

Equine rotavirus infection

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This review briefly describes the virus classification, clinical signs, epidemiology, diagnosis, disinfection, and vaccines related equine group A rotavirus (RVA) infection. Equine RVA is one of the most important pathogens causing diarrhoea in foals. The main transmission route is faecal–oral, and the clinical signs are diarrhoea, fever, lethargy, and anorexia (decreased suckling). Some human RVA rapid antigen detection kits based on the principles of the immunochromatographic assay are useful for the diagnosis of equine RVA infection. The kits are used in daily clinical practice because of their rapidity and ease of handling. Equine RVA is a non-enveloped virus and is more resistant to disinfectants than enveloped viruses such as equine influenza virus and equine herpesvirus. Although amphoteric soaps and quaternary ammonium compounds are commonly used in veterinary hygiene, they are generally ineffective against equine RVA. Alcohol products, aldehydes, and chlorine- and iodine-based compounds are effective against equine RVA. Inactivated vaccines have been used for equine RVA infection in some countries. Pregnant mares are intramuscularly inoculated with a vaccine, and thus their colostrum has abundant antibodies against RVA at the time of birth. According to G and P classification defined in accordance with the VP7 and VP4 genes, respectively, the predominant equine RVAs circulating in horse populations globally are G3P[12] and G14P[12] equine RVAs, but the vaccines contain only the G3P[12] equine RVA strain. Ideally, a G14P[12] equine RVA should be added as a vaccine strain to obtain a better vaccine effect.

Key words: diarrhoea, equine rotavirus, foal, horse, review

Equine group A rotavirus (RVA) is one of the most important pathogens causing diarrhoea in foals [8, 19]. Equine RVA was first detected by electron microscopy in a diarrhoeic sample collected from a foal in 1975 in the United Kingdom [18], and it was first isolated in 1981 by using MA-104 cells [32]. Only two genotypes of equine RVA have been circulating recently among horse populations in many countries [58]. Many methods are used to diagnose equine RVA infection, and inactivated vaccines are commercially available to prevent rotaviral diarrhoea in foals in some countries [1]. This review briefly describes the virus classification, clinical signs, epidemiology, diagnosis,

disinfection, and vaccines related to equine RVA.

Classification of RVAs

Rotaviruses belong to the genus *Rotavirus* in the family *Reoviridae* and are non-enveloped viruses with 11 double-stranded RNA genome segments [17]. Equine RVA particles have an icosahedral structure with a diameter of 70 to 80 nm (Fig. 1) [30]. The 11 genome segments code structural proteins (VP1 to VP4, VP6 and VP7) and non-structural proteins (NSP1 to NSP6). One segment codes one protein, except in the case of the 11th segment, which codes both NSP 5 and NSP6 [17]. Rotaviruses are classified into groups A to J according to the intermediate capsid protein VP6 [2]. To date, there has been no report of the detection of rotaviruses other than those of group A in horses [1]. VP7 and VP4, which are outer capsid proteins encoded by the ninth and fourth segments, respectively, elicit neutralizing antibodies and are used to classify RVAs into G (Glycoprotein) and P (Protease-sensitive) types, respectively [17]. The G and P classification system has been used for a long

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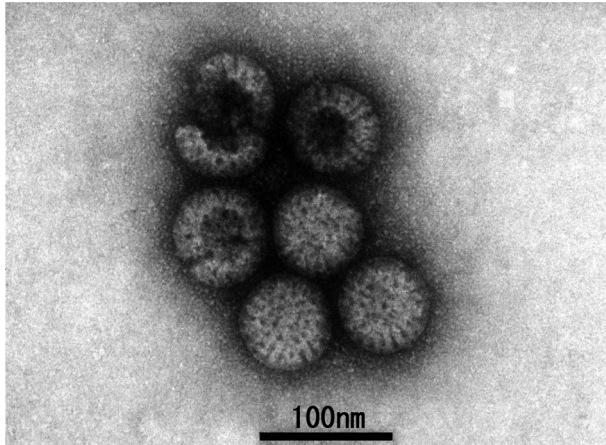


Fig. 1. Equine group A rotavirus particles under an electron microscope (photograph provided by the Equine Research Institute, Japan Racing Association).

time for surveillance of equine RVAs. A whole-genome classification system using the following formula was proposed in 2008: Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, representing the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genotypes, respectively [41]. Whole-genome sequencing can now be easily performed by using next-generation sequencing technology, and there have been increasing numbers of studies determining all 11 genome segments. In 2011, it was proposed that names of RVA strains should be described as follows: group/species of origin/country of identification/common name/year of identification/G and P type [42].

Clinical Signs

Equine RVA infection is usually reported in foals aged 6 months or younger, and the mortality rate is relatively low [61]. However, foals aged 2 weeks or younger generally show more severe clinical signs and have a high risk of death [61]. The main transmission route is faecal–oral. In experimental challenge studies, foals show clinical signs after 1 to 4 days of infection [27, 33], and the average duration of diarrhoea is 2.3 days (range, 1–9 days) [15]. Infected foals excrete virus in the faeces for 1 to 12 days, with or without clinical signs [1]. The main clinical signs are diarrhoea (Fig. 2), fever, lethargy, and anorexia (decreased suckling); infected foals do not always have a fever [61]. Dehydration due to diarrhoea is often observed, and fluid therapy should be instituted for dehydrated foals [61]. There is no specific antiviral agent for equine RVA infection, and symptomatic treatments are given for affected foals according to their clinical signs. Because a relationship between equine RVA infection and gastroduodenal ulcer

has been suggested [61, 63], anti-gastric-ulcer medicines such as proton pump inhibitors or histamine H₂-receptor antagonists (H₂ blockers) are sometimes prophylactically administered.

Epidemiology

Equine RVA is ubiquitous and has been detected around the world [1], and most adult horses have antibodies against it [13, 25]. According to G and P classification, six G (G3, G5, G8, G10, G13, and G14) types and six P (P[1], P[3], P[7], P[11], P[12], and P[18]) genotypes have been reported in RVAs collected from horses [1]. Combinations of G and P types include G3P[3] [21], G3P[12] [28, 37], G5P[7] [11, 29], G8P[1] [37], G10P[11] [36, 37], G13P[18] [5], and G14P[12] [6, 37]. Of them, G3P[12] and G14P[12] equine RVAs are predominantly circulating in horse populations in many countries [58]. The equine G3 type is further classified into subtypes G3A and G3B on the basis of cross-neutralization assays using monoclonal antibodies [7] and phylogenetic analysis [12]. G3A equine RVAs have been detected in Argentina [21], Australia [4], European countries [12, 16, 39, 47, 57], and the United States [10]. In Japan, only G3B equine RVAs were detected from 1982 onward [20, 56, 65], but in 2016, G3A equine RVAs suddenly began to be detected in addition to G3B equine RVAs [51]. Molecular analyses showed that the Japanese G3A equine RVAs were closely related to North American G3A equine RVAs detected in 2017, and the Japanese G3A equine RVAs would therefore most likely have originated from North American G3A equine RVAs [51]. Reports from Argentina [46] and Japan [51, 56] show that G3 or G14 equine RVAs were alternately prevalent every few years (Fig. 3). Argentinian and Japanese vaccines contain G3P[12] equine RVAs as vaccine strains. The cyclic patterns of G genotypes suggest that the vaccines do not crucially affect the main G genotypes of equine RVA circulating each year. Whole-genome analysis shows that the genotype constellations of G3P[12] equine RVAs can be assigned to G3-P[12]-I6-R2-C2-M3-A10-N2-T3-E2/E12-H7, and those of G14P[12] equine RVAs are classified as G14-P[12]-I2-R2-C2-M3-A10-N2-T3-E2/E12-H7 [40, 51, 54]. G3P[12] and G14P[12] equine RVAs have largely more conserved genotype constellations compared with those of other animals' RVAs. Interestingly, the G3 and G14 genotypes of VP7 are strongly associated with the I6 and I2 genotypes of VP6, respectively [44]. The E12 genotype of NSP4 has been detected only in Argentinian equine RVAs, whereas the E2 genotype has been detected in other countries [24, 38, 40, 51, 54].

Unusual genotype G13P[18] equine RVAs were isolated in 1991 in the United Kingdom (RVA/Horse-tc/GBR/



Fig. 2. Diarrhoea in a foal infected with equine group A rotavirus (photographs taken by Dr. Yoshiro Endo, Hidaka Training and Research Center, Japan Racing Association).

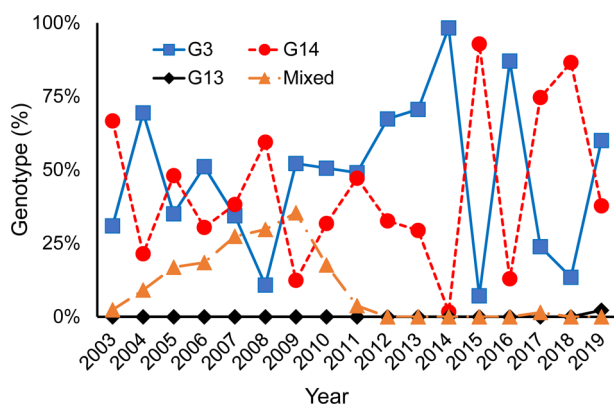


Fig. 3. Percentages of G genotypes (G3, G14, G13, and mixed infection with G3 and G14) relative to the total numbers of positive samples collected from 2003 to 2019 in Japan. The figure was prepared from epidemiological data for 2003–2008 [56], 2012–2018 [51], and 2019 [52]. The data for 2009–2011 are published for the first time in this paper. G13 was only detected once, in 2019.

L338/1991/G13P[18]) [5] and in 2019 in Japan (RVA/Horse-tc/JPN/MK9/2019/G13P[18]) [52]. G13P[18] RVAs have never been detected in animals other than horses and have a unique genotype constellation: G13-P[18]-I6-R9-C9-M6-A6-N9-T12-E14-H11 [40, 52]. Based on these single instances of G13P[18] RVA isolation in the United Kingdom and Japan, G13P[18] RVAs are likely to be only accidentally detected and are unlikely to be prevalent.

Cross-species transmission rarely occurs from other animals to horses. To date, G8P[1], G10P[11], G3P[3],

G5P[7] and G6P[5] equine RVAs are considered to have originated from RVAs of other animals. G8P[1] and G10P[11] RVAs are likely to have been derived from bovine RVAs [34, 37]. Complete genome analyses suggest that gene segments of strain RVA/Horse-wt/E3198/2008/G3P[3] were derived from feline and canine RVAs [45]; those of strain RVA/Horse-tc/GBR/H-1/1975/G5P[7] came from porcine RVA [23], and those of strain RVA/Horse-tc/JPN/OH-4/1982/G6P[5] came from bovine and bovine-like human RVAs [24]. These G/P genotypes other than G3P[12] and G14P[12] are not likely to be established among horse populations.

Diagnosis

Faecal samples and rectal swabs are used for the diagnosis of equine RVA infection. Virus isolation is the gold standard of diagnosis, as is the case in other viral infections. MA-104 cells derived from Rhesus monkey kidney [32] and Caco-2 cells from human colon adenocarcinoma [55] are used to isolate equine RVAs. The cells are rotationally cultured with a medium containing trypsin and are incubated for 5 to 7 days [32]. The efficiency of virus isolation is improved by adding trypsin to serum-free medium. It is rather difficult to isolate equine RVA, and a viral cytopathic effect is usually observed in several passages. Because virus isolation takes a long time, requires laborious steps, and has a low success rate, it is seldom used other than for research purposes.

Diarrheal samples collected from infected foals usually contain a lot of the virus, and RVA particles can be observed directly under an electron microscope [18, 30]. However,

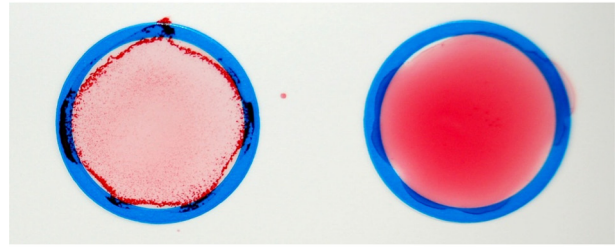
this method requires expensive equipment and sophisticated skills, and it is therefore not usually used for the diagnosis of equine RVA.

Some rapid antigen detection kits for human RVA are also useful for diagnosing equine RVA infection [14, 31, 43, 53, 61]. These kits do not require expensive equipment or special techniques, and they can yield results within 15 min. They are based on the principles of the latex agglutination assay (Fig. 4A) or immunochromatographic assay (Fig. 4B). Many kits employ antibodies against VP6 protein to detect RVAs, because VP6 protein is highly conserved among RVAs [61]. Not all of these human kits can be used to diagnose equine RVA, and it is important to evaluate whether a kit can detect equine RVAs before using it. Some latex agglutination assay kits are reported to be useful for the diagnosis of equine RVA infection [14, 31]; however, these kits are less sensitive than immunochromatographic assay kits, and their results are more difficult to judge [53]. Therefore, in Japan, an immunochromatographic assay kit (Dipstick ‘Eiken’ Rota, Eiken Chemical Co., Ltd., Tokyo, Japan) is widely used in daily clinical practice.

Reverse transcription polymerase chain reaction (RT-PCR) is used to detect equine RVA RNA [20, 22, 26, 65]. In 2001, Tsunemitsu *et al.* reported the development of a semi-nested RT-PCR assay for detecting the VP7 gene and distinguishing between G3 and G14 equine RVAs (Fig. 5) [65]. This semi-nested RT-PCR assay has helped to facilitate epidemiological studies in Japan. Recently, a real-time RT-PCR assay has been developed to detect G3 and G14 equine RVA RNAs [9]. The real-time RT-PCR assay targets VP7 genes to distinguish between G3 and G14, as well as NSP3 genes to detect both G3 and G14 RVAs. It can be applied to laboratory diagnosis and epidemiological studies, because it is generally sensitive in detecting viral genes and has less risk of contamination than semi-nested RT-PCR.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) has also been developed for the detection of equine RVA RNA [49]. The RT-LAMP assay targets the P[12] genotype of the VP4 gene—the predominant P genotype globally—and can therefore detect equine RVAs irrespective of the G3 and G14 genotypes. The RT-LAMP assay is performed in 60 min under isothermal conditions (60°C), and the results can be judged with the naked eye on the basis of the turbidity or fluorescence of the reaction mixture (Fig. 6). The RT-LAMP assay can be performed without expensive equipment or gel electrophoresis after RT-PCR; it should therefore be useful for the diagnosis of equine RVA in diagnostic laboratories.

(A) A latex agglutination assay kit



(B) An immunochromatographic assay kit

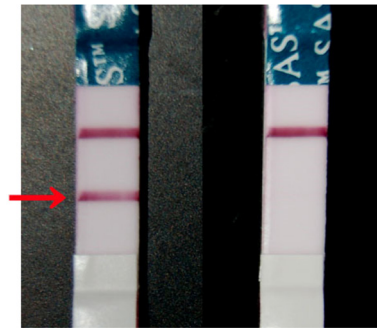


Fig. 4. (A) A latex agglutination assay kit (Rotalex Dry, Sekisui Medical Co., Ltd., Tokyo, Japan) and (B) an immunochromatographic assay kit (Dipstick ‘Eiken’ Rota, Eiken Chemical Co., Ltd., Tokyo, Japan). The results on the left for both kits are positive for equine group A rotavirus antigen. Rotalex Dry is not available in 2021.

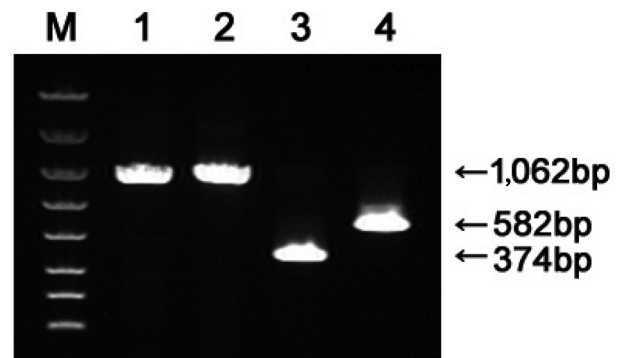


Fig. 5. Semi-nested reverse transcription polymerase chain reaction (RT-PCR). Results are shown for the first RT-PCR when using G3 (lane 1) and G14 (lane 2) genotypes, and those for the second PCR are shown when using G3 (lane 3) and G14 (lane 4) genotypes. In the first RT-PCR (lanes 1 and 2), both bands appear at around 1,062 base pairs (bp), and therefore the G3 and G14 genotypes are indistinguishable. In the second PCR, the G3 and G14 genotypes appear at around 374 bp (lane 3) and 582 bp (lane 4), respectively. The G3 and G14 genotypes can be distinguished by these differences in the band positions.

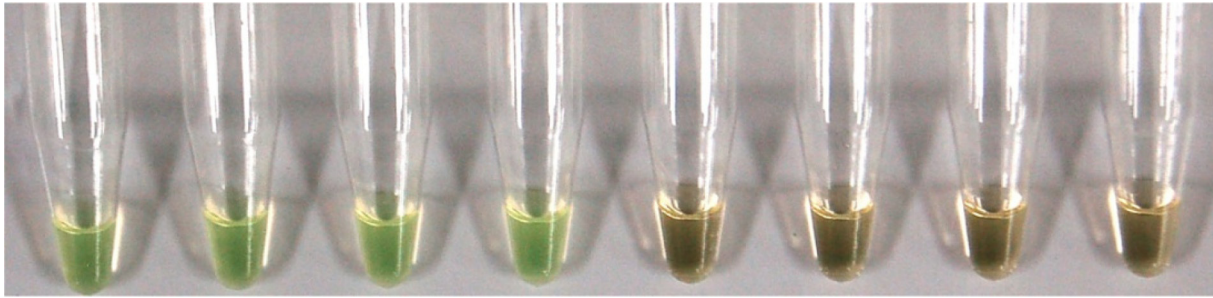


Fig. 6. RT-LAMP (reverse transcription loop-mediated isothermal amplification). The left four green tubes are positive for equine group A rotavirus gene, and the right four brown tubes are negative.

Disinfection

Diarrhoeic foals infected with equine RVA excrete huge numbers of virus particles. Although we have no data regarding the stability of equine RVA, bovine RVA is stable for several months in the environment [66], and only a small amount of porcine RVA can cause diarrhoea in piglets [59]. Therefore, RVA is highly contagious, and contaminated livestock barns must be disinfected with effective chemicals to prevent outbreaks. Equine RVA is a non-enveloped virus and is more resistant to disinfectants than enveloped viruses such as equine influenza virus [67] and equine herpesvirus [64]. Amphoteric soaps and quaternary ammonium compounds are commonly used in veterinary hygiene, but they are generally ineffective against equine RVA [48].

Alcohol products such as ethanol and isopropanol are effective against human RVA [62] and therefore should also be effective against equine RVA. They are useful for hand disinfection and the disinfection of farming tools. Aldehydes and chlorine- and iodine-based compounds are also effective against equine RVA [48]. Although the virucidal effects of chlorine- and iodine-based disinfectants are not greatly affected by temperature and reaction time, they are reduced by the presence of organic matter [48]. Organic matter such as faeces needs to be removed before a contaminated barn is disinfected with chlorine- or iodine-based disinfectants. When chlorine- or iodine-based disinfectants are used in foot mats, the disinfectants should be replaced frequently to prevent increases in the amounts of organic matter present. Glutaraldehyde is an aldehyde and is effective against equine RVA, but low temperatures or short reaction times, or both, greatly reduce its virucidal effect [48]. Glutaraldehyde needs warmer temperatures and long reaction times. It is harmful to animals, including humans, and those handling it should be careful to use it according to the manufacturer's instructions.

Vaccines

Inactivated vaccines have been used against equine RVA infection in some countries. Pregnant mares are inoculated intramuscularly with a vaccine, so that their colostrum contains abundant antibodies against RVA at the time of birth. Their neonates can acquire passive immunity via the colostrum [61]. Because rotaviral diarrhoea can occur in foals less than 1 month old, which have immature immune systems, it is considered that direct vaccination of foals is ineffective against equine RVA infection. To our knowledge, three inactivated vaccines are available globally. A vaccine containing strain RVA/Horse-tc/GBR/H-2/1976/G3P[12] (Zoetis, Parsippany, NJ, U.S.A.) is available in the United States, New Zealand, Australia and several European countries [1, 39]. In Argentina, a trivalent vaccine containing RVA/Horse-tc/GBR/H-2/1976/G3P[12], RVA/Simian-tc/ZAF/SA11/1958/G3P[2] and RVA/Cow-tc/USA/NCDV-Lincoln/1967/G6P[1] has been available since 1996 [46]. In Japan, a vaccine has been commercially available since 2001 [35]; strain RVA/Horse-tc/JPN/HO-5/1982/G3P[12] (Nisseiken, Tokyo, Japan) has been used as the vaccine strain because only G3P[12] RVAs were predominant until the early 1990s [36].

Field trials have shown that the three vaccines significantly increase neutralizing antibody titres in mares and foals [3, 35, 60]. Two studies have shown that vaccination of mares reduces the duration of diarrhoea and eases clinical signs in foals [3, 35]. One study demonstrated that the incidence of rotaviral diarrhoea was lower in a vaccinated group than in an unvaccinated group, although the difference was not significant [60]. These studies suggest that the vaccines cannot completely prevent equine RVA infection but can reduce the duration of diarrhoea and ease clinical signs. Vaccine effectiveness in foals may be limited by passive immunity, and a different method of immunization may be needed to improve vaccine efficacy.

In general, co-occurrence of the G and P genotypes is

important for making an effective vaccine. As no study had evaluated in detail the effectiveness of the G3P[12] vaccine against G14P[12] equine RVAs, we evaluated it by using sera from vaccinated pregnant mares [55] and a suckling mouse model [50]. Sera from pregnant mares inoculated with the Japanese G3P[12] vaccine had neutralizing antibodies against not only G3P[12] equine RVAs but also G14P[12] equine RVAs, although the antibody titres against G14P[12] equine RVAs were lower than those against G3P[12] equine RVAs [55]. In the suckling mouse model, the G3P[12] vaccine was effective against G3P[12] equine RVA strains but was less effective against G14P[12] equine strains; the G14P[12] vaccine was effective against both G3P[12] and G14P[12] equine RVA strains [50]. These reports suggest that the vaccine containing a G3P[12] equine RVA strain is only partially effective against G14P[12] equine RVAs, and the co-occurrence of the genotype P[12] is likely to contribute to vaccine effectiveness. Ideally, it would be desirable to add a G14P[12] equine RVA as a vaccine strain to obtain a better vaccine effect.

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