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The causes of infectious diseases have been studied for well over 100 years and, for well over half of that time, much research has been directed at the ‘filterable agents’, or viruses. A massive body of information has accumulated on the aetiology of viral diseases and the viruses that cause them and one might perhaps be forgiven for thinking that there is little left to learn. But of course biological surprises invariably catch us out. Viruses unexpectedly emerge in new situations through genetic reassortment, or by recombination of virus genes, or by jumping the species barrier. Recent severe outbreaks of severe acute respiratory syndrome (SARS) coronavirus and H5N1 influenza are just two reminders of our vulnerability.

The review on animal noroviruses by Etienne Thiry and colleagues, published in this issue of *The Veterinary Journal*, reinforces that there are still viruses out there that we have simply just failed to recognise up to now (Scipioni et al., 2008). Since their characterisation in the early 1990s, human noroviruses have hit the headlines in the popular press, not for the severity of the disease they cause, but because of their association with ‘winter vomiting disease’ syndrome, which sweeps through hospital wards, cruise ships and other closed communities. The layman might ask why, after so many years of virus research, is it only now that we hear about these ‘new’ viruses? This leads us to think about how we detect viruses, and, in particular, those viruses that inhabit the gut.

Ever since man’s activities have been recorded, it has been evident that mastery of tools and techniques has been instrumental to his advancement. It is similar with our understanding of infectious diseases. Inoculation of animals with material passed through filters that retained bacteria demonstrated the existence of viruses. Then came the egg, a sterile package of different cell types, which allowed for the identification of a number of quite different viruses, for example, influenza and pox viruses. Eggs are still used today to produce influenza vaccines. The next major technical revolution, and one that led to an explosion of viral knowledge, was the ability to grow and maintain a range of cell types in the laboratory. A huge number of viruses were identified by their ability to produce a variety of cytopathic effects that could be visualised using the light microscope.

The inoculation into cell cultures of faecal filtrates taken from diarrhoeal disease often resulted in the isolation of reoviruses and enteroviruses. However, application of Koch’s postulates by inoculation of susceptible animals with material grown in cell cultures failed to confirm these viruses as pathogens (Murphy et al., 1999). Up to the 1970s, viruses were suspected of causing much diarrhoeal disease but proof was hard to come by. Something was clearly missing.

The electron microscope (EM) is a highly sophisticated and expensive piece of equipment. When used to examine fluids from infected eggs or cell cultures after negative staining (Brenner and Horne, 1959), the previously unforeseen, but exquisite, structure of viruses was revealed – from the orderly nature of adenoviruses to the complex structure of poxviruses. Application of EM to the causes of diarrhoeal disease took until the late 1960s, which is, perhaps, not surprising considering the nature of the material to be examined and the perceived requirement for highly purified material for EM. Once applied to faecal material, however, unrecognised viruses revealed themselves, and often in large numbers. Rotaviruses, astroviruses and calici-like viruses were identified for the first time – the latter two being quaintly called ‘fuzzy wuzzies’ because of their indistinct appearance (Saif, 1990).

Initially, rotaviruses were refractory to culture in the laboratory, thus explaining why they had not been identified previously using eggs and cell culture methodologies. The key to their culture was the inclusion of trypsin in the culture medium which cleaved the VP4 protein allowing multiple rounds of virus replication (Saif, 1990). Immunostaining of infected cell cultures played a useful role in the study of rotaviruses and astroviruses, but not of the calici-like viruses, which still remain refractory to routine culture in vitro. The application of immune electron microscopy proved particularly useful in revealing in faeces the very
low numbers of the human Norwalk agent, the virus we now know as human norovirus (Greenberg et al., 1990).

The key technique to elucidate the role of calici-like viruses in animal and human diarrhoeal disease has been the application of genetic methodologies. Elucidation of the Norwalk norovirus gene sequence (Jiang et al., 1993; Lambden et al., 1993) and the application of the now ubiquitous reverse transcriptase polymerase chain reaction (RT-PCR) revealed diarrhoeal viruses in man and animals which are refractory to culture in vitro. The bovine calici-like viruses identified in association with diarrhoeal disease in the 1970s by animal inoculation and EM have been characterised as belonging not only to the new norovirus genus in the family *Caliciviridae*, as reviewed by Scipioni et al. (2008), but also to a second previously unrecognized genus, the proposed Nabovirus genus, in the family *Caliciviridae* (Oliver et al., 2006).

Thus, although we can be fairly confident that we have assigned the correct viral pathogens to many of the diseases that plague us, identification of previously unrecognised viruses using the technique of the day does raise the question of how much of the viral iceberg we have seen so far. Current advertising material for the new third edition of the massive five-volume *Encyclopedia of Virology* edited by Brian Mahy and Marc van Regenmortel, claims it has been updated with a 50% increase in identified and accepted viruses since the year 2000 (Mahy and van Regenmortel, 2008)! Only when the next new technique emerges will our eyes be opened further on the viral causes of infectious diseases.

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