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Torovirus

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Glossary

Colostrum-deprived (CD) calves Conventional calves that did not receive antibody-rich first milk or colostrum during the first 6–12 h of life, therefore lacking of passive immunity from the mother (calves are hypogammaglobulinemic individuals when they born).

Gnotobiotic (Gn) calves Animals that are obtained and maintained in germ-free environments, in which the composition of any associated microbial flora, if present, is fully defined.

History

Equine torovirus (EToV), originally referred to as Berne virus, was accidentally isolated in equine kidney cells in 1972 from a rectal swab taken from a horse with diarrhea. EToV is the only torovirus that has been propagated in cell culture, in lines of equine dermis or embryonic mule skin cells, where it causes a cytopathic effect that results in cell lysis. While Berne virus was not neutralized by antisera against known equine viruses, serologic cross-reactions were observed in neutralization tests and enzyme-linked immunosorbent assay (ELISA) using sera from calves that had been experimentally infected with morphologically similar particles, then named ‘Breda’ viruses.

The first Breda virus, now called bovine torovirus (BToV), was discovered in 1979 during an investigation in a dairy herd in Breda (Iowa), in which severe neonatal calf diarrhea had been a problem for three consecutive years. After this initial report, other strains of BToV were identified in beef calves from Ohio, and in a colostrum-deprived

(CD) calf from Iowa. Despite repeated attempts, BToV cannot be adapted to grow in cell or tissue cultures and must be passaged in gnotobiotic (Gn) calves, which has hampered its biochemical, biophysical, and molecular characterization. Most of the studies on the pathogenesis and pathology of torovirus infections have been done in BToV-infected Gn and CD calves, as well as in limited field studies; in contrast, most of the biochemistry and morphogenesis data on toroviruses are based on EToV studies.

In 1984, torovirus-like (TVL) particles were detected in the feces from human patients with gastroenteritis by using electron microscopy (EM). Since then, reports of human toroviruses (HToVs) in children and adults with acute diarrhea have appeared in several countries. TVL particles have also been detected in fecal samples from pigs, and named porcine torovirus (PToV). Proof that the observed structures were not artifacts was obtained when toroviral RNA sequences were found in the feces of piglets and in stools from humans with diarrhea. In recent years, TVL particles have also been detected in turkeys and have been associated with a ‘stunting syndrome’.

Taxonomy and Classification

Toroviruses are single-stranded, positive-polarity RNA viruses with a peplomer-bearing envelope. The term *torus* (Latin) refers to the circular convex molding in the form of a doughnut that some columns or pilaster have at their bases; indeed, it was the unique biconcave disk and C-shape of the virion in the extracellular environment that suggested this naming. Since 1992, the genus *Torovirus* has been included with the genus *Coronavirus* and the newly recognized genus *Bafinivirus* in the family *Coronaviridae*, based on similarities in genomic organization and replication strategies. Toro- and coronaviruses are also

ancestrally related: their polymerase and envelope genes diverged from those of a common predecessor. Because of their inclusion in the family *Coronaviridae*, the nomenclature for coronavirus genes, mRNAs, and structural proteins have also been applied to toroviruses. However, the lack of sequence homology in the structural genes and the absence of antigenic relatedness with coronaviruses justify their taxonomic position as a separate genus. The International Committee on Taxonomy of Viruses (ICTV) presently recognizes four species in the genus *Torovirus*: *Equine torovirus*, *Bovine torovirus*, *Porcine torovirus*, and *Human torovirus*.

The families *Coronaviridae* and *Arteriviridae*, as well as the new family *Roniviridae*, are the constituents of the order *Nidovirales*, the second order in animal virology (after the order *Mononegavirales*). This assignment is based on their similar basic genomic organization and common replication strategy: the synthesis of a 3' co-terminal nested set of subgenomic mRNAs, and the possession of two open reading frames (ORFs) connected by a frameshift site to express a replicase directly from the genomic RNA. This nested set of mRNAs was the foundation for the name of the order *Nidovirales* (from Latin *nidus*, 'the nest').

Virion Properties

Torovirus particles possess a nucleocapsid with helical symmetry coiled into a hollow tube (diameter 23 nm, average length 104 nm, periodicity 4.5 nm). Extracellular, negatively stained torovirus virions are generally observed as kidney- or C-shaped particles (105–140 nm × 12–40 nm). They can also be seen as spherical or oval particles

(89 ± 7 nm × 75 ± 9 nm) or rod-shaped virions (35 nm × 170 nm), depending on the different orientations of the virions with respect to the electron beam. A graphic representation of a torovirion is shown in **Figure 1**. A tightly fitting envelope, 11 nm thick, surrounds the virion structure bearing prominent drumstick-shaped peplomers (17–24 nm), and a fringe of shorter spikes (8–10 nm), which represent the spike and the hemagglutinin-esterase (HE) proteins, respectively. Intracellularly, toroviruses are observed as elongated tubules with rounded ends (rod-shaped virions, 35–42 nm × 80–105 nm), located in the cytoplasm of infected intestinal cells.

Genome

The torovirus genome consists of a single-stranded, polyadenylated RNA of positive (messenger) polarity, which is about 28.5 kbp in length. Recently, the BToV genome has been completely sequenced, and it has been shown to comprise 28 475 nt and contain six ORFs (see **Figure 1**), each of which encodes a known protein. ORF1a and ORF1b are the most 5' proximal reading frames and constitute the replicase (RNA-dependent RNA polymerase) gene, which is expressed as a large precursor protein directly from the genomic RNA by a ribosomal frameshift mechanism, similar to other nidoviruses. The large product of these ORFs is apparently involved in the synthesis of a negative-strand RNA and the onset of genomic and subgenomic RNA synthesis. The other four ORFs correspond to structural protein genes and are expressed by the production of a 3'

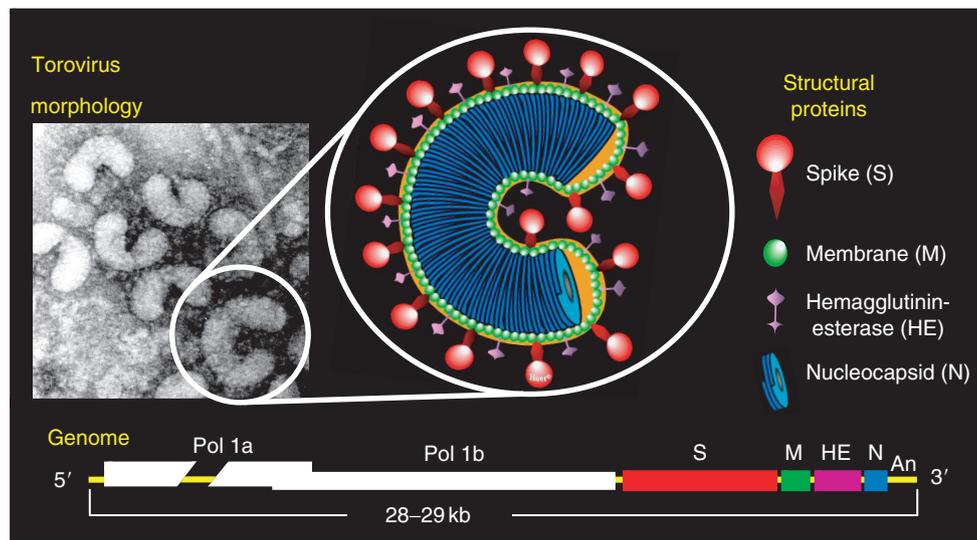


Figure 1 Typical extracellular morphology of a torovirus as visualized by immune electron microscopy (IEM), with a graphic representation of torovirus virion and its structural proteins and genome. Modified with permission from Hoet AE (in press) Torovirus pathogenesis and immune responses. In: Perlman S, Snijder EJ, and Gallagher TM (eds.) *The Nidoviruses*, copyright 2008 by the ASM Press.

co-terminal nested set of four mRNAs. The BToV ORF2 (4752 nt), ORF3 (702 nt), ORF4 (1248–1251 nt), and ORF5 (504 nt) encode the spike (S), membrane (M), HE, and nucleocapsid (N) proteins, respectively.

Proteins

Proteins with molecular weights of 20, 22, 37, and 80–100 kDa have been identified in EToV virions. Detergent treatment of virions releases the 22, 37, and 80–100 kDa proteins, which indicates their association with the envelope. Similar polypeptides of 20, 37, 85, and 105 kDa have been identified in purified BToV by means of surface radioiodination. The nucleocapsid (N) protein (167 aa) is the most abundant polypeptide found in the virion (80–84%) with a predicted molecular weight of 18.3–19.2 kDa. Blotting experiments performed with EToV have identified this internal protein as the only RNA-binding polypeptide in the virion. The M glycoprotein (233 aa, 26.5 kDa) is the second most abundant protein (~13%) and is probably associated with the envelope. Computer analysis has revealed characteristics of a class III membrane protein lacking a cleaved signal sequence, but containing three successive transmembrane α -helices in the N-terminal half. The M protein accumulates in intracellular membranes, predominantly those of the endoplasmic reticulum and is believed to play a role in assembly, maturation, and nucleocapsid recognition during the budding process.

The heterogeneous, N-glycosylated, 80–100 kDa protein is recognized by both neutralizing and hemagglutination-inhibiting monoclonal antibodies and is therefore identified as the spike (S) protein projecting from the virion surface. The spike (S) gene encodes an apoprotein (1581 aa) with a molecular weight of about 178 kDa. The deduced amino acid sequence contains domains typical of type I membrane glycoproteins: an N-terminal signal sequence, a putative C-terminal transmembrane anchor, and a cytoplasmic tail. The HE is a class I membrane N-glycosylated protein (416–417 aa) of 65 kDa that is also located in the BToV envelope. It has an N-terminal signal sequence, a C-terminal transmembrane domain, several N-glycosylation sites, and a putative 'FGDS' motif which displays acetyltransferase activity specific for *N*-acetyl-9-*O*-acetylneuraminic acid. The HE protein could be an additional receptor-binding protein (next to the spike protein) but without a function in viral entry. EToV virions lack an HE protein, only possessing a partial sequence in ORF4.

Physical Properties

Buoyant densities of 1.16, 1.17, 1.18, and 1.14 g ml⁻¹ have been reported for the virions of EToV, BToV serotype 1,

BToV serotype 2, and HToV, respectively. EToV is remarkably stable in the environment, and relatively resistant to phospholipase C, trypsin, chymotrypsin, and even deoxycholate; however, Triton X-100 and organic solvents destroy its infectivity. EToV is stable within a wide range of pH, being inactivated only below pH 2.5 or above pH 10.3. BToV1 appears to be less stable than both EToV and BToV2 as changes in its sedimentation behavior and density have been observed after prolonged storage at -70 °C. The infectivity of a fecal preparation containing BToV1 has been reported to be lost completely after 3 weeks at 4 °C, whereas EToV in cell-free supernatant remained stable for 92 days. Storage of toroviruses at -20 to -70 °C helps to preserve infectivity. However, even at these temperatures the viruses will deteriorate, though at a slower rate. Repeated cycles of freezing and thawing of purified BToV2 results in loss of peplomers and disintegration of virions.

Replication and Morphogenesis

Because it can be grown in cell culture, the morphogenesis of EToV has been most extensively studied. Ultrastructural and immunofluorescence (IF) studies on intestinal cells from BToV-infected calves have shown similarities with EToV morphogenesis.

Attachment, Entry, and Uncoating

Attachment to the apical surface of enterocytes is apparently mediated through the spike proteins, but the HE proteins may be involved as well. Entry or penetration of BToV into enterocytes is apparently by receptor-mediated endocytosis. Lysosomal degradation in vesicles containing the virus is probably responsible for uncoating and subsequent release of the BToV RNA. The site at which this occurs has not yet been determined.

EToV replication occurs in the cytoplasm. Preformed tubular capsids bud through membranes of the Golgi stack and of the endoplasmic reticulum. A host cell nuclear function seems to be required since ultraviolet (UV) pre-irradiation of cells, actinomycin D, and α -amanitin have been reported to reduce virus yields. The replication cycle takes around 10–12 h to complete.

Transcription

In EToV-infected cells, five virus-specific, polyadenylated mRNAs are found with sizes of >20.0, 7.5, 2.1, 1.4, and 0.8 kbp. Northern (RNA) blot hybridizations with restriction fragments from cDNA clones have shown that the five EToV mRNAs form a 3' co-terminal nested set. Sequence analysis has revealed the presence of four complete ORFs with initiation codons coinciding with the

5' ends of EToV RNAs 2–5, respectively; RNA 5 is contiguous on the consensus sequence. EToV RNAs 1–3 are transcribed independently, as has been shown by UV transcription mapping. The genes for M, HE, and N are preceded by short noncoding 'intergenic' regions, containing a transcription-regulating element (TRE) conforming to the consensus sequence 5' (C)ACN_{3–4}CUUUAGA 3'. A copy of this sequence is also present at the extreme 5' terminus of the genome. In contrast, the S gene overlaps with the replicase gene and the N-terminal 28 residues of S are in fact encoded by an internal (–1) reading frame within ORF1b; moreover, there is no TRE. The production of this 3' co-terminal nested set of mRNAs characterizes the toroviruses and justifies their inclusion into the order *Nidovirales*.

Translation

No RNA-dependent RNA polymerase is found in torovirus virions. The torovirus replicase is probably translated as soon as the RNA is liberated. Translation yields two large polyproteins, from which by proteolytic cleavage the various subunits of the viral replicase/transcriptase are derived, as well as accessory proteins of as yet unknown function. Downstream of ORF1b, there are the genes for the structural proteins S, M, HE, and N (as ordered from 5' to 3'); these are translated from four subgenomic mRNAs, numbered 2–5 (with the genomic RNA as RNA1).

Post-Translational Processing

The N-glycosylated S protein is derived from the processing of a 200 kDa precursor present in infected cells, but not in virions. Eighteen potential N-glycosylation sites, two heptad repeat domains, and a possible 'trypsin-like' cleavage site exist in the spike protein amino acid sequence. The mature S protein consists of two subunits and their electrophoretic mobility upon endoglycosidase F treatment suggests that the predicted cleavage site is functional *in vivo*. The heptad repeat domains are probably involved in the generation of an intrachain coiled-coil secondary structure; similar interchain interactions can play a role in the formation of the observed S protein dimers. The intra- and interchain coiled-coil interactions may stabilize the stalk of the torovirus peplomers.

Assembly, Budding, Egress, and Maturation

About 10 h after infection, EToV particles are observed within parts of the unaltered Golgi apparatus, and extracellularly. At that time, tubular structures of variable length, diameter, and electron density appear in the cytoplasm, and also in the nucleus of infected cells, probably representing preformed nucleocapsids. It is unknown

whether the accumulation of nucleocapsids in the nucleus reflects a nuclear phase in the replication of EToV or some sort of defective assembly. Viruses predominantly bud into the lumen of Golgi cisternae. The preformed nucleocapsid tubules approach the Golgi membrane with one of the rounded ends oriented toward the membrane and attach to it laterally. During budding, the nucleocapsid is apparently stabilized, leading to a higher electron density and a constant diameter (23 nm). Release into the intestinal lumen is probably through reverse pinocytosis. Virus maturation apparently occurs intracellularly during the egress process, where the virus nucleocapsid appearance changes from a straight rod (intracellular) into a torus shape (extracellular). The characteristic torus morphology of BToV is only observed in extracellular viral particles or in vacuoles near the cell surface, and never in the cytoplasm.

Geographic Distribution

In cattle, toroviruses have been detected by ELISA, reverse-transcriptase polymerase chain reaction (RT-PCR), and/or immune electron microscopy (IEM) in Austria, Belgium, Canada, Costa Rica, France, Germany, Great Britain, Hungary, Japan, Netherlands, New Zealand, South Africa, and the USA. BToV-seropositive cattle have also been reported in Belgium, France, Germany, India, Switzerland, United Kingdom, and the USA, with seroprevalence ranging between 55% and 94.6%. Most adult horses tested in Switzerland possess neutralizing antibodies to EToV. HToV appears to occur in Canada, Brazil, France, Great Britain, India, the Netherlands, and the USA.

Host Range

Neutralizing antibodies to EToV have been found in sera from horses, cattle, goats, sheep, pigs, rabbits, and feral mice, but not in humans or in carnivores. The host range of BToV appears to be restricted to cattle; however, seropositive reactions to BToV have also been detected in several ungulate species.

TVL particles have been observed in the feces of children and adults with diarrhea. Interestingly, these can be aggregated after the addition of anti-BToV calf sera, can be detected by using a BToV antigen capture ELISA, and the hemagglutination of rat erythrocytes by TVL particles can be inhibited by BToV antisera. These observations indicate antigenic cross-reactivity between HToV and BToV, and may point to a zoonotic connection. TLVs have also been seen in fecal samples of cats and dogs but neither serologic nor molecular identification has been obtained.

Genetic Relationships

There is limited information available on genetic variation amongst toroviruses. The complete genome sequence has been reported only for BToV. However, partial genome sequences have been obtained and used to chart the genetic diversity. In general, there is little divergence (20–40%) among known genotypes of EToV, BToV, PToV, and HToV in the (S, M, HE, and N) genes. Phylogenetic analyses have shown that all BToV strains are closely related, whether they are of European or American origin. Also, all PToV variants form a distinct genetic cluster. However, BToV and PToV sequences are sufficiently different to be assigned as different genotypes. HToVs show a high degree of similarity to New World BToVs (83%), and less with European strains of BToV (73%) and PToV (56%).

Antigenic Properties

In addition to the typical torovirus morphology, BToV, EToV, HToV, PToV, and Lyon-4 virus (Breda-like BToV detected in France) share common antigens. Currently, only one strain of EToV has been isolated, and all attempts to obtain a second equine isolate have been fruitless. Two strains of BToV have been reported in addition to the original isolate described by Gerald Woode and colleagues. One of the strains was detected in feces from a 5-month-old diarrheal calf in Ohio; the second (Iowa strain) was recovered from a 2-day-old experimental animal. On the basis of their cross-reactivity in ELISA, IEM, and hemagglutination/hemagglutination inhibition (HA/HI) assays using rat or mouse erythrocytes, the three isolates were assigned to two serotypes: BToV1, represented by the Iowa 1 isolate, and BToV2, comprising the Ohio and the second Iowa isolate.

Antigenic cross-reactivity has been demonstrated by ELISA, IEM, HI, and immunoblotting between BToV and HToV, indicating a close relationship. Several authors reported that HToV particles detected in humans with persistent diarrhea, and morphologically similar to BToV, could be agglutinated by BToV antiserum; stronger reactions are observed when BToV-2 antisera are used. Hyperimmune sera to BToV have also been used to detect toroviruses in humans by ELISA and IEM.

Epidemiology

Several epidemiological studies have demonstrated a high seroprevalence of BToV antibodies in several different groups of cattle, indicating that the virus may circulate with high frequency in these populations. BToV have also been detected by ELISA and/or RT-PCR in cases of gastroenteritis in cattle. Up to 44% of the BToV-positive

samples from these cases did not contain other major enteric pathogens. Calves up to 4 months of age are highly susceptible to diarrhea induced by BToV, especially those below 3 weeks of age. The virus has also been recognized in 5–6-month-old beef calves arriving from sales barns. Intermittent BToV shedding can occur in young calves during the first 10 months of life. Older calves and adult animals can also shed BToV at different ages, perhaps by intermittent subclinical infections or by contracting new BToV infections. Levels of maternal BToV-specific antibodies circulating in the calf influence the clinical outcome of the infection; a seronegative neonatal calf is about seven times more likely to develop diarrhea than a seropositive calf.

Transmission and Tissue Tropism

It has been suggested that the transmission of BToV is via the oral/nasal route by direct contact with contaminated feces or nasopharyngeal secretions. Oral inoculation of calves with BToV has been shown to induce diarrhea with virus shed in feces under experimental conditions. The nasal route is another possible pathway for entry as BToV antigen and viral RNA have been detected in the nasal secretions of feedlot calves. Additionally, diarrhea has been induced after intranasal inoculation in Gn and CD calves. For bovine coronavirus (BCoV), respiratory tract infections have been reported to occur prior to enteric infections, indicating the possible importance of this route of transmission in the spread and pathogenesis of this distantly related group of enteric nidoviruses. It is possible that BToV, like BCoV, could initially replicate in nasal epithelial cells, and thereby amplify before being swallowed and infecting the intestinal tract. This hypothesis for BToV pathogenesis should be further studied.

BToV has a tissue tropism for enterocytes located from the lower half of the villi extending into the crypts, affecting the caudal portion of the small intestine (mid-jejunum through ileum) and the large intestine. Infection of other types of cells and organs by BToV has not been reported.

Pathogenesis and Clinical Features of Infection

All BToV strains are pathogenic, causing mild to profuse diarrhea in experimentally and naturally infected young calves. Twenty-four to seventy-two hours post exposure, the first clinical signs are observed (mild fever, depression, weakness, and anorexia), followed by a greenish-yellow to bright yellow watery diarrhea that lasts for 3–5 days. Calves may develop severe dehydration and die. Fecal virus shedding begins 24–72 h post infection, coinciding with the onset of diarrhea, and lasts for 2–6 days. Shedding

peaks around 3–4 days post infection. Mixed infections with other enteric viruses, such as rotaviruses or astroviruses, result in a more severe watery diarrhea than is induced by either virus alone. In CD calves with a normal intestinal flora, diarrhea is generally more severe than in Gn calves. Sporadic and recurrent shedding of BToV can last for up to 4 months. BToV has also been detected in nasal samples. Further studies are needed to analyze BToV replication and shedding in the respiratory tract as well as its role in respiratory pathologies.

EToV seroconversion occurs in horses between 10 and 12 months of age, without the appearance of symptoms. Experimentally infected animals (intravenous route) have been reported to seroconvert without clinical signs. Oral infection experiments in horses have not been reported to date.

Several studies have shown an association of HToV infection with diarrhea in children. In one study, 35% of children with enteritis shed HToVs in their feces, but only 14.5% of the asymptomatic controls shed the virus (statistically significant difference – odds ratio 3.1). Affected children showed watery diarrhea, vomiting three to four times daily, and dehydration as a consequence; neither fever, nor the presence of other enteric pathogens, was recorded. Recently, fecal excretion of HToV has been associated with nosocomial infections in infants with necrotizing enterocolitis (NEC). Immunocompromised children appear to be highly susceptible to disease following HToV infection.

Pathology and Histopathology

The target organs of BToV in calves are the lower half or two-thirds of the small intestine and the entire large intestine, particularly the spiral colon. There is little macroscopic evidence of the infection. Histological examination shows villous atrophy and epithelial desquamation in randomly scattered areas from the mid-jejunum to the lower small intestine, as well as areas of necrosis in the large intestine. As shown by IF, both crypt and villus epithelial cells contain antigen. The watery diarrhea is probably a result of the loss of reabsorptive capacity of the colonic mucosa, combined with malabsorption in the small intestine. The germinal centers of Peyer's patches are depleted of lymphocytes and may occasionally show fresh hemorrhage. The dome epithelial cells, including the M-cells, display the same cytopathic changes as seen in the absorptive cells of the villi. Virions are found in cells of both the small and large intestine, and between enterocytes at the basal and lateral plasma membranes. In macrophages of the lamina propria, virions in various stages of degradation are found.

Antigen is detected as early as 48 h after infection in epithelial cells of the lower half of the villi and crypts of

the affected areas, as well as in dome epithelium. Fluorescence is cytoplasmic (although a few nuclei may be faintly stained) and generally most pronounced in the intestines with the least tissue damage. The mid-jejunum is infected first and the infection eventually reaches the large intestine. Diagnosis by IF should be performed preferentially on sections of the large intestine from calves killed after the onset of diarrhea (i.e., several days after infection of the epithelium).

Immune Response

Up to the age of 4 months, all calves in a sentinel experiment regularly excreted BToV in their feces. They showed early serum IgM responses despite the presence of IgG1 isotype maternal antibodies, but no IgA seroconversion. Antibody titers then decreased below detection; persistent IgG1 titers developed in only a few animals. After introduction into the dairy herd at 10 months of age, all calves developed diarrhea and shed virus. Seroconversion for all antibody isotypes was observed at this stage, indicating lack of mucosal memory. In contrast, coronavirus infection in the presence of maternal antibodies leads to isotype switch and a memory response.

Prevention and Control

There are no specific preventive measures for this virus; however, general hygiene, biosecurity practices, and the intake of adequate, protective amounts of colostrum can be used to prevent BToV infections. There are no reports on the effects of disinfection or heat sterilization on toroviruses.

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See also: Coronaviruses: General Features; Coronaviruses: Molecular Biology.

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Tospovirus

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Glossary

Ambisense genome Viral RNA genome with open reading frames in both the viral- and viral complementary (vc) sense on the same genome segment.

Envelope Membrane-like structure that packages genome segments.

IGR The intergenic region is the untranslated, A-U rich region found between the two open reading frames on the S and M RNA segments.

Negative sense genome Viral RNA genome that codes for proteins in the vc sense. Transcription of vc mRNA is required for translation of viral proteins.

Nucleocapsid Viral RNA encapsidated in the nucleoprotein.

Protoplast Plant cell lacking its cell wall.

RNP Ribonucleoprotein complex consisting of the viral RNA genome segment, nucleoprotein, and a small number of polymerase molecules.

Virion Quasispherical structure containing the viral genome and bounded by a membrane-like envelope.

Tospovirus were obtained through investigation of TSWV even after the discovery of additional viruses in the genus (**Table 1**). Biological investigations beginning in the 1940s revealed a virus that had an unusually large host range and occurred in nature as a complex mixture of phenotypic isolates. However, it was one of the least stable viruses and most difficult plant viruses to mechanically transmit. Although the enveloped virions were observed in the 1960s, molecular characterization and elucidation of the genome organization were not completed until the early 1990s. The virus was shown to be vectored by thrips in the 1930s and later transmitted in a persistent manner. Thrips were demonstrated to be a host for replication of the virus and that replication was required for transmission in the early 1990s. Later it was recognized that limited, localized replication may occur in thrips that does not result in the thrips becoming viruliferous. Advances in gene function and cellular biology have been limited due to the absence of a robust *in vitro* plant or thrips cell culture system, and lack of an efficient reverse genetics system. However, limited progress has been made utilizing gene expression systems and classical viral genetics.

History

Diseases now known to be caused by tomato spotted wilt virus (TSWV) were first reported in 1915 and were shown to be of viral etiology by 1930. This taxon of plant viruses was categorized as a monotypic virus group consisting of a single virus (TSWV) until the report of impatiens necrotic spot virus (INSV) in 1991. Thus, most of the characteristics which define the genus

Taxonomy and Classification

Tospoviruses constitute the only genus of plant-infecting viruses in the family *Bunyaviridae*; however, these viruses share many molecular characteristics typical of other members of this virus family. They have an enveloped virion containing the viral genome which is distributed among three RNA segments that replicate in a manner consistent with that of other negative strand viruses. All three segments have highly conserved, complementary