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Author(s)	MIWA, Norinaga
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Detection and enumeration of enterotoxigenic *Clostridium perfringens*  
in intestinal contents of livestock and meat using newly developed  
nested polymerase chain reaction method

Norinaga Miwa

Shizuoka Institute of Environment and Hygiene  
4-27-2, Kitaando, Shizuoka 420, Japan

*Clostridium perfringens* is a significant causative agent of human food poisoning. A major vehicle causing food poisoning due to *C. perfringens* is meat, which may be contaminated with organisms in intestinal contents of livestock. The symptoms associated with the food poisoning are caused by an enterotoxin produced by enterotoxigenic *C. perfringens*, which may be present together with nonenterotoxigenic cells in the intestinal contents of livestock and meat. Therefore, the detection and enumeration of enterotoxigenic *C. perfringens*, rather than those of the total enterotoxigenic and nonenterotoxigenic *C. perfringens*, are needed in relation to food poisoning. In this study, the incidence and number of enterotoxigenic *C. perfringens* in the intestinal contents of livestock and meat were determined by the most probable number (MPN) method combined with a newly developed nested polymerase chain reaction (nested PCR).

The specificity of the nested PCR was confirmed by restriction endonucleases digestion of the amplified products with enterotoxigenic *C. perfringens*, and by the absence of amplified products with nonenterotoxigenic *C. perfringens* or any other bacterial strains. The sensitivity of the nested PCR was determined by using inoculated samples. The *C. perfringens* enterotoxin gene in the intestinal contents of livestock and

meat was detected by the nested PCR after culturing of the sample at an initial sample inoculum of fewer than 10 CFU/g of enterotoxigenic *C. perfringens*, with or without a nonenterotoxigenic strain.

The MPN method was combined with the nested PCR after culturing of the sample for the enumeration of enterotoxigenic *C. perfringens*, and the usefulness of the method was ascertained by comparison with the plate count method using inoculated samples. By this method, the incidence and number of indigenous enterotoxigenic *C. perfringens* in the intestinal contents of livestock and meat were determined, and compared with those of the total enterotoxigenic and nonenterotoxigenic *C. perfringens* determined by the conventional MPN method. Intestinal contents of broiler chickens showed a relatively high incidence and number of enterotoxigenic *C. perfringens* than those of cattle and swine. As for meat, enterotoxigenic *C. perfringens* was found in beef and chicken, the latter showing a high incidence and number, whereas not in pork. A small number of enterotoxigenic cells of *C. perfringens* co-existed with a large number of nonenterotoxigenic cells in the intestinal contents of livestock and meat. This is the first quantitative study of enterotoxigenic *C. perfringens* in intestinal contents of livestock and meat.