ISSN 1466-2523

Animal Health Research Reviews **16**(1); 83–87
doi:10.1017/S1466252315000092

Pestivirus control programs: how far have we come and where are we going?

Volker Moennig* and Paul Becher

Department of Infectious Diseases, Institute for Virology, University of Veterinary Medicine, Bünteweg 17, D-30559 Hannover, Germany

Accepted 16 March 2015

Abstract

Classical swine fever (CSF) is endemic in large parts of the world and it is a major threat to the pig industry in general. Vaccination and stamping out have been the most successful tools for the control and elimination of the disease. The systematic use of modified live vaccines (MLV), which are very efficacious and safe, has often preceded the elimination of CSF from regions or countries. Oral vaccination using MLV is a powerful tool for the elimination of CSF from wild boar populations. Bovine virus diarrhea (BVD) is endemic in bovine populations worldwide and programs for its control are only slowly gaining ground. With two genotypes BVD virus (BVDV) is genetically more diverse than CSF virus (CSFV). BVDV crosses the placenta of pregnant cattle resulting in the birth of persistently infected (PI) calves. PI animals shed enormous amounts of virus for the rest of their lives and they are the reservoir for the spread of BVDV in cattle populations. They are the main reason for the failure of conventional control strategies based on vaccination only. In Europe two different approaches for the successful control of BVD are being used: Elimination of PI animals without or with the optional use of vaccines, respectively.

Keywords: bovine viral diarrhea, classical swine fever, pestivirus control

Classical swine fever

Classical swine fever (CSF) is one of the most severe infectious diseases of pigs with high mortality rates. Control programs for CSF have always been an economic necessity, and due to progress in vaccinology, laboratory diagnosis, and epidemiology they have been improved continuously. In some parts of the world, in particular Australia and North America, the infection had been eliminated several decades ago. However, it took long to eliminate it from the European Union (EU) and it is still prevalent in Eastern parts of Europe, Asia, South America, the Caribbean, and parts of Africa. In affected countries, endemic situations in backyard pig holdings and wild boar populations are of particular concern. Countries free of CSF face the constant threat of reintroduction, especially through illegal imports of fresh pork products, tourism, hunting, and swill feeding. The latter has been banned in the EU. During the past two decades the EU experienced a series of reintroductions of CSF with serious socio-economic consequences (Stegeman *et al.*, 2000). The overall damage amounted to several billions of Euro.

*Corresponding author. E-mail: volker.moennig@tiho-hannover.de

Diagnosis

The first line of defense is the rapid clinical diagnosis of primary CSF outbreaks. Complicating factors are the slow spread of the virus in herds and the atypical symptoms which can be confused with other porcine infectious diseases, e.g. porcine circovirus 2 infections and/or porcine respiratory and reproductive syndrome. The late detection of index cases typically results in a prolonged 'high risk period', i.e. the time between introduction and detection of CSFV. All the more, it is important to have an arsenal of sensitive and specific laboratory diagnostic tools at hand. Laboratory diagnostic methods have been improved greatly during the last 20 years. Classical tissue culture-based methods for the detection of virus or antibodies are only used in special cases. Enzyme-linked immonosorbent assays (ELISA) are routinely applied for screening for virus-specific antibodies, and polymerase chain reaction after reverse transcription (RT–PCR) is the method of choice for the rapid detection of CSFV. The methods are described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals and the EU Diagnostic Manual (Anonymus, 2002). In Western Europe and many other countries, a network of national CSF reference laboratories exists, and regular meetings and

proficiency tests organized by the OIE and EU Reference Laboratory in Hannover, Germany have contributed to a reliable diagnostic performance (Floegel-Niesmann and Moennig, 2004).

Vaccination

Current modified live vaccines (MLV) against CSF are widely used for CSF control. In general these vaccines – which should be produced in accordance with the OIE manual mentioned above – are highly efficacious and safe. They elicit a rapid and long-lasting solid immunity. A widely used vaccine is derived from the lapinized China (C) strain. Vaccination is routinely applied in countries with endemic CSF and its systematic use might lead to the elimination of the infection (Terpstra and Robijns, 1977). In the EU, where prophylactic vaccination is banned, emergency vaccination can be implemented in cases of severe outbreaks in domestic pigs. However, current MLV elicit the full spectrum of antibodies and vaccinated pigs cannot be distinguished from infected pigs by serological methods. Therefore the use of MLV is followed by severe trade restrictions. The availability of safe and effective DIVA (Differentiating Infected from Vaccinated Animals) vaccines and a corresponding laboratory diagnostic test could solve this dilemma, provided that DIVA vaccination becomes an internationally accepted method for emergency vaccination without disruption of trade.

Control

CSF is listed by the World Organisation for Animal Health (OIE) and it is required that its occurrence has to be reported to OIE. An effective treatment is neither available nor attempted. In endemic regions, prophylactic vaccination is often used to prevent the spread of the infection. Once CSF is under control, vaccination can be stopped while surveillance is continued. The OIE Terrestrial Animal Health Code defines the requirements for a country or a zone to be considered free of the disease. In disease-free areas, new outbreaks are controlled by early detection, stamping out, movement control, safe disposal of carcasses, and cleaning and disinfection. In the wake of the introduction of the internal common market, the EU had to make provisions for a guaranteed uniform animal health status. Since the vaccination policy was heterogeneous among member states, a decision had to be made whether to vaccinate against CSF or not. After some analysis, it was decided to ban prophylactic vaccination. Emergency vaccination had to be approved by the EU Commission, and there were no provisions for the vaccination of wild boar, since it was assumed that CSF outbreaks in wild boar would be self-limiting. Following this policy change, a number of outbreaks in domestic pigs with heavy losses struck several member states, e.g. Belgium, Germany, The Netherlands, and Spain. Typically areas with high densities of pigs (>1000 pigs/km^2) were worst hit.

In addition, the historical observation that CSF outbreaks in wild boar are self-limiting proved to be wrong (Terpstra, 1987). In 1992 outbreaks in Northern Germany spread rapidly and became endemic. The areas affected had a high density of wild boar and the causative virus was of moderate virulence. In a substantial number of cases the infection spilled over to domestic pig holdings. For the perpetuation of the infection susceptible animals play a key role, i.e. young animals which are no longer protected by maternal antibodies. Many if not most older animals in endemic areas were seropositive because they had survived a field infection. Therefore the goal of any control effort must be the reduction of susceptible animals in order to keep the reproduction of infection $R_0 < 1$. In areas with a low density of wild boar, hunting measures targeting young wild boar might be sufficient to reduce the number of susceptible animals, thus leading to the elimination of the infection. Hygienic measures, e.g. the safe disposal of carcasses of shot animals, should be part of the program. However, hygienic measures taken by hunters and increased hunting alone proved to be insufficient for the control of the infection in areas with high densities of animals. In those cases oral immunization was shown to be an appropriate method to reduce the number of susceptible wild boar that ultimately leads to the elimination of CSF (von Ruden *et al.*, 2008). The current protocol for oral immunization of wild boar (Kaden and Lange, 2001; Kaden *et al.*, 2001) has been successfully applied, e.g. in Germany, France, and Slovakia. However, unlike oral vaccination of foxes against rabies, baiting has to take into account the behavior of wild boar, e.g. baits cannot be distributed by planes, instead distinct feeding places have to be identified and baits have to be covered with a thin layer of earth. Environmental temperatures should be low in order to ensure the survival of vaccine virus.

The negative experiences made in the first years of the new non vaccination legislation prompted the EU to amend the regulations (Anonymus, 2001). Stamping out and movement restrictions in protection and surveillance zones remained central elements of control, but emergency vaccination of domestic pigs was facilitated, the use of DIVA vaccines and appropriate tests is an option and unrestricted marketing of products of DIVA-vaccinated animals is possible. However, barriers to vaccination are still high because the majority of member states have to agree to the measures and the reaction of trade partners is unpredictable. Emergency vaccination was so far used only once to control CSF outbreaks in the backyard pig population in Romania between 2007 and 2009 (Anonymus, 2006). A high degree of vaccine coverage was reached and after stopping vaccination by the end of 2009 no new CSF outbreaks have occurred in Romania.

In addition to the amended legislation EU member states have improved their abilities to respond to CSF outbreaks more efficiently.

Epidemiological situation

Except for minor outbreaks in Lithuania and Latvia, the EU has been free of CSF in recent years. However, reintroduction of CSFV remains a constant threat.

https://doi.org/10.1017/S1466252315000092 Published online by Cambridge University Press

Bovine virus diarrhea virus (BVDV)

In contrast to CSFV, BVDV is antigenically more diverse with its two genotypes (BVDV-1 and -2) (Ridpath *et al.*, 1994) and several antigenic groups within the genotypes. The naturally occurring BVDV is noncytopathic (ncp). Cytopathic (cp) variants occasionally arise *de novo* in persistently infected (PI) animals by various kinds of mutations and are crucial for pathogenesis of fatal mucosal disease in these PI animals (Becher and Tautz, 2011). Acute postnatal infections are mostly mild with fever and little or no noticeable clinical signs. However, a transient immunosuppression may pave the way for accompanying infectious agents thus triggering respiratory or enteric disease. Occasionally virulent variants arise and cause severe hemorrhagic disease with high fatalities (Rebhun *et al.*, 1989). So far, only BVDV-2 were the source for highly virulent variants (Jenckel *et al.*, 2014).

Most of the economic damage is done by the interference of BVDV with bovine reproduction. Transient infertility, resorption, abortions, stillbirths, malformations, and the generation of PI calves are common effects of BVDV infections.

Diagnosis

Untypical clinical signs or their absence in acutely infected and PI animals make a reliable clinical diagnosis of BVD impossible. Therefore laboratory diagnosis of BVDV is of prime importance for the control of BVD. For a long time only tissue culture based diagnostic methods were available for the demonstration of virus or virus-specific antibodies. Virus isolation on susceptible bovine cells and neutralization assays were expensive, time-consuming and labor intensive. This changed with the development of a first antigen capture ELISA (AgC-ELISA) for the detection of BVDV (Gottschalk *et al.*, 1992) and ELISAs for the demonstration of virus-specific antibodies (Bottcher *et al.*, 1993). Both tests were relatively inexpensive and they allowed the testing of large numbers of animals. With the availability of mass screening tools first control programs were initiated (Bitsch and Ronsholt, 1995).

Vaccination

Compared to CSF the development of vaccines against BVD is more complicated due to the relatively wide antigenic diversity among BVDV field strains (Van Oirschot *et al.*, 1999; Ridpath, 2005, 2013). The first vaccine against BVD was a MLV based on a cpBVDV developed by Coggins *et al.* (1961). In the following years, many more vaccines followed. Most of them were also MLV. These vaccines were quite efficacious; however, they had intrinsic safety problems. During production of these vaccines ncpBVDV accidentally present in fetal calf serum used for the tissue culture medium sometimes contaminated vaccine batches. In order to prevent damage by live vaccine virus or ncp virus contaminants the use of these vaccines was restricted to non-pregnant animals, a condition that was difficult to comply with in the field. As a response to the safety problems of MLV, killed vaccines were developed. These usually had an excellent safety record; however, in general their efficacy was inferior to that of MLV (Zimmer *et al.*, 2002). Another problem of killed vaccines became apparent when attempts were made to increase efficacy by very strong adjuvants: Bioprocess impurities elicited the formation of maternal alloantibodies in vaccinated dams, which caused Bovine Neonatal Pancytopenia (BNP) in newborn calves at the age of about 10 days. The condition was characterized by spontaneous bleeding and severe anemia with an almost complete destruction of the red bone marrow (Deutskens *et al.*, 2011).

Initially, the main target of prophylactic vaccination was the prevention of clinical signs. It took many years until the significance of PI animals for the epidemiology of BVD and the importance of prevention of fetal infections were recognized. The immune response of many vaccines was too weak to prevent fetal infections. Consequently, the main target for prophylactic vaccination has shifted towards fetal protection which is now the main parameter for newly developed vaccines.

Vaccines usually are an inexpensive and very effective tool for the control of animal virus diseases, e.g., CSF, rinderpest, rabies, and pseudorabies. However, in the case of BVD some 50 years of vaccination have not changed the epidemiological situation (O'Rourke, 2002). In most parts of the world, today's BVD prevalence is as high as it was before vaccination was being introduced. There are several possible reasons for the failure of vaccination to effectively control the infection: (a) the unsystematic use of vaccines, i.e. the vaccination of single herds instead of whole regions or countries; (b) the failure to remove PI cattle before vaccination and the impact of huge amounts of virus shed by PI animals; (c) the inability of vaccination to effectively prevent fetal infections.

Control

The history of BVD control is very different from that of CSF mainly because the damage caused by BVDV infections was long underestimated due to the stealthy nature of the disease. In 1946 BVD was described as a relatively inconspicuous viral diarrhea (Olafson *et al.*, 1946) and it took several decades until the complete extent of damage caused by BVDV was fully appreciated. With BVD endemic in cattle populations worldwide, and a lack of proper diagnostic tools, it was widely believed that control is impossible, and it took almost half a century from BVD's discovery until the elimination of the infection from the first countries that had started systematic control programs.

The first voluntary control programs for BVD were developed in the late 1980s in the German federal state of Lower Saxony. Farmers were encouraged to participate in a program that was based on the identification and removal of PI cattle after testing of individual animals. The public animal insurance paid for the AgC-ELISAs used for the identification of PI cattle and culled animals were compensated. The program initially was successful on a herd basis. However, PI-free herds rapidly became seronegative and thus fully susceptible to BVDV

https://doi.org/10.1017/S1466252315000092 Published online by Cambridge University Press

infections. Because the program was voluntary, only a fraction of cattle holders participated and others, among them probably those with less optimal management, still had infected herds. Reintroduction of BVDV into susceptible herds was frequent and caused major damage, and the animal insurance decided to change the by-laws of the control program: participating farmers had to vaccinate their herds after removal of PI cattle. The recommended method for immunization was administration of a killed vaccine followed 4 weeks later by injection of a MLV. This two-step vaccination had been shown to be most effective in terms of duration of immunity and fetal protection (Frey *et al.*, 2002).

Systematic, i.e. compulsory control/eradication schemes were first developed in Scandinavian countries in the early 1990s. Depending on cattle density, seroprevalence in these countries varied from about 1% in Finland to about 50% in Denmark (Moennig *et al.*, 2005). Due to a historically restrictive attitude in Scandinavia to cattle vaccines in general, BVD vaccination had never been a control option. Therefore, the occurrence of BVDV-specific antibodies was always indicative for natural BVDV infection. Herds were tested for BVDV-specific antibodies using bulk milk samples. Herds with high antibody levels were suspected to have an active BVDV infection and all animals were retested individually using AgC-ELISA. After removal of PI cattle, strict biosecurity was another important feature of the Scandinavian control programs. The use of vaccines was banned. Before the programs were launched, great efforts were made to educate and to motivate farmers. The cattle industry strongly supported the programs and cooperation between industry and authorities was close (Houe, 1999; Lindberg and Alenius, 1999). All Scandinavian programs have been very successful, and it took about 10 years for the countries to become free from BVD (Sandvik, 2004). The next country to successfully adopt the Scandinavian control approach was Austria (Rossmanith *et al.*, 2005, 2010). Switzerland chose a different approach, since seroprevalence in the country as result of natural infection and widespread vaccination was very high. Within one year (2008–2009) the whole Swiss cattle population was tested for BVDV. During the following 4 years (2009–2013) all newborn calves were tested for BVDV using ear notch samples. At present a serological surveillance program is in place using bulk milk samples or individual blood samples of young stock. After 6 years only a handful of herds were still infected (Bachofen *et al.*, 2013). In Austria and Switzerland, vaccination is banned.

The systematic control effort in Germany differed from the other European programs because vaccination is an additional tool of the program. In 2004 BVD (PI animals) became notifiable, and in January, 2011 the systematic control program was started. The central element is testing of all newborn calves using ear notch samples. In addition, animals to be moved and mothers of PI calves have to be tested. PI animals have to be destroyed. All newborn calves in infected herds have to be tested for 12 months after identification of the last PI animal in the herd. Based on the negative experiences with fully susceptible herds in preceding voluntary programs, authorities may order vaccination as an accompanying measure in cattle-dense areas where infectious pressure is still high. The status of PI cattle-free herds is 'BVD unsuspected', i.e. there may be seropositive animals due to past infection or vaccination, but no BVDV. Results of the control effort are encouraging: the prevalence of PI animals dropped from 0.55% in early 2011 to 0.07% in the early 2014. Similar programs are in place in Belgium, Scotland and Ireland.

Epidemiological situation

In most countries of the world BVD is still ubiquitous, even though it is an acknowledged fact that it is one of the economically most important viral infections of cattle. The economic benefits of BVD elimination are obvious and prospects for the elimination of the infection are good provided that there is the political will to do so.

Conclusions

During the last 25 years the development of sensitive and inexpensive laboratory diagnostic methods has facilitated the design and implementation of highly effective control schemes for CSF and BVD. Although prophylactic vaccination against CSF is banned in most countries, it is a valuable tool in emergencies. For the control of CSF in dense wild boar and feral pig populations oral vaccination using conventional MLV is indispensable. The disadvantage of current CSF MLV is their lack of a marker which makes a distinction between infected and vaccinated animals impossible. Hopefully the ongoing development of a DIVA MLV will fill this gap (Beer *et al.*, 2007).

Currently there are two different approaches successfully being used for BVD control: systematic removal of PI cattle from national cattle populations (1) without vaccination and (2) accompanied by vaccination. Strict biosecurity measures have to be an integral part of any successful control program. There are still deficits in the availability of efficacious live vaccines that are safe for all cattle, irrespective of pregnancy, and that confer effective fetal protection against both genotypes of BVDV.

References

Anonymus (2001). COUNCIL DIRECTIVE 2001/89/EC on Community measures for the control of classical swine fever. *Official Journal of the European Communities L* **316**: 5–37.

Anonymus (2002). COMMISSION DECISION approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever. *Official Journal of the European Communities L* **39**: 71–88.

Anonymus (2006). COMMISSION DECISION approving the plans for the eradication of classical swine fever in feral pigs and the emergency vaccination of those pigs and of pigs in holdings against that disease in Romania. *Official Journal of the European Union L* **329**: 34–37.

Bachofen C, Stalder H, Vogt HR, Wegmuller M, Schweizer M, Zanoni R and Peterhans E (2013). Bovine viral diarrhea (BVD): from

https://doi.org/10.1017/S1466252315000092 Published online by Cambridge University Press

biology to control. *Berliner und Munchener tierarztliche Wochenschrift* **126**: 452–461.

Becher P and Tautz N (2011). RNA recombination in pestiviruses: cellular RNA sequences in viral genomes highlight the role of host factors for viral persistence and lethal disease. *RNA biology* **8**: 216–224.

Beer M, Reimann I, Hoffmann B and Depner K (2007). Novel marker vaccines against classical swine fever. *Vaccine* **25**: 5665–5670.

Bitsch V and Ronsholt L (1995). Control of bovine viral diarrhea virus infection without vaccines. *Veterinary Clinics of North America: Food Animal Practice* **11**: 627–640.

Bottcher J, Gottschalk E, Greiser-Wilke I, Moennig V, Bommeli W and Liess B (1993). Diagnosis of bovine virus diarrhoea by two enzyme-linked immunosorbent assays. *Revue Scientifique et Technique* **12**: 461–469.

Coggins L, Gillespie JH, Robson DS, Thompson JD, Phillips WV, Wagner WC and Baker JA (1961). Attenuation of virus diarrhea virus (strain Oregon C24 V) for vaccine purposes. *The Cornell Veterinarian* **51**: 539–545.

Deutskens F, Lamp B, Riedel CM, Wentz E, Lochnit G, Doll K, Thiel HJ and Rumenapf T (2011). Vaccine-induced antibodies linked to bovine neonatal pancytopenia (BNP) recognize cattle major histocompatibility complex class I (MHC I). *Veterinary Research* **42**: 97.

Floegel-Niesmann G and Moennig V (2004). Quality management in reference tests for the diagnosis of classical swine fever. *Revue Scientifique et Technique* **23**: 895–903.

Frey HR, Eicken K, Grummer B, Kenklies S, Oguzoglu TC and Moennig V (2002). Foetal protection against bovine virus diarrhoea virus after two-step vaccination. *Journal of Veterinary Medicine* **49**: 489–493.

Gottschalk EE, Greiser-Wilke I, Frey HR, Liess B and Moennig V (1992). An antigen capture test for the detection of cattle viremic with bovine viral diarrhoea virus–a comparison with BVD virus isolation from buffy coat cells in bovine kidney cells. *Zentralbl Veterinarmed* **B39**: 467–472.

Houe H (1999). Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Veterinary Microbiology* **64**: 89–107.

Jenckel M, Hoper D, Schirrmeier H, Reimann I, Goller KV, Hoffmann B and Beer M (2014). Mixed Triple: allied viruses in unique isolates of recent highly virulent type 2 bovine viral diarrhea virus (BVDV-2) detected by deep sequencing. *Journal of Virology* **88**: 6983–6992.

Kaden V and Lange B (2001). Oral immunisation against classical swine fever (CSF): onset and duration of immunity. *Veterinary Microbiology* **82**: 301–310.

Kaden V, Schurig U and Steyer H (2001). Oral immunization of pigs against classical swine fever. Course of the disease and virus transmission after simultaneous vaccination and infection. *Acta Virology* **45**: 23–29.

Lindberg AL and Alenius S (1999). Principles for eradication of bovine viral diarrhoea virus (BVDV) infections in cattle populations. *Veterinary Microbiology* **64**: 197–222.

Moennig V, Houe H and Lindberg A (2005). BVD control in Europe: current status and perspectives. *Animal Health Research Reviews* **6**: 63–74.

O'Rourke K (2002). BVDV: 40 years of effort and the disease still has a firm hold. *Journal of the American Veterinary Medical Association* **220**: 1770–1773.

Olafson R, MacCallum AD and Fox FH (1946). An apparently new transmissible disease of cattle. *Cornell Veterinarian* **36**: 205–213.

Rebhun WC, French TW, Perdrizet JA, Dubovi EJ, Dill SG and Karcher LF (1989). Thrombocytopenia associated with acute bovine virus diarrhea infection in cattle. *Journal of Veterinary Internal Medicine* **3**: 42–46.

Ridpath JF (2005). Practical significance of heterogeneity among BVDV strains: impact of biotype and genotype on U.S. control programs. *Preventive veterinary medicine* **72**: 17–30; discussion 215–9.

Ridpath JF (2013). Immunology of BVDV vaccines. *Biologicals* **41**: 14–19.

Ridpath JF, Bolin SR and Dubovi EJ (1994). Segregation of bovine viral diarrhea virus into genotypes. *Virology* **205**: 66–74.

Rossmanith W, Deinhofer M, Janacek R, Trampler R and Wilhelm E (2010). Voluntary and compulsory eradication of bovine viral diarrhoea virus in Lower Austria. *Veterinary Microbiology* **142**: 143–149.

Rossmanith W, Janacek R and Wilhelm E (2005). Control of BVDV-infection on common grassland–the key for successful BVDV-eradication in Lower Austria. *Preventive Veterinary Medicine* **72**: 133–137; discussion 215–9.

Sandvik T (2004). Progress of control and prevention programs for bovine viral diarrhea virus in Europe. *Veterinary Clinics of North America: Food Animal Practice* **20**: 151–169.

Stegeman A, Elbers A, de Smit H, Moser H, Smak J and Pluimers F (2000). The 1997–1998 epidemic of classical swine fever in the netherlands. *Veterinary Microbiology* **73**: 183–196.

Terpstra C (1987). Epizootiology of swine fever. *Veterinary Quarterly* **9** (suppl. 1): 50S–60S.

Terpstra C and Robijns KG (1977). Experience with regional vaccination against swine fever in enzootic areas for limited periods using C-strain virus. *Tijdschr Diergeneesked* **102**: 106–112.

Van Oirschot JT, Bruschke CJ and van Rijn PA (1999). Vaccination of cattle against bovine viral diarrhoea. *Veterinary Microbiology* **64**: 169–183.

von Ruden S, Staubach C, Kaden V, Hess RG, Blicke J, Kuhne S, Sonnenburg J, Frohlich A, Teuffert J and Moennig V (2008). Retrospective analysis of the oral immunisation of wild boar populations against classical swine fever virus (CSFV) in region Eifel of Rhineland-Palatinate. *Veterinary Microbiology* **132**: 29–38.

Zimmer GM, Wentink GH, Bruschke C, Westenbrink FJ, Brinkhof J and De GI (2002). Failure of foetal protection after vaccination against an experimental infection with bovine virus diarrhea virus. *Veterinary Microbiology* **89**: 255–265.