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1 **Zoonotic aspects of infections with noroviruses and**
2 **sapoviruses**

3
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13

14 **1 Summary**

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

26 **2 Introduction**

27 Noro- and sapoviruses belong to the virus family *Caliciviridae* and constitute their own
28 genera *Norovirus* and *Sapovirus* within this family. Noroviruses as the most important
29 causative agents of non-bacterial epidemic human gastroenteritis are important for public
30 health. Sapoviruses play a minor role as causative agents of gastroenteritis mainly in young
31 children. The detection of noroviruses and sapoviruses in the faeces of healthy as well as
32 diseased farm animals increased public interest in a possible zoonotic transmission of these
33 viruses (Saif et al., 1980; van der Poel et al., 1996; Sugieda et al., 1998; Dastjerdi et al.,
34 1999; Liu et al., 1999).

35 Virus isolates from animals and humans belong to the same two genera. This raises
36 the questions whether transmission of these viruses between animal and man and vice versa
37 occurs and whether animals represent a reservoir for enteric disease in man (Guo et al.,
38 2001a). The aim of this review is to summarize the respective literature and to evaluate the
39 zoonotic aspects.

40 3 History

41 **Noroviruses.** Symptoms of norovirus infections in humans have already been
42 described in 1929 as “winter vomiting disease”. This term refers to the typical seasonality of
43 the infection as well as the high percentage of patients suffering from projectile vomiting. At
44 that time the disease could not be related to any known bacterial or parasitic agent. Later
45 experimental infection of volunteers with stool filtrates showed that viruses are responsible
46 for the disease, but the causative agent remained unknown. It took 29 years until Kapikian
47 and co-workers were able to demonstrate viral particles with a size of 27 to 32 nm in a stool
48 sample collected during an outbreak of acute gastroenteritis at a school in
49 Norwalk/Ohio/USA by immune electron microscopy using convalescent sera taken 4 years
50 earlier (Kapikian et al., 1972). Noroviruses do not exhibit the typical calicivirus morphology
51 with cup-shaped surface depressions and were thus first called “small round structured
52 viruses” (SRSV). Examination of the Norwalk virus proteins showed their similarity to
53 caliciviruses. Cloning of the viral genome and its characterization finally proved that
54 noroviruses belong to the caliciviruses. Since then they form their own genus within the
55 family *Caliciviridae* initially called “*Norwalk-like viruses*” and later “*Norovirus*” (Green et al.,
56 2000; Koopmans et al., 2005).

57 Noroviruses have also been identified in animals. Bovine noroviruses were already
58 found in 1976 (Newbury agent-1) and 1980 (Jena virus) in faeces of cattle suffering from
59 diarrhoea (Woode and Bridger, 1978; Günther and Otto, 1987). Both samples contained viral
60 particles exhibiting a feathery appearance in electron microscopy (Bridger et al., 1984;
61 Günther and Otto, 1987). It took again several years until molecular studies demonstrated
62 that these viruses also belong to the family *Caliciviridae* (Dastjerdi et al., 1999; Liu et al.,
63 1999).

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

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

72 Recently, noroviruses with tropism for the intestinal tract have also been described in a
73 captive lion cub and a canine pup; in both cases the animals showed enteritis (Martella et al.,
74 2007; Martella et al., 2008b).

75
76 **Sapoviruses.** Morphologically typical caliciviruses in humans have been depicted for
77 the first time in Great Britain in 1975. The prototype strain Sapporo virus was detected in
78 1977 during an outbreak of acute gastroenteritis in an infant home in Sapporo/Japan (Chiba
79 et al., 1979). Two years later faecal samples which originated from an outbreak at a school in
80 London were examined by immune electron microscopy and a morphological similarity with
81 caliciviruses was demonstrated. Due to this observation the viruses were designated as
82 “typical human caliciviruses” (Pringle, 1998). The name was changed first into “*Sapporo-like*
83 *viruses*” (Green et al., 2000) and later into “*Sapovirus*” (Koopmans et al., 2005).

84 Animal sapoviruses comprise the porcine enteric sapovirus as well as the mink
85 enteric sapovirus. The porcine enteric sapovirus strain Cowden was isolated from faeces of a
86 27 day old piglet (Saif et al., 1980). Until now the Cowden strain is the only member within
87 the genus which can be propagated in cell culture. Initially called porcine enteric calicivirus
88 the resulting abbreviation “PEC” is still commonly used.

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

94 4 The infectious agents

95 **Taxonomy and phylogeny.** Noroviruses and sapoviruses belong to the virus family
96 *Caliciviridae*. Based on sequence data and phylogenetic analyses the virus family is currently
97 divided into 4 genera: *Vesivirus*, *Lagovirus*, *Norovirus* and *Sapovirus* (Koopmans et al.,
98 2005). Two additional genera are currently discussed: Two virus strains detected in faecal
99 samples of cattle called Newbury agent-1 (Bridger et al., 1984) and Nebraska (Smiley et al.,
100 2002) cluster together in a separate group for which the genus names *Becovirus* or
101 *Nabovirus* have been proposed (Oliver et al., 2006). Another calicivirus detected in faeces
102 from a rhesus monkey may lead to the establishment of a putative sixth genus with the
103 suggested name *Recovirus* (Farkas et al., 2008).

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

109
110 Noroviruses are subdivided into 5 genogroups (Zheng et al., 2006). Human viruses
111 are located in genogroups I, II and IV. Genogroup I comprises the prototype strain Norwalk
112 virus and is further subdivided into 8 genotypes. Genogroup II contains 19 genotypes, where
113 genotypes 11, 18 and 19 include porcine strains (Wang et al., 2005a). Bovine noroviruses
114 build their own genogroup III which is divided into two genotypes. Murine noroviruses are
115 located in genogroup V. Phan and co-workers suggested two additional genogroups VI and
116 VII (Phan et al., 2007).

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

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

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

127

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

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

137

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

150 polymerase) polyprotein is not further processed. The structural proteins are synthesized
151 from the subgenomic RNA. As shown for rabbit hemorrhagic disease virus (RHDV) belonging
152 to the genus *Lagovirus* the calicivirus minor capsid protein VP2 is expressed via reinitiation
153 of protein synthesis after termination of translation of the preceding VP1 gene (Meyers,
154 2007).

155 **5 Disease in animals and man**

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

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

177 diarrhoea and xylose malabsorption after an incubation time of 12-24 hours (Woode and
178 Bridger, 1978). Histologically, villous atrophy in the small intestine dominated (Hall et al.,
179 1984). From the second day after onset of clinical disease viral particles were found in
180 faeces (Dastjerdi et al., 1999).

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

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

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

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

197

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

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

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

217 **6 Therapy and diagnostic issues**

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

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

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

249
250 **Disinfection.** As expected for non-enveloped viruses calciviruses exhibit relatively
251 high tenacity. They remain infectious for several weeks in the environment and are resistant
252 to heating up to 60°C and 10 ppm of chloride used for disinfection of drinking water.
253 Inactivation is accomplished by using non-ionizing radiation (UV-radiation) and ionizing
254 radiation (γ -radiation) (de Rouda Husman et al., 2004). A reduction of the virus titre has also
255 been achieved using ethanol, higher temperature and pH-inactivation as well as higher
256 chloride concentrations; for additional information see Duizer and co-workers (Duizer et al.,
257 2004).

258

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

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

274 **7 Conclusions**

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

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

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

293
294 **Detection of antibodies.** Antibodies against bovine noroviruses could be detected in
295 human serum samples using an ELISA based on recombinant bovine norovirus capsid
296 protein expressed via VEE (Venezuelan equine encephalitis) replicons (Widdowson et al.,
297 2005). Interestingly, IgG reactivity was more common in veterinarians (28%) compared to the
298 general population (20%). Since there was no evidence for cross-reactivity between the
299 capsid protein of bovine and human noroviruses a specific response against the bovine viral
300 antigen was suggested. In another study Han and colleagues were not able to show cross-
301 reactivity between bovine and human noroviruses using VLPs (Han et al., 2005).

302 Antibodies against human noroviruses have been found in swine (Farkas et al.,
303 2005). Interestingly, antibody titres against genogroup I viruses (test virus: Norwalk virus)
304 were higher than those against genogroup II viruses (test virus: Hawaii virus). Importantly all
305 porcine noroviruses known so far belong to genogroup II. This finding can be explained by
306 cross-species spread of enteric caliciviruses. However it can not be excluded that the
307 antibodies were directed against a yet undiscovered porcine norovirus (Scipioni et al., 2008).

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

310
311 **Virus detection.** Already about 25 years ago zoonotic transmission of enteric
312 caliciviruses was suspected after residents in an old persons home got gastroenteritis 24 h
313 after the proprietors dog had been sick (Humphrey et al., 1984).

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

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

324

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

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

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

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

344

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

355 Adaptation of human noro- and sapoviruses to animals and vice versa remains
356 difficult to study. For some norovirus strains a connection between susceptibility to infection
357 and the presence of histo-blood group antigens has been established (Donaldson et al.,
358 2008). Interestingly there has been a first report about successful binding of recombinant
359 norovirus-like particles to histo-blood group antigen on epithelial cells of pig gastrointestinal
360 tissue (Tian et al., 2007) which was also reported after infection of gnotobiotic pigs with
361 human noroviruses (Cheetham et al., 2006). On the other hand, porcine norovirus-like
362 particles did not bind to human major histo-blood group types in saliva samples (Farkas et
363 al., 2005).

364

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

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

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

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

383 **8 Future prospects**

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

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

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

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

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406

407 **Conflict of interest statement**

408 None.

409 **References**

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585 Figures

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

587 Phylogenetic tree of members of the family *Caliciviridae* based on nucleic acid sequences
 588 from the RdRp encoding region (227 b). Sequence comparison was performed using the
 589 HUSAR package provided by the DKFZ, Heidelberg, Germany. Strains of animal origin within
 590 the genera *Norovirus* and *Sapovirus* are highlighted by boxes. Phylogenetic distances were
 591 calculated using the Kimura-2-parameter method; the tree was made based on the
 592 neighbor-joining method. A bootstrap analysis with 1000 replicates was included, numbers
 593 indicate bootstrap values. Branch lengths are proportional to genetic distances.
 594 GenBank accession numbers: Genus *Norovirus*: aj011099 (Jena), u04469 (DesertShield),
 595 eu224456 (Dog), af093797 (Norwalk), af414427 (SaintCloud), ab074892 (Po/SW43), l07418
 596 (Southampton), af312518 (Erfurt007), af414426 (FortLauderdale), u07611 (Hawaii),
 597 ef450827 (Lion), x86557 (Lordsdale), ay228235 (MNV1), af097917 (Newbury agent-2).
 598 Genus *Sapovirus*: af405715 (Arg39), af488718 (Gorosgal), af435811 (Houston7-1181),
 599 u95643 (HoustonDCC), hcu67859 (Houston27), ay237420 (MC10), af338407 (MEC),
 600 eu221477 (Po/It), ay615811 (Po/NL), ay974192 (Po/OH/JJ681), u73124 (Parkville),
 601 af182760 (PECCowden), ay289186 (PECKorean), ay425671 (Po/OH/LL14), u50825
 602 (PretoriaMK17), u65427 (Sapporo), u43287 (Vanderbijlpark). Genus *Vesivirus*: nc_004542
 603 (canineCV), nc_001481 (FCV1), u15301 (SMSV-S1), u76874 (VESV1), af091736 (VESV-
 604 Pan1). Genus *Lagovirus*: nc_002615 (EBHSV1), nc_001543 (RHDV-FRG). Not classified:
 605 ay082891 (Nebraska), dq013304 (Newbury agent-1).

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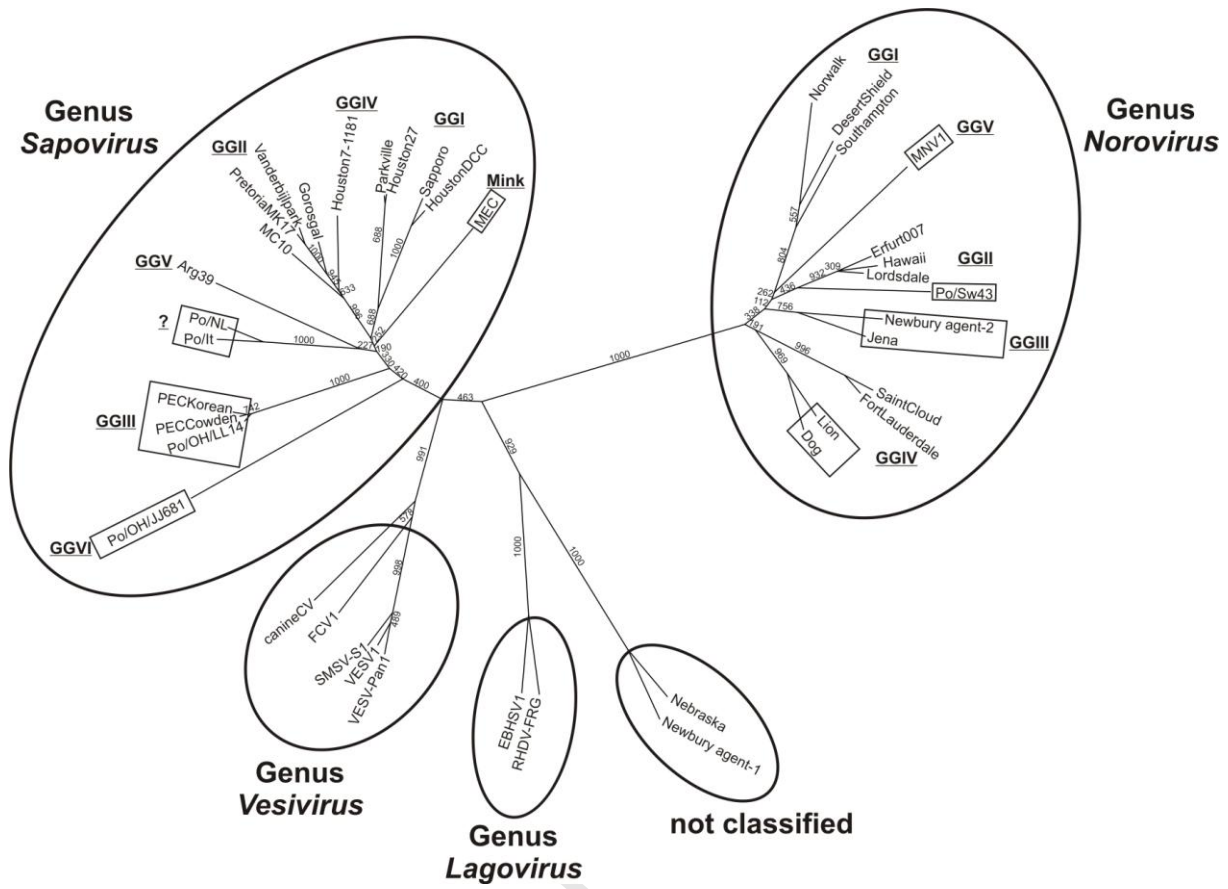
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608 Figure 2: Electron micrographs of bovine noroviruses (A) and porcine sapoviruses (B).

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

612

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

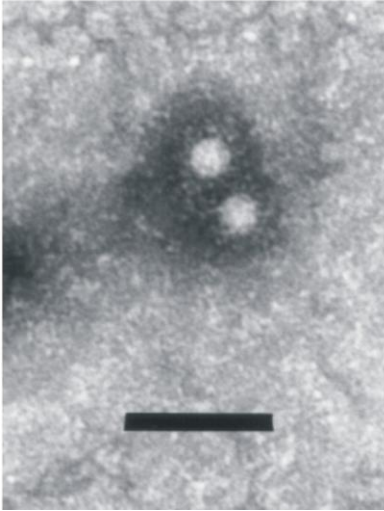


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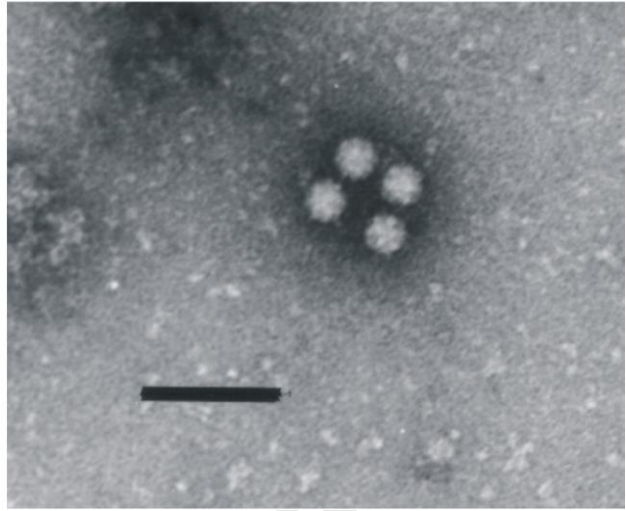
Accepted

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

(A)



(B)



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