

Detection of *Torovirus*-like particles from calves with diarrhea

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송아지 설사 분변에서 *Torovirus* 검출

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초 록 : *Torovirus*-like particles이 심급성의 설사로 폐사한 송아지의 분변으로부터 검출되었다. 설사로 폐사한 송아지는 2주령으로 설사증상을 보인 5두중 2두였으며 심한 설사증상을 보인지 24시간만에 급성폐사를 나타내었다. 나머지 3두도 심한 설사증상을 보였으나 항생제 및 수액요법으로 치료를 한 후 회복되었다. 전자현미경으로 관찰된 바이러스 입자는 *Woode et al*이 관찰한 *Torovirus* 입자와 동일하였으며 그 크기는 원형입자일 경우 70~90nm의 크기로 8~10nm의 Peplomer를 가지고 있었다. 그러나 바이러스이 입자형태는 소, 돼지 그리고 사람에서 보고된 *Torovirus*와 마찬가지로 다양하였으며 Tube 형태로 된 것은 길이가 약 150nm에 달하기도 했다. 이 보고는 국내에서의 송아지 *Torovirus* 감염증의 첫 보고이며 국내에서도 *Torovirus*가 존재한다는 것을 입증한 것이다.

Key words : *Torovirus*, calf, diarrhea, electronmicrograph.

Introduction

Torovirus has been recognized as a new emerging pathogen in animals include equine, bovine and swine^{1-4,6}. Berne virus is prototype *Torovirus* was isolated from horse with diarrhea in Switzerland. In US, Breda virus with two different serotype have been isolated from calves with diarrhea in 1972 and 1985, respectively^{4,5,7}. In pigs *torovirus* has been

isolated from 3 week old piglets suffering from severe gastroenteritis. *Torovirus*-like particles were also have been found in the fecal smaples of children and adults with gastroenteritis in United Kingdom. Several viral pathogens associated with diarrhea in piglets were recognized and extensive research revealed a significance of these viral pathogens. Several viral pathogens associated with diarrhea in piglets were recognized and extensive research revealed a significance of these viral pathogens. Evolution study re-

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revealed that torovirus derived from coronavirus⁶. Torovirus-like particles were detected from fecal samples of 2 week old calves with diarrhea. The virus particles were pleomorphic and short spikes which distinct from that of *Coronavirus*. Size of the particles were 70~90nm in diameter and majority of the particles were spherical shape with 8~20nm of peplomers. Five calves developed diarrhea and two calves were died 24 hours after clinical onset of diarrhea. There was no intestinal contents found in ileum and no febrile signs observed. Histologically, villi were shortened and mucosal layer was diffusely necrotized. Electron microscopy indicate that there were mixed infection of bovine rotavirus and torovirus-like particles. The virus particles were pleomorphic and short spikes were distinct from that of Coronavirus. Size of the particles were 70~90nm in diameter and majority of the particles were spherical shape. This report is the first report on the torovirus detection in calves in Korea based on my knowledge.

Materials and Methods

Fecal samples : Fecal samples were obtained from five calves with diarrhea and two calves were died 24 hours after clinical onset of diarrhea. There was no intestinal contents found in an ileum of the dead calves and no febrile signs observed from calves survived after severe diarrhea. Villi were shortened and mucosal layer was diffusely necrotized. Fecal materials were collected from small intestine of dead calves and calves with diarrhea. The fecal materials collected were resuspended in distilled water for the electronmicroscopy to examine viral particles and detection of other enteric pathogens.

Electronmicroscopy : To prepared fecal samples for the electronmicroscopy intestinal content or fecal materials were resuspended in distilled water and centrifuged for 10 minutes in microcentrifuge at 4°C. Supernatants were collected and negatively stained with phosphotungstic acid and examined under the electronmicroscope for the presence of virus particles. To compare morphology of torovirus with coronavirus, transmissible gastroenteritis virus samples were prepared separately and examined.

Results

Pleomorphic torovirus-like particles were distinct from that of coronavirus. The particles were similar to that of torovirus particles in other animals. Size of the particles detected were ranged from 70~90nm in diameter(Fig 1, 2) and small particles were also detected from same sample(Fig 1). Long tubular structure with around 150nm in length was present in the diarrheic fecal sample(Fig 1). Peplomers(Fig 1, 2) were closely attached to the viral membrane protein than that of coronavirus(Fig 3), closely attached to the viral membrane and column shaped. This column shaped peplomers were distinct from that of coronavirus which has club shaped peplomers. There were heterogeneous populations of particles were detected in the samples with various sizes and shapes. These electronmicrograph indicate that virus particles detected from calves with diarrhea were distinct from coronavirus particles. These particles were similar to torovirus particles detected from animals and human.

Discussion

There are several agents with associated with diarrhea in animals need to be investigated on their pathogenicity and significance of the agents causing diarrhea in animals. Torovirus has been known as a causative agent responsible for the diarrhea in animals and human but there is no report has been made in Korea. Torovirus has been detected in calves with diarrhea but simultaneously rotavirus particles were found in some of fecal sample. This mixed infection need to be clarified and scrutinize their roles in calves. Particles detected in intestinal content and fecal materials were similar to torovirus particles detected in other animal species. Morphology of the torovirus particles detected from fecal samples was distinct from coronavirus which is common pathogen in calves with diarrhea. Peplomers of the torovirus like particles were closely attached to the viral membrane protein than that of coronavirus(Fig 2, 3). Overall comparison of the particle size showed torovirus-like particles were smaller than coronavirus particles. No clear evidence has

been made for the torovirus as a causative agent for the death and diarrhea of the submitted calves but in one calves there was no other pathogens present in the samples indicated that torovirus-like particles may an agent responsible for the diarrhea. Further investigation will need to be made to collect more information on pathogens involved in diarrhea in calves as well as other species of animals include human beings. Torovirus particles present in intestinal con-

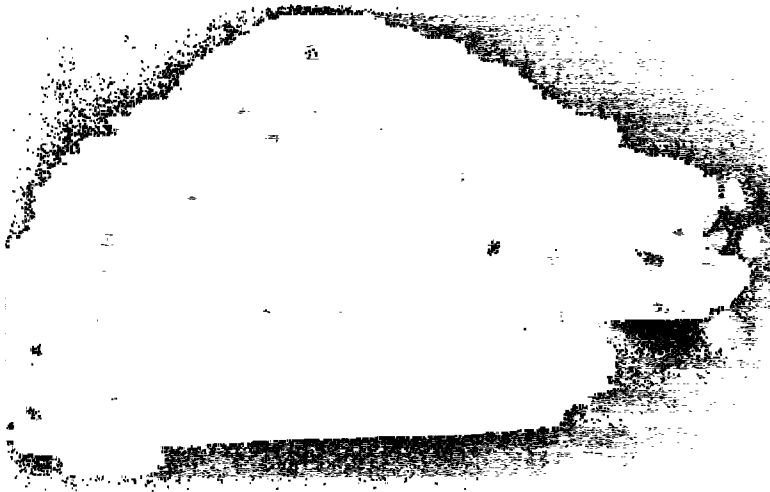
tents were distinct from coronavirus and rotavirus particles. In this smaples no pathogenic bacterial pathogens were detected. Vaccine failures in calves in the field may indicate that torovirus as one of a causative agent for claf diarrhea. To solve this problems we need further investigation on the pathogenesis, molecular biology and virological studies of the torovirus.

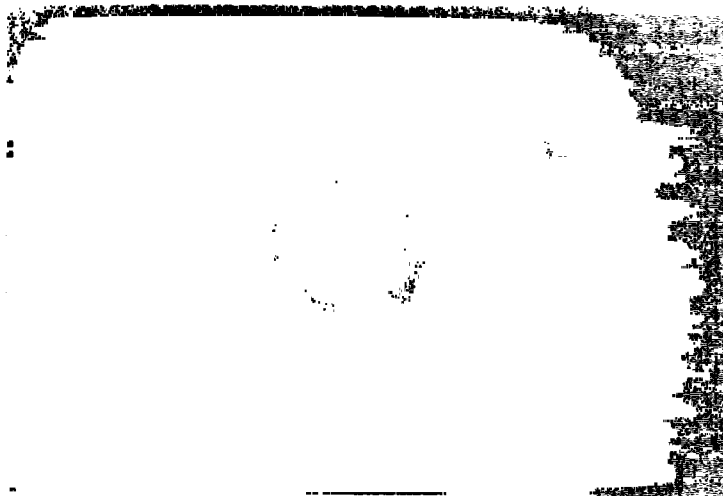
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Fig 1. Electronmicrograph of torovirus-like particles from fecal smaples of diarrheic calves. Various sized ranges from 70 to 90nm and different shapes were found.

Fig 2. Electronmicrograph of torovirus-like particles with 70~90nm in diameter and closely attached short peplomers to the viral membrane protein (8~10nm in length) were distinct from that of coronavirus particles.

Fig 3. Electromicrograph of coronavirus particles with 130nm in diameter and characteristic club shaped spikes.





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