



## Screening of Indian Pigs for Porcine Kobuvirus (PKV): Is it Matters?

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### Abstract

The porcine kobuvirus (PKV) is suspected to cause large outbreaks of diarrhea and dehydration along with vomiting in suckling piglets with a high morbidity rate. Despite of its global importance only few studies are available on the pathology of PKV. The present study was conducted with the objective to investigate the pathology of PKV in naturally infected Indian pigs. A total of 41 intestinal content and tissue samples from dead pigs were collected from post-mortem facility of, ICAR-IVRI during April 2019 to July 2020 and subjected to histopathological examination and molecular investigation. The 3D region of PKV was amplified using RT-PCR and 3D gene based Genetic characterization of Indian isolate of the PKV was done. A total of six out of 41 necropsied piglets were found positive for the PKV with the thickening and clouding of brain meninges, congestion of brain, pneumonia along with catarrhal and haemorrhagic enteritis as common gross lesions. Mild to moderate perivascular cuffing, congestion of meningeal and cerebral vessels, focal gliosis and neuronophagia along with mild to severe interstitial pneumonia and emphysema was observed as common microscopic findings. Intestinal mucosa revealed severe inflammatory changes along with sloughing of villi. This study reported the first pathological investigation of PKV from Indian pigs. In addition, the molecular characterization revealed maximum identity of 91 - 93% with Chinese strain of PKV, FJ459905 and Indian isolate of PKV, MH394282. Further explorative surveillance along with epidemiological studies is highly warranted to find out the real impact of the PKV on Indian pigs.

**Keywords:** Indian Pigs; Porcine Kobuvirus; Pathology; PKV

The Kobuvirus is a single-stranded, positive-sense RNA virus classified under the family *Picornaviridae* and composed of small, non-enveloped viruses [1,2]. The genus Kobuvirus consists of six species viz. Aichivirus A, Aichivirus B, Aichivirus C, Aichivirus D, Aichivirus E and Aichivirus F, infecting a wide range of hosts like pigs, sheep, goats, dogs, cats, cattle, ferrets, bats, and humans [3,4]. The Aichivirus C is the only species under the genus Kobuvirus reported to infect pigs, therefore known as "porcine kobuvirus (PKV)" [3]. The PKV was first identified in Hungary from faecal

samples of pig in 2008 followed by China [5,6]. Thereafter, PKV was subsequently reported from Asia, Africa, Europe and United states [7,8]. The PKV has been reported to be detected from both healthy [9] and diarrhetic pigs [10]. Moreover, the prevalence of PKV in domestic pigs was reported from 13-99% [11] which decreases with advancing age [12]. Despite of widespread circulation of PKV among global pig population very few studies are available on pathology and pathogenesis of this important swine pathogen. Currently the PKV may be considered as the best example of a neglected pathogen with a great potential to cause huge economic impact.

We conducted the study aimed to investigate the real impact of porcine kobuvirus (PKV) in naturally infected Indian pigs. A total of 41 naturally died piglets presented to the post-mortem facility of ICAR- Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India for systematic necropsy examination were included in the study. All the presented animals were systematically necropsied and appropriate samples were collected in the ice and 10% neutral buffered formalin for molecular and histopathological examination respectively. The tissues were processed for the histopathological screening by using routine haematoxylin and eosin staining. The collected samples were subjected to RT-PCR screening using specific primers forward: 5'-TGGAC GACCAGCTCTTCCT-TAAACAC-3' and reverse: 5'-AGTGCAAGTGCAAGTCTGGGTTGCAGC-CAACA-3' [5]. In our study tissues from six out of 41 necropsied piglets were found positive for the PKV and exhibited specific amplicons of 495 bp in 1.5% agarose gel. The samples were also screened for the porcine sapelovirus (PSV) and porcine teschovirus (PTV) to rule out the involvement of these viruses in the pathology. The diarrhoea and dehydration were observed as the most common clinical signs and piglets of less than 1 month of age were primarily found affected. On necropsy, the clouding and thickening of brain meninges, mild to severe interstitial pneumonia along with severe haemorrhagic and catarrhal enteritis was observed as common gross lesions. In addition, microscopic lesions included mild to moderate perivascular cuffing, congestion of meningeal and cerebral vessels, neuronophagia and focal gliosis in the brain. In the lungs, mild to severe interstitial pneumonia along with variable degree of emphysema was noticed. Intestinal mucosa exhibited severe inflammatory changes along with sloughing of villi. Mild to severe depletion of Peyer's patches of the ileum was observed as a common finding. We could not stain the PKV antigen in the positive tissues due to unavailability of specific primary antibodies which could be attempted in near future to establish tissue tropism and pathogenesis. Phylogenetic analysis of the 3D region revealed that Indian isolates of this study viz. MW018696 and MT946377 are closely related to the other Indian isolates viz. MH394282/India/2015 and MH394279/India/2015. The genetic characterization revealed maximum identity of 91 - 93% with Indian isolate of PKV, MH394282 and Chinese strain of PKV FJ459905. The circulation of PKV among Indian pig population is rarely studied and our study revealed the first pathological investigation of PKV in Indian pigs.

The PKV is suspected to cause large outbreaks of diarrhea and associated dehydration which may be aggravated with vomiting in young suckling piglets with a morbidity rate of 80-100% and mortality of 50-90% in China [13]. The large outbreaks of diarrhoea with highly significant mortality rate may contribute to greater economic losses. Despite of such a significant impact on global swine industry, studies on PKV in piglets are not widely conducted in India and other parts of world. Lack of information on PKV pathology, pathogenesis and unavailability of a vaccine for protection of piglets from the PKV together raised the concern of further studies on the virus. Our study suggests that the studies directed towards molecular and pathological investigation of PKV is highly warranted in Indian pigs to minimize the economic losses associated with high mortality among Indian piglets. In addition, further explorative studies in suitable animal model will definitely provide better insights of PKV pathology and pathogenesis.

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### Conflicts of Interest

The authors declare that they have no conflict of interests.

### Ethics Approval

Not applicable, as samples were not collected from the live animals.

### Consent to Participate

Not applicable.

### Consent for Publication

Not applicable.

### Availability of Data and Material

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

### Code Availability

Not applicable.

### Authors' Contributions

Shailesh Kumar Patel: conceptualization, data curation, writing-original draft. Mamta Pathak: writing-review and editing. Alok Singh: writing-review and editing. Aditya Agrawal: proof-reading and editing. Jigyasa Rana: proof-reading and editing. G Saikumar: conceptualization, writing-original draft, proof-reading and editing.

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