The role of transmission electron microscopy in the study of gastroenteritis viruses

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The technique of transmission electron microscopy (TEM) was crucial in the discovery of the major viral causes of gastroenteritis in humans (norovirus, rotavirus, astrovirus, sapovirus, adenovirus) and subsequently played a valuable role in the detection of these viruses. In the 21st century, TEM continues to play a role in the understanding of viral gastroenteritis but chiefly in a research role rather than in a diagnostic context.

Introduction

It was only with the development of transmission electron microscopy (TEM) that virus structure could be studied in detail[1] and TEM has had a significant impact on the development of virology in general[2,3]. It is notable that all the major human viral causes of gastroenteritis, including norovirus, rotavirus, astrovirus and sapovirus, were discovered by TEM[3] and TEM was also important in establishing the role of adenovirus in human gastroenteritis[4,5]. Although TEM initially played an important role in the diagnosis of these gastroenteritis viruses, it has now been largely supplanted by other procedures such as the polymerase chain reaction (PCR) and enzyme immunoassay (EIA) techniques. Nevertheless, TEM continues to play a valuable research role in the identification and characterisation of viruses associated with the gastrointestinal tract in both humans and animals. The following review briefly summarises the historical role of TEM in gastroenteritis virus identification and its current role in viral gastroenteritis research.

TEM methods

TEM methodology includes a number of techniques which are of particular value for virological studies[3,6]. Negative staining TEM allows the examination of particulate material including determination of structure and size of particles and has proved important in virological studies. The technique can be combined with immuno methods to facilitate viral detection or to localise viral antigens. Thin sectioning TEM involves the fixation and embedment of relevant material such as virus-infected tissues or cells. The material is then thinly sliced and virus-cell interactions, including stages of viral replication, can be viewed in the slices. The technique can be combined with immuno methods to localise specific antigens. TEM cryomicroscopy enables the three-dimensional reconstruction of molecular structures and is proving valuable in the analysis of viral architecture.

Negative staining TEM in the discovery, identification and characterisation of virus and virus-like particles associated with the gastrointestinal tract

Negative staining methods were used in the initial identification of a number of viruses associated with human gastroenteritis including norovirus[7] (Figure 1), astrovirus[8-10] and sapovirus[11] and played a key role in the identification of human gastroenteritis-associated adenovirus[4]. The method is still useful in surveys of viral gastroenteritis[12].

Negative staining TEM has proved to be a valuable approach for the detection of faecal viruses in a variety of animals[13-15] and this approach remains relevant today. For example, negative staining TEM was recently used to detect astrovirus-like particles in pigs[16], astrovirus-like virus in cheetahs[17] and astrovirus-like virus in dogs[18]. Negative staining TEM was also used to identify rotavirus in an English red squirrel[19] and has also been used to identify norovirus-like particles in monkeys[20].

Negative staining TEM is a valuable technique to monitor the presence of recombinant norovirus virus-like particles[21-23]. The method is also useful in evaluating the sensitivity and specificity of commercial virus detection kits. For example, in this laboratory it has proved useful in evaluating the sensitivity and specificity of two norovirus EIA detection kits[24,25] as well as the specificity of a norovirus immunochromatographic detection kit[26]. Similarly, negative staining TEM was used in the evaluation of a commercial multiplex reverse transcription-PCR for the detection of adenovirus, rotavirus and norovirus[27].

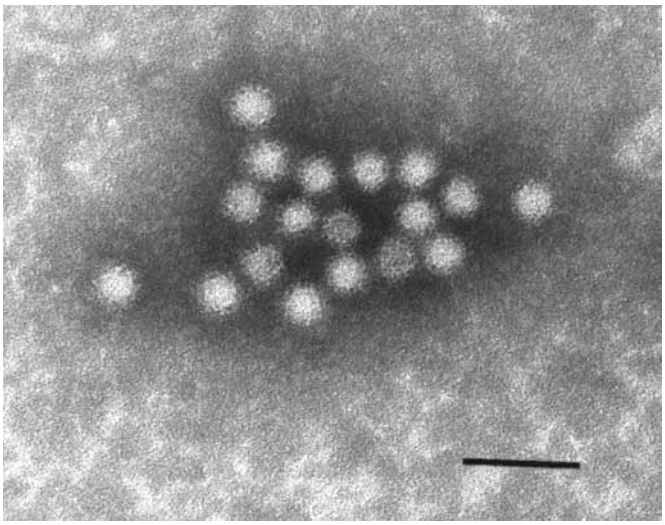

Figure 1. Negative staining electron micrograph of norovirus, the first major cause of human gastroenteritis to be discovered by TEM[7]. Bar represents 100 nm.

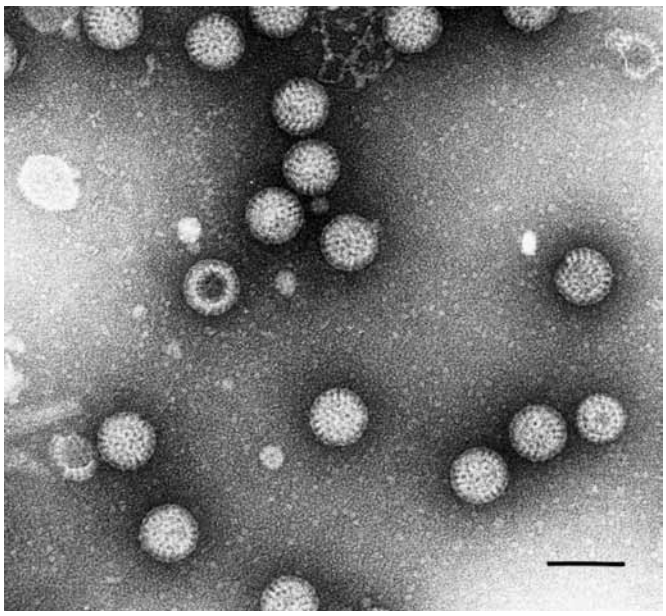

Figure 2. Negative staining electron micrograph of rotavirus, the second major cause of human gastroenteritis to be discovered by TEM[28]. Bar represents 100 nm.

Thin sectioning TEM

Thin sectioning TEM was the method used to discover human rotavirus[28] (Figure 2) and has been widely used in research studies on viruses such as rotavirus[29,30], astrovirus[31,32] and, more recently, murine norovirus[33,34].

TEM cryomicroscopy

In recent years, TEM cryomicroscopy methods have enabled high-resolution studies of virus morphology[6] and the methodology has contributed to an understanding of the structure of both rotavirus[35] and norovirus[36,37].

Conclusion

TEM methodology permits the visualisation of viruses and virus-like particles as well as their interaction with cells. It remains an important research approach and will continue to be so in the future.

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Biography

John Marshall is a scientist in VIDRL with particular interests in electron microscopy and viral gastroenteritis. His current research focuses on viral morphology in general, diagnosis of viral gastroenteritis and the molecular epidemiology of norovirus.