

Pneumatosis cystoides intestinalis in a rabbit doe (*Oryctolagus cuniculus*)

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Abstract. Pneumatosis cystoides intestinalis (PCI) is an infrequent condition of animals characterized by the existence of numerous thin-walled, gas-filled cystic structures within the intestinal wall and adjacent lymph nodes. Microscopically, the cystic structures appear to be dilated lymphatics located in the lamina propria, submucosa, muscularis, subserosa, mesentery, and mesenteric lymph nodes. This report describes a case of pneumatosis cystoides intestinalis in a rabbit doe from an organic farm where 20 rabbit does were fed ad libitum with a natural diet consisting of whole barley, pea beans, alfalfa hay, and a pelleted vitamin–mineral blend. A combination of nutritional, bacterial, and other factors are hypothesized as possible predisposing factors in the development of PCI.

Key words: Intestinal emphysema; organic farm; pneumatosis cystoides intestinalis; rabbits.

Pneumatosis cystoides intestinalis (PCI) is an uncommon disorder of animals characterized by the presence of gas-filled cysts within the wall of some parts of the gastrointestinal tract. Similar cysts are found frequently in the mesentery adjacent to the affected intestine and are found occasionally in other organs of the abdominal cavity. Pneumatosis cystoides intestinalis is also known as pneumatosis intestinalis, intestinal emphysema, cystic lymphopneumatosis, and abdominal or intestinal gas cysts.¹¹ The condition has been described infrequently in animals and is usually an incidental finding at slaughter in healthy swine.⁷ A similar condition also occurs in humans, where both fulminant and benign forms exist.⁶

Although numerous explanations have been advanced as to the cause of this condition, the etiology remains unknown.⁹ This report describes a case of PCI in a rabbit doe from an organic farm and discusses various etiologic factors.

A 15-month-old female New Zealand white rabbit (*Oryctolagus cuniculus*) was housed with 19 other female rabbits in an organic farm in Spain. The does were fed ad libitum with a diet consisting of whole barley, pea beans, alfalfa hay, and a pelleted vitamin–mineral blend. A mixture of the concentrate ingredients (whole barley 39%, pea beans 58.5%, and pelleted vitamin–mineral blend 2.5%) was placed in a feeder, whereas alfalfa hay was offered separately in another feeder. The animals selectively ingested more barley than pea, thus effectively consuming a diet that was not of the expected composition. Table 1 shows that the nutritive value of the diet differed from current recommendations for

breeding rabbit does, especially because of its very high starch content.

The 20 rabbits exhibited normal appetite but they had chronic weight loss, probably because of inadequate dietary energy content. The doe in this case presented with lethargy and apparent inactivity. No other clinical abnormalities were observed. The doe was euthanized by an intravenous injection of barbiturate,^a following the Cardenal Herrera-CEU University ethical guidelines. A complete necropsy was performed and any observed gross lesions were recorded. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, cut at 4 μ m, and stained with hematoxylin and eosin, van Gieson trichromic, Periodic acid-Schiff, and Gram methods.

At necropsy, multiple gas-filled cysts, 1–5 mm in diameter, were present in the distal part of the jejunum and ileum, giving the bowel a spongy touch on palpation. Cysts were not observed in the mesenteric lymphatic vessels or in the mesenteric lymph nodes. The intestinal mucosa had an irregular surface because of the presence of numerous small gas-filled cysts (Fig. 1). With the exception of the small intestine, the internal organs appeared normal at the time of necropsy.

Histologically, the small intestinal lesions consisted of numerous, variably sized cystic structures in the mucosa and submucosa. The cysts were located mainly in the lymphatic vessels of the lamina propria, resulting in a marked deformation of the intestinal villi (Fig. 2). In the jejunal submucosa, cysts displaced portions of the Peyer patches. In some portions, connective tissue separating adjacent cysts contained an inflammatory infiltrate characterized by the pres-

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Table 1. Nutritional value of consumed diet and current recommendations for breeding rabbit does (g/kg).

	Consumed diet*	Current recommendations ³
Digestible energy (MJ/kg)	10.7	11.1
Digestible protein	112	129
Crude fiber	131	135
Starch	282	180

* Calculated from a measured average daily intake of barley (113 g), pea (96 g), alfalfa hay (138 g), and vitamin–mineral blend (7 g), and tables of nutritional values of feed ingredients.⁴

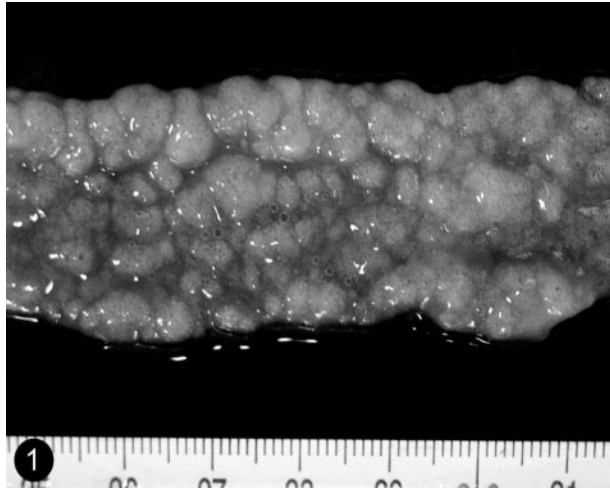


Figure 1. Opened gut. The ileum mucosa shows an irregular surface because of the existence of numerous gas cysts within the intestinal wall.



Figure 2. A section of ileum. Several vesicles mainly located in mucosa and submucosa. Dilatation of lymphatic vessels and a moderate infiltrate of mononuclear cells are in the lamina propria. The cysts located in the intestinal serosa are smaller and fewer in number. HE. Bar = 240 μ m.

ence of lymphocytes, macrophages, and plasma cells. In the most severely affected areas, there was also mild goblet cells hyperplasia. Cysts were also seen in muscular and serosal layers but were smaller and fewer in number. Several gut sections showed numerous gram-positive bacilli in the intestinal lumen, but these organisms were not associated with the cysts. No concurrent disorders of the gastrointestinal tract were present in this animal, and no histopathologic lesions were observed in other organs.

Pneumatosis cystoides intestinalis is a rare condition in animals. It is found mainly in slaughter-aged pigs, although the condition has also been reported in sheep,⁷ chickens,⁵ and laboratory rats.¹² To our knowledge, this is the first report of PCI in rabbits. The lesion has not been associated with clinical disease.⁹ The rabbit doe in this report was lethargic and exhibited chronic weight loss. This weight loss might have been because of malnutrition caused by an unbalanced diet or by malabsorption caused by extensive involvement of the intestinal mucosa in this cystic process, or both. The histopathologic lesions observed in this rabbit were most prominent in the mucosa of the small intestine. This location plays an important role in the nutrient absorption. Neither weight loss nor other clinical signs has been observed in pigs with similar or more extensive intestinal lesions.¹⁰

There was a clear distension of lymphatic vessels of the mucosal lamina propria forming cystic structures. It has been hypothesized in 1 experimental report that the mucosa is affected severely because of gases absorbed directly from the intestinal lumen.¹⁰ Höer et al.⁶ reported 2 cases of PCI in humans in which they hypothesized that gas followed a breach of mucosal integrity. Ulcers, erosions, or other microscopic lesions were not observed in the mucosa of this rabbit doe.

Although numerous explanations have been advanced as to the cause of this condition, the etiology remains unknown.⁹ In the past decades, the bacterial theory gained support⁸ when PCI was reproduced in laboratory animals after inoculation with *Clostridium perfringens*, *C. tertium*,¹² and *Escherichia coli*.¹⁰ It is hypothesized that intestinal emphy-

sema observed in this study may have been the consequence of a combination of bacterial and nutritional causes. Indeed, it is known that some enterobacteria (*C. perfringens*, *C. tertium*, *E. coli*) induce marked gastric dilation, congestion, and intestinal emphysema in gnotobiotic pigs⁸ and rats.¹⁰ It has been reported that an increase in dietary starch level affects fermentation activity in the hindgut. High-starch diets lead to a rise in lactate concentration in the intestinal lumen and a fall in the molar proportion of butyrate and propionate, as well as a drop in the lipopolysaccharide content. These variations could indicate a change in the microflora, with stimulation of the gram-positive bacteria to the detriment of the gram-negative bacteria.²

It is proposed that the PCI may have been caused by an intestinal dysbiosis resulting from a high multiplication of bacteria promoted by a diet rich in readily fermentable carbohydrates. Indeed, it has been reported in humans and pigs that host factors such as carbohydrate intolerance may also have a significant role either alone¹ or in combination with bacterial enteric pathogens.¹⁰

Although only 1 rabbit doe was studied, and it was not possible to determine whether all organic farm rabbits were affected with PCI, lower dietary starch level and pelleting (to avoid selective intake of feed ingredients) were recommended to resolve malnutritional problems of the remaining animals and to prevent new cases of PCI. One month after the change in the diet, the animals had fattened up, and no rabbit doe deaths have occurred in the farm.

In conclusion, the etiology of PCI in this rabbit is not clear, although a combination of bacterial, nutritional, and perhaps host factors should be considered. An unbalanced diet might have been the leading factor. Pneumatosis cystoides intestinalis should be part of the differential diagnosis when assessing intestinal pathology in rabbits.

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Sources and manufacturers

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A method for detecting complex vertebral malformation in Holstein calves using polymerase chain reaction–primer introduced restriction analysis

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Abstract. Complex vertebral malformation (CVM), a hereditary lethal disease in Holstein calves, is characterized by complex anomalies of the vertebral column and limbs in an aborted fetus and in prematurely born, stillborn, and neonatal calves. The mode of inheritance of CVM is autosomal recessive, and CVM is caused by a point mutation from G to T at nucleotide position 559 of the *bovine solute carrier family 35 member 3* (*SLC35A3*) gene. Although an allele-specific polymerase chain reaction (AS-PCR) is a useful method for diagnosis of CVM, the AS-PCR requires selected DNA polymerases and strictly controlled reaction conditions to obtain reliable results. Therefore, an alternative screening method for the CVM gene would be useful. Polymerase chain reaction–primer introduced restriction analysis (PCR-PIRA) is a method that can be used for detecting a single nucleotide mutation in any gene without a restriction site around the mutation site. In this study, primers were designed to introduce *Pst*I or *Eco*T22 sites into PCR products from the wild-type and CVM alleles, respectively. The wild-type allele, a heterozygote, and a homozygote of the CVM allele could be discriminated by restriction fragment length polymorphism analysis. Specific introduction of restriction sites into PCR products depending on the change in a single nucleotide of template was shown using a variety of DNA polymerases and PCR machines. Therefore, the PCR-PIRA technique using primers designed in this study might provide a more useful method for extensive screening of CVM.

Key words: Complex vertebral malformation; diagnosis; Holstein; PCR-PIRA.

Complex vertebral malformation (CVM), a familial lethal syndrome in Holstein calves, has been reported in stillborn,

aborted, and preterm calves.¹ Affected calves are characterized by shortened cervical and thoracic regions of the vertebral column, bilateral symmetric contraction of the metatarsophalangeal joints, and symmetric arthrogryposis.^{1,3,8,11} Multiple hemivertebrae, scoliosis, and synostosis of the vertebral column have also been reported.^{1,3,8,11} Complex vertebral malformation was first identified and characterized in Holstein cattle in Denmark.¹ Two common ancestors in pedigrees of CVM were found; both were elite sires of US Holstein origin. Because of the widespread international use of

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