Experimental Infection of Calves with *Escherichia coli* O157:H7

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ABSTRACT. Three 3-month-old Japanese Black calves were experimentally infected with *Escherichia coli* O157:H7 to define the magnitude (CFU/g) and duration of fecal shedding of the organism. In two of the three calves, fecal shedding of *E. coli* O157:H7 ceased in 5 and 9 weeks. The remaining calf continued shedding *E. coli* O157:H7 for more than 31 weeks, and the magnitude of the shedding ranged from 10^1 to 10^4 CFU/g of feces. The possibility is suggested that a percentage of animals naturally infected with *E. coli* O157:H7 on farms may become long-term shedders, transmitting the organism to other animals in the herd and to the proximate environment.—KEY WORDS: cattle, *Escherichia coli* O157:H7.

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Escherichia coli O157:H7 was first identified as a human pathogen following two geographically separate outbreaks of hemorrhagic colitis in the United States in 1982 [14]. Cattle have been implicated as a source of infection and a cause of the human disease [9, 13]. A recent national survey performed by the Ministry of Agriculture, Forestry and Fisheries of Japan revealed that 0.62% of cattle on randomly selected farms fecally shed *E. coli* O157 [12]. The experimental infection of calves and adult cattle with *E. coli* O157:H7 has revealed that shedding of the organism varies widely among animals of the same age group but persists longer in calves than in adults, and that previous infection does not prevent re-infection with the same strain of *E. coli* O157:H7 [4]. Neonatal calves experimentally infected with *E. coli* O157:H7 developed diarrhea and enterocolitis with attaching and effacing lesions [6], though the organism did not appear to be pathogenic in calves older than 3 weeks [3, 4]. Little is known of the duration or magnitude of fecal shedding of *E. coli* O157:H7 in naturally infected cattle. Besser *et al.* [2] reported that the duration of detected excretion of *E. coli* O157:H7 by individual cattle was shorter than one month in 63% of cattle, and the fecal shedding of *E. coli* O157:H7 was most common in warm weather [8, 10]. It was reported that the magnitude of fecal shedding of *E. coli* O157:H7 by naturally infected cattle was low and estimated at 4 to 43 CFU/g by the most probable number (MPN) method [1]. The objective of this study was to define the magnitude and duration of fecal shedding of *E. coli* O157:H7 in experimentally infected cattle.

Three 3-month-old Japanese Black calves were housed individually and had no contact with each other. Calves were housed in segregated barns in accordance with the guidelines of animal experiment defined by the National Institute of Animal Health of Japan. All animals were culture negative for *E. coli* O157 and O26, *Salmonella*, *Clostridium perfringens*, and *Campylobacter* species prior to inoculation. Calves of similar sizes (ca. 90 kg) were fed twice a day (4 kg of dry matter per day) and had free access to water. The feed used was a mixture of 50% of cut rice plant straw and 50% grain.

The challenge organism was a nalidixic acid- and rifampicin-resistant derivative of *Escherichia coli* O157:H7 strain MN157 (*E. coli* MN157), an isolate from a calf with diarrhea. *E. coli* MN157 was inoculated in Trypticase soy broth (Beckton Dickinson Microbiology Systems, Cockeysville, MD, U.S.A.) and incubated at 37°C for 7 hr with shaking (120 rpm). For the subject calves, 10 m*l* of the log-phase culture (10^9 CFU/m*l*) added to 40 m*l* of commercial long-life milk was administered orally with a plastic syringe equipped with a stomach tube. After the challenge, feces were collected weekly, and on selected days directly from the rectum at 9:00 a.m. before feeding. For direct plating, 2.0 g of feces was added to 18.0 m*l* of diluent and homogenized with a Lab-Blender 80 (Seward Laboratory UAC House, London, England). The homogenate was filtered through a nylon mesh (pore size = 500 μm) to remove undigested fibrous debris. Serial 10-fold dilutions were made and 0.1 m*l* of each dilution was spread on NRSMAC (sorbitol MacConkey agar [Eiken Chemical Co., Ltd., Tokyo, Japan] containing nalidixic acid [20 μg/m*l*] and rifampicin [20 μg/m*l*]). Concurrently, 2 m*l* of each 10^{-1} dilution was spread on five well-dried NRSMAC plates. The limit of detection of *E. coli* MN157 by the direct plating method described above was 1 CFU/g of feces. Additionally, the MPN method was employed to detect shedding at levels lower than those detectable by direct plating count. Ten, 1.0, and 0.1 g of feces were added to 100 m*l*, 10 m*l*, and 10 m*l* of mEC broth (Eiken Chemical Co., Ltd.), respectively. Five sets of enrichment culture were prepared. After enrichment overnight at 37°C, 0.1 m*l* of each culture was spread on NRSMAC agar. A limit of two CFU of *E. coli* MN157/100 g of feces was detectable by the MPN method. Fecal samples having an MPN value below 2 were regarded as *E. coli* MN157-negative. The presence of *E. coli* MN157 on NRSMAC plate was tested after overnight incubation at 37°C. Selected sorbitol-negative colonies typical of *E. coli* O157:H7 were tested for the O157 antigen by the slide agglutination test with anti-O157 serum (Denka Seiken Co., Ltd., Tokyo).

Following oral inoculation with $10^{10.72}$ CFU of *E. coli* MN157, all animals began fecal shedding of the organism by the next day, and the magnitude of shedding was maintained at a high level ranging from 10^4 to 10^6 CFU/g up to 7 days post-inoculation (data not shown) and the subject calves remained healthy during this period. Calf No. 1 exhibited watery diarrhea that continued for 3 days between the 1st and 2nd week after infection. *Clostridium perfringens* was suspected as a causative agent of the diarrhea, and was successfully isolated in the range from 10^4 to 10^6 CFU/g of feces during this period. The animal recovered spontaneously without any medical treatment. Fecal shedding of *E. coli* MN157 by calf No. 1 decreased linearly and was undetectable at the 5th week. The level of fecal shedding of *E. coli* MN157 at the 3rd and 4th week were 5 and 2 CFU/100 g feces, respectively. In this case, temporal infectious diarrhea might have caused alterations in the physiological or bacteriological circumstances in the intestine that led to diminution of *E. coli* MN157. No other clinical disorders were observed after this episode in any of the animals. In calf No. 2, fecal shedding of *E. coli* MN157 continued for 8 weeks and ceased at the 9th week. In this case, a remarkable temporal increase reaching 10^5 CFU/g was observed at the 7th week, though no explanation of this phenomenon or of the sudden disappearance of the organism was given. Re-shedding of *E. coli* MN157 from these two calves was not observed.

In contrast to these two calves, calf No. 3 exhibited long-term fecal shedding, with a magnitude ranging from 10^1 to 10^4 CFU/g of feces (Fig. 1). The level of fecal shedding from the 3rd to the 20th weeks ranged from 10^2 to 10^3 CFU/g, though the levels after the 20th week tended to vary widely. Follow-up survey of fecal shedding of all animals was suspended at the 31st week after experimental infection.

On the whole, the three calves experimentally infected with *E. coli* O157:H7 exhibited different patterns of fecal shedding. The present study indicates that a percentage of the animals naturally infected with *E. coli* O157:H7 on farms may easily become long-term shedders transmitting the organism to other animals in the herd and to the proximate environment. Several factors can induce alterations in physiological or bacteriological circumstances in the gastrointestinal tract; sporadic diarrhea due to some other causative agent like *Cl. perfringens* as observed in this study, heat stress in warm weather [8, 10], dietary stress such as high proportional grain feeding [7], fasting [5], and transportation stress, all or any of which may affect the fecal shedding of *E. coli* O157:H7 by cattle.

The goal of our study is to find a measure for reducing or eliminating the fecal shedding of *E. coli* O157:H7 by cattle, with the eventual goal of preventing the entry of the pathogen into the human food chain. Several control strategies to prevent contamination of carcasses and meat with *E. coli* O157:H7 were proposed. Improvement of slaughtering and processing procedures, employment of the hazard analysis critical control point (HACCP) system, and improved management of livestock on farms appear to be feasible approaches. The presence of *E. coli* O157:H7 is confined to the gastrointestinal tract in inoculated calves [3, 4], and the primary sites of localization and proliferation of *E. coli* O157:H7 in the calves are the forestomachs [3]; thus, factors influencing the proliferation of *E. coli* O157:H7 in the forestomachs may ultimately determine the magnitude of fecal shedding [11]. Use of probiotic bacteria to modify the gastrointestinal ecological environment seems to be the most practical measure to eliminate *E. coli* O157:H7 from the gastrointestinal tract of cattle on farms. Further studies to isolate potential probiotic bacteria and to evaluate their efficacy to reduce or eliminate *E. coli* O157:H7 from infected cattle are in progress.

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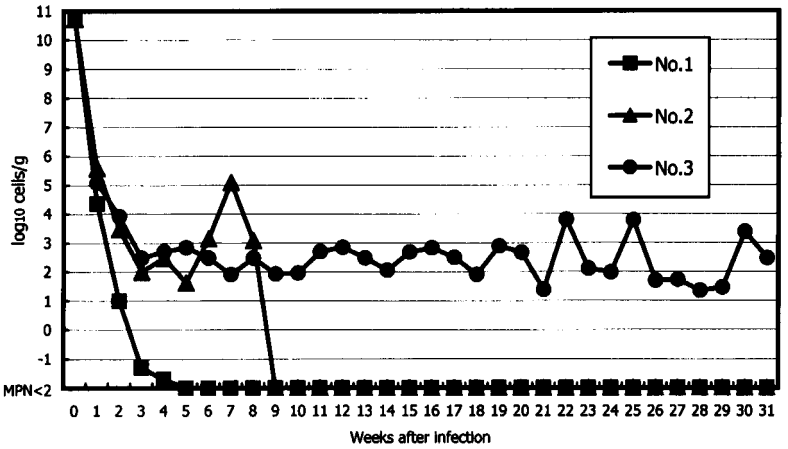

Fig. 1. Time course of fecal shedding of *E. coli* O157:H7 in experimentally infected calves.

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