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# **Zoonotic aspects of infections with noroviruses and sapoviruses**

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## 1 Summary

The close genetic relationship of noroviruses and sapoviruses found in animals and humans has raised the question whether these viruses have a zoonotic potential. Transmission from animals to humans and vice versa would have far-reaching consequences for epidemiology and food safety. So far animal noro- and sapoviruses have not been found in humans. However detection of human noroviruses in animals as well as simultaneous presence of animal and human viruses in bivalve molluscs suggest a risk of transmission. Furthermore, antibodies against animal noroviruses were detected in humans as well as antibodies against human noroviruses in swine. Experimental infection of gnotobiotic calves and pigs with human noroviruses demonstrated that virus replication and seroconversion can occur. Accordingly the possible role of noro- and sapoviruses as zoonotic agents needs to be further investigated.

## 2 Introduction

Noro- and sapoviruses belong to the virus family *Caliciviridae* and constitute their own genera *Norovirus* and *Sapovirus* within this family. Noroviruses as the most important causative agents of non-bacterial epidemic human gastroenteritis are important for public health. Sapoviruses play a minor role as causative agents of gastroenteritis mainly in young children. The detection of noroviruses and sapoviruses in the faeces of healthy as well as diseased farm animals increased public interest in a possible zoonotic transmission of these viruses (Saif et al., 1980; van der Poel et al., 1996; Sugieda et al., 1998; Dastjerdi et al., 1999; Liu et al., 1999).

Virus isolates from animals and humans belong to the same two genera. This raises the questions whether transmission of these viruses between animal and man and vice versa occurs and whether animals represent a reservoir for enteric disease in man (Guo et al., 2001a). The aim of this review is to summarize the respective literature and to evaluate the zoonotic aspects.

### 3 History

**Noroviruses.** Symptoms of norovirus infections in humans have already been described in 1929 as “winter vomiting disease”. This term refers to the typical seasonality of the infection as well as the high percentage of patients suffering from projectile vomiting. At that time the disease could not be related to any known bacterial or parasitic agent. Later experimental infection of volunteers with stool filtrates showed that viruses are responsible for the disease, but the causative agent remained unknown. It took 29 years until Kapikian and co-workers were able to demonstrate viral particles with a size of 27 to 32 nm in a stool sample collected during an outbreak of acute gastroenteritis at a school in Norwalk/Ohio/USA by immune electron microscopy using convalescent sera taken 4 years earlier (Kapikian et al., 1972). Noroviruses do not exhibit the typical calicivirus morphology with cup-shaped surface depressions and were thus first called “small round structured viruses” (SRSV). Examination of the Norwalk virus proteins showed their similarity to caliciviruses. Cloning of the viral genome and its characterization finally proved that noroviruses belong to the caliciviruses. Since then they form their own genus within the family *Caliciviridae* initially called “Norwalk-like viruses” and later “Norovirus” (Green et al., 2000; Koopmans et al., 2005).

Noroviruses have also been identified in animals. Bovine noroviruses were already found in 1976 (Newbury agent-1) and 1980 (Jena virus) in faeces of cattle suffering from diarrhoea (Woode and Bridger, 1978; Günther and Otto, 1987). Both samples contained viral particles exhibiting a feathery appearance in electron microscopy (Bridger et al., 1984; Günther and Otto, 1987). It took again several years until molecular studies demonstrated that these viruses also belong to the family *Caliciviridae* (Dastjerdi et al., 1999; Liu et al., 1999).

Porcine noroviruses were detected in the Netherlands (van der Poel et al., 1996) and in faecal samples from clinically healthy swine in Japan (Sugieda et al., 1998). Additional representatives of the genus *Norovirus* in animals are the murine noroviruses isolated during an infection of immunocompromised laboratory mice (Karst et al., 2003). The causative

agent called murine norovirus 1 (MNV-1) led to a lethal disease with encephalitis, vasculitis, meningitis, hepatitis and pneumonia. In the meantime three additional murine noroviruses (MNV-2, 3 and 4) were identified which cause persistent infection and prolonged faecal shedding (Hsu et al., 2006).

Recently, noroviruses with tropism for the intestinal tract have also been described in a captive lion cub and a canine pup; in both cases the animals showed enteritis (Martella et al., 2007; Martella et al., 2008b).

**Sapoviruses.** Morphologically typical caliciviruses in humans have been depicted for the first time in Great Britain in 1975. The prototype strain Sapporo virus was detected in 1977 during an outbreak of acute gastroenteritis in an infant home in Sapporo/Japan (Chiba et al., 1979). Two years later faecal samples which originated from an outbreak at a school in London were examined by immune electron microscopy and a morphological similarity with caliciviruses was demonstrated. Due to this observation the viruses were designated as “typical human caliciviruses” (Pringle, 1998). The name was changed first into “*Sapporo-like viruses*” (Green et al., 2000) and later into “*Sapovirus*” (Koopmans et al., 2005).

Animal sapoviruses comprise the porcine enteric sapovirus as well as the mink enteric sapovirus. The porcine enteric sapovirus strain Cowden was isolated from faeces of a 27 day old piglet (Saif et al., 1980). Until now the Cowden strain is the only member within the genus which can be propagated in cell culture. Initially called porcine enteric calicivirus the resulting abbreviation “PEC” is still commonly used.

The mink enteric sapovirus, first called mink enteric calicivirus (“MEC”), was detected in connection with an outbreak of diarrhoea at a mink farm in the USA. Typical caliciviral particles were found using immune electron microscopy of faecal samples with anti-PEC hyperimmune sera. Phylogenetic analyses grouped the agent into the genus *Sapovirus* (Guo et al., 2001a).

## 4 The infectious agents

**Taxonomy and phylogeny.** Noroviruses and sapoviruses belong to the virus family *Caliciviridae*. Based on sequence data and phylogenetic analyses the virus family is currently divided into 4 genera: *Vesivirus*, *Lagovirus*, *Norovirus* and *Sapovirus* (Koopmans et al., 2005). Two additional genera are currently discussed: Two virus strains detected in faecal samples of cattle called Newbury agent-1 (Bridger et al., 1984) and Nebraska (Smiley et al., 2002) cluster together in a separate group for which the genus names *Becovirus* or *Nabovirus* have been proposed (Oliver et al., 2006). Another calicivirus detected in faeces from a rhesus monkey may lead to the establishment of a putative sixth genus with the suggested name *Recovirus* (Farkas et al., 2008).

According to the nomenclature of the *International Committee on Taxonomy of Viruses* (ICTV) the genera *Norovirus* and *Sapovirus* contain as type species Norwalk Virus and Sapporo Virus respectively. Based on sequence analyses comprising conserved regions within the RNA-dependent RNA-polymerase (RdRp) and the capsid gene both species are further subdivided into genogroups and genotypes.

Noroviruses are subdivided into 5 genogroups (Zheng et al., 2006). Human viruses are located in genogroups I, II and IV. Genogroup I comprises the prototype strain Norwalk virus and is further subdivided into 8 genotypes. Genogroup II contains 19 genotypes, where genotypes 11, 18 and 19 include porcine strains (Wang et al., 2005a). Bovine noroviruses build their own genogroup III which is divided into two genotypes. Murine noroviruses are located in genogroup V. Phan and co-workers suggested two additional genogroups VI and VII (Phan et al., 2007).

For sapovirus classification five genogroups are generally accepted. Genogroups I, II, IV and V include human viruses, genogroup III porcine enteric sapoviruses with the Cowden strain (Farkas et al., 2004). Several strains from pigs which do not fit in genogroup III have been suggested as members of new genogroups VI and VII (Wang et al., 2005b; Martella et al., 2008a). Until now the high genetic diversity (Wang et al., 2005b) has complicated the

classification of these viruses. Mink enteric sapoviruses are not classified yet but clearly form their own separate cluster.

Figure 1 displays a phylogenetic tree of members of the family *Caliciviridae* with special emphasis on the genera *Norovirus* and *Sapovirus*. It is expected that more genogroups will be established once additional sequence data are submitted to GenBank.

**Morphology.** Calicivirions consist of an icosahedral capsid composed of 90 dimers of a single capsid protein VP1 forming 32 cup-shaped depressions which led to the name derived from the latin word “calix” for cup or goblet (Thiel and König, 1999). These depressions give caliciviruses in a certain view a star-of-David-like appearance in electron microscopy. Noroviruses differ from this since they show a feathery appearance. Figure 2 displays electron micrographs of bovine noroviruses and porcine sapoviruses.

Caliciviruses are non-enveloped, 27 to 40 nm in diameter and exhibit a T=3 icosahedral symmetry. Several three-dimensional structures have been determined using cryo-electron microscopy and computer image processing techniques (Prasad et al., 1994).

**Molecular biology.** The caliciviral genome consists of a single stranded RNA with positive polarity and a size of 7.4 to 7.7 kb. A protein (VPg) is covalently linked to the 5′ end of the 3′ polyadenylated RNA. After infection of cells an additional 3′ coterminal RNA with a size between 2.2 and 2.4 kb is transcribed from the viral genome (Thiel and König, 1999). The genome of caliciviruses is organized in 2-3 open reading frames (ORFs). For noroviruses, ORF1 encodes the non-structural proteins; ORFs 2 and 3 encode the structural proteins VP1 and VP2. The sapoviral genome is organized differently: ORF1 encodes the non-structural proteins as well as the capsid protein VP1; ORF2 encodes the minor structural protein VP2. Translation of the non-structural proteins termed NS1 to NS7 of caliciviruses is required for viral replication. They show similarities to the picornaviruses, namely with regard to certain sequence motifs and genome organization. The polyproteins are co- and posttranslationally cleaved by the proteinase (NS6). For sapoviruses, the NS6-7 (proteinase-



polymerase) polyprotein is not further processed. The structural proteins are synthesized from the subgenomic RNA. As shown for rabbit hemorrhagic disease virus (RHDV) belonging to the genus *Lagovirus* the calicivirus minor capsid protein VP2 is expressed via reinitiation of protein synthesis after termination of translation of the preceding VP1 gene (Meyers, 2007).

## 5 Disease in animals and man

**Noroviruses.** In the 1980s human noroviruses were held responsible for 19-42% of all cases of acute, non-bacterial human gastroenteritis. In the 1990s 23 million cases per year were estimated within the USA (Mead et al., 1999). Today noroviruses are still the most common cause for non-bacterial human gastroenteritis (Green, 2007). Elderly people, children and immunocompromised individuals are most susceptible. After an incubation time of 15-36 hours an acute, self-limiting diarrhoea develops. Characteristics of a norovirus infection is the projectile vomiting which occurs mainly during the first day of disease and was described by Kaplan in context of the so-called "Kaplan criteria" (Kaplan et al., 1982). These criteria which are still valid today include a mean incubation time of 24-48 hours, a mean duration of disease of 12-60 hours, no detection of bacterial or parasitic agents in stool samples as well as vomitus in more than 50% of the patients. Further symptoms are fever, nausea, abdominal pain and cramps (Rockx et al., 2002). Experiments in human volunteers showed histologically broadening and blunting of the villi of the proximal small intestine while the mucosa remained intact (Green, 2007). Virus excretion can remain for up to 3 weeks. The transmission route is faecal-oral but vomit is also infectious, leading to aerogen infection as well as infection via contaminated surfaces. Immunocompromised individuals e.g. after organ transplantation may stay chronically infected. A case of norovirus-associated encephalitis was reported in a 23 months old child in Japan.

Bovine noroviruses are associated with diarrhoea mainly in calves. The Jena strain could be passaged 11 times in cattle fostered without colostrum and led to diarrhoea at each passage (Liu et al., 1999). The second prototype strain, Newbury agent-2, induced anorexia,

diarrhoea and xylose malabsorption after an incubation time of 12-24 hours (Woode and Bridger, 1978). Histologically, villous atrophy in the small intestine dominated (Hall et al., 1984). From the second day after onset of clinical disease viral particles were found in faeces (Dastjerdi et al., 1999).

Until date, porcine noroviruses have been exclusively detected in faecal samples of adult swine without clinical signs (van der Poel et al., 1996; Sugieda et al., 1998; Scipioni et al., 2008). However, in experiments with gnotobiotic pigs mild diarrhoea occurred (Wang et al., 2005a).

Recently, noroviruses have also been isolated from the intestinal content of a captive lion cub euthanized because of severe haemorrhagic enteritis. However, the pathogenic potential and origin of this virus still have to be elucidated (Martella et al., 2007).

The canine norovirus was detected in the faeces of a pup suffering from vomiting and diarrhoea together with canine parvovirus (CPV). The respective norovirus could be detected for 22 days which indicates viral replication in the pup (Martella et al., 2008b). Since CPV alone can cause severe symptoms the pathogenicity of canine norovirus is unknown.

For animal noroviruses serious clinical signs are only observed under certain conditions (Scipioni et al., 2008). For example, immunocompromised mice show severe histopathological changes after infection with murine noroviruses. The authors state that noroviruses in bovines should be regarded as benign pathogens which may facilitate or complicate gastroenteritis especially in neonates.

**Sapoviruses.** Human sapovirus infections occur less frequently than norovirus infections. In the majority of cases patients show no clinical symptoms. Sporadic cases as well as outbreaks of sapovirus infections have been reported. Mainly children under 5 years (Chiba and Nakata, 2000) and elderly people are affected. Clinical symptoms are diarrhoea, vomiting and fever, whereas the projectile vomiting typical for norovirus disease and seasonality is absent. In the majority of cases the virus excretion lasts only for two weeks (Rockx et al., 2002).

The porcine enteric sapovirus strain Cowden was isolated from a diarrhoeic piglet. Infection of gnotobiotic pigs with stool filtrate derived from this animal induced diarrhoea and virus excretion. Histologically, villous atrophy in the small intestine could be shown (Saif et al., 1980). These results were also confirmed by further infection experiments where villous atrophy and crypt hyperplasia were seen. Interestingly, sapovirus specific RNA could also be detected in sera of orally infected pigs which suggested the occurrence of viraemia (Guo et al., 2001b).

Mink enteric calicivirus was detected for the first time during an outbreak of diarrhoea at a mink farm in the USA. Initially only elderly and subsequently also young animals showed clinical disease. The feeding of animals with raw swine livers and kidneys was discussed as source of infection, but this could not be confirmed. After phylogenetic analyses the agent could be identified as a sapovirus different from known sapovirus strains (Guo et al., 2001a).

## 6 Therapy and diagnostic issues

**Therapy.** The therapy of noro- and sapovirus induced disease is reduced to symptomatic treatment. In man the fluid loss is equalized via oral or parenteral administration of electrolytes, the symptoms are reduced by antidiarrhoeica as well as antiemetics. Norovirus outbreaks such as epidemics e.g. in hospital wards, on cruise ships and at catered events have a high economic impact, especially due to the subsequent absence of employees (Mead et al., 1999). In the USA, noroviruses are categorized as “biodefense category B priority pathogen”. Hygiene measures currently represent the main objectives to avoid infection and thereby disease. One major point is avoidance of contamination of food and water as well as reduction of virus spread after outbreaks. This is managed by consequent sanitary measure guidelines, e.g. issued by the Center of Disease Control (CDC). These guidelines should be followed closely especially because the dose required for infection is less than 100 infectious virions. In Europe, a consortium of 12 laboratories called “Foodborne Viruses in Europe Group” has joined with the aim of early identification of

common-source foodborne virus outbreaks by collecting epidemiological as well as nucleotide sequence data (Koopmans et al., 2003).

The high numbers of detected and estimated norovirus infections emphasize the need for protection against disease, e.g. via vaccination. Until now, a commercial vaccine is not available. Approaches for vaccine development against norovirus induced disease were stimulated by the successful immunization of rabbits with rabbit haemorrhagic disease virus (RHDV). Rabbits vaccinated with the RHDV capsid protein expressed via the baculovirus expression system showed a high humoral immune response and were protected against disease. Vaccines against human norovirus-induced disease have already been tested in mice, gnotobiotic calves and phase I clinical studies in man. Such vaccines are based on virus-like particles (VLPs) which self-assemble after expression of the capsid protein by the baculovirus system, but also after expression in plants such as potatoes, tomatoes or tobacco. Target groups for norovirus vaccines are especially elderly people, children, pregnant women, travellers, immunocompromised patients as well as the military (Estes et al., 2000). Major obstacles for vaccine development are the high heterogeneity of noroviruses. Furthermore it has been demonstrated that norovirus immunity is complicated and probably confounded by pre-existing exposure histories and variable immune responses (Donaldson et al., 2008).

**Disinfection.** As expected for non-enveloped viruses caliciviruses exhibit relatively high tenacity. They remain infectious for several weeks in the environment and are resistant to heating up to 60°C and 10 ppm of chloride used for disinfection of drinking water. Inactivation is accomplished by using non-ionizing radiation (UV-radiation) and ionizing radiation ( $\gamma$ -radiation) (de Rouda Husman et al., 2004). A reduction of the virus titre has also been achieved using ethanol, higher temperature and pH-inactivation as well as higher chloride concentrations; for additional information see Duizer and co-workers (Duizer et al., 2004).

**Diagnostic issues.** A tentative diagnosis of noro- and sapovirus infection in humans can be based on clinical symptoms, especially for noroviruses, by using the Kaplan criteria described above (Kaplan et al., 1982). In the past electron microscopy together with immune electron microscopy using reconvalescent sera were the methods of choice for diagnosis of human calicivirus disease (Kapikian et al., 1972). Because noroviruses lack the typical calicivirus morphology and the method is not very sensitive, RT-PCR and ELISA systems have largely replaced EM diagnosis.

A confirmation of norovirus infection is usually done by conventional or real time RT-PCR with stool samples. Universal primers which supposedly detect all isolates of either genus or primers specific for genogroups are frequently employed. In cases of outbreaks, the RT-PCR products can be sequenced to reveal epidemiologic infection chains. Alternatively ELISA systems are commercially available which allow antigen detection with stool samples from humans. Animal viruses are either detected via RT-PCR or non commercial antigen ELISAs. Moreover, antibody ELISAs are used to screen the antibody prevalence in the respective species.

## 7 Conclusions

**Noro- and sapoviruses as zoonotic agents?** British scientists performed a qualitative risk assessment of the emerging zoonotic potential of animal diseases. Within this study, bovine noroviruses have been categorized in level 0 (level 0: not zoonotic until level 4: confirmed zoonosis). This conclusion was based on sequence data for several thousand strains obtained from infected humans where no porcine or bovine sequences have been found (Palmer et al., 2005). Animal viruses have not been detected in human stool samples until date. We question the conclusions of the study mentioned above and state that the importance of animal noro- and sapoviruses as infectious agents in humans is not clear so far. Arguments which support a risk for interspecies transmission are outlined below. Within this review, the term zoonosis is used based on the EASAC (European Academies Science Advisory Council) nomenclature defining zoonosis as any infection that is naturally

transmissible, directly or indirectly, between vertebrate animals and humans with some agents causing disease both in the animal host and in humans and others commensal in the animal host.

While zoonotic transmission of noro- and sapoviruses can not be excluded it is likely to be a rare event. Infection of humans with these viruses occurs mainly via contaminated food or water and by person-to-person transmission. Whether transmission via food of animal origin or by contact with diseased animals is possible remains uncertain.

**Detection of antibodies.** Antibodies against bovine noroviruses could be detected in human serum samples using an ELISA based on recombinant bovine norovirus capsid protein expressed via VEE (Venezuelan equine encephalitis) replicons (Widdowson et al., 2005). Interestingly, IgG reactivity was more common in veterinarians (28%) compared to the general population (20%). Since there was no evidence for cross-reactivity between the capsid protein of bovine and human noroviruses a specific response against the bovine viral antigen was suggested. In another study Han and colleagues were not able to show cross-reactivity between bovine and human noroviruses using VLPs (Han et al., 2005).

Antibodies against human noroviruses have been found in swine (Farkas et al., 2005). Interestingly, antibody titres against genogroup I viruses (test virus: Norwalk virus) were higher than those against genogroup II viruses (test virus: Hawaii virus). Importantly all porcine noroviruses known so far belong to genogroup II. This finding can be explained by cross-species spread of enteric caliciviruses. However it can not be excluded that the antibodies were directed against a yet undiscovered porcine norovirus (Scipioni et al., 2008).

It should be noted that the mere detection of antibodies argues for an infection which does not necessarily lead to disease.

**Virus detection.** Already about 25 years ago zoonotic transmission of enteric caliciviruses was suspected after residents in an old persons home got gastroenteritis 24 h after the proprietors dog had been sick (Humphrey et al., 1984).

Humans can be infected with caliciviruses eating bivalve molluscs. Shellfish are able to concentrate virus from contaminated water due to their high filtration capability. As they are often eaten raw, there is a considerable incidence of infection. In oysters, besides human strains bovine and porcine noroviruses as well as porcine sapoviruses could be identified (Costantini et al., 2006) indicating a possible source of co-infection of man with human and animal viruses.

In animal faecal samples genogroup II noroviruses could be identified belonging to genotype 4 most dominant in humans. Genogroup II.4-like noroviral RNA was also detected in a retail raw pork sample. This finding indicates another possible route for zoonotic transmission of noroviruses to humans via the food chain (Mattison et al., 2007).

**Recombinants between animal and human caliciviruses?** Recombination is a common event in noro- and sapoviruses (Bull et al., 2007; Hansman et al., 2007). Recombination happens mainly within genogroups, but intergenogroup recombination also occurs. For noroviruses intragenogroup recombinants arise frequently where donor and acceptor viral RNA replicate in the same host species (Jiang et al., 1999; Han et al., 2004; Wang et al., 2005a). Recently Phan and co-workers reported for the first time intergenogroup recombination between two human norovirus strains (Phan et al., 2007). With porcine noroviruses clustering in genogroup II together with human isolates the emergence of recombinants with a broader host spectrum after double infection of a host can not be excluded (Wang et al., 2005a).

Hansman and co-workers described recombination between representatives of different genogroups, namely genogroup II and IV of the genus *Sapovirus*. They also emphasized the possibility of recombination including representatives of genogroup III (porcine enteric sapoviruses) (Hansman et al., 2005).

Co-infection with human and animal viruses may lead to recombination and result in generation of new viruses with unknown pathogenic potential and altered species tropism for both animals and humans. Examples for emerging virus variants after recombination are

found in other positive strand RNA viruses such as BVDV (Bovine Viral Diarrhoea Virus) and also negative strand RNA viruses such as influenza virus.

**Tissue tropism of noro- and sapoviruses.** The molecular basis for the species and tissue tropism of noro- and sapoviruses is not known. In this regard identification and characterization of the cellular receptor(s) will be a major breakthrough. Replication of noroviruses is thought to occur in the small intestine but might not be restricted to enterocytes (Scipioni et al., 2008). Investigations are complicated by the fact that the murine norovirus and the sapovirus PEC Cowden strain are the only members of both genera which can be cultured. In cell culture murine noroviruses show a tropism for dendritic cells and macrophages. Recently infection and replication of human noroviruses in a physiologically relevant 3-dimensional organoid model of human small intestinal epithelium could be shown (Straub et al., 2007).

Adaptation of human noro- and sapoviruses to animals and vice versa remains difficult to study. For some norovirus strains a connection between susceptibility to infection and the presence of histo-blood group antigens has been established (Donaldson et al., 2008). Interestingly there has been a first report about successful binding of recombinant norovirus-like particles to histo-blood group antigen on epithelial cells of pig gastrointestinal tissue (Tian et al., 2007) which was also reported after infection of gnotobiotic pigs with human noroviruses (Cheetham et al., 2006). On the other hand, porcine norovirus-like particles did not bind to human major histo-blood group types in saliva samples (Farkas et al., 2005).

**Interspecies infection experiments.** The possibility of noro- and sapovirus transmission from man to animal can be tested by animal experiments. Such experiments have been performed using human noroviruses (genogroup II strain HS66) and gnotobiotic calves. The infection with stool suspensions containing the human isolate induced a mild diarrhoea. Viral replication in enterocytes could be shown accompanied by excretion of virus.



Lesions in the proximal small intestine were similar but less severe than described for Newbury agent-2. An immune response was shown by detection of antibodies and by modified cytokine profiles (Souza et al., 2008).

Pathogenesis of a genogroup II human norovirus was studied in gnotobiotic pigs (Cheetham et al., 2006). Infected animals shed virus for up to 4 days, and 13 out of 22 animals seroconverted. In some animals infection of duodenal and jejunal enterocytes could be demonstrated by immunofluorescence using a monoclonal antibody against human norovirus genogroup II capsid protein.

Experimental infection of non-human primates with human noroviruses was only successful in rhesus macaques, whereas little or none virus shedding was detectable in common marmosets, cotton top tamarins and cynomolgus macaques (Rockx et al., 2005). No clinical symptoms occurred in any of the animals, although the inoculum used had been shown to infect human volunteers and to induce disease.

## 8 Future prospects

Transmission of noroviruses from animals to man will have wide-ranging implications for our understanding of the epidemiology and transmission of noroviruses and for food safety (Widdowson et al., 2005). Calicivirus transmission between different host species has been reported for vesiviruses and cannot be excluded for noro- and sapoviruses; however there is no proof until date.

The recent detection of animal noroviruses belonging to genogroup IV in a dog pup and a lion cub (Martella et al., 2007; Martella et al., 2008b) show that genetically closely related viruses can be found in different species. Animal experiments may be performed to test if these viruses show an enlarged host range. Moreover the search for noro- and sapoviruses should be extended to animal species not known to be susceptible for noro- or sapovirus infection so far (Martella et al., 2007; Martella et al., 2008b). Endemic infections of companion animals with agents such as canine norovirus would imply considerable risk for infection in humans due to the close contact with these animals. Further studies along this

line are required addressing the question of zoonotic potential as well as the pathogenesis of the agents for animals and man.

During outbreaks epidemiological data such as the source of infection, examination of food and conclusions on infection chains as well as viral nucleic acid sequences are collected. It would be interesting to collect additional information concerning the possibility of infection by food of animal origin, farm animals and companion animals. If interspecies transmission or generation of human-animal recombinants occur these may lead to an increase of noro- and sapovirus infections with major implications for public health.

#### **Conflict of interest statement**

None.

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## Figures

Figure 1: Phylogenetic tree of members of the family *Caliciviridae*.

Phylogenetic tree of members of the family *Caliciviridae* based on nucleic acid sequences from the RdRp encoding region (227 b). Sequence comparison was performed using the HUSAR package provided by the DKFZ, Heidelberg, Germany. Strains of animal origin within the genera *Norovirus* and *Sapovirus* are highlighted by boxes. Phylogenetic distances were calculated using the Kimura-2-parameter method; the tree was made based on the neighbor-joining method. A bootstrap analysis with 1000 replicates was included, numbers indicate bootstrap values. Branch lengths are proportional to genetic distances.

GenBank accession numbers: Genus *Norovirus*: aj011099 (Jena), u04469 (DesertShield), eu224456 (Dog), af093797 (Norwalk), af414427 (SaintCloud), ab074892 (Po/SW43), l07418 (Southampton), af312518 (Erfurt007), af414426 (FortLauderdale), u07611 (Hawaii), ef450827 (Lion), x86557 (Lordsdale), ay228235 (MNV1), af097917 (Newbury agent-2).

Genus *Sapovirus*: af405715 (Arg39), af488718 (Gorosgal), af435811 (Houston7-1181), u95643 (HoustonDCC), hcu67859 (Houston27), ay237420 (MC10), af338407 (MEC), eu221477 (Po/It), ay615811 (Po/NL), ay974192 (Po/OH/JJ681), u73124 (Parkville), af182760 (PECCowden), ay289186 (PECKorean), ay425671 (Po/OH/LL14), u50825 (PretoriaMK17), u65427 (Sapporo), u43287 (Vanderbijlpark). Genus *Vesivirus*: nc\_004542 (canineCV), nc\_001481 (FCV1), u15301 (SMSV-S1), u76874 (VESV1), af091736 (VESV-Pan1). Genus *Lagovirus*: nc\_002615 (EBHSV1), nc\_001543 (RHDV-FRG). Not classified: ay082891 (Nebraska), dq013304 (Newbury agent-1).

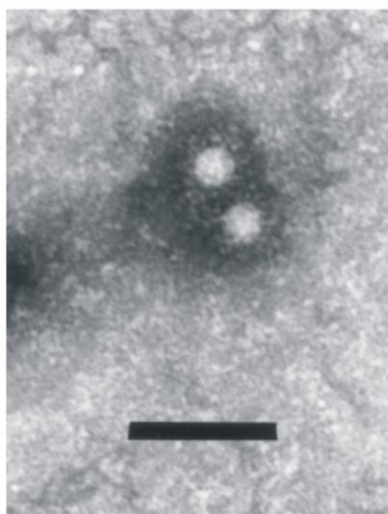
Figure 2: Electron micrographs of bovine noroviruses (A) and porcine sapoviruses (B).

Negative staining of (A) norovirus particles and (B) sapovirus particles out of faecal samples of infected animals with 2% methylamine tungstate. Samples were preincubated with specific antisera. Bars represent 100 nm.

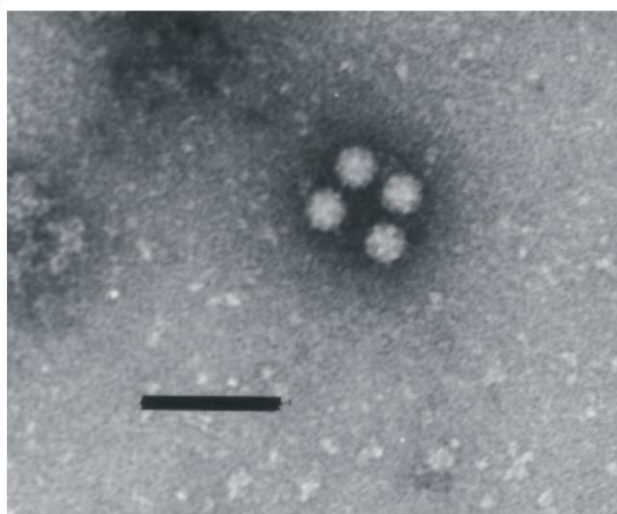


5 Figure 2: Electron micrographs of bovine noroviruses (A) and porcine sapoviruses (B).

(A)



(B)



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