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Abstract

Nopadon Pirarat* Theerayuth Kaewamatawong Somporn Techangamsuwan

A RETROSPECTIVE IMMUNOHISTOCHEMISTRY STUDY ON FELINE PANLEUKOPENIA VIRUS, INDUCED ENTERITIS, IN CATS

The retrospective study of 30 cases records using formalin-fixed, paraffin-embedded intestines, from Feline Panleukopenia (FPL) infected cats using histopathological criteria as seen by the Department of Pathology, Faculty of Veterinary Sciences, Chulalongkorn University between 1997-2001, was undertaken. The objective of the study was to examine FPLV infection in feline intestines showing characteristic lesions of parvovirus enteritis and to describe the relationship between the intestinal microscopic lesions of FPL in affected cats, with the location of FPL antigens, using immunohistochemical methods. The most striking histological lesions were villous atrophy and epithelial cell necrosis 96.67%. The lumen of crypts were often greatly reduced in diameter or occasionally were widely distended with mucus, 56.67%. Degenerative and hyperplastic changes were noted in the crypt epithelium, 90%. Secondary complications with bacteria and fungal organisms were commonly seen, 66.67%, with inflammatory cells infiltrating into the lamina propria, 60%. Immunohistochemistry demonstrated FPLV antigens in 12 out of 30 cases, (40%) and were mostly detected in infected, crypt epithelium, 36.67%, smooth muscle cells, 13.33% and villous epithelium, 6.67% which correlated with the histopathological criteria.

Keywords: feline panleukopenia, histopathology, immunohistochemistry

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บทคัดย่อ

นพลดิษฐ์ ศรีสุทธิ์ แก้ววลีพระ ธนาบรรณาธิบดี

การศึกษาข้อมูลหลังรักษาที่ถูกให้เกิดล้าสัณห์โดยวิธีอภิปรายในสถิติคณิต

ศึกษาข้อมูลหลังรักษาล้าสัณห์โดยวิธีที่เป็นตัวของโรครักษาจำแนก จำนวน 30 ตัวอย่าง ณ ภาควิชาพยาธิวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ระหว่างปี พ.ศ. 2539-2544 จุดประสงค์เพื่อวิจัยความสัมพันธ์ระหว่างรายรุกค่าล้าสัณห์วิทยาของล้าสัณห์และลำดับการปรากฏของข้อรักษาที่มีโดยวิธีอภิปรายในสถิติคณิต ผลทางพยาธิวิทยาพบว่าการคลื่นลมและภาวะของล้าสัณห์ยุ่งยากเกิดขึ้น ร้อยละ 96.67 การคลื่นลมขยายจากต่ำเพียงสูงถึงต่ำจนถึงสูงของล้าสัณห์ปริมาณและเฉลี่ยน้อย ร้อยละ 56.67 การเป็นโรครักษาฟักตัดการรักษาที่มีข้อรักษาเหล่านี้ ไว้รักษาที่เหล่านั้น 12 ตัวอย่างจากทั้งหมด 30 ตัวอย่าง ร้อยละ 40 โดยพบวิสัยทัศน์ยุ่งยากปริมาณที่ต่ำเกือบเท่ากับ เรายละ 36.67 ในกลุ่มเดี๋ยวนี้ข้อมูลเหล่านี้ ร้อยละ 13.33 และข้อมูลทางสถิติคณิต ร้อยละ 6.67 ข้อมูลคลื่นลมด้านล้าสัณห์วิทยาที่พบ

คำสำคัญ: ใช้ที่เหล่านั้น ล้าสัณห์วิทยา อภิปรายในสถิติคณิต

Introduction

Feline panleukopenia (FPL), infectious, feline enteritis, is considered the most important infectious disease of cats. The causative agent is a highly contagious, heat-stable parvovirus (Parrish, 1994). FPLV is a host-range variant of feline parvovirus in a subgroup consisting of canine parvovirus, mink enteritis virus, raccoon parvovirus and blue fox parvovirus (Horiiuchi et al., 1997; Steinel et al., 2000). Cats of any age can be susceptible but the predilection is for cats under 2 years of age (Langheinrich et al., 1971). Incubation periods of the disease are 3-7 days. This precedes sudden clinical signs of anorexia, vomiting, abdominal pain and diarrhea. Diarrhea is an usual finding, except in peracute cases (Parrish, 1994).

The classical lesions of FPLV infection are necrosis of the intestinal epithelium beginning in the crypts, villous atrophy, necrosis of lymphoid tissue and depletion of lymphocytes (Waldvogel et al., 1992). Distinctive histologic lesions of FPL are attributed to the pathogenesis of the viral replication required for rapidly dividing cells. Infected cells must proceed through the DNA
synthesis phase of the cell cycle before parvovirus replication can occur (Nelson et al., 1979).

The purpose of this present study was to examine the FPLV infection in feline intestines with characteristic lesions of parvovirus enteritis and to describe the relationship between the intestinal microscopic lesions of FPL in affected cats with the location of FPL antigens using immunohistochemical method.

**Materials and Methods**

A retrospective study of 30 cases of FPL using formalin-fixed, paraffin-embedded, intestinal samples, from infected cats using the histopathological criteria of Nelson et al., (1979) at the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, between 1997-2001 was undertaken. The formalin-fixed samples of small intestine were sliced into 0.5 cm thick, serial sections and routinely histopathologically, processed and embedded in paraffin wax. The blocks were sectioned 5-6 μm thick and stained with hematoxylin and eosin (H&E).

**Immunohistochemistry**

Immunohistochemical identification of FPL viral antigen, using peroxidase-anti-peroxidase (PAP), was performed on formalin-fixed, paraffin embedded intestine tissues, using silane-coated slides. Tissue sections were deparaffinized and hydrated by immersion in xylene, graded alcohols and distilled water. Endogenous peroxidase activity was blocked by incubating sections in 3% hydrogen peroxide solution for 30 min. Sections were incubated with skim milk, to block any specific reaction. Sections were gently drained and incubated with monoclonal anti-FPLV antibody (courtesy by Dr.Y. Une), at a dilution 1: 500, in PBS at 4°C, overnight. After another rinse in PBS buffer solution, sections were consecutively incubated with commercial, peroxidase, conjugated, antibody polymer (Nichirei, Japan), stained with 3-amino-9-ethylcarbazole solution (AEC) and counterstained with methyl green. The slides were observed under a light microscope. A section of normal cat intestine was used as a negative control and an internal positive control for checking technical errors, was also performed, using PBS, instead of specific primary antibody. The immunoreactivity was evaluated by observing the positive dark red color of AEC chromogen which contrasted with the light green background of methyl green.
Results

Table 1. Histopathological lesions induced by FPLV

<table>
<thead>
<tr>
<th>Histopathological lesions</th>
<th>Positive cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballooning epithelium</td>
<td>90.00% (27/30)</td>
</tr>
<tr>
<td>Distorted luminal crypts</td>
<td>56.67% (17/30)</td>
</tr>
<tr>
<td>Intranuclear inclusion bodies</td>
<td>50.00% (15/30)</td>
</tr>
<tr>
<td>Intramucosal organisms</td>
<td>66.67% (20/30)</td>
</tr>
<tr>
<td>- Bacteria</td>
<td>60.00% (18/30)</td>
</tr>
<tr>
<td>- Fungi</td>
<td>6.67% (2/30)</td>
</tr>
<tr>
<td>Infiltration of inflammatory cells</td>
<td>60.00% (18/30)</td>
</tr>
<tr>
<td>Goblet cell hyperplasia</td>
<td>30.00% (9/30)</td>
</tr>
<tr>
<td>Villous necrosis</td>
<td>96.67% (29/30)</td>
</tr>
</tbody>
</table>

Table 2. Positive viral antigen and distribution of antigenic locations

<table>
<thead>
<tr>
<th>Antigenic locations</th>
<th>Positive IHC cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degenerated villous epithelium</td>
<td>6.67% (2/30)</td>
</tr>
<tr>
<td>Crypt epithelium</td>
<td>36.67% (11/30)</td>
</tr>
<tr>
<td>Smooth muscle of tunica muscularis</td>
<td>13.33% (4/30)</td>
</tr>
</tbody>
</table>

Cats below 5 month-old can be susceptible to FPLV (22/30) whereas only two 6 to 11 month-old and four 1 to 2 year-old were seen. No cats over 3 year-old were observed and no age was available for two cats. There were no significant differences in sex of FPLV infected cats, 8 males, 14 females with 8 unknown. The intestines showed various degree of autolysis ranging from the loss of the villous epithelium to the disappearance of the nucleus of the cells in the lamina propria mucosae. The predominant features of the intestinal lesions were extensive necrosis of crypt epithelium, (96.67%) with retraction of the lamina propria and shortening of the villi (Fig.1). The lumina of the crypts were often greatly reduced in diameter or occasionally were widely distended with mucus, (56.67%). Degenerative and hyperplastic changes were noted in the epithelium, (90%) with evidence of secondary intramucosal organisms, (66.67%). Secondary bacterial and fungal contamination
Figure 1. Extensive necrosis of epithelium crypts, crypt distortion (large arrow), retraction of the lamina propria, and shortening of villi (small arrow). (small H&E, intestine, bar = 100 µm)

Figure 2. Positive immunoreactivity of FPLV antigens in villous epithelium (IHC, AEC stain, bar = 50 µm)
were detected in 60% and 6.67% of cases respectively. Inflammatory cell infiltration, (60%) was mainly composed of mononuclear lymphocytes. A number of neutrophilic responses were also seen, especially in cases of secondary bacterial and fungal infection. Hyperplasia of the epithelium was indicated by increased cellularity, cellular hypertrophy and increased cytoplasmic basophilia. Crypt enterocytes had either an abundant, light, eosinophilic cytoplasm and swollen, vesicular nuclei with prominent nucleoli or the cells were shrunken and had pyknotic or fragmented nuclei. In some areas of degenerated intestinal epithelium, goblet cell hyperplasia was also in evidence, (30%). There was an apparent syncytial formation in the intestinal crypts, characterized by clustering of the cell nuclei and a loss of discernible boundaries. Specific intranuclear inclusion bodies were also observed (50%) (Table 1).

Immunohistochemical detection of FPLV were possible in 12 out of 30 cases, (40%). FPLV antigens were mostly detected in infected crypt epithelium, (36.67%), some smooth muscle cells, (13.33%) and mucosal epithelium, (6.67%) (Table 2) (Fig. 2). The location sites of positive reaction were mostly detected in the cytoplasm of degenerated crypt epithelium. Some nuclear positive cells were occasionally seen. The positive immunoreactivity observed in crypt epithelium and degenerated villous epithelium corresponded with histopathological changes such as, hyperplastic crypt epithelium, intranuclear inclusion bodies in crypt epithelium and occasionally in degenerated epithelial cells. In contrast with the positive reaction in smooth muscle cells, no evidence of hemorrhage or intranuclear inclusion bodies in smooth muscle cells was observed.

Discussion

In the present study, The most striking intestinal lesions were an extensive necrosis of crypt epithelium with retraction of the lamina propria and shortening of the villi, as seen by other investigators (Langheinrich et al., 1971; Nelson et al., 1979). Histopathological characterization, such as crypt dilatation, crypt epithelium degeneration, vanishing crypts and villous atrophy were not seen only in FPLV induced enteritis but also in feline, leukemia virus (FeLV), associated enteritis (Reinacher, 1987).

Because the pathognomonic lesions of FPLV infection were mainly observed in the intestine, by histopathological criteria and formalin-fixed intestines are the only convenient archival specimens for conducting retrospective study, the tissues of choice, selected in the present study were only small intestine, especially the jejunum. Furthermore, various autolytic changes on the top of the villous epithelium were easily observed in the intestines infected with FPLV. The retrospective cases studied were unavoidably conducted without any selection.

According to Reinacher (1987), the mean age animals suspected of contracting FPLV is about 6 months, whereas cats with FeLV-associated enteritis are more than 2 years of age, on average. The common intestinal complication of the initial viral insult was an invasion of damaged mucosa by
bacteria and fungi (Ikegami et al., 1999). The number with a positive immunohistochemical reaction was relatively low when compared with the typical histopathological lesions. The inability to detect FPLV might have been due to prolonged storage in 10% formalin, severe autolytic changes or ischemia and cell death which initiated the release of lysosomal, proteolytic enzymes that irreversibly alter molecular structure and antigenicity. Furthermore, the virus may have already been eliminated or at least reduced below detection level, by the time the animal died (Waldvogel et al., 1992). The FPLV antigens seen in the present study were mostly detected in the crypt epithelium whereas mature villous epithelium was rarely positive, when compared with the histopathological picture. This might be due to the dead and degenerated epithelial changes and the virus being already been eliminated, by the time the animal died, according to Waldvogel (1992). In organs known as replication sites of parvovirus, Weissenbock and Burtscher (1991) claimed that the dorsal epithelial cells of the tongue was the most reactive site, for detecting FPLV antigen by immunofluorescence and histopathological methods. The stratified epithelium of the tongue has a more rigid integrity than the columnar epithelium of the intestine which is easily broken down and autolysed.

Monoclonal, anti-FPLV antibody used in the study was also positive in canine intestines infected with canine parvovirus (Une, unpublished data). Immunohistochemical results in this study could not differentiate FPLV from canine parvovirus infection because of homologous DNA sequences and identical antigenic properties amongst the subgroup. Furthermore because of similarity of the histopathological lesions and evidence of canine parvovirus isolation from cats, the question arises whether canine parvovirus is a pathogen for cats and is still under discussion (Mochizuki et al., 1996).

According to other investigators, viral antigen in most cases was found in the cytoplasm of the intestinal epithelial cells. This may indicate that virus and viral components are diffused into the cytoplasm after the breakdown of the nuclear membranes of degenerated cells (Waldvogel et al., 1992). The immunohistochemical positive reaction seen in this study was not only observed in young, active, crypt epithelium, but also in tissues with low or no mitotic activity such as smooth muscle cells and mucosal villous epithelium, as reported by Weissenbock and Burtscher (1991). The question allows us to postulate the parvoviruses exclusive affinity to rapidly dividing cells. Several conditions have been mentioned to be a likely cause of similar lesions, such as feline leukemia virus (Waldvogel et al., 1992). Definitive confirmation of the etiology by demonstrating the antigen within the lesions is currently the most useful method that can be achieved.

**Acknowledgement**

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References


