures should be performed in the Class II-A2 cabinets following standard microbiological practices. Laboratory personnel should wear protective laboratory coats, gowns, uniforms, gloves, appropriate eye and face protection to protect them from infectious aerosol or splashes. During the post-analytical phase,
which is the main cause of environmental risk, the hazard is controlled by proper decontamination of the plastic/solid/liquid waste generated during the procedures (Table). In addition, the laboratory personnel and field workers should be immunized with two doses of KFD vaccine with interval of one month as the immunogenic response produced by formalin-killed tissue culture-derived KFD vaccine is short lived. Expansion in the geographical area of KFD is an important issue necessitating for increase in laboratory facilities. Similar approach is being used for COVID-19 diagnosis during the current pandemic.

However, handling animal samples, growing the virus or performing a necropsy on the KFD-suspected animals still need higher BSLs as these specimens possess higher viral load. BSL-2 laboratories with inadequate infrastructure not equipped to meet required risk mitigation need to be upgraded.

| Table. Risk assessment for providing diagnosis for Kyasanur forest disease in laboratory and hospital settings |
|---|---|---|---|---|
| Phase | Process | Possible risk | Mitigation | Remarks |
| --- | --- | --- | --- | --- |
| Risk assessment in laboratory settings | | | | |
| Pre-analytical | Sample collection | Needle stick injury | Minimize use of needles and sharps | Use of needle-free devices, Vaccination of staff and testing antibody titre
Post-exposure prophylaxis as a contingency plan |
| | Spill | Spill kits | Proper management of solid and liquid waste, plastic ware and PPE |
| | Sample transport, receiving | Leakage or spill of the receptacles | Regular drills for spill management | Trained personnel and carrier assigned for sample transport |
| | Sample aliquoting | Aerosol generation | Standard GMP | Use of Class II A2 cabinet and barrier tips, Dedicated set of equipment, Unidirectional workflow |
| Analytical | Test procedures | Aerosol generation during ELISA | Sample inactivation at 56°C before testing | Sample handling in biosafety cabinet, Liquid and solid waste efficiently treated within laboratory, Volume used for test is less as it is a clinical sample |
| | | Aerosol generation during Q-RT-PCR | Use of chaotropic agents | Waste generated during the procedure plastic/solid/ liquid is autoclaved before leaving the laboratory Use of barrier tips/dedicated equipment Volume used is small |
| Post-analytical | Autoclave to discard the spill material | Leakage/aerosol | Do not overload autoclave. Use of a tray to keep the biohazard bags containing the material | No human to human transmission is recorded via droplets or droplets nuclei/body fluids Hospitals need to have comprehensive waste management and programme for decontamination using well-defined procedures |

PPE: Personal protective equipment, ELISA: Enzyme-linked immunosorbent assay; RT-PCR, reverse transcription polymerase chain reaction; GMP, good medical practice
Way forward

Anthropogenic impact influences environmental changes leading to alteration in ecological niches of the host, vector and/or pathogen for zoonotic pathogens. Considering the low mortality among human population and limited availability of high containment laboratories (BSL-3 and BSL-4), it is imperative to re-assess the BSL for diagnosis. High-risk steps during diagnosis involve percutaneous exposure of KFDV during the sample collection. This can be mitigated through proper use as well as disposal of sharps and by KFDV vaccination with periodical checking of the antibody titres.

The transmission of KFDV is by vectors, not by body fluids. In hospital settings, while handling patients with gastrointestinal symptoms or bleeding manifestations, the risk can be mitigated with the use of PPE and standard microbiological practices (Table). Risk associated with accidental splashes can be controlled through effective spill management process.

The network of virus diagnostic and research laboratories across the country is well equipped with BSL-2 facilities and trained workforce. This network can be used as a multi-sectorial ‘one health approach’ for disease surveillance as well as for control in naïve and affected areas. The paradigm-shifting convening the change in the risk group level of KFDV from high containment to BSL-2 will be a milestone in early detection and further controlling the spread of virus to naïve areas, making the diagnosis of KFD cost-effective. Training of staff for handling the clinical samples as well as strict adherence to uniform standard operating procedures (SOPs) will strengthen the diagnostic and surveillance capacity of the country.

To conclude, there is a gap in scientific evidence on infectivity and transmissibility of KFDV in different hosts. Research in this area needs to be prioritized. Till then, handling of animal samples, large-volume and live virus amplification activities must be restricted to containment laboratories.

Financial support & sponsorship: Financial support was provided to the first author (DTM) by the ICMR supporting him as Chair-Virology and Zoonoses.

Conflicts of Interest: None.

Devendra T. Mourya1*, Ashok Munivenkatappa2, Reshma Kulkarni2, Pragya D. Yadav3, Nivedita Gupta4 & Manju Rahi5

1Chair, Virology & Zoonoses, Indian Council of Medical Research, 2Diagnostic Virology Group BSL-4 Laboratory, 3Maximum Containment Laboratory, ICMR-National Institute of Virology, Pune 411 021, Maharashtra, 4ICMR-National Institute of Virology, Bangalore Unit, RGICD Premises, Bengaluru 560 029, Karnataka & 5Division of Epidemiology & Communicable Diseases, Indian Council of Medical Research, New Delhi 110 029, India

*For correspondence: dtmourya@gmail.com

Received October 12, 2019

References

1. Mourya DT, Yadav PD, Ullas PT, Bhardwaj SD, Sahay RR, Chadha MS, et al. Emerging/re-emerging viral diseases & new viruses on the Indian horizon. Indian J Med Res 2019; 149 : 447-67.

2. Boshell J, Rajagopalan PK, Goverdhan MK, Pavri KM. The isolation of Kyasanur forest disease virus from small mammals of the Sagar-Sorab forests, Nysore State, India: 1961-1964. Indian J Med Res 1968; 56 : 569-72.

3. Rajagopalan PK, Paul SD, Sreenivasan MA. Involvement of Rattus blanfordi (Rodentia: Muridae) in the natural cycle of Kyasanur Forest disease virus. Indian J Med Res 1969; 57 : 999-1002.

4. Singh BB, Gajadhar AA. Role of India’s wildlife in the emergence and re-emergence of zoonotic pathogens, risk factors and public health implications. Acta Trop 2014; 138 : 67-77.

5. National Centre for Disease Control. CDAlert. Kyasanur Forest Disease: A public health concern. Available from: https://www.idsp.nic.in/WriteReadData/1892a/60398414361527247979.pdf, accessed on October 1, 2020.

6. Patil DY, Yadav PD, Shete AM, Nuchina J, Meti R, Bhattad D, et al. Occupational exposure of cashew nut workers to Kyasanur Forest disease in Goa, India. Int J Infect Dis 2017; 61 : 67-9.

7. Mourya DT, Yadav PD, Mehla R, Barde PV, Yergolkar PN, Kumar SR, et al. Diagnosis of Kyasanur forest disease by nested RT-PCR, real-time RT-PCR and IgM capture ELISA. J Virol Methods 2012; 186 : 49-54.

8. Padbidri VS, Wairagkar NS, Joshi GD, Umarani UB, Risbud AR, Gaikwad DL, et al. A serological survey of arboviral diseases among the human population of the Andaman and Nicobar Islands, India. Southeast Asian J Trop Med Public Health 2002; 33 : 794-800.

9. Murhekar MV, Kasabi GS, Mehendale SM, Mourya DT, Yadav PD, Tandale BV. On the transmission pattern of Kyasanur Forest disease (KFD) in India. Infect Dis Poverty 2015; 4 : 37.
10. Mourya DT, Yadav PD, Ullas PT, Bhardwaj SD, Sahay RR, Chadha MS, et al. Emerging/re-emerging viral diseases & new viruses on the Indian horizon. Indian J Med Res 2019; 149: 447-67.

11. Mourya DT, Yadav PD, Patil DY. Expediency of dengue illness classification: The Sri Lankan perspective Highly infectious tick-borne viral diseases: Kyasanur forest disease and Crimean-Congo haemorrhagic fever in India. WHO South East Asia J Public Health 2014; 3: 8-21.

12. Mourya DT, Sapkal GN, Yadav PD. Difference in vector ticks dropping rhythm governs the epidemiology of Crimean-Congo haemorrhagic fever & Kyasanur forest disease in India. Indian J Med Res 2016; 144: 633-5.

13. ICD10Data.com. 2021 ICD-10-CM Diagnosis Code A98.2. Kyasanur Forest Disease. Available from: https://www.icd10data.com/ICD10CM/Codes/A00-B99/A90-A99/A98/-A98.2, accessed on October 1, 2020.

14. Centers for Disease Control and Preventions. Biosafety in microbiological and biomedical laboratories 5th edition. Available from: https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF, accessed on October 1, 2020.

15. World Health Organization. Laboratory biosafety manual – third edition. Available from: https://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/, accessed on October 1, 2020.

16. Munivenkatappa A, Sahay RR, Yadav PD, Viswanathan R, Mourya DT. Clinical & epidemiological significance of Kyasanur forest disease. Indian J Med Res 2018; 148: 145-50.

17. Holbrook MR. Kyasanur forest disease. Antiviral Res 2012; 96: 353-62.

18. World Health Organization. Laboratory biosafety manual fourth edition and associated monographs: Risk assessment. Available from: file:///C:/Users/Dell/Downloads/WHO%20risk%20assessment%20(1).pdf, accessed on October 1, 2020.

19. World Health Organization. Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products. Technical Report, Series No. 924. Geneva: WHO; 2004.

20. Mourya DT, Sapkal G, Yadav PD, M Belani SK, Shete A, Gupta N. Biorisk assessment for infrastructure & biosafety requirements for the laboratories providing coronavirus SARS-CoV-2/(COVID-19) diagnosis. Indian J Med Res 2020; 151: 172-6.

21. Hassell JM, Begon M, Ward MJ, Fèvre EM. Urbanization and disease emergence: Dynamics at the Wildlife-Livestock-human interface. Trends Ecol Evol 2017; 32: 55-67.

22. Department of Health Research, Ministry of Health & Family Welfare, Government of India. Establishment of a network of laboratories for managing epidemics and natural calamities (VRDL). Available from: https://dhr.gov.in/schemes/establishment-network-laboratories-managing-epidemics-and-natural-calamities, accessed on October 1, 2020.