Histological and Ultrastructural Findings in Commercial Bred Rabbits Exhibiting Severe Diarrhea

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Summary

The objective of this study was to describe histological and ultrastructural findings in the intestine of 8 rabbits exhibiting severe diarrhea, a problem which occasionally severely effects commerceila rabbit breading operations.. Microscopic lesions from the small and large intestines were very similar to those described in the mucoid enteritis complex. Histological lesions included mucoid neutrophilic or lymphocytic-plasmacytic enteritis with atropy and fusion of the villi, hyperplasia of the goblet cells and submucosal and serosal edema in the small intestine. *Escherichia coli* was isolated from 3 animals. The ultrastructural study revealed in addition to hyperplasia of goblet cells, loss of microvilli in the small intestine. Furthermore pseudonuclear inclusions surrounded by true nuclear membrane and free intranuclear and intracytoplasmic virus-like particles were observed in all rabbits. It was concluded that the diarrhea had a multi-factorial etiology, probably due to change in feed, bacterial proliferation and bacterial coinfection with enteric virus.

Introduction

The enteritis complex, or mucoid enteritis, in fattened pet and breeding rabbits (*Oryctolagus cuniculus*) has been associated with several factors, one main factor being a high-carbohydrate low-fiber diet. These types of diets can cause the proliferation of bacteria such as enteropathogenic *Escherichia coli* (*Sinkovics 1976; Meshorer 1976; Peeters et al., 1988a*), production of bacterial toxins by agents like *Clostridium perfringens, Clostridium difficile and Clostridium spiriforme* (*Butt et al., 1994*), proliferation of protozoans like *Eimeria intestinalis, Eimeria flavescens, Eimeria magna, Eimeria media, Eimeria perforans and Cryptosporidium parvoum* (*Peeters et al., 1988b*) and can establish a coinfection with different enteric viruses.

All the above agents cause damage to the intestinal mucosa provoking anorexia, diarrhea, dehydration, polydypsia in addition to the abnormal feces and finally death (*Wilber 1999a; Nieddu et al., 2000*). Gross lesions are characterized by gastric distention by fluid and gas, distention of the jejunum by translucent, watery fluid, cecal impaction by dry contents and gas, and distention of the sacculated colon by the characteristic clear, gelatinous mucoid exudate. Microscopically a striking goblet cell hyperplasia is observed in the jejunal, ileal and colonic mucosa with minimal inflammatory cell infiltrates composed mostly of neutrophils, lymphocytes and plasma cells. In the colon, the crypts and lumen are distended with mucus. There are minimal to absent lesions in the cecum (*Wilber, 1999a*). Differential diagnosis of the mucoid enteritis complex must include any infectious or management problem that results in disruption of the normal microbial environment causing diarrhea. *Campylobacter*-like bacteria and *Lawsonia intracellularis* have been associated with colonization of

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enterocytes in young rabbits; nevertheless some histologic changes are different to the mucoid enteritis complex. Histologic changes caused by *Lawsonia intracellularis* are located mainly in the ileum. They consist of marked thickening of the mucosa associated with hyperplasia of the villous and crypt epithelium and inflammatory cell infiltrates composed mostly of histiocytes with few lymphocytes, plasma cells, and occasional groups of neutrophils (*Duhamel et al., 1998*). On the other hand, intestinal lesions described by *Campylobacter*-like bacteria infection are similars to the lesions caused by *Lawsonia intracellularis*, consisting of multifocal to diffuse epithelial proliferation and accumulation of histiocytes and lymphocytes in the lamina propria of the small intestine, cecum, and sacculated colon. Erosive and suppurative cecocolitis can also be observed (*Schoeb & Fox, 1990*).

Recently in Europe, epizootic enterocolitis or mucous enteropathy has also been reported but without reaching a conclusion as to the viral pathogens involved (*Jones & Duff, 2001*). According to an electron microscopy study (*Nieddu et. al., 2000*) in Italy, of 1067 rabbits with an enteropathy; 41.9% were infected with rotavirus, 25.6% with coronavirus-like virus, 21.1% with parvovirus, and 10.3% with enterovirus-like virus. Adenovirus, calcivirus and reovirus were also sporadically found in 30 cases. Two to three different viruses in the same sample were also found; the most frequent association included coronavirus and parvovirus. A high percentage of a mixed viral infection with a coronavirus-like and enterovirus was found in this study, suggesting that these agents might potentially be pathogenic to rabbits. Coronavirus has been previously demonstrated in rabbits with diarrhea, and with high prevalence in seroepidemiologic studies (*Lapierre et al., 1980; Descoteaux, 1985; Deeb et al., 1993; Nieddu et. al., 2000*). Rotavirus has been isolated several times from rabbits with enteritis, but its pathogenicity has been considered low (*Schoeb et al., 1986; DiGiacomo et al., 1986; Thouless et al., 1988; Nieddu et al., 2000*). It has been demonstrated that the infection by rotavirus is widespread in fattened rabbits, and could be considered endemic in these populations (*Thouless et al., 1988*). Thouless et al. (*1996*) showed that mortality due to acute diarrhea is higher when enteropathogenic *Escherichia coli* and rotavirus occur in association, rather than when these two agents act separately.

In mice and dogs, ultrastructural changes in the intestine attributed to infection by rotavirus in mice and dogs consist of swollen villus epithelial cells, denuded foci on intestinal villi, slight to moderate villus atrophy, constriction of villus bases, edema of the lamina propria and vacuolation of enterocytes, suggesting that diarrhea results from malabsorption of fluid by damaged villi and hypersecretion of ions released from increased numbers of dividing cells at villus-crypt borders (*Johnson et al., 1986; Osborne et al., 1998*). It has been considered that parvovirus has low pathogenicity, since it has also been isolated from the intestine of healthy rabbits (*Matsunaga & Chino, 1981*). Therefore, the significance of parvovirus in the enteritis complex is unknown. Adenovirus, reovirus and calicivirus in diarrheic rabbits have been sporadically reported (*Nieddu et al., 2000*). Paramixovirus and herpesvirus have not been reported as enteric agents in rabbits.

Since enteropathies in rabbits usually have a multifactor etiology, the objective of this study was to describe histological and ultrastructural findings in the intestine of rabbits exhibiting severe diarrhea.

Case presentation

The study focused on 8 fattened rabbits with severe diarrhea from four different farms (4 cases) in central Mexico. These rabbits were euthanized and a post mortem study was performed at the Pathology Department of the College of Veterinary Medicine, National Autonomous University of Mexico. The farms experienced spontaneous high rates of mortality associated with severe diarrhea from November 2001 to June 2002. Housing and care were apparently adequate but all farms had changed

from a commercial brand feed containing from 16% to 18% of fiber to another one with 14.5% of fiber. The case specifics are summarized in Table 1.

On physical examination, all animals exhibited a distended abdomen and brownish/greenish mucoid fecal material staining the perianal region. All animals had a gastric and intestinal gas distention, a high mucous content of the stomach, small and large intestines, and brownish-greenish semi-liquid contents of the small intestine. In seven rabbits (cases 1, 2 and 3) cecal contents appeared brown and partially dehydrated with a mucus component. In one rabbit (case 4) the cecum was filled by a brown watery and mucous content. The other organs and tissues did not present significant gross lesions. Samples of heart, lung, liver, spleen, kidneys, brain, stomach and intestines were fixed in 10% neutral buffered formalin and embedded in paraffin wax, and sections (3 µm) were stained with haematoxylin and eosin (HE).

Extra samples of intestines were taken from cases 1 and 4 for a general bacteriological study. Samples of 1-3 mm x 1-3 mm of small and large intestines were fixed in 2.5% glutaraldehyde for the electron microscopy study. Afterwards, the tissues were rinsed with a phosphate buffer solution, post fixed in 1% osmium tetraoxide for an hour, and were rinsed again with a phosphate buffer solution in order to eliminate the osmium tetraoxide excess. The tissues were dehydrated with acetone in an ascending concentration, embedded in Epon and polymerized at 60 °C for 24 hours. Semi-thin and ultra-thin sections were cut using an ultramicrotome. Semi-thin 200 to 300 nm thick sections were stained with blue toluidine to be observed through the light microscope. Ultra-thin sections of 70 to 100 nm thick were stained with uranyl acetate and lead citrate for observation in the transmission electron microscope (Zeiss EM 900).

Results of case 1

Histopathology

In both animals, a mucoid and severe diffuse neutrophilic enteritis with desquamation, atrophy and fusion of the villi in the small intestine was observed (Figure 1). Abundant Gram-negative bacilli in the border of the villi along with a moderate submucosal and serosal edema were observed in the small intestine. Hyperplasia of the goblet cells and cytoplasmatic vacuolization in epithelial cells were observed in the large intestine. The other organs did not present significant lesions.

Ultrastructure

In addition to hyperplasia of goblet and epithelial cells, loss of microvilli were observed in the small

Table 1. Features of the 4 cases

Case	Breed	Age	Sex	Number of animals in the farm	Percent of mortality by week (%)	Origin (State)	Date of remission for diagnostic
1	1.-New Zealand 2.-California	55 days	Males	750	25	Michoacán	November 2001
2	3.-New Zealand 4.-New Zealand 5.-New Zealand	37 days	Males	800	19	Distrito Federal	March 2002
3	6.-New Zealand 7.-New Zealand	70 days	Males	600	9	Hidalgo	April 2002
4	8.-New Zealand	45 days	Males	400	75	Estado de México	June 2002

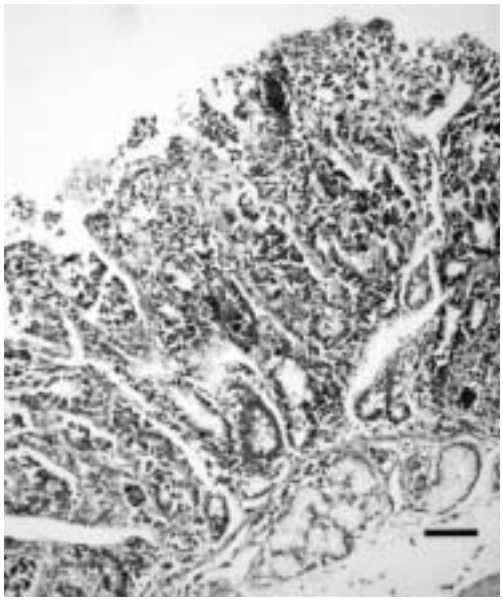

Figure 1. Neutrophilic infiltration of the duodenal mucosa with atrophy and desquamation of the villi. H&E (Hematoxilin & Eosin). *Bar* 150 µm.

intestine. Furthermore, in the small intestine numerous free virus-like particles were observed in the nucleus and in the cytoplasm of the enterocytes,

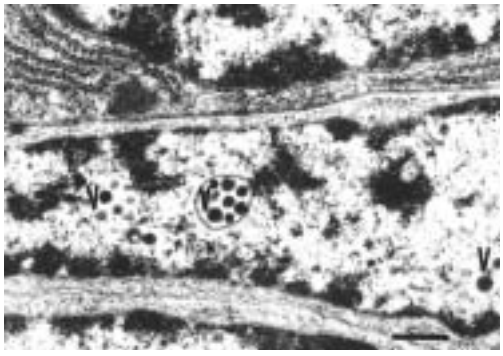

Figure 2. Electron micrograph showing an epithelial cell nucleus with free virus-like particles and a pseudonuclear inclusion surrounded by true nuclear membrane. V = Virus-like particles. *Bar* 0.6 µm.

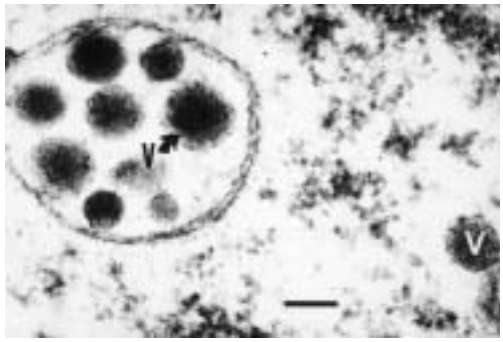

Figure 3. High magnification of a pseudonuclear inclusion surrounded by true nuclear membrane with virus-like particles. V = Virus-like particles. *Bar* 0.15 µm.

like pseudonuclear inclusions surrounded by true nuclear membrane (Figures 2 and 3). These virus-like particles appeared nearly spherical or hexagonal in outline, were from 80 to 180 nm in outer diameter and composed of a dense core with less dense thin tegument in some of them. Numerous smaller virus-like particles were also found in the cytoplasm of the enterocytes in the small intestine, and some also in the large intestine. These virus-like particles were almost spherical, from 50 to 84 nm in diameter, composed of a tegument and a

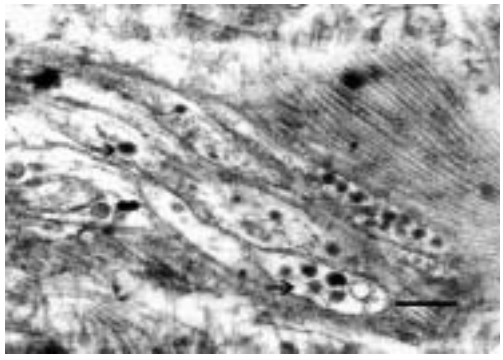

Figure 4. Electron micrograph showing numerous virus-like particles found in the cytoplasm of the enterocytes (arrowheads). *Bar* 0.4 µm.

dense core (Figure 4). Abundant intracellular bacteria were also found in the enterocytes and macrophages.

Results of case 2

Histopathology

A mucoid and lymphoplasmocytic enteritis was observed in these animals; it was slightly diffuse with atrophy and fusion of the villi. Hyperplasia of the goblet cells was observed in the large intestine (Figure 5). The other organs did not present significant lesions.

Ultrastructure

A rupture of the nuclear membranes and apoptosis of epithelial cells was noted in the small intestine. Intranuclear virus-like particles were also found and resembled those presented in the above mentioned case. In some segments, microvilli were completely absent.

Results of case 3

Histopathology

A diffuse moderate to severe lymphoplasmocytic enteritis with both atrophy and fusion of the villi was observed. The rest of the organs did not present significant lesions.

Ultrastructure

Hyperplasia of goblet cells and virus-like particles in the nucleus and in the cytoplasm of epithelial cells were observed in the small intestine, consistent with the findings of the above mentioned cases.

Results of case 4

Histopathology:

A slight diffuse lymphoplasmocytic enteritis with both atrophy and fusion of the villi, and severe edema in the submucosa of the large intestine were noted in this animal (Figure 6). Likewise, abundant

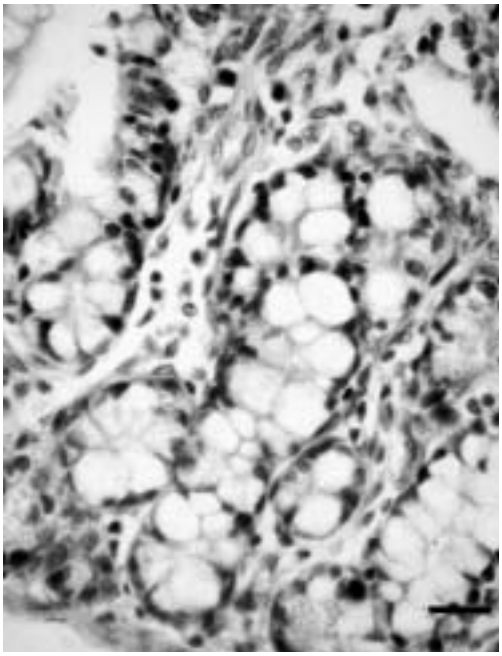

Figure 5. Cecal mucosa showing deeper part of crypts with numerous goblet cells. H&E *Bar* 40 μm.

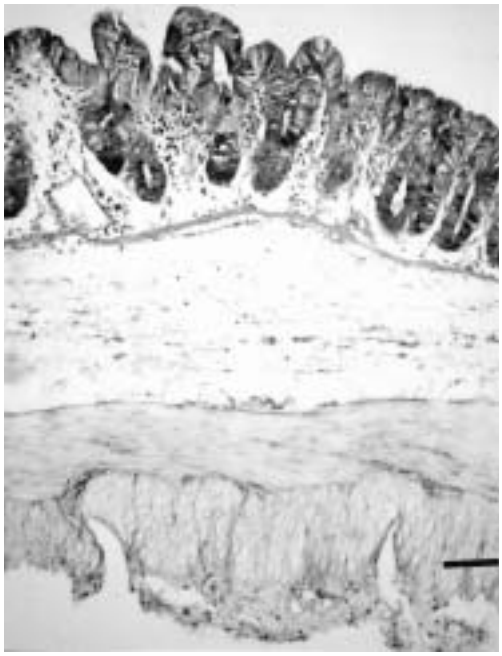

Figure 6. Severe edema in the submucosa and mucosa of the colon. PAS (Periodic acid-Schiff). *Bar* 150 μm.

Gram-negative bacilli were observed in the villi. The rest of the organs did not present significant lesions.

Ultrastructure

Hyperplasia of goblet and plasma cells, and abundant lymphocytes were observed. There were also virus-like particles in the nucleus and in the cytoplasm of the epithelial cells of this rabbit that shared similar characteristics with the above mentioned cases. These virus-like particles were observed isolated or forming groups of 2 to 9.

Bacteriology

Abundant *Escherichia coli* was isolated in pure culture from cases 1 and 4.

Discussion

In these four cases, the gross and microscopic lesions in the small and large intestines were very similar to those described in the mucoid enteritis or mucoid enteritis complex. Thus it has been reported that the accumulation of mucous in the large intestine is a consequence of constipation or an intestinal obstruction in rabbits with this disorder; this has also been demonstrated experimentally by binding the cecum (*Sinkovics, 1976; Hotchkiss & Merrit, 1996*). According to the microscopic lesions we saw, the neutrophilic enteritis found in the rabbits in case 1 was consistent with an acute presentation, whereas the lymphoplasmocytic enteritis in the rest of the cases was consistent with a subacute presentation of mucoid enteritis and probably with rotavirus infection, as has been described (*Meshorer, 1976; Thouless et al., 1988*). The fusion and atrophy of the villi, hyperplasia of goblet cells and edema in the lamina propria and submucosa were consistent with lesions previously reported in rabbits which had died from mucoid enteritis with subacute presentations and rotavirus infection (*Meshorer, 1976; Johnson et al., 1986; Thouless et al., 1988; Osborne et al., 1988*). The most commonly found ultrastructural changes in the intestine of all the examined rabbits included hyperplasia of goblet cells and loss of intestinal microvilli, that was most likely due to the bacterial and viral coinfection present in these cases.

The ultrastructural characteristics of the virus-like particles found in the cytoplasm of the enterocytes were similar to rotavirus. The numerous rotavirus-like particles found in the intestine of five rabbits corroborate reports in seroepidemiologic studies and viral isolation which mention that roratavirus could be considered endemic in large populations of commercial rabbits. Rotavirus may proliferate in special conditions, such as low fiber feeding, and occur in association with bacterial agents thus causing acute diarrhea resulting in high mortality (*DiGiacomo & Thouless, 1986; Thouless et al., 1988; Thouless et al. 1996; Nieddu, 2000*). In addition, the fusion and atrophy of the villi are typical intestinal lesions caused by this virus, and although enteric coronavirus infection might cause similar lesions, coronavirus-like particles were not found in these rabbits (*Schoeb et al., 1986*). The sudden onset, rapid spread, and high mortality could have been associated with the introduction of rotavirus into colonies not previously exposed and with low immunity (*Schoeb et al., 1986*).

An unusual finding which was observed in all rabbits, was the presence of numerous virus-like particles in the nucleus of the enterocytes. Their location and morphological characteristics are similar to herpesvirus (*Ohtsuki et al., 1999*), but an immunoelectronmicroscopy study would be needed to confirm this supposition. Herpesvirus-like viral infections have also been found in a rabbit in Canada with post mortem findings such as pulmonary congestion and edema, multifocal splenic, renal, gastric, and intestinal hemorrhage, and hydropericardium. Microscopically, necrosis focus and hemorrhage were evident in the different organs (*Swan et al., 1991; Wilber, 1999b*). Although, enteritis associated with herpesvirus in rabbits apparently has not been reported, different types of enteric herpesvirus have been found in humans and other species like bovines, birds and cats (*Waber et al., 1981; Henry et al., 1986; Leprince, et al., 1993;*

Goodwin et al., 1995; Roperto et al., 2000; Shawky & Schat, 2002). In broiler chicks with diarrhea and enterotyphlitis, intralesional herpesvirus, reovirus-like virus particles and bacteria have been found in their small and large intestines (*Goodwin et al., 1995*). The significance of the herpesvirus-like particles found in these rabbits, is not known. This study could be the basis for future studies to determine if these herpesvirus-like particles are saprophytic or could be pathogenic. Enteric coronavirus, parvovirus, enterovirus, adenovirus, calicivirus or reovirus were not detected in these rabbits. It has been observed that laboratory rabbits are susceptible to the experimental infection by the Sendai virus (paramyxovirus), but that viral replication has been confined to the upper respiratory apparatus and rabbits appear asymptomatic (*Machii et al., 1989; Wilber, 1999b*).

The severe diarrhea and high mortality rates in these cases could be associated with several factors, one being a change of feed. The farms had fed the same fiber content brand for years before changing to a lower fiber content brand. Possibly the change of the fiber content in pellets has been one of these factors. Adequate fiber content is necessary for normal intestinal motility in rabbits and when fiber is scare, constipation, mucous accumulation, pH changes in the cecum, and fermentation, due principally to coliform bacteria, can result. Although abundant quantities of *Escherichia coli* were isolated from three animals (cases 1 and 4), pathogenicity studies were not performed; therefore the role of this bacterium as responsible for the diarrhea is not clear. The rotavirus-like particles could be an important cause of enteric disease in these cases, and they should be considered in the differential diagnosis of diarrhea, especially in young rabbits. Apparently there are no previous reports of herpesvirus-like particles from rabbits exhibiting severe diarrhea. Nevertheless their importance as primary agents and their pathogenic role in enteritis of rabbits needs to be further investigated. It was concluded that the diarrhea had a multi-factorial etiology, probably due to change in feed, bacterial proliferation and bacterial coinfection with enteric virus.

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