Pathological observations of herpes virus infection in a Labrador pup

Nishchal Dutta, Jasmine Banga, Sidhartha Deshmukh and Harmanjit Singh Banga

Abstract
This pathological study deals with 7 day old pup which suffered from canine herpes virus (CHV) infection. Macroscopically, typical multifocal patches of haemorrhages with white necrotic centre in lungs along with various other organs were observed. The knowledge of its transmission was not known till the presence of typical intra nuclear inclusion bodies in all major organs including endothelial cells of blood vessels was noted microscopically. The pathologic changes in eye, cerebral parenchyma, endothelial cell lining of blood vessels and myocardial cells, besides major splanchnic organs clearly demonstrated possible haematogenous transmission of viral particle following in transplacental infection. Interestingly, vivid pathologic description of ocular and cerebral changes, in addition to the myocardial and endothelial cells involvement has been attempted to deliver the first ever information following naturally occurring infection of canine herpes virus in neonatal pup in India.

Keywords: Canine herpes virus, intra nuclear inclusion bodies, labrador pup, pathology

Introduction
Canine herpes virus infection (CHV) in neonatal pup is generally characterized by widespread congestion, multifocal haemorrhage (s) along with necrotizing lesion (s). This virus usually affects pup lesser than 3 weeks of age [3, 5] with resultant heavy mortality infecting various organs such as liver, kidneys, lungs and intestines [7, 8] and gradual resistance at later ages of life. The infectious agent is almost omnipresent across the globe and has been reported from many countries [6, 11, 13]. The disease seems to be acquired through transplacental route [13] and apparently healthy pups are born with surviving infections in utero, which later succumb to death, depending upon host susceptibility. The probable reason behind increased mortality following post-natal period could be cited as interference with the development of organs in various ways by acute viral infections [13] during ante-natal period and also as a result to sudden exposure to increased environmental temperature following birth, which results in to clinical manifestation of CHV. Interestingly, this report endeavour to describe the histological observation of exceptional appearance of typical intra nuclear eosinophilic viral inclusion bodies in almost all organs of the body with special reference to central nervous system (CNS) and optic nerve, observed from a dead Labrador pup which perhaps died following transplacental infections with canine herpes virus infection. We believe the present case is an example of transplacental infection from the observation of massive presence of inclusion bodies in all splanchnic organs, including brain and focal granulomatous lesion of optic neuritis in a week old pup. We believe this report to be first of its kind from India with an evidence of inclusion bodies in all most all major organs of body, including endothelial cells of blood vessels. The incidence of canine herpes virus infection in young and small aged pups has not yet been reported from India. The present study gave an insight to confirmed transmission of virus particle to various organs and suggesting transplacental migration. We suggest in future, the probable mother dogs should undergo prior screening for antibodies to CHV to avoid unexpected deaths of neonatal or infant puppies in kennels.

Materials and Methods
Case History: Six pups were born and all of them died in span of week time. The presented case was of 7 day old pup which had a clinical history of epistaxis with shallow breathing. The mortality started after five to six days of birth on a regular basis, and continued till the last pup which survived for few days and died just before its presentation to Necropsy annexe of...
Department of Veterinary Pathology, GADVASU, Ludhiana. The animal was necropsied and all major splanchnic organs were collected in 10% neutral buffered formalin. Gross pathological recording(s) was carried out, while collecting representative tissue samples from various organs for histological analysis. Tissue samples were processed as per the routine histological procedure and were paraffin embedded, finally sectioned at 4μm thickness. After sectioning, specimens were stained with haematoxylin and eosin (H-E). The sectioned slides were further subjected to Triple Shorr’s staining for the demonstration of inclusion bodies in different organ(s) and Masson’s Trichome for demonstration of fibrous tissue proliferation in optic nerve. Gram’s staining was also performed to rule out the evidence of bacterial organism(s).

Results
There was no a clinical history of canine herpes virus infection in mother dog. However, the presence of respiratory distress with shallow breathing and having bleeding from nose, implied to a possible infections of canine herpes virus in a pup. The principal macroscopic findings noted were marked congestion and haemorrhages on the epicardial surface. The lungs typically had multifocal patches of haemorrhages with white necrotic centre, mainly over the diaphragmatic lobes and on apical lobes. There was presence of frothy and amber coloured fluid in the bronchioles. The kidneys were characteristically swollen and nephrotic and appeared to be hyperaemic. The liver revealed enlarged haemorrhagic lesions at multifocal places with areas of pale discolouration. Additionally, the swollen liver exhibited characteristics embossed rib impressions on its surface. The stomach, especially the fundic part was found to be thick and oedematous and seen as a highly folded rugal mucosa. The cerebral parenchyma was highly hyperaemic and oedematous with an evidence of swollen gyri (gyrus) throughout its surface. Surprisingly, the ocular (gross) changes, noted in the case was not fair enough to be considered as changes produced during the course of canine herpetic infections in developing neonatal pups, however mild congestion and swollen orbit was observed. The microscopic findings revealed disseminated areas of hyperaemia and haemorrhages in various organs, which are considered to be fundamental lesion, in the present study.

Cerebrum: In cerebrum, hyperaemia, massive oedema of neuropil and swollen neurons in parenchyma were consistently observed. There was irregular and small indistinct focal gliosis in the neuropil. The swollen neurons particularly, pyramidal cells exhibited at least one or two characteristics intra nuclear acidophilic inclusion bodies with or without clear halo within nucleus (Fig. 1), which mimicked to “Mickey Mouse eye” appearance in the affected nuclei.

![Fig 1: Cerebrum, swollen neuron showing typical intra-nuclear inclusion bodies (Arrow) within nucleus. H&E Bar = 20 μm.](image1)

The neurons appeared faint with fair loss of eosinophilic cytoplasmic staining and certain loss to dendritic connectivity and parallel arrangement. At some places, ghost of neurons and condensation of affected nervous nuclei were observed. The vascular endothelial cells of cerebral parenchyma were often found to be oedematous with intermittent presence of inclusion bodies in the nuclei (Fig. 2).

![Fig 2: Cerebrum, scattered presence of swollen vascular endothelial cell (Arrows) containing intra nuclear inclusion bodies. H&E Bar = 20 μm.](image2)

However, perivascular cuffing with mononuclear cells was not discernible at any place. The areas of small and scattered gliosis were characterized by gamastocytic astrocytes proliferation. The leptomeninges revealed marked hyperaemia with infiltration of mononuclear cells with obvious disorientation and depletion of horizontal cells of cajal from cerebral cortex.

Eye: Amazingly, the section revealed marked damage to choroid and sclera layer(s) with an evidence of focal actinotic granuloma in the nerve fibre of the peri-orbital tissue. The lesion was characterized by central areas of homogenous eosinophilic mass and oedematous spaces with well margined fibrous tissue proliferation besides, marked mononuclear cell infiltration(s). Some of the Schwann cells...
were seen to be enlarged with oedematous fluid resembling with gamastocytic astrocytes. Interestingly, mononuclear cells, especially the macrophages were found to be approaching to the zones of eosinophilic clumps to efface out the degenerated material. At occasional places of peri-orbital granuloma, deposition of faint basophilic granular substances within the degenerated nerve cells was observed, adjacent to optic tract. On Gram’s staining, those granular bodies appeared as prominent bluish vesiculated bodies restricted to only degenerated nerve cells of granulamatus lesion and does not reveal any bacterial organism(s). The evidence of intra-nuclear eosinophilic inclusion bodies in endothelial cells and Schwann cells (Fig. 3) of peri-orbital nervous tissue was often observed. The peri-orbital proliferative fibrous tissue revealed green staining on Masson’s Trichome stain.

**Lungs:** In lung, mild desquamation of septal and bronchial epithelial cells was prominent and the alveolar lumen had haemorrhagic areas. The bronchiolar epithelial cells typically showed presence of intra-nuclear acidophilic inclusion bodies (Fig. 4) with characteristic loss of cilia. The infected epithelial cells were found to be deserted by the neighbouring healthy cells with resultant migration by few mononuclear cells in the sub epithelial region at many instances. In addition to it, mild multifocal interstitial pneumonia was observed throughout the parenchyma.

**Kidney:** Renal lesion was seen in cortex or medullary zone, involving glomeruli, tubules and collecting ducts. Tubular epithelial cells and some glomerular cells exhibited vacuolar degeneration along with areas of haemorrhages. At various places, haemosiderin laden swollen tubular epithelial cells were noted. The proximal and distal convoluted tubules containing intra nuclear inclusion bodies (Fig. 5) were found to be swollen and dissociated from their customary configured luminal framework with resultant movement of mononuclear cells in the interstitial tissue spaces.

**Liver:** Every hepatocyte revealed marked albminous degeneration with characteristic presence of multiple eosinophilic inclusion bodies (Fig. 6). The endothelial cells of hepatic blood vessels appeared oedematous with evidence of inclusion bodies in them. Swelling of sinusoidal lining of hepatic parenchyma was also noted. Very small areas of necrosis along with haemorrhages were irregularly seen throughout the parenchyma. Amazingly, peculiar peri-cellular accumulation of mononuclear cells around the hepatocytes laden with inclusion bodies was observed. The areas of extra medullary haemopoeisis were also apparent at some places.
Fig 6: Liver, Hepatocytes showing marked aluminous degeneration with characteristic presence of multiple eosinophilic inclusion bodies having “Mickey Mouse eye” appearance (Arrows). Tripple Shorr’s Bar=20 µm.

Pancreas: Comparatively in pancreatic acinar cells remarkably large sized inclusion bodies (Fig. 7) were noted, which resulted in to mark swelling of cells. The affected pancreatic acinar structure resembles to ruined fabric matrix with loss of internal strength and associated disorientation of architectural framework.

Fig 7: Pancreas, Acinar cells showing variable and remarkably large sized inclusion bodies (Arrows) having ruined pancreatic matrix. H&E Bar = 20 µm.

Stomach: In fundic stomach, the parietal cells revealed degenerative changes with presence of eosinophilic inclusion bodies (Fig. 8) followed by characteristic individualization and detachment with consequent focal necrosis.

Fig 8: Stomach, Parietal cells containing inclusion bodies (Big Arrow) and characteristics detachment of cells and consequent process of focal necrosis (Small Arrow). H&E Bar = 20 µm.

Heart: In heart, atrial valvular epithelial cell lining and myocardial cells revealed intra-nuclear acidophilic inclusion bodies (Fig. 9 and 10). At several places, the affected muscle fibres showed intermittent weakening of cross striations. Interestingly, all the examined organs exhibited positive saffron to red brown coloured inclusion bodies on Tripple Shorr’s staining.
Interestingly, the age of the diseased pup in the present case in question to an age group of 2-3 weeks from a mother (bitch), which had no clinical history of infections with canine herpes virus (CHV) or Canine distemper (CD). The gross pathology recorded in the present case, somehow specify towards systemic disease of neonatal pup which was characterized by hyperaemia and haemorrhagic lesions in a wide variety of organs. It is reported that, neonatal systemic herpetic infections are mostly slated to be hyperaemic and haemorrhagic and acquired through birth canal [17]. In newly born puppies with established herpes virus infections, virus particle are often associated with uneven existence in varied organs with irregular evidence of viral shedding [21] in addition to mal development of certain sensitive organs like eye, central nervous system [9]. From the view point of histopathology, in our case, the exceptionally high and accessible observation of viral inclusions in every cell from every major organ was surprising and such findings have never been reported before. The ocular lesion comprises of focal granulamatus lesion in the optic nerve, which was well maintained with fibrous tissue proliferation suggests to chronic progression of diseases, and deliberately cue to preferential involvement of the central nervous system, during in utero. It is recognized that the canine herpes virus has a tendency to produce severe ocular inflammation [1] with relative evidence of regressive changes, with or without the involvement of central nervous system. However, the ocular lesion in the present study do not corroborate to any resultant agenesia or mal development following infection. The central nervous system which includes cerebral parenchyma exhibited marked congestion with swollen gyri (gyrus) on gross observation and showed massive presence of intra nuclear acidophilic inclusion in all the pyramidal cells without any major inflammatory cell infiltration or accumulation in neuropil on histological observation. It is conditionally observed in one previous study [8] that, during severe CHV infection, the intensity of inflammatory reaction didn’t reach high and very irregular amount of necrotic and haemorrhagic lesions was observed in the involved organs. We, believe the suitable reason to this observation, could be the disturbances in cellular migration with respect to viral seating in the nerve cells. Acute viral infections are intent to cause disturbance in the differentiation of tissues and cellular migration resulting in altered embryogenesis. However, in the present study, the cerebral parenchyma was found to be relatively mature at the time of infection and showed minimal alteration like disorientation and depletion of horizontal cell of cajal, just beneath the meninges. The unusual occurrence of bluish vesiculated granular bodies in the degenerated neurons of optic nerve remains obscure and similar observation was made by[9], from a pup transplacentally infected with canine herpes virus. The widespread evidence of acidophilic inclusion bodies in the pulmonary cells, myocardial cells, hepatic and renal cells along with parietal cells of fundic stomach and pancreatic acinar cell suggest to serious systemic migration of virus particle through haematogenous route. The associated evidence of intra nuclear inclusion in myocardial cells, atrial lining epithelial cells and occurrence of inclusion bodies in endothelial cells of all most every blood vessels of organ suggested to viral haematogenous excretion and considered to be an extraordinary findings and not being reported earlier. The evidence of inclusion bodies in endothelial lining and myocardial cells from the present case confirms to the migration of viral particle through blood

Discussion

The present study elaborated the unusual involvement of central nervous tissue with ocular changes in canine herpes virus infection from a Labrador pup. The widespread involvement of splanchnic organs in the present case also set out to suggest marked systemic load of infections in newly born pup. The route of infection and incidence of progression of the disease in the given case since prenatal stage is undefined; however, the consequent history of mortality among littermates along with ocular lesions and evidence of acidophilic intra-nuclear inclusion bodies in neurons of cerebral parenchyma and other splanchnic organs showed characteristics lesions of canine herpes virus infection. The other pathological abnormalities associated with various organs are almost identical to those seen in canine herpes virus infection as described earlier [9, 14, 20]. Therefore, we purported reasonably to suppose this case as a canine herpes virus infection which may have been acquired in utero (transplacental) rather than after birth. Although direct evidence to CHV infections was not obtained in the present case, through any of isolation or immunological assays. The age of an animal at the time of infection with virus often decides the pattern of the diseases [16] and under both natural and experimental condition, the disease affects mainly young pups less than 3 weeks of age with heavy systemic infections [2, 4] and resultant mortality, while dogs over 3 weeks of age exhibit disease with variable severity and less mortality [19],

Fig 9: Heart. Nuclei of myocardial cells showing acidophilic inclusion bodies (Arrow) and loss of cross striation by affected fibres. Tripple Shorr’s Bar=20 µm.

Fig 10: Heart. Atrial valvular epithelial lining containing intra nuclear inclusion bodies (Arrow). Tripple Shorr’s Bar=20 µm.
vessels with subsequent systemic dissemination to various organs of the body. Hypertrophy of vascular endothelial cell considered possible dissemination of canine herpes virus through blood vessels was observed [14], but failed to mention the appearance of typical acidophilic inclusion bodies in endothelial lining. The infrequent and occasional peri-cellular infiltration of leucocytes around the affected cells also indicated to the disturbance in the chemotaxis, induced by early viral particles. Among splanchnic organs, the pancreatic acinar cells revealed the disturbances in embryogenesis which was characterized by inadequate framing of acinar cell around central lumen and disjointed architectural framework. The other histopathological appearance in lungs, liver, heart, stomach and kidney were more or less similar to the earlier observations made by several workers [10, 20, 21].

Conclusion
There is an under-reporting of canine herpes virus infection in India probably due to poor testing tendency by the breeders. Majority of them pretends to deflect situational demand by showing oblivion to the circumstances. Incidentally we have come across with unique description of pathological changes due to canine herpes virus infection affecting central nervous system and ocular tissues in a young pup infected via trans placental route. This is a first ever study that demonstrated the confirmed haematogenous spread of virus particle in various organs of the body possibly acquired during in utero development of pup. We therefore suggest in future, prior to breeding and whelping, the expected mother dogs should sincerely be subjected to mandatory screening of antibodies against CHV to avoid unexpected deaths of neonatal or infant puppies in kennels to avoid losses.

Acknowledgment
Authors are thankful to Dean, Khalsa College of Veterinary and Animal Science, Amritsar and Director of Research, GADVASU, Ludhiana for extending their support at every steps of making this case report.

References
6. Cornwell HJC, Wright NG, Campbell RSF, Roberts RJ, Reid A. Neonatal disease in the dog associated with a herpes like virus. Veterinary Record. 1966; 79:661-662.
7. Cornwell HJC, Wright NG. Neonatal canine herpes virus infection; A review of present knowledge. Veterinary Record. 1969; 84:2-6.