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Case Report

Colibacillosis in a New Zealand white rabbit (*Oryctolagus cuniculus*)

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Abstract

Colibacillosis is a disease caused by *Escherichia coli* in a variety of animals, including humans. Rabbit colibacillosis is infrequent or with an incipient description in Chile. Here, we describe an *E. coli* case in a white New Zealand rabbit at an animal facility in Santiago, Chile. Necropsy, histology, bacteriology, and 16S sequencing indicated an *E. coli* systemic infection. Phylogenetic analysis suggested that this *E. coli* J305 isolate is closely related to *Shigella* spp.

Key words: Rabbit; *Escherichia coli;* colibacillosis; New Zealand.

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Introduction

Escherichia coli is a common commensal bacterium of the gastrointestinal tract of warm-blooded animals [1-4]. However, in an immune-suppressed host, certain *E. coli* strains can become virulent and cause diarrheal and extraintestinal diseases [2]. Digestive infections represent one of the main pathological problems and are responsible for significant economic losses in rabbit breeding facilities [5]. The most common *E. coli* serovar found in rabbits is enteropathogenic *Escherichia coli* (EPEC), which causes watery diarrhea that can be mucoid or bloody, along with dehydration and lethargy [4]. Many factors are involved in the development of an infection. Stress and diet are main factors that affect the output of a gastrointestinal infection. The intestinal hypomotility syndrome, also known as gastrointestinal stasis, is characterized by anorexia, decreased or no stool production, and a gastrointestinal tract (stomach and/or cecum) filled with food and/or gas. This condition triggers intestinal bacterial overgrowth and can consequently lead to septicemia and death [6]. Here, we describe an intestinal hypomotility syndrome with fatal colibacillosis caused by *E. coli* in a white New Zealand rabbit after a personnel change at an animal facility in Santiago, Chile.

Case Report

New Zealand rabbits between four and six months of age were purchased from the Chilean Public Health Institute, Santiago, Chile. The rabbits were treated for parasites upon arrival at the facility and maintained under an environmental enrichment program [7]. The animals were fed with conventional hay pellets. After a veterinary personnel replacement, a one-year-old laboratory New Zealand rabbit presented eye-nose mucopurulent discharge, dehydration, and lack of appetite. The rabbit was isolated and treated with physiological serum. Two days after treatment, it was found dead. Necropsy was performed within six hours of death, and samples of liver and lung were fixed in 4% formalin for histological analysis.

The stomach was distended, and the content was composed mainly of dried food, pellets, and fur. The liver was pale with a yellowish color, and the gallbladder was distended and full. Additionally, the lungs were hyperemic, and a frothy hemorrhagic fluid was found in the bronchi and trachea, suggesting pneumonia. These findings indicated that the cause of death was a systemic infection [3]. The stomach and liver were characteristic of hepatic lipidosis, which has been reported as a common cause of anorexia [8]. Furthermore, the stomach contents and gas accumulation in the cecum indicated hypomotility (Figure 1A). The yellow mucus content in the small

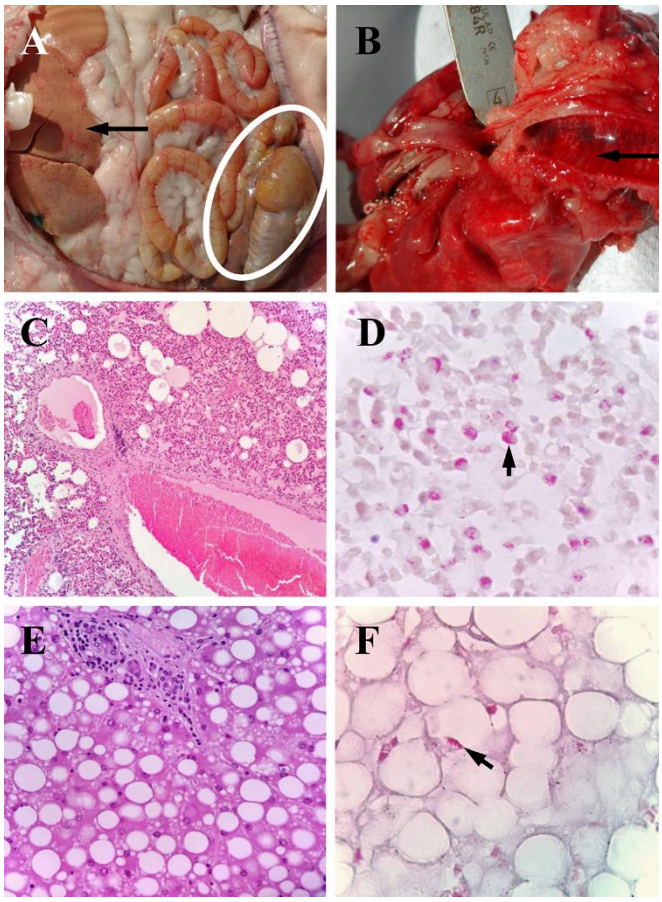
intestine and dried feces around the anus denoted diarrhea likely caused by an infectious agent.

Histopathological lesions in the lung were compatible with severe hemorrhagic bronchopneumonia (Figures 1B and 1C). Tissue Gram stain of the tissues revealed multiple Gram-negative rods inside the lung macrophages (Figure 1D). The liver presented severe steatosis and Gram-negative rods inside the macrophages (Figures 1E and 1F).

Sterile swabs from the lungs, liver, and intestines were cultured in CHAB (cysteine heart and blood agar), and MacConkey agar (Difco, Franklin Lakes, USA). Homogenous single colonies were obseved in the diferent media and samples. Fifteen single colonies were isolated and thesed for identification. Gram stain revealed that all the isolates corresponded to short Gram-negative rods (Figure 2A). Bacteriological identification using the API 20E system (bioMériux, Marcy I'Etoile, France) indicated that all these isolates corresponded to *Escherichia coli* (API20E code 514457217; very good identification; 99.4% *E. coli* 1) (Figure 2C).

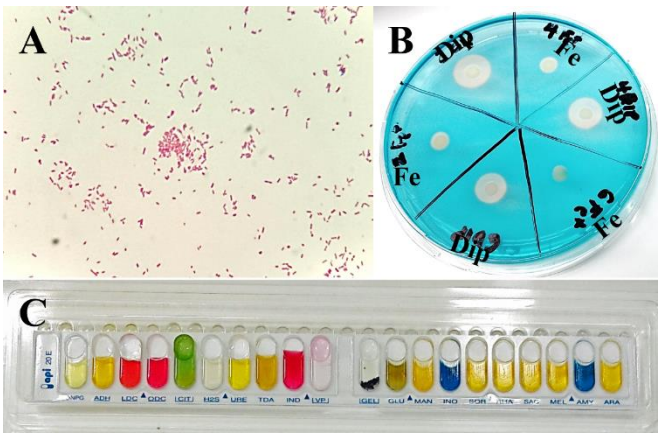
16S PCR amplification and sequencing using the universal primers 27F AGAGTTTGATCMTGGCTCAG and 1492R GGTTACCTTGTTACGACTT [9] corroborated the bacteriological identification of *E. coli* J305 (KY040364). These results confirmed a systemic infection caused by *E. coli*. Phylogenetic analysis [10-12] showed that the *E. coli* J305 isolated was closely related to *Shigella* spp. (Figure 3). Due to the

Figure 1. Rabbit necropsy and histology.



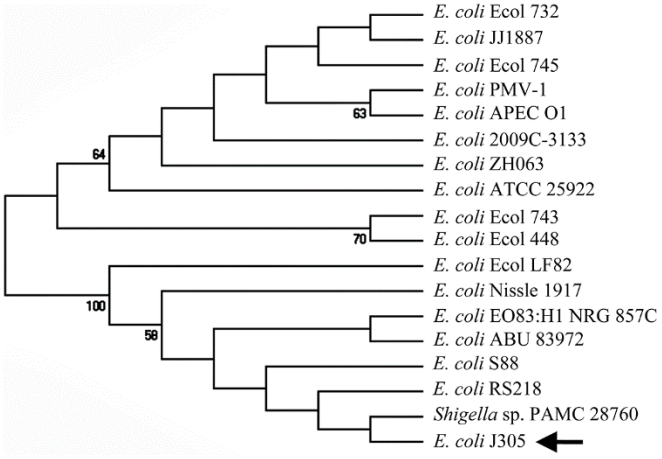
A: *In situ* examination pale liver (arrow) and dilated cecum with local hemorrhagic (circle); B; Macroscopic examination of the lung. Trachea congested, filled with hemorrhagic fluid (arrow); C: Lung H&E stained (100X) showing emphysema, edema, hemorrhage, and congestion of blood vessels; D: Lung Gram stained (1,000X). Gram-negative bacteria inside lung macrophages (arrow); E: Liver H&E stained (400X). Optically empty vesicles inside hepatocytes, which have the nucleus displaced towards the periphery; F: Liver Gram stained (1,000X). Gram-negative organisms inside liver macrophages (arrow).

Figure 2. Phenotype of *E. coli* J305.



A: Gram stain of *E. coli* J305; B: Siderophere synthesis dependent of iron availability. *E. coli* J305 grown under iron limitation (Dip; 4,4′−Dipyridyl 150 µM) and iron rich conditions (Fe; $FeSO_4$ 150 µM). The yellow ring indicates the synthesis of siderophores; C: API20E profile.

Figure 3. Evolutionary relationships of *E. coli* J305 16S sequence (KY040364).



The evolutionary history was inferred using the neighbor-joining method mediated MEGA7 [12].

infrequent isolation of *E. coli* virulent strains from rabbits, some virulent factors were evaluated. Briefly, type I fimbria [13], siderophore synthesis [14,15], motility, and hemolytic activity were evaluated. Type I fimbria found in the *E. coli* J305 is an important adhesin required for tissue colonization, and it was expressed in most of the extraintestinal pathogenic *E. coli* (ExPEC). *E. coli* J305 did not show hemolytic activity in CHAB, in contrast to *Staphylococcus aureus* (ATCC 12600) that was used as a positive control. Nevertheless, the hemolytic activity does not necessarily correlate with virulence [16]. Secretion of siderophores is an important virulent factor detected in *E. coli* J305, which was increased under iron-limited conditions (Figure 2B).

Enrofloxacin (Baytril) is a common antibiotic used in veterinary medicine [4,17]. Even though *E. coli* has been demonstrated to be sensitive to fluoroquinolones [18], the MIC of *E. coli* J305 to enrofloxacin was evaluated based on the standard methods [19]. An MIC of < 0.008 μg/mL was found, similar to the reported MIC for *E. coli* isolates (MIC ≤ 0.016–0.031 μg/mL) [20]. As a prophylactic measure, all the rabbits in the animal facility were given a dose of 5 mg/kg of enrofloxacin for seven days and their diet was supplemented with fresh hay to increase fiber consumption and prevent possible obstructions [21]. The animals were monitored for food and water consumption and any signs of diarrhea or respiratory distress. The cages were cleaned with chlorine and aspersed with alcohol 70%. The other rabbits did not present any signs of disease after this episode.

All research involving laboratory animals was conducted as per the protocol approved by the bioethics and biosafety committee of the Universidad Mayor.

Discussion

Several pathotypes of enteric/diarrheagenic *E. coli* give rise to gastroenteritis but rarely cause disease outside the intestinal tract. On the other hand, ExPEC strains colonize the gut without apparent consequence, but they have the capacity to disseminate and colonize other host niches, including the blood, the central nervous system, and the urinary tract, resulting in a systemic disease [6]. Although the pathogenesis of colibacillosis suggests a fecal-oral origin, *E. coli* has been found in the gastrointestinal tract of rabbits as a part of the microflora [1,2,17,22]. Stress, lack of exercise, excessive grooming, and a low-fiber diet possibly triggered hypomotility [8], resulting in a stasis of gastrointestinal content causing overgrowth of pathogenic and/or opportunistic bacteria such as *E. coli*, giving rise to septicemia, organ failure, and, consequently, death [6,23]. Lesions similar to the ones found in the lungs of the rabbit are commonly described in poultry affected by colibacillosis [21]. This study showed that rabbits carry pathogenic *E. coli* that can be a potential cause of diarrhea and death for the animal and a source of infection for humans. A more extensive study is required to determine the virulence nature of *E. coli* rabbit isolates in Chile. It is important to continue the study of zoonotic *E. coli* infections in humans, as some strains have been shown to be resistant to antibiotics [24].

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