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TOROVIRUS DETECTION IN FAECAL SPECIMENS OF CALVES AND PIGS IN HUNGARY: SHORT COMMUNICATION

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Bovine torovirus is an established aetiological agent of disease in cattle, while porcine torovirus has only been isolated from healthy animals. Evidence for the presence of torovirus has been described in several European countries and also in the United States. A survey was performed to detect toroviruses in Hungary by means of sampling ten swine and nine bovine herds. Rectal swabs and faecal specimens were collected from diarrhoeic calves and from weaned piglets. The samples were tested by the reverse transcription-polymerase chain reaction (RT-PCR) using torovirus-specific primers and the positive samples were further examined by electron microscopy (EM). Torovirus was detected in 4 diarrhoeic calves (out of 111) and in 10 healthy weaned pigs (out of 200 tested), representing two of the 9 calf herds and two of the 10 pig herds tested. This is the first report of exact diagnosis of torovirus in Hungary.

Key words: Porcine, bovine, torovirus, RT-PCR

The *Torovirus* genus is a member of the family *Coronaviridae*, order *Nidovirales* (Cavanagh, 1997). There are four species in the genus: the equine torovirus (ETV, formerly Berne virus; Weiss et al., 1983), the bovine torovirus (BoTV, or Breda virus; Woode et al., 1985), the porcine torovirus (PoTV; Kroneman et al., 1998) and the human torovirus (HTV; Duckmanton et al., 1997). The toroviruses are enveloped single-stranded RNA viruses, about 25–30 kilobases in size. The appearance of virions is often pleiomorphic. The particles can be spherical, elongated, oval or kidney shaped. Members of the genus have close genetic and morphologic similarity. With the exception of ETV the toroviruses do not grow in tissue culture (Cavanagh, 1997).

The aetiological role of BoTV has been associated with diarrhoea in calves (Liebler et al., 1992; Duckmanton et al., 1998; Perez et al., 1998), usually under 60 days of age (Duckmanton et al., 1998). Two serotypes of BoTV are distinguished on the basis of haemagglutination inhibition, ELISA and immunoelectron microscopy tests (Woode et al., 1985). According to serological sur-

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veys BoTV is widespread in Europe and in the United States as well (Weiss et al., 1984; Woode et al., 1985; Brown et al., 1987; Koopmans et al., 1989). The pathogenic potential of porcine torovirus has not yet been tested. However, torovirus infection is as common in pigs as in cattle and horses (Weiss et al., 1984; Kroneman et al., 1998). According to Kroneman et al. (1998), shedding of porcine torovirus begins shortly after weaning and lasts for one to nine days. The presence of torovirus in Hungary has not been proved so far, although the so-called 'Breda agent' was detected by electron microscopy (EM) in faecal samples of diarrhoeic calves as early as 1986 (Nagy et al., 1986). However, exact diagnosis had to wait molecular analysis, as described in this paper.

In order to give convincing evidence for the presence of toroviruses in Hungary a survey was planned. Ten swine and nine bovine herds were selected randomly as study groups from three counties in Eastern Hungary. Altogether 200 pig and 111 bovine samples were tested. Rectal swabs and faecal specimens were collected from diarrhoeic calves under 60 days of age and from healthy piglets after weaning. The age of the sampled animals was determined according to the literature, i.e. bovine torovirus was detected in calves under 60 days of age (Duckmanton et al., 1998), while shedding of porcine torovirus has been reported to begin shortly after weaning and last for one to nine days (Kroneman et al., 1998).

The screening procedure was carried out by using an RT-PCR assay. RNA was extracted from rectal swabs and faecal samples applying the guanidinium isothiocyanate silica method (Boom et al., 1990). The conditions of the RT-PCR were as described Kroneman et al. (1998). The primers (oligonucleotides 293 and 294) targeting the highly conserved 3'NTR of the torovirus genome were kindly provided by M. C. Horzinek. The 135 bp PCR products were sequenced in both directions using an ABI PRISM sequencing device. The obtained nucleotide sequences were analysed by the BLASTN program (Altschul et al., 1997). The RT-PCR positive faecal specimens were examined by negative-contrast EM (Duckmanton et al., 1998) to give further evidence for the presence of torovirus particles.

The RT-PCR assay yielded a product of the predicted size (135 bp) in four calf and ten pig specimens. Altogether four herds proved to be positive for toroviruses by RT-PCR. Two pig herds (each represented by 20 specimens) contained eight and two RT-PCR positive samples, respectively. Similarly, two cattle herds (represented by 15 and 10 samples) contained two positive samples each. The positive calves were under 30 days of age while the positive pig samples originated from 29–35 days old weaned piglets. Sequence analysis of the PCR products identified the fragments as torovirus genomic sequences based on the nucleotide sequence data deposited in the GenBank. The EM investigations of the RT-PCR positive samples showed the presence of kidney-shaped virions 102×50 nm in size, bearing surface projections that are characteristic of *Coronaviridae* (Fig. 1).

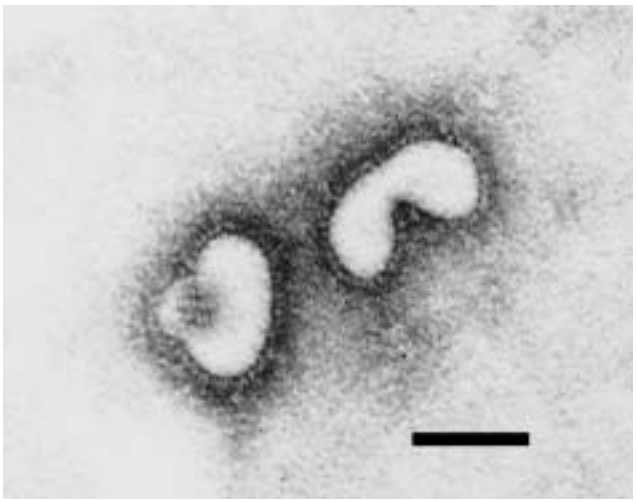

Fig. 1. Pleiomorphic torovirus virions from a faecal sample of a diarrhoeic calf. One virion shows the characteristic kidney shape. Transmission electron micrograph. Bar = 73 nm

RT-PCR proved to be a useful tool for the detection of toroviruses. Primers targeting the highly conserved 3'NTR region of the torovirus genome (approximately 88% sequence identity between different toroviruses, Kroneman et al., 1998) are adequate for performing screening studies in different animal species. The proportion of torovirus-positive samples in diarrhoeic calves is in agreement with the data of Liebler et al. (1992) from Germany, but it is lower than the positivity rate reported in Canada (Duckmanton et al., 1998). In this study, our primary goal was to answer the question whether toroviruses could be detected in the Hungarian pig and cattle farms by molecular analysis. The presence of the virus calls for further studies in order to reveal the prevalence of toroviruses in Hungary. Additionally, it is important to collect and analyse data of our own and compare it to those available in the literature and to examine the pathogenicity of toroviruses, especially in pig populations.

This is the first exact report of the detection of toroviruses in Hungary that serves as additional data on the extended presence of these viruses in Europe.

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