Rotaviruses from poultry flocks can exchange genes with rotaviruses from mammals - the risk of infection for humans is low

Summary research report on the BfR research project “Characterisation of the zoonotic potential of rotaviruses in poultry” by the BfR from 17 August 2020

Avian rotaviruses are widespread in food-producing poultry flocks. However, they are only distantly related to the rotaviruses that occur in mammals and humans and which can lead to disease in them. In the BfR research project “Characterisation of the zoonotic potential of rotaviruses in poultry”, funded by the German Research Foundation (DFG), Professor Dr. Reimar Johne and his research group showed that rotaviruses of birds can exchange genetic material with rotaviruses of mammals, which can lead to the development of new types of rotaviruses. The scientists, however, consider the risk of developing such virus types, known as “reassortants”[1], in nature as being low. This is because the new viruses capable of replication only appeared in a few cases under laboratory conditions. In addition, it was difficult for them to efficiently reproduce. The risk of infection for humans is therefore estimated to be relatively low. Nevertheless, studies on rotavirus diversity in humans should in future also include distantly related rotaviruses such as those of birds in order to be able to determine the occurrence of new types at an early stage.

In contrast to rotaviruses of mammals, rotaviruses of birds have been only scarcely studied until now. The aim of the BfR was to analyse the genetic diversity of rotaviruses, especially in birds, and to determine their potential for transmission and adaptation to mammals and humans. An important question was whether the rotaviruses of birds exchange genetic material with those of mammals, which could possibly result in new types of rotaviruses. The project identified a wide variety of different known and previously unknown rotavirus species and types in both birds and mammals. The genome of these viruses was usually completely sequenced using newly developed methods, which made it possible to precisely characterise their properties and their relationship to known rotaviruses. Overall, the analyses indicate that a broad repertoire of divergent rotavirus strains must be expected in the animal kingdom, which could possibly be transmitted directly to humans or, through the exchange of genome segments, could lead to the formation of new rotaviruses. For the rotaviruses of birds, however, this appears to be very rare.

Rotaviruses are among the most common pathogens causing gastrointestinal diseases in humans and in numerous animal species. There are many different types of rotaviruses that are constantly evolving through mutations and the exchange of genome segments. Human diseases are mainly caused by human rotaviruses. But it is also known that certain rotaviruses can be transmitted from animals to humans and vice versa. Vaccines against human rotavirus diseases have been around since 2006 and are generally very effective. However, if new rotaviruses containing genetic material from animals develop, the immunity acquired by vaccination against such infections could become ineffective.

1 Topic, initial questions and objectives of the research project

Rotaviruses are among the most common pathogens causing gastrointestinal diseases in humans and numerous animal species. Mutual transmissions between mammals and humans have been described frequently. As a result, there are a large number of different types

[1] Reassortants are new virus types that arise from the exchange of genome segments from different parent viruses of the same species.

of rotaviruses that are constantly developing through mutations and the exchange of genome segments. Vaccines against human rotavirus diseases have been available since 2006 and have been recommended for use throughout Germany since 2013. Even if the general effectiveness of the vaccines has meanwhile been proven, it remains unclear whether these vaccines show a consistent efficacy against the multitude of individual circulating rotavirus types. It is possible that - as observed with influenza viruses - a selection of certain virus types will take place through vaccination.

Especially rotaviruses from animals, which are genetically and anti-genetically only distantly related to those of humans, are possible candidates for the occurrence and selection of new types of rotavirus in humans. In contrast to the rotaviruses of mammals, bird rotaviruses have so far been only scarcely studied. Our preliminary work showed that these are only distantly related to the mammalian and human rotaviruses. Nevertheless, there was evidence of isolated transmissions of genetic material from avian rotaviruses to mammals and humans. Our preliminary work also showed that the avian group A rotaviruses are capable of exchanging genome segments with one another, which could result in novel antigen combinations of rotaviruses. However, it was not known how easily avian rotaviruses can be transferred to mammals and whether genome segments can be exchanged between avian and mammalian rotaviruses so that novel antigen combinations of human rotaviruses arise.

The aim of the project was therefore to determine the genetic diversity of avian rotaviruses, to investigate their potential for zoonotic transmission to mammals and for the exchange of genome segments with mammalian rotaviruses. Genome analyses of field strains as well as cell culture studies and modern reverse genetic systems should be used for this purpose. Some of the investigation techniques had to be newly developed for the project.

Specific sub-goals of the project were:

1. Characterisation of additional avian rotaviruses, whereby the genetic diversity of bird rotaviruses is analysed and their relationship to mammalian rotaviruses determined. The results should show which types of rotavirus can be expected in birds and which evolutionary relationships exist with other rotaviruses.

2. To determine the ability of poultry rotaviruses to exchange genetic material with mammalian rotaviruses. This also includes studies on the susceptibility of mammalian cells for infection with avian rotaviruses, which is a prerequisite for reassortment. The results should show whether mammals are likely to be infected with avian rotaviruses and whether reassortment can result in new types of viruses that are adapted to mammals.

3. The characterisation of reassortants that have genome parts of avian and mammalian rotaviruses. An estimate should be provided of how well the reassortants can multiply in avian and mammalian cells and whether adaptations, e.g. in other genome segments, occur via their passaging. The aspects examined should, amongst others, clarify to what extent the occurrence of avian/mammalian reassortants and their spread in the human population are likely.

4. Establishment of a reverse genetic system (RGS) for avian rotaviruses. Attempts should be made to generate avian rotaviruses or reassortants of mammalian and avian rotaviruses from cloned plasmids. The availability of a reverse genetic system would enable the production of specifically modified rotaviruses and thus represent a starting point for more targeted investigations into their molecular biology, pathogenicity mechanisms and vaccine development.

Overall, the results should enable assessment of the zoonotic potential of avian rotaviruses, identify relevant avian rotavirus types and thus contribute to the development of broadly effective vaccines.

2 Performed Work

All planned investigations of sub-goal 1 “Characterisation of avian rotaviruses” have been performed. The genomes of various rotavirus isolates were sequenced and the distribution of avian rotaviruses in farm poultry was also examined. Due to the rapid technical development in the field of genome sequencing, Next Generation Sequencing (NGS) techniques for rotaviruses have been newly developed and applied. Initially, 454-based sequencing and later Illumina-based NGS techniques were used.

In sub-goal 2, “Determination of the reassortment ability”, field strains with reassorted genome constellations were examined and infection tests were performed on mammalian cell cultures using avian rotaviruses. Reverse genetics avian/mammalian rotavirus reassortants were also generated.

In sub-goal 3 “Characterisation of the reassortants”, growth kinetics in cell cultures were investigated and electron microscopic investigations were carried out to characterise particles.

In sub-goal 4 “Establishing a reverse genetic system (RGS)”, all planned plasmids and bacmids were created. A helper virus-dependent and a helper virus-independent RGS were established and these systems were used to produce reassortants.

3 Results achieved and discussion with regard to the relevant state of research, potential application perspectives and possible follow-up studies

3.1. Characterisation of other rotaviruses

3.1.1. Establishment of new techniques for the detection and characterisation of rotaviruses

New PCR-based detection systems have been developed to detect avian rotaviruses in field samples. This included real-time RT-PCR systems for the detection of avian RVA strains as well as for RVD strains [3]. Due to the heterology of the sequences, separate protocols had to be developed for the respective chicken and turkey RVAs. The protocols showed high sensitivity and specificity and were used in a first study on the spread of avian rotaviruses in farm poultry [3].

Various NGS systems have been developed for the genome characterisation of rotaviruses. Initially, this was done with the help of the Roche-454 technique, which was the first time that whole genomes of avian rotavirus isolates from cell cultures were sequenced using NGS [4]. In a later phase, the corresponding laboratory protocols were converted to the newer Illumina technology. This made it possible to characterise rotavirus genomes from faecal samples (from shrews) [8] as well as from cell culture supernatants (with avian RVA strains) [9]. In conjunction with a bioinformatics pipeline that has been developed, the technology allows genome sequencing of a very wide range of rotaviruses without the use of specific primers, which also enabled first identification of the new rotaviruses K and L from shrews. Almost the entire genome of the rotaviruses was sequenced, only the sequencing of the genome sequence ends, which was only about 50% complete, still needs to be optimised.

3.1.2 Distribution of rotaviruses from birds and mammals

With the help of the developed PCR systems, samples from chickens and turkeys from various European countries and from Bangladesh were examined [3]. RVA was found in 16% of the samples and RVD in 39% of the samples. Evidence was provided in both animal species and in all countries examined. This indicates a widespread distribution of RVA and RVD in poultry flocks worldwide. RVF and RVG were detected in only 2% of the samples using classic gel electrophoresis techniques. Even if this technique is less sensitive than PCR, the results indicate that these viruses play a rather subordinate role in avian rotavirus infections.

An examination of faecal samples from shrews from Germany led to the identification of a broad spectrum of new RVA strains and the new types of rotavirus, designated as RVK and RVL (see 3.1.3) [8]. Using classic PCR protocols, RVA was detected in 22%, RVK in 11% and RVL in 15% of the samples. The first data on the spread of rotaviruses in shrews indicate that these could represent an important reservoir for rotaviruses and should be further investigated. Rotaviruses with sequence homologies to avian rotaviruses were not found in these animals.

3.1.3. Genome characterisation of avian and mammalian rotaviruses

In the project, genomes of avian rotaviruses in particular were further characterised by genome sequencing. Before the start of the project, only a few complete genomes of avian RVA strains and a VP6 sequence of an RVD strain were available. For the first time, the complete genome sequences of one strain of RVD [1], RVF and RVG [5] have been sequenced, which subsequently became the reference strains for these rotavirus species. Both the percentages of the sequence identities of the coding regions and the conserved sequences at the genome ends confirmed the classification of these viruses as separate rotavirus species.

The genome sequence data obtained were then used for phylogenetic analyses of the relationship with other rotavirus species [5]. It was found that the rotavirus species fall into two large phylogenetic groups, one containing RVA, RVC, RVD and RVF, the other RVB, RVG and RVH. The viruses of both groups also differ greatly in the sequences at the genome ends, which makes an exchange of genome segments between these two groups impossible. An adaptation to the host appears plausible for RVD, RVF and RVG, since these have so far only been found in birds.

For a better and easier classification of rotaviruses into different types, the data were used to build a new classification system that is based exclusively on sequence analyses [2]. For this purpose, a cut-off value of 53% was determined for the amino acid sequences of the VP6 gene, under which a rotavirus belongs to a new species. The system is completely consistent with the previous one using antibody reactivities and genomic electrophoresis data. It has been proved by the ICTV and has meanwhile been used to classify various newly discovered rotaviruses.

In contrast to the species-specific RVD, RVF and RVG, RVA strains are often found in both mammals and birds, which could make cross-transmission possible. A more detailed phylogenetic analysis shows, however, that the avian and mammalian RVA isolates largely cluster into separate subgroups, meaning that frequent reciprocal transfers and exchanges of genome segments are apparently rare. However, the genome analysis we performed on an RVA isolate from a pheasant gives clear indications that reassortment between the two

groups is possible and obviously takes place in nature [4]. This isolate consisted of 10 genome segments closely related to avian rotaviruses, while the genome segment coding for VP4 was closely related to that of mammalian rotaviruses. The total genomes of 4 further RVA isolates from chickens were completely sequenced in a later part of the project, which provided further evidence of a reassortment between different avian RVA strains, but not with those of the mammals [9]. Exchanges of genetic material between mammalian and avian rotaviruses are obviously possible in principle, but they obviously only occur very rarely.

The methods established in the project were also used to examine shrews for rotaviruses [8]. New RVA genotypes were detected, which have significantly expanded the genetic spectrum of RVA strains. The RVA sequences did not cluster directly with avian rotaviruses, but their branch in the phylogenetic tree originated at the root before the avian and mammalian rotavirus branches divided. Surprisingly, almost complete genomes of two previously unknown rotavirus species (RVK and RVL) were also identified. The results show that unexpectedly diverse rotaviruses also occur in mammals, which - similar to avian rotaviruses - have the potential to induce strong antigenic changes in human strains through reassortment.

3.2. Determination of the ability of rotaviruses in poultry to exchange genetic material with mammalian rotaviruses

3.2.1. Identification of avian/mammalian RVA reassortants in field samples

As already described in Section 3.1.3, an RVA strain from a pheasant was identified which contained a VP4 gene with high sequence identity to mammalian rotaviruses in a genome otherwise consisting of avian RVA segments [4]. The strain therefore probably represents a reassortant that has a VP4 gene from an (unknown) mammalian rotavirus and the rest of the genome from a typical pheasant rotavirus. This indicates the possibility of reassortment between avian and mammalian RVA strains under field conditions. Since this is the only published reassortant of this kind, such a reassortment seems to take place very rarely.

3.2.2. Double infection experiments in cell culture

Various studies have been performed on the preparation of reassortants by double infection with avian and mammalian RVA strains. Overall, no reassortants were obtained in several experiments. Unfortunately, mammalian-mammalian rotavirus reassortants that have already been described by others could not be generated with this technique, which indicates a technical problem with the establishment of the method in the laboratory. The results can therefore not be evaluated and interpreted.

3.2.3. Generation of reassortants by reverse genetics

Various attempts have been made to generate reassortants with genome segments from avian and mammalian RVA strains using RGSs. This was achieved for the first time when a monkey rotavirus (SA11) with a temperature-sensitive VP4 gene was used in the project and it was possible to exchange this gene with a VP4 from a chicken RVA strain [6]. The resulting reassortant replicated stably and was further characterised.

After the availability of a completely plasmid-based RGS for the SA11 strain, which was developed and provided by a Japanese work group, further experiments for the production of reassortants were performed in the project. It was possible to reproduce the previously produced reassortant with a VP4 gene from a chicken RVA strain and another one with the VP4 from the pheasant RVA strain [9]. In spite of the close relationship with the chicken RVA

strain, however, it was not possible to produce a reassortant with the VP4 gene from a turkey RVA strain. Interestingly, the production of VP4 reassortants from different mammalian strains succeeded, while in most of the cases tested no reassortants with VP4 genes could be created from human strains [7]. So far it remains unclear which determinants dictate whether reassortment between different rotavirus strains is possible.

Another area of the project tried to produce mono-reassortants of the SA11 virus, each containing one of the 11 genome segments of the chicken RVA strain [10]. Aside from the VP4 reassortant, which was already known, this was only possible for the VP3 gene, as all other genome segments did not lead to the formation of infectious viruses. Here, too, the underlying mechanisms are not known. However, the results indicate that apparently only a few combinations permit the formation of infectious reassortants from avian and mammalian RVA strains, which could explain the small number of such reassortants found in the field.

3.3. Characterisation of reassortants

3.3.1. Investigation of the growth in cell culture

The reassortants described in Section 3.2.3. were examined in cell culture with regard to the growth kinetics. The VP4 (chicken) reassortant showed slower growth and with lower endpoint titers as compared to the two parent viruses (chicken rotavirus and SA11 strain) [6, 7]. This was observed both in the monkey kidney cell line MA104 and in embryonic chicken fibroblast cells. The SA11 virus grew best on the MA104 cells, while the chicken parent virus reproduced best on the chicken cells, suggesting an adaptation to the host species. The other VP4 (pheasant) reassortant [7] and the VP3 (chicken) reassortant [10] also replicated more slowly on MA104 cells than the SA11 virus. All avian/mammalian rotavirus reassortants produced in the laboratory reproduced in a stable and reproducible manner over many cell culture passages. However, the results indicate that they have a lower fitness than the original circulating viruses.

3.3.2. Investigations of genome changes through frequent transition in cell culture

The experiments on genome changes of the VP4 (chicken) reassortant during frequent passaging in MA104 cells had to be terminated without clear results. The first encouraging results with an increase in replication after 10 cell culture transitions led to the identification of a point mutation in the VP4 gene by means of Sanger sequencing. Unfortunately, it was not possible to confirm this result by further sequencing using NGS. The results can therefore not be evaluated and interpreted. Due to time constraints it was not possible to repeat the experiment and test different experimental conditions (e.g. further passages, different MOIs, isolation of individual clones by means of plaque assay, other cell lines).

3.3.3. Structural studies with electron microscopy

All generated reassortants were examined using transmission electron microscopy (TEM) [7, 10]. In all cases, typical triple-shelled virus particles were detected that did not differ in their structure from the parent viruses.

A high proportion of double-shell capsids was found in the TEM analysis of another bird RVA strain from a chicken [9]. Genomic analysis of this strain showed that it contained an unusual avian VP4 gene. The replication kinetics of this strain were also significantly slower than that

of the other avian RVA strains examined. The results may indicate that VP4 reassortants sometimes form unstable capsids that result in a lower rate of replication.

3.4. Establishment of a reverse genetic system for avian rotaviruses

3.4.1. Experiments to establish an RGS for an avian rotavirus

For this purpose, complete cDNAs were first produced for all 11 genome segments of a chicken RVA strain, which were then cloned into plasmids with regulatory sequences. The plasmids had a T7 RNA polymerase promoter upstream of the genome segment sequence and an HDV ribozyme sequence downstream, which guarantee nucleotide-precise start and end sequences. The system is identical to that described for SA11. Unfortunately, after all 11 plasmids were transfected in combination (also together with helper plasmids from the SA11 system), no infectious progeny viruses could be generated in several experiments [10].

Since it could be assumed that the transfection of 11 plasmids leads to the uptake of a complete set of plasmids by a cell in only a few cases, efforts were made to reduce the number of plasmids required. For this purpose, the genome segments together with the functional regions from the individual plasmids were cloned one after the other into a bacmid. In the end, one bacmid was obtained that contained all structural protein segments and another with all non-structural protein segments. Unfortunately, after both bacmids were transfected, again no infectious progeny virus was produced (not published).

In order to determine the reasons for this, both the plasmids and the bacmids were sequenced again, with no mutations being found. Since the sequences were originally based on Sanger sequencing of PCR products from cell culture virus, the original virus was sequenced again using NGS [9]. Here, isolated nucleotide exchanges were found, which, however, should not impair the functionality of the genes after a detailed analysis. At least for the VP3 and VP4 genes, functionality could also be demonstrated directly through the production of viable reassortants. Overall, it remains unclear why the system could not produce any infectious viruses. It must be noted, however, that functional RGSs have only been developed for two rotavirus strains (SA11 and a human rotavirus), which indicates general problems with the current systems. In the case of avian rotavirus, the comparatively slow propagation kinetics should be taken into account, which may have contributed to an inefficient RGS.

3.4.2. Generation of mammalian/avian RVA reassortants using RGS

Various RGSs based on the simian rotavirus SA11 were established in the laboratory during the project and successfully used to produce various mammalian/avian RVA reassortants [6]. First, a temperature-sensitive (ts) SA11 mutant was used, which had a defect in the VP4 gene, which led to inefficient reproduction at higher temperatures. This effect was used to exchange this VP4 gene with one from a chicken RVA. For this purpose, the plasmid with the VP4 (chicken) gene was transfected into T7-RNA-expressing cells and subsequently infected with the ts-SA11 mutant. After an initial incubation at 31 °C, both ts-SA11 viruses and VP4 (chicken) reassortants were able to form. The released viruses were then incubated on MA104 cells at 39 °C, and only the reassortant was able to multiply efficiently. The system enabled the production of this reassortant, but it was complex and only limited to the VP4 exchange.

In a later area of the project, the plasmid-based system developed by a Japanese work group was used [7, 10]. This consists of 11 plasmids that carry the respective genome segments for the SA11 virus, two plasmids that express subunits for a vaccinia virus capping enzyme and one plasmid that produces a cell fusion protein. All plasmids are transfected into a T7-expressing cell line, in which infectious SA1 virus can form. Reassortants can be generated by exchanging individual plasmids. The system was successfully established in the laboratory and it was possible to produce various reassortants (see Section 3.2.3.).

4 Conclusions

The project identified a wide variety of different rotavirus species and strains in both birds and mammals. Overall, the spectrum of known rotavirus species was expanded to include rotaviruses K and L from shrews, whilst the previously known avian rotavirus species D, F and G were genetically characterised for the first time. The project also identified and characterised new genotypes for the RVA strains in both mammals and birds. Overall, a broad catalogue of divergent RVA strains should be expected in the animal kingdom, which could possibly be transmitted directly to humans or through reassortment could lead to the formation of new antigenic mixed rotaviruses.

The project showed that bird RVA strains are widespread in farmed poultry. The predominantly separate phylogenetic clustering of avian and mammalian RVA strains indicates a largely separate evolution of both virus groups without frequent virus transmissions between hosts. However, the project identified a reassortant in a pheasant that carried a mammalian RVA-like VP4 gene, showing that an exchange of genetic material between the two groups is also possible under field conditions.

The in-vitro analyses performed consistently show that mammalian RVA reassortants can be produced with bird RVA VP4 genes that are then stably reproduced in cell cultures. The identified reduced replication efficiency, however, indicates a lower fitness compared to the parent viruses. In addition to the VP4 gene, it was only possible to exchange the VP3 gene in-vitro, whilst exchanging other genes did not lead to viable viruses.

Overall, the investigations show that the generation of mammalian rotavirus reassortants with genetic material from avian rotaviruses is possible in principle. However, the risk of generation- appears to be low, because replication-competent reassortants appear to be limited to the exchange of the VP3 and VP4 genes and the resulting reassortants exhibit poor fitness. Since, however, important antigenic determinants may also be altered when the VP4 gene is exchanged, their development should be considered, especially in the case of broad vaccine use, which could lead to the selection of such strains. The systems for monitoring rotavirus diversity in the human population should therefore be able to identify genetic material from divergent (including avian) RVA strains. The techniques developed within the project, especially the NGS and RGS-based methods, can help in the future to better and more comprehensively characterise rotavirus genomes and to investigate the effects of genome changes on their biological behaviour in a more targeted manner.

5 Publications from the research project

a) Papers that appeared or were finally accepted in publications with scientific quality assurance at the time the report was prepared:

1. Trojnar, E., Otto, P., Roth, B., Reetz, J., und Johne, R. (2010): The genome segments of group D rotavirus possess group A-like conserved termini but encode group-specific proteins. J. Virol., 84, 10254-10265.

2. Matthijnssens, J., Otto, P.H., Ciarlet, M., Desselberger, U., Van Ranst, M., Johne, R. (2012): VP6 sequence-based cut-off values as a criterion for rotavirus species demarcation. Arch. Virol., 157, 1177–1182.

3. Otto, P., Ahmed, M.U., Hotzel, H., Machnowska, P., Reetz, J., Roth, B., Trojnar, E., Johne, R. (2012): Detection of avian rotaviruses of groups A, D, F and G in diseased chickens and turkeys from Europe and Bangladesh. Vet. Microbiol., 156, 8–15.

4. Trojnar, E., Sachsenröder, J., Twardziok, S., Reetz, J., Otto, P.H., Johne, R. (2013): Identification of an avian group A rotavirus containing a novel VP4 gene of close relationship to those of mammalian rotaviruses. J. Gen. Virol., 94, 136-142.

5. Kindler, E., Trojnar, E., Heckel, G., Otto, P.H., Johne, R. (2013): Analysis of rotavirus species diversity and evolution including the newly determined full-length genome sequences of rotavirus F and G. Infect. Genet. Evol. 14:58-67.

6. Johne, R., Reetz, J., Kaufer, B.B., Trojnar, E. (2016): Generation of an avian/mammalian rotavirus reassortant using a helpervirus-dependent reverse genetics system. J. Virol., 90, 1439-1443.

7. Falkenhagen, A., Patzina-Mehling, C., Rückner, A., Vahlenkamp, T.W., Johne, R. (2019): Generation of Simian Rotavirus Reassortants with Diverse VP4 Genes Using Reverse Genetics. J. Gen. Virol., 100, 1595-1604.

8. Johne, R., Tausch, S.H., Grützke, J., Falkenhagen, A. Patzina-Mehling, C., Beer, M., Höper, D., Ulrich, R.G. (2019): Distantly Related Rotaviruses in Common Shrews, Germany, 2004-2014. Emerg. Inf. Dis., 25, 2310-2314.

9. Patzina-Mehling, C., Falkenhagen, A., Gadicherla, A.K., Grützke, J., Tausch, S.H., Johne, R. (2020): Whole genome sequence analysis of cell culture-adapted rotavirus A strains from chicken. Infect. Genet. Evol., 81, 104275.

10. Patzina-Mehling, C., Falkenhagen, A., Trojnar, E., Gadicherla, A.K., Johne, R. (2020): Potential of avian and mammalian species A rotaviruses to reassort as explored by plasmid only-based reverse genetics. Virus Res., https://doi.org/10.1016/j.virusres.2020.198027.

b) Work on scientific qualification:

Eva Trojnar: „Charakterisierung aviärer Rotaviren und Untersuchungen zu ihrem zoonotischen Potenzial“
Dissertation zur Erlangung des akademischen Grades des Doktors der Naturwissenschaften, Freie Universität Berlin, 2013.

Further information on the topic of rotaviruses from the BfR website

https://www.bfr.bund.de/en/a-z_index/rotaviruses-202731.html

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