

A steadily changing virus

Without continual growth and progress, such words as improvement, achievement, and success have no meaning.

Benjamin Franklin

Dr Norbert Nowotny and colleagues were among the first in Europe to compare the nucleotide sequences of viruses found in hares and rabbits. These sequences are the genetic blueprints of viruses and so revealed a lot about the origins and history of the pathogens. Sequencing involved extracting RNA, including the viral RNA, from tissues of infected rabbits and hares and then using reverse transcription PCR (RT-PCR) to make DNA copies (complementary DNA or cDNA). The cDNA is more stable than RNA and can be produced in sufficient quantity to sequence the component nucleotides accurately. Although automated sequencing was being increasingly used at the time, the work was a major undertaking with the technology available.

Initially, the researchers' interest lay in exploring whether or not RHDV and the related hare virus EBHSV really were distinct lineages of viruses (Nowotny *et al.* 1997). Working mainly with the genetic sequences that determined the structure of the virus coat protein, they found that there was 89–100% similarity between viruses collected from rabbits. Similarly, viruses collected from hares were also closely related. In contrast, there was only 52–60% similarity when viruses from rabbits were compared with those from the hares. Nowotny and his associates concluded that the separation of RHDV and EBHSV into two separate virus species was justified.

While the hare viruses were found to be fairly uniform, those from rabbits aggregated naturally into three groups. Dr Nowotny explained during a conference in New Zealand (Nowotny *et al.* 1999) that the groups came from three areas: Eastern Europe, Central Europe, and Spain and France. As the Spanish isolates were close to Korean and Mexican isolates and close to Chinese samples, this suggested a separate introduction into Europe. Although he did not speculate, it seemed possible that some viruses had arrived in Europe from the Eastern Bloc countries. Viruses from Italy were mixed – similar to those in both Eastern and Central Europe. Furthermore, the sample from United Kingdom was close to a sample from Belgium, suggesting viruses in Britain had originated in neighbouring cross-Channel countries.

Such a picture, although limited and perhaps oversimplified, was consistent with the idea that RHDV may have arrived in Europe by several different routes. It opened up the possibility of viruses arriving through Eastern and Central Europe or even from China through intermediate countries such as Mexico.

Dr Ghislaine Le Gall at the French Food Safety Agency (AFSSA) in Ploufragan pursued similar studies. Samples that arrived in her laboratory, mostly from domestic rabbit farms, confirmed that there were several recognisable sub-groups of the virus, but more importantly