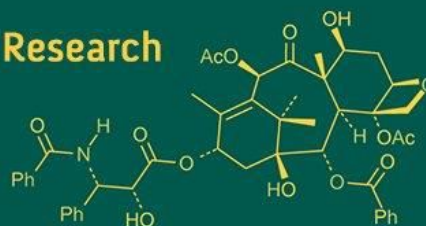
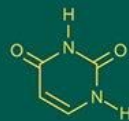
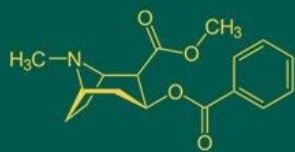


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Pathomorphological observations in pigs infected with Porcine Circovirus type-2

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Abstract

Porcine circovirus type-2 (PCV-2) represents a significant viral economic disease affecting pigs, particularly linked to the porcine respiratory disease complex (PRDC). The present study examines PCV-2 in pigs with respiratory infections, utilising PCR-based confirmation. Histopathological alterations in the lungs, tonsils, bronchial lymph nodes, spleen, heart, kidneys and brain tissues were thoroughly analysed. Post-mortem observations in cases infected with PCV-2 included a rough hair coat, erythematous patches on the abdomen and perineum, tan-mottled and non-collapsible lungs with a rubbery texture, mild to moderate congestion in the tonsils, bronchial lymph nodes, spleen and myocardium, slightly congested and swollen kidneys, as well as mild to moderate cerebral congestion. Significant histological findings in the lungs included interstitial and broncho-interstitial pneumonia, lymphocyte depletion in lymphoid organs, the presence of giant cells and syncytia formation. This study indicates a higher prevalence of PCV-2 infection in pigs with notable lesions in both respiratory and lymphoid organs.

Keywords: Lungs, lymphoid depletion, pneumonia, respiratory lesions

Introduction

Porcine respiratory disease complex (PRDC) is a multi-causal infection in pigs, primarily caused by Porcine circovirus type-2 (PCV-2), Porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, and Influenza A viruses (Pomorska-Mól and Dors, 2022) ^[11]. The porcine circovirus associated disease (PCVAD) causes significant economic losses to the piggy sector and principally it is caused by PCV-2. Infected weanlings show wasting, swollen lymph nodes, jaundice and weight loss. PCV-2 is a member of *Circoviridae* (Genus *Circovirus*), a single-stranded DNA virus with a circular genome of 1767-1768 nucleotides (Hamel *et al.*, 1998) ^[5]. PCV-2 infection is associated with disorders like reproductive failure, enteritis, pneumonia, and necrotizing dermatitis. Infected hosts exhibit immunosuppression due to lymphoid depletion, typical of PCVAD co-infection, increasing the risk of bacterial and viral infections (Ramamoorthy and Meng, 2009) ^[13].

PCV-2 infections significantly impact pig farming economics due to higher mortality, reduced weight gain, increased feed costs, more condemnations at slaughterhouses and elevated treatment and labour expenses, resulting in financial losses for producers. Additionally, PCV-2 respiratory infections are a major health concern in modern pig farming (Yaeger and Alstine, 2019) ^[21]. Symptoms associated with PCV-2 in pigs are weakness, breathing difficulties, swollen lymph nodes, diarrhoea and pallor or icterus and also PCV-2 is associated with conditions such as pneumonia, hepatitis, nephritis, myocarditis, enteritis and pancreatitis. The complete manifestation of the disease syndrome may also require the involvement of other agents, including porcine parvovirus or the PRRS virus (Allan and Ellis, 2000) ^[1]. Porcine circovirus type-2 (PCV-2) is a widely prevalent pathogen in swine populations; therefore, the present study was undertaken to elucidate its role in swine respiratory infections and to characterise the pathological changes induced by PCV-2 in affected organs of pigs.

Materials and Methods

Sample collection: Pigs with respiratory infections were included for the confirmation of PCV-2 using PCR from January to December 2022 in Hassan district of Karnataka, India.

Samples (108) were collected along with data on age, gender, breed, gross lesions in lungs and other organs. Representative tissue samples from organs showing gross lesions were collected in 10% neutral buffered formalin and subjected to routine histopathology. Tissues were processed using the paraffin embedding technique, sections of 5 microns thickness were cut and stained using hematoxylin and eosin as well as Page Green method for inclusion bodies (Suvarna *et al.*, 2018) [19]. Tissues (lungs, tonsils of the soft palate, lymph nodes, heart and spleen) were collected and stored at -20 °C until molecular analysis.

Polymerase chain reaction

The HiPurA® Mammalian Genomic DNA Purification Kit (HiMedia Laboratories Pvt. Ltd) was used to extract total DNA from organ tissue samples, after which PCR was conducted using the PCV-2 specific nucleocapsid gene, which measures 481 bp.

Results

A total of 108 samples were included in the study, of which 82 were collected from slaughterhouses located in and around the Hassan region, while the remaining 26 were obtained from the Department of Veterinary Pathology, Veterinary College, Hassan, and were submitted by pig farms in the surrounding areas. Gross examination of the 108 pig carcasses revealed that 87 animals predominantly exhibited respiratory-related lesions, 19 showed lesions associated with the digestive system, and two presented with reproductive lesions.

Among the 87 animals with respiratory-related lesions, 61 were PCR-positive for PCV-2, accounting for an occurrence of 70.11%.

Histopathology

Lungs: Pneumonic lesions were observed in 87 of the 108 lung tissue samples, representing 80.55%. Based on morphological characteristics, extent of lobe involvement, lung consistency, and the presence of exudate, these inflammatory lesions were classified as bronchopneumonia, interstitial pneumonia or broncho-interstitial pneumonia. In the present study, 61 of the 87 cases were confirmed as PCV-2 positive by PCR.

Interstitial pneumonia: Of the 61 PCR-confirmed PCV-2-positive cases, 36 exhibited interstitial pneumonia. The lungs were tan and mottled in appearance, non-collapsed, rubbery in consistency and showed mild rib impressions (Fig. 1). They exhibited varying degrees of ecchymotic hemorrhages, congestion and thickened interlobular septa. Histopathological analysis showed a rapid inflammatory response in the interstitial spaces, thickened by oedematous eosinophilic fluid, red blood cells, and mainly mononuclear inflammatory cells. Lymphocytolysis was also observed, resulting in depletion and reduced cellularity of the lymphoid tissue associated with the bronchial and bronchiolar mucosa.

Broncho-interstitial pneumonia: In the present study, 24 of the 61 cases exhibited broncho-interstitial pneumonia. The lungs were non-collapsible, edematous and showed varying degrees of congestion and patchy consolidation. Histopathological analysis revealed expanded interstitial spaces filled with eosinophilic fluid and inflammatory cells, mainly mononuclear. Alveolar capillaries were blood-filled,

with eosinophilic proteinaceous exudate noted in some cases (Fig. 2 and 3). Bronchial and bronchiolar epithelial cells exhibited hyperplasia, with the lumen filled with hemorrhagic exudate, fibrin and inflammatory cells (Fig 4). In severely affected lungs, bronchial epithelial cells showed degeneration and the lumen contained fibrino-hemorrhagic exudate (Fig 5). The cilia of the bronchial lining epithelium were occasionally absent. Bronchial-related lymphoid tissue was decreased. Intracytoplasmic inclusion bodies were occasionally seen in the lining epithelial cells of the bronchiole and bronchus (Fig 6).

Bronchopneumonia: One case of bronchopneumonia was observed in this study. The cranio-ventral lung region was reddish-brown, firm, and congested with extensive consolidation patches. The surface displayed subtle multifocal foci in tan to grey. Incision revealed varying exudate levels in the bronchi and bronchioles. Histopathological analysis showed inflammation in the alveoli, bronchioles and bronchus, with neutrophils and some lymphocytes infiltrating around the broncho-alveolar junction, bronchi, bronchioles and alveolar lumen (Fig 7).

Tonsils: Of the 61 PCV-2-positive cases, 21 (34.42%) exhibited oedema, congestion, and haemorrhages. Histopathological analysis showed congestion and lymphoid cell depletion in tonsillar lymphoid follicles, with no clear distinction between follicular and para-follicular cells. The tonsillar crypts were thickened, displaying degenerative changes in the epithelial lining, necrosis, and multifocal fibrous tissue growth. Lymphocytolysis reduced the size of the tonsillar lymphatic nodule, while moderate haemorrhage and congestion were noted in the subcapsular areas and tonsils. Some affected tonsils contained giant cells, epithelioid cells and histiocytes (Fig 8). Occasional syncytial development inside the tonsillar crypts and in the germinal core of the tonsillar lymphoid follicle (Fig 9).

Bronchial Lymph Nodes: In 34 of 61 positive cases (55.7%), bronchial lymph nodes were enlarged with mild to severe congestion. Histopathology revealed varying degrees of lymphocytic depletion in the lymphoid follicles, especially in the peripheral regions and germinal center (Fig 10). Increased mitotic cells were noted in the germinal center of lymphoid follicles. Syncytia formation occurred in the medullary region, with fewer lymphoid cells around blood vessels.

Spleen: The spleen showed congestion and slight discoloration. Histopathology indicated mild to moderate lymphocyte depletion, especially in the white pulp, with degenerating lymphocytes in both the white and red pulp (Fig 11). This depletion made the trabeculae more visible.

Heart: In 8 of 61 PCV-2 positive cases (13.11%), the heart showed slight enlargement and mild to moderate epicardial vascular congestion. Histopathology indicated mild vascular congestion and hemorrhage, with swollen myocardial fibers losing eosinophilia due to fiber disruption and mild mononuclear cell infiltration.

Kidneys: Out of 61 cases, 5 (8.19%) exhibited kidney congestion, moderate discoloration and capsular thickening. Histopathological analysis revealed mild congestion in

cortical and medullary areas, cellular edema, eosinophilia, and increased granularity in tubular epithelial cells. Some tubular cells showed severe swelling, narrowing the lumen, with mild macrophage infiltration around degenerating tubules.

Brain: Of 61 cases, two (3.27%) showed mild to moderate cerebral congestion, with histopathology revealing congested small blood vessels and mild perivascular mononuclear cell infiltration (Fig 12).



Fig 1: Lung showing mottled meaty appearance with rib imprints

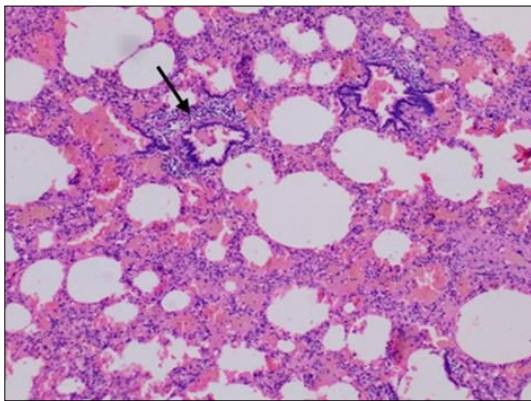


Fig 2: Lung: Inflammatory changes in interstitial spaces and bronchioles; severe haemorrhages with BAL depletion (arrow) (H&E, X100)

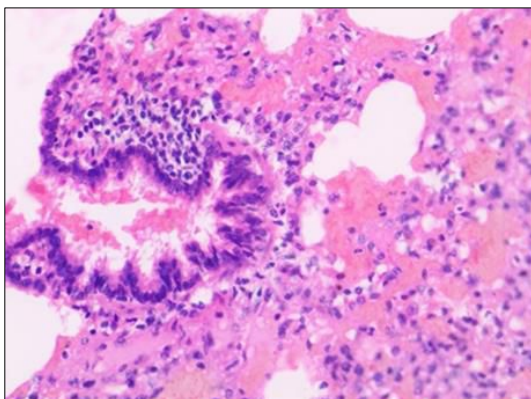


Fig 3: Lung: Inflammatory changes in interstitial space and bronchiole; inflammatory exudate in bronchiolar lumen and BAL depletion (H&E, X400)

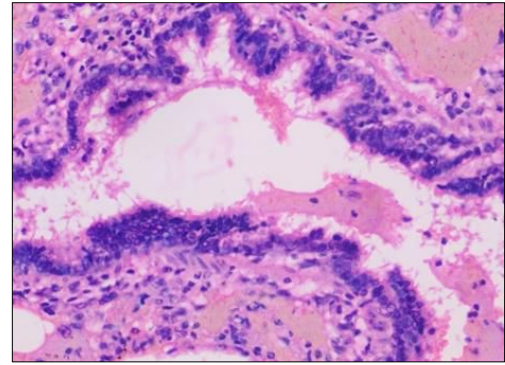


Fig 4: Bronchiole: Fibrin rich inflammatory exudate in lumen, hyperplastic changes in lining epithelium (H&E, X400)

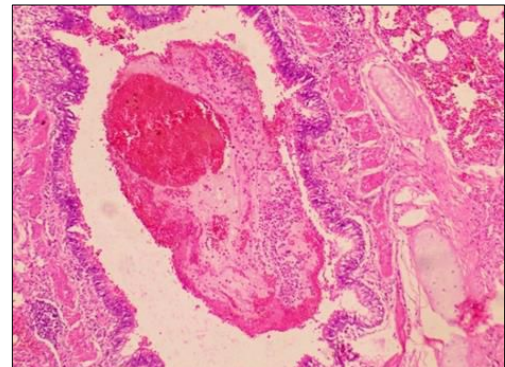


Fig 5: Bronchus: Accumulation of fibrino-hemorrhagic exudate in lumen (H&E, X100)

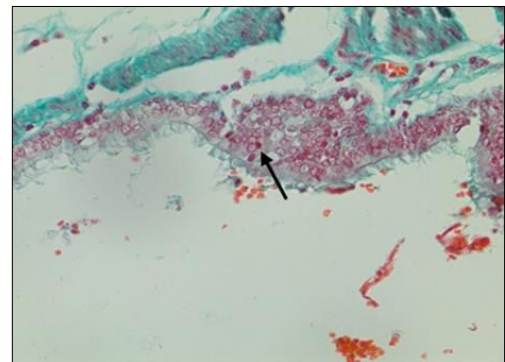


Fig 6: Lung: Orange coloured intracytoplasmic inclusion bodies (arrow) in bronchial lining epithelial cells (PGM, X400)

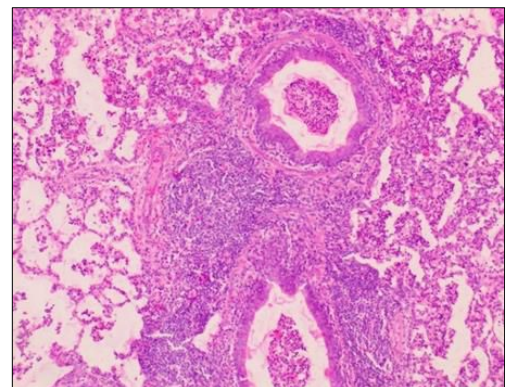


Fig 7: Lung: Inflammatory changes in bronchioles and alveoli with hemorrhages, hyperplasia of BAL (H&E, X100)

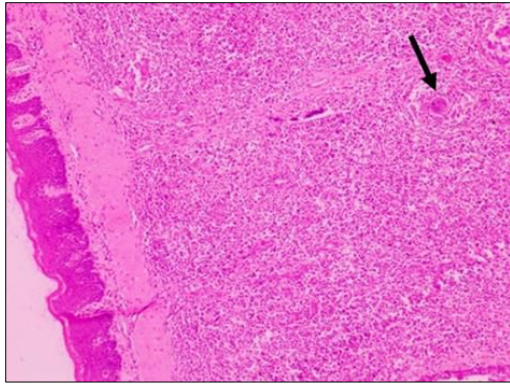


Fig 8: Tonsil: Lymphoid depletion and giant cell formation (arrow) in lymphoid follicle (H&E, X100)

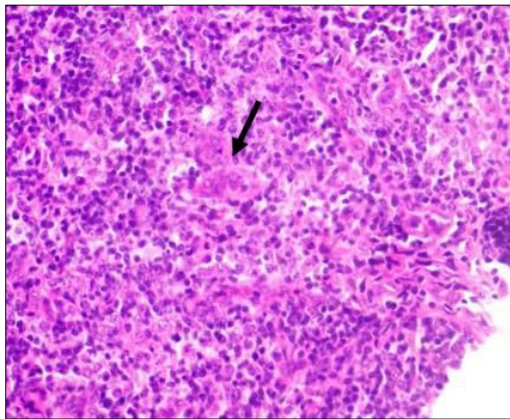


Fig 9: Tonsil: Syncytia formation (arrow) (H&E, X400)

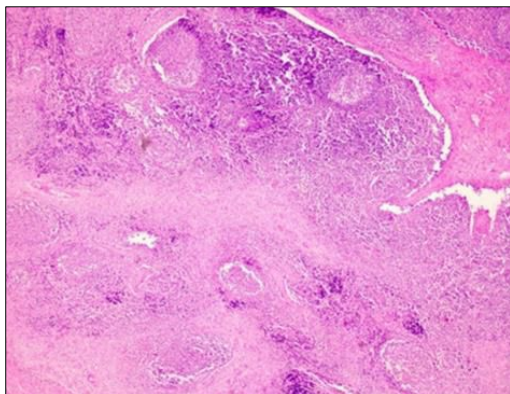


Fig 10: Bronchial lymph node: Depletion of lymphocytes in cortex and medulla (H&E, X40)

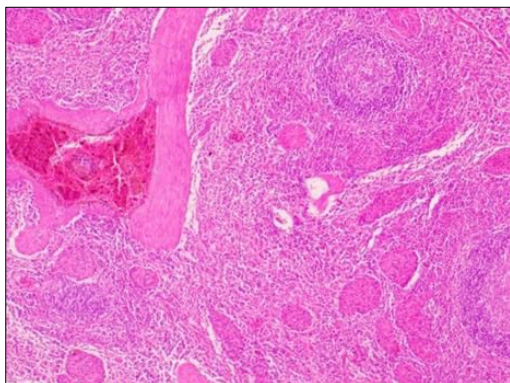


Fig 11: Spleen: Depletion of lymphocytes in white and red pulp. Trabecular arterial congestion (H&E, X100)

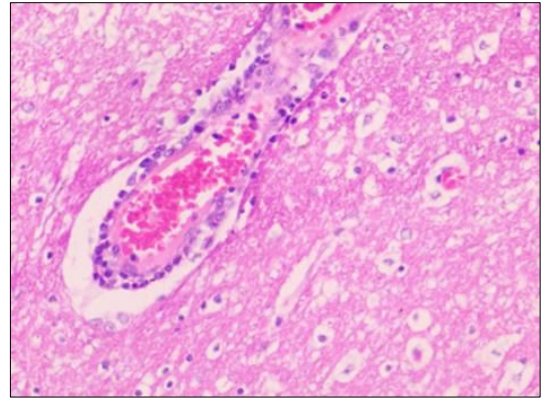


Fig 12: Brain: Perivascular infiltration of mononuclear cells around congested blood vessel (H&E, X400)

Discussion

The current investigation was undertaken with the objective of analysing the pathophysiology of respiratory infections in pigs in the Hassan district, with a particular focus on Porcine circovirus- 2. These results correspond with previous studies that highlight significant respiratory problems in pigs from the Hassan region, with similar observations made by scholars nationwide.

The study identified 61 PCV-2 positive cases via PCR testing, including 36 interstitial pneumonia, 24 broncho-interstitial pneumonia, and 1 broncho pneumonia case. Interstitial pneumonia is the most prevalent type linked to PCV-2 infection (Ellis *et al.*, 1999; Keerthana *et al.*, 2019; Sairam *et al.*, 2019) [2,6,15].

All PCV-2 positive piglets showed pneumonic changes, either as consolidation in the cranio-ventral lobes or a meaty texture indicating bronchopneumonia or interstitial pneumonia. Pneumonic lesions were the main gross findings in suckling and nursery piglets with PCV-2 infection (Szeredi and Szentirmai, 2008) [20]. The current study's histopathological findings correlate well with previous publications (Segalés *et al.*, 2005; Kim and Chae, 2004; Sairam *et al.*, 2019) [16,7,15]. In severely affected lungs, bronchial epithelial cells revealed degenerative changes a severe degree of hemorrhage associated with hemosiderin, and the lumen was filled with fibrino-haemorrhagic exudate (Keerthana *et al.*, 2019) [6].

The present study showed mild to moderate tonsillar congestion in PCV-2 affected pigs. According to previous reported findings, the lymphoid organs typically affected with PCV-2 infections were the mesenteric lymph nodes, tonsils and spleen (Keerthana *et al.*, 2019) [6]. Histopathologically, the tonsillar lymphoid follicles revealed varying degrees of congestion and lymphoid cell depletion due to lymphocytolysis, with lack of distinct demarcation between follicular and parafollicular cells (Sairam *et al.*, 2019) [15]. PCV-2-associated lymphoid depletion is not always associated with clinical manifestation and can be detected in subclinically infected pigs (Quintana *et al.*, 2001) [12]. Few affected tonsils showed giant cells and epithelioid cells scattered in the lymphoid follicles along with histiocytes (Madson *et al.*, 2009) [9].

The affected lymph nodes in the current study were enlarged, with mild to severe congestion and haemorrhages. Although the pathogenesis of PCV-2 associated disease is not fully known, direct virus induced injury to the lymphoid system following PCV-2 infection is an essential aspect. The fate of

the infection is determined by host variables, co-infections and host immunomodulation (Opriessnig *et al.*, 2006) [10]. Lymphocyte depletion noticed could be due to lysis of virus infected cells. Monocyte infiltrations in the lymphocyte depleted areas are mainly due to clear the cell debris and virus (Ghebremariam and Gruys, 2005) [4]. Even in the absence of extensive organ damage, changes in lymphoid tissue including variable lymphoid depletion associated with large, often multinucleated inclusion body containing macrophages are considered to be almost pathognomonic of PCV-2 infection (Ellis, 2014) [3]. The present investigation revealed granulomatous inflammation with a considerable number of histiocytes dispersed throughout, particularly in the medullary area (Sairam *et al.*, 2019) [15]. The presence of histiocytes and eosinophils in the lymph node can be attributed to viral aetiology and their capacity to produce a variety of antiviral and immune regulatory cytokines may be advantageous against viral infection (Stevenson *et al.*, 2001) [18]. Other researchers have reported variable lymphocytic depletion with loss of follicular architecture, multifocal to diffuse and mild to severe histiocytic infiltration and occasionally necrotizing lesions in lymphoid follicles with the occurrence of sharply demarcated, spherical, amphophilic to basophilic cytoplasmic inclusions of PCV-2 in histiocytic cells as distinct lesions of PCV-2 infection (Rosell *et al.*, 1999; Segalés *et al.*, 2005) [14, 16]. However, such inclusion bodies were not observed in the present study.

Grossly, spleens of the affected pigs showed slightly congestion with slight discolouration in some areas. Histopathologically, spleen revealed mild to moderate depletion of lymphocytes and showed varying degree of degenerating lymphocytes both in white pulp and the red pulp. However, depletion was very prominent in the white pulp region (Keerthana *et al.*, 2019; Rosell *et al.*, 1999) [6, 14]. Multinucleate syncytia were a common finding in lymphoid organs and lymphoid depletion of germinal centres and surrounding areas. Lymphoid depletion in the lymphoid organs could be due to the affinity of the virus to immune cells followed by replication and lysis of virus infected cells (Ghebremariam and Gruys, 2005) [4].

The myocardial fibres were swollen in few areas with infiltration of RBCs in between the fibres. The heart diseases characterised by severe fibrosis and/or necrotizing myocarditis was the hallmark of PCV-2 affected reproductive diseases (Opriessnig *et al.*, 2006; Madson *et al.*, 2009) [10, 9]. The kidneys were enlarged with greyish-white streaks or spots in chronic cases of PCV-2 (Kumar *et al.*, 2017; Opriessnig *et al.*, 2006) [8, 10]. Multifocal interstitial nephritis with vasculitis, lymphohistiocytic infiltrations and pyelitis in the kidney has been reported earlier (Keerthana *et al.*, 2019; Sairam *et al.*, 2019) [6, 15]. In this study, pathomorphological changes in the brain, such as mild to moderate congestion, were observed. Infection with PCV-2 has been linked to cerebellar lymphohistiocytic vasculitis with haemorrhages or lymphohistiocytic meningitis (Segalés, 2012) [17].

Conclusions

The current investigation reveals that PCV-2 is one of the most common respiratory infections in pigs in Karnataka's Hassan district. The PCV-2 virus is known to cause immunosuppression in piglets due to lymphocyte depletion, which may predispose them to other opportunistic pathogens and aggravate the condition. Pig farmers must therefore

implement excellent management procedures in order to reduce secondary opportunistic infections.

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