

<Case Report>

## A case of nonserotypable *Escherichia coli* infection in a Korean rabbit farm

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### Abstract

Rabbits are highly susceptible to colibacillosis, and no treatment is able to control the disease effectively. Rabbits raised in a farm in Chonbuk province presenting persistent diarrhea and death were submitted for diagnosis. Ninety percent of the infected animals died; weanlings suffered the most mortality. Necropsies showed prominent hemorrhagic foci along the intestinal and cecal serosae. In histopathological examination, rod-shaped bacteria were observed in the necrotic areas of the tips of villi in the small intestine and neutrophils infiltration was found around the necrotic villous areas. The affected animals consistently yielded *Escherichia coli* isolates from the intestines. The isolated organism was atypically indole-negative and was nonserotypable using 62 known O group-typing sera. Further microbiological and epidemiological works to recognize and control colibacillosis infection in farmed rabbits in Korea is therefore critical.

**Key words** : Colibacillosis, Hemorrhagic enteritis, Nonserotypable *E. coli*, Rabbits

### INTRODUCTION

Rabbits are highly susceptible to *Escherichia (E.) coli* infection and the disease is considered a devastating problem in large commercial farms. After weaning, rabbits normally do not have any *E. coli* strains present in their gastrointestinal tracts, or, if *E. coli* is present, it is usually present at very low levels (Blanco et al, 1996; Marlier et al, 2006; Gallois et al, 2008). Rabbits are, however, used as an animal model for *E. coli* proliferation (Griffin et al, 1990; Nataro and Kaper, 1998; Brunder et al, 1999; Garcia et al, 2002; Agin et al, 2005). The ceca of rabbits produce abundant volatile fatty acids (VFAs) that inhibit *E. coli* growth. Alteration of the pH of the gastrointestinal tract or suppression of cecal production of VFAs by stress or infection may al-

low *E. coli* to proliferate and cause a clinical infection (Blanco et al, 1996; Blanco et al, 1997; Pontello et al, 2003). Rabbit colibacillosis is insidious and treatment is often unsuccessful (Blanco et al, 1997; Marlier et al, 2006).

### CASE REPORT

Rabbits raised in a farm that had been operational for 5 years in Chonbuk province, Korea, were submitted to Laboratory of Pathology, College of Veterinary Medicine, Chonbuk National University for clinicopathological diagnosis because of insidious mortality over the period of a year. The farm at the time of the investigation was home to a total of 2,000 rabbits; 500~600 weanlings, 500~600 infant rabbits, 300 parent rabbits, while the remaining rabbits were sexually mature adults.

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The young rabbits had an average body weight of 1.2 kg while the adult and market-age animals had an average weight of 3 kg. The animals were regularly dewormed with albendazole, vaccinated against epidemic hemorrhagic fever and fed with alfalfa and pellets. The farmer-owner noticed increased mortality, mostly among weanling rabbits, in the early spring of 2005. Clinical signs included diarrhea, inappetence, weakness, a crouching stance, slow mobility, abdominal swelling and death. Almost 90% of the morbid animals (30% of the total population) died. Antibiotics failed to contain the mortalities. Necropsy of total six weaned animals (first necropsy: two weaned rabbits, second necropsy: four weaned rabbits) was individually performed on January 25 and February 2, 2006. Tissue samples of the main internal organs were collected for histopathological and bacteriological isolation. For histopathological evaluation, samples were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin and cut into 5- $\mu$ m sections. Samples from all tissues were stained with hematoxylin and eosin (H&E).

Hemorrhagic foci along the intestinal and cecal serosa were the most striking lesions seen in the necropsied rabbits. The rest of the internal organs remained intact. Based on histopathological examination, rod-shaped bacteria were observed in the necrotic areas of the tips of villi in the small intestine and neutrophils infiltration was found around the necrotic villous areas (Fig. 1). Such bacteria were only found in the necrotic tissues

with inflammation indicating that severe enteritis occurred by the bacterial infection.

*E. coli* was consistently isolated from hemorrhagic intestinal lesions. Fresh isolates grew abundantly on eosin methylene blue (EMB) agar and showed beta-hemolysis on blood agar. Attempts to serotype the isolate using 62 different *E. coli* group O sera (Denka-Seiken, 43; LDR *E. coli*, 18 and 038) were unsuccessful. The biochemical properties of the isolate as determined by API system 20NE test were consistent with a reference *E. coli* strain.

## DISCUSSION

Attempts to treat rabbit colibacillosis are usually unsuccessful and the indicated method of control is total eradication. Antimicrobial susceptibility assays have shown that only fluoroquinolone is effective against rabbit colibacillosis (Pisoni et al, 2004) and recently, another report recommended the use of apramycin (Morel et al, 2008) and medium chain fatty acids (Gallois et al, 2008). Enteropathogenic *E. coli* strains can be differentiated from nonpathogenic ones on the basis of serotype and biotype identification and the determination of virulence factors (Blanco et al, 1996; Brunder et al, 1999; Pisoni et al, 2004). Previous studies have reported nonserotypable pathogenic *E. coli* in pigs and poultry (Hunter et al, 1994; Edens et al, 1997; Pontello et al,

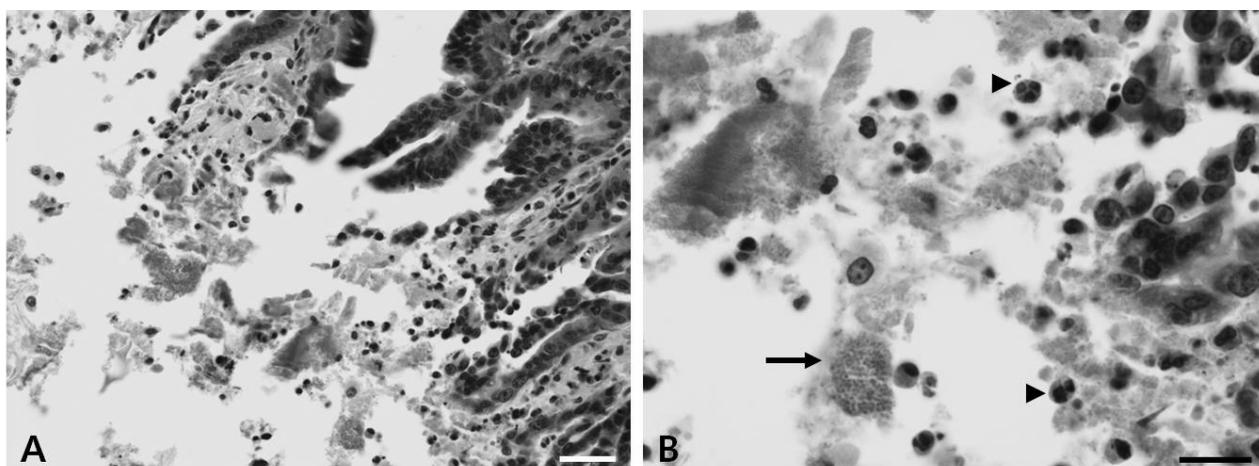


Fig. 1. Rod-shaped bacteria (arrow) were observed in the necrotic areas of the tips of villi in the small intestine and neutrophils (arrowheads) infiltration was found around the necrotic villous areas. (A)  $\times 400$ , scale bar=20  $\mu$ m, (B)  $\times 1,000$ , scale bar=10  $\mu$ m, H&E staining.

2003). However, this case is the first report of an unusual nonserotypable *E. coli* isolated from diarrheic rabbits.

The most effective strategy to control rabbit colibacillosis is culling and thorough disinfection of infected premises. Rabbit colibacillosis must be distinguished from that caused by enterotoxaemia and other bacterial, parasitic, or viral pathogens (García et al, 2002; Pisoni et al, 2004, Agin et al, 2005). This case of persistent diarrhea may serve as a reference for understanding underlying refractory enteritis in farmed rabbits in Korea. Further molecular and virulence characterization of this nonserotypable *E. coli* strain is critical.

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## REFERENCES

- Agin TS, Zhu C, Johnson LA, Thate TE, Yang Z, Boedeker EC. 2005. Protection against haemorrhagic colitis in an animal model by oral immunization with isogenic rabbit enteropathogenic *Escherichia coli* attenuated by truncating intimin. *Infect Immun* 73: 6608-6619.
- Blanco JE, Blanco M, Blanco J, Mora A, Balaguer L, Mouriño M, Juárez A, Jansen WH. 1996. O serogroups, biotypes and *eae* genes in *Escherichia coli* strains isolated from diarrhoeic and healthy rabbits. *J Clin Microbiol* 34: 3101-3107.
- Blanco JE, Blanco M, Blanco J, Mora A, Balaguer L, Cuervo L, Balsalobre C, Muñoa F. 1997. Prevalence and characteristics of enteropathogenic *Escherichia coli* with *eae* gene in diarrheic rabbits. *Microbiol Immunol* 41: 77-82.
- Brunder W, Schmidt H, Frosch M, Karch H. 1999. The large plasmids of Shiga-toxin-producing *Escherichia coli* (STEC) are highly variable genetic elements. *Microbiology* 145: 1005-1014.
- Edens FW, Qureshi RA, Parkhurst CR, Qureshi MA, Havenstein GB, Casas IA. 1997. Characterization of two *Escherichia coli* isolates associated with poult enteritis and mortality syndrome. *Poult Sci* 76: 1665-1673.
- Gallois M, Gidenne T, Orenge J, Caubet C, Tasca C, Milon A, Boullier S. 2008. Testing the efficacy of medium chain fatty acids against rabbit colibacillosis. *Vet Microbiol* 131: 192-198.
- García A, Marini RP, Feng Y, Vitsky A, Knox KA, Taylor NS, Schauer DB, Fox JG. 2002. A naturally occurring rabbit model of enterohaemorrhagic *Escherichia coli*-induced disease. *J Infect Dis* 186: 1682-1686.
- Griffin PM, Olmstead LC, Petras RE. 1990. *Escherichia coli* O157:H7-associated colitis. A clinical and histological study of 11 cases. *Gastroenterology* 99: 142-149.
- Hunter JE, Bennett M, Hart CA, Shelley JC, Walton JR. 1994. Apramycin-resistant *Escherichia coli* isolated from pigs and a stockman. *Epidemiol Infect* 112: 473-480.
- Nataro JP, Kaper JB. 1998. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 11: 142-201.
- Marlier D, Dewrée R, Lassence C, Licois D, Maimil J, Coudert P, Meulemans L, Ducatelle R, Vindevogel H. 2006. Infectious agents associated with epizootic rabbit enteropathy: isolation and attempts to reproduce the syndrome. *Vet J* 172: 493-500.
- Morel SA, Bostvironnois C, Limet A. 2008. Apramycin in the control of enteritis associated with *Escherichia coli* in rabbits. Proceedings of the 9th World Rabbit Congress-June 10-13, Verona, Italy.
- Pisoni AM, Piccirillo A, Gallazi D, Agnoletti F, Grilli G. 2004. Biotype and susceptibility to antimicrobial agents of rabbit *Escherichia coli*. Proceedings of the 8th World Rabbit Congress. Convention Center, Puebla City-September 7-10, Puebla, Mexico.
- Pontello M, Bersani C, Colmegna S, Cantoni C. 2003. Verocytotoxin producing *Escherichia coli* in foodstuffs of animal origin. *Eur J Epidemiol* 18: 157-160.