Minireview Article

Genetic Diversity of Bat Rotaviruses.

ABSTRACT

Rotaviruses are the most common cause of diarrhea in children and animals. Bats are considered reservoirs of many viruses with zoonotic impact worldwide. Rotaviruses have been detected in bats and many of those strains that have been identified globally share high homology with rotavirus strains identified in animals and humans, demonstrating that roles are being created in interspecies transmission and genetic rearrangement in a large number of occasions, which is producing rotavirus genetic diversity. The current effort to characterize strains of rotavirus in bats would help expand knowledge about the great genetic diversity of rotaviruses and could also suggest a bat origin for several unusual rotavirus strains detected in humans and animals. This is a review of the different strains of rotavirus that have been detected in bats globally, where bats have been identified as a possible zoonotic potential in the transmission of rotavirus to animals and humans; and possible anthropozoonosis events are revealed.

Keywords: Bats rotavirus, human rotavirus, genetic diversity, zoonosis.

INTRODUCTION

Rotaviruses cause diarrheal disease primarily in children 6 to 24 months of age [1]. Studies carried out in Latin America and around the world reveal detection of unusual strains of rotavirus in humans, as well as detection of possible rotavirus zoonoses in cattle, pigs, goats, alpacas, cats, and dogs, which reveals that rotavirus presents several events of genetic rearrangements [2,3].

Emerging viral diseases are on the rise, periodically, a new virus keeps those responsible for health busy due to several factors, among them, the most important is the transmission of viruses by bats [4]. This phenomenon is occurring due to climate change, food, radioactive particles emitted, mosquitoes, rodents, pigs, birds, changes and movement of the human population, the constant increase in population and its mobility. Periodically, a new virus occupies the front pages, favored by demographic pressure, climate change and migratory phenomena [4]. “Increased contact between humans and wild bats will further increase the risk of zoonosis, guaranteeing increased attention to these viruses” [4,5].

“Increased research on coronaviruses in bats after Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), allowed very rapid identification of SARS-CoV-2” [4 , 6]. “This turns out to be an excellent example of the

importance of knowing the viruses that are harbored in wildlife in general; being bats, considered great reservoirs of emerging viral pathogens" [4,6].

"Currently, there are eight recognized Rotavirus (RV, family Reoviridae, genus Rotavirus) species (RV, family Reoviridae, genus Rotavirus) and one proposal (RVA to RVH and RVI, respectively). Among these, RVA to RVC, RVE, RVH, and RVI are known to infect mammals, and RVA is the most widespread species in most, if not all, mammalian hosts" [1,2]. "Current surveillance studies on rotavirus focus on humans and, to a lesser extent, domestic animals, and pets" [2]. "Recent studies have identified new rotavirus genotypes in many captive and free-ranging wild animals" [5]. "However, relatively little is known about the prevalence of rotavirus in wildlife" [5]. "The first report of group A rotavirus identified in bats was detected in the species *Eidolon helvum*, which was captured in Kenya, finding the new genotype G25P (6)-I15-Rx-C8-Mx-Ax-N8-T11-E2- H10" [7]. There are very few studies conducted in Europe, Asia, and Latin America [8,9,10]. Some studies have shown that bats appear to serve as reservoirs for multiple genotypes of group A rotaviruses, which could pose a veterinary and public health risk [2,8].

"The current effort to characterize strains of rotavirus in bats would aid insight into the great genetic diversity of rotaviruses and might also suggest a bat origin for rotaviruses in bats that may occur more frequently than currently believed" [11]. This study is a review of the different studies that have been carried out on the identification of rotavirus strains that have been detected in bats globally.

BATS AS VIRUS RESERVOIRS

Bats make up one of the largest and most diverse animal orders on the planet. Its numerous species represent approximately 20% of all known mammalian species [12].

Before attacking humans, viruses can remain confined to an animal reservoir for a long time, usually wild birds or bats [4]; To reach the human being, it needs intermediate hosts closer to man, such as pigs, poultry or mosquitoes, in addition to favorable conditions for its transmission such as: climate, humidity, temperature, atmospheric pressure, nutrients, etc. among others; and deforestation leads to bringing wild animals closer to inhabited areas [4,5,6].

The countries that present factors that are considered important in the appearance of an emerging virus in an area, in which the disease had not manifested itself before, is because they present: poor health structure, as well as deforestation, which facilitates contact with the jungle, which increases the opportunities for wild animals to come into contact with livestock animals and humans [4,5,6].

Bats have a series of characteristics that allow them to harbor and be reservoirs of many emerging viruses, such as, having small bodies and using large amounts of energy to propel their flight; yet, many species of bats exceed 25 -30 years of life [13]. Long periods of infectivity over a long life increase the chances that bats will transmit their viruses to other animals, they are likely to live with other types of bats in dense living conditions which greatly increases viral spread [13]. They have significant differences in their system that could give them the ability to carry and spread a viral load without dying from it or showing symptoms of infection [14]. Humans and other mammals have only a few different types of interferons, with bats exhibiting as many as 12 different types of these signaling molecules [14]. Also, most importantly, the interferon system, which is mainly

turned off in the absence of a threat in other mammals, is always activated in bat cells, which means that cells in bats can start their response against a virus threat immediately [14]. Viruses that overcome this defense, can mutate to produce viruses that ignore interferon signaling in other mammals [14].

"It is well known that bats are reservoirs for zoonotic viruses, such as filoviruses (Ebola and Marburg viruses), henipaviruses (Hendra and Nipah viruses), and coronaviruses (including severe acute respiratory syndrome coronavirus SARS-CoV" [15].

Current surveillance studies on rotavirus focus on humans [2]. Recent studies have identified new rotavirus genotypes in many wild animals, cattle, pigs, goats, alpacas, cats, and dogs [2,5], however, relatively little is known about the prevalence of rotavirus in wildlife.

IDENTIFICATION OF ROTAVIRUSES IN BATS

America

Very few studies have been carried out in Latin America on rotavirus surveillance in bats. After conducting a bibliographic review, it is revealed that in Latin America there are only data reported in countries such as Costa Rica and Brazil [8]. A study involving the collection of fecal samples from bats in several countries, including Costa Rica, sampled in a region of this country called Orosi, where feces samples from different bats were collected to carry out group A rotavirus identification studies through genome sequencing including all rotavirus structural and non-structural proteins [8]. The result of this research in Costa Rica showed a surprising genetic diversity of rotaviruses, detecting a close genetic relationship of several bat rotaviruses with other mammals [8]. The researchers emphasized that this strain diversity will continue to grow genetically and geographically [8]. It is important to emphasize, that in this study, a single rotavirus sequence never before reported was identified in Costa Rica in the feces of the bat *Carollia perspicillata* : RVA/Bat-wt/CRC/KCR10-93/2010/G20P47; scientists showed that this strain has nucleotide similarities grouping phylogenetically with VP6, VP1, VP2, VP3, NSP2 and NSP4 proteins with a study conducted in Suriname by Esona MD et al., [16], whose strain was identified as RVA/ Human-wt/SUR/2014735512/2013/G20P28 , identified in a child. Also, Esona showed that this strain detected in Suriname showed similarities with bat strains already reported [16] and also that this strain: RVA/Human-wt/ SUR /2014735512/2013/G20P28 shared high homology with the rotavirus strain detected in humans in Ecuador [17] for the VP6 protein: RVA/Human-wt/ECU/ Ecu534 /2006/G20P28, and also shares homology with the strain of rotavirus detected in Brazil in bats: RVA/Bat-wt/3081/BRA/2013/G20Px [18]. Another identified finding further revealed that (P [47]) has been identified in bats in Ghana (RVA/Bat-wt/GHA/K212/2009/G30P47), and Cameroon (RVA/Bat-wt/CMR/BatLy17/ 2014/G30P47) and that are closely related to this strain identified from Costa Rica that also presents VP4 P [47]; [8] thus they suggest that this is possibly a recent genetic rearrangement event [8] . On the other hand, Simsek C et al., [8] suggests that the same genotype detected among Ghanaian and Costa Rican bat rotaviruses, which are found more than 9,000 km apart, could be explained by the great flight capacity of bats. (Figure 1).

Approximately 300 species of bats are found in South America [19]. Studies carried out in Brazil demonstrate the role of rotavirus epidemiology in bats and provide evidence of rotavirus rearrangement events and interspecies transmission [20]. "In the first study to identify rotavirus in bats in Brazil, 29 species of bats belonging to three families (*Molossidae, Phyllostomidae and Vespertilionidae*) were collected from 73 cities in the state of Sao Paulo, Brazil, detecting and identifying the RVA/Bat-wt/ strains. BRA/4754/2013/G3P[3] and RVA/Bat-

wt/BRA/3081/2013/G20P[X]" [21]. According to the phylogenetics designed by the researchers, the strain RVA/Bat-wt/BRA/4754/2013/G3P [3] had a constellation of genotypes of G3-P[3]-Ix-Rx-Cx-Mx-Ax -Nx- T3-E3-H6, similar to strains found in bats, felines, canines, humans, apes, and horses [22, 23]. This BRA/4754 strain has homology to other bat strains identified in China, which also share homology to human rotavirus strains identified in China and horse rotavirus in Argentina (Figure 2). The RVA/Bat-wt/BRA/3081/2013/G20P[X] strain was detected in an adult male of the species Glossophaga soricina, a nectarivorous bat found in the neotropics, from Mexico to northern Argentina; this G20 genotype was also detected in Costa Rica, but in the bat Carollia perspicillata [8]. Another study carried out in Brazil, in the Amazon region, from October 2014 to April 2016, analyzed fecal samples from wild and domestic animals belonging to three areas of a fragmented forest, analysis was performed for the NSP3 gene by qPCR qualitative, in the following animals: birds, canines, bats, cattle, horses, small rodents, pigs and cats [5]. Regarding Bats, the species Carollia perspicillata, Desmodus rotundus, Uroderma bilobata, Artibeus lituratus, Artibeus Planirostus, Diaemus iyoug and Glossophagine were positive for RVA [5]. Of all the animals sampled and where rotavirus analyzes were performed, the highest percentage of rotavirus positivity was detected in bats [5]. The P genotype [4] was detected in samples from bats, dogs, pigs and felines [5]. This genotype is not common in animals, being more detected in human and environmental samples in various parts of the world and Brazil [5]. (Figure 1).

EUROPE

In France, in the year 2009-10, species of insectivorous bats were collected, detecting rotavirus in the Myotis *mystacinus*. Phylogenetic analysis based on the partial sequence of the VP1 and VP2 genes emerged that this virus was more closely related to group A rotavirus [24]. The authors emphasize the need for more studies since gene sequencing was partial [24]. In Switzerland in the year 2018-20 in three samples of *Myotis daubentonii*, similarity of 92 and 93% of a porcine rotavirus H (KT962037) were detected for the rotavirus NSP5 segment; On the other hand, samples extracted from *Rhinolophus hipposiderus*, *Rhinolophus ferrumequinum*, *Pipistrellus pipistrellus*, *Pipistrellus kuhlii* showed high homology in VP2 to bat rotavirus (strain KJ020892) and also to human rotavirus (KF835897) [25]. Four similarities were identified with different rotavirus A VP1 segments from bats, humans, and pigs (MN433617; MH238214; KF690125; EF583033); demonstrating again events of genetic rearrangements [25]. In Germany, in the bat species *Pipistrellus pipistrellus*, rotavirus was detected with a specific sequence detected in Germany, access MN851290, named "Hannover bat rotavirus" (HbrV) [26]. The bat rotavirus identified in this study was detected in group 5, which in *Pipistrellus pipistrellus* bats, phylogenetic reconstruction assigns the sequence of the bat rotavirus distinct but related to type A rotaviruses discovered in France; however, the bat rotavirus identified in France is similarly related to group A rotaviruses [26]. In Denmark, Rotavirus H reads were identified in the M. dasycneme sample data and partial genome sequences of 9 of the 11 genome segments assembled. These sequences resembled the *M. daubentonii rotavirus H* detected in bats in Switzerland in 2019 (MT815963) [25], with about 93% identity [27]. In Bulgaria, the G3P3 genotypes identified as strains RVA/Bat-wt/BGR/BB89-15/2008/G3P[3] and RVA/Bat-wt/BGR/BR89-60/2008/G3P[3] were identical or very similar to the bat RVA strains MSLH14 from China for the proteins VP6, VP1, VP2, VP3 and also similar to a partially sequenced strain of bats from Brazil BRA/4754, a bat from China, a horse from Argentina (Horse -wt/ARG/E3198) and a human from China (Human-tc/CHN/L621) [8]. This study reveals that there is high homology of the Bulgarian strains for VP7, VP4 with Zimbabwean bat strains (LUS12-14) from Gabon, USA Simian strain (USA/RRV1975), and Hungarian dog (HUN/135) [8]. For proteins NSP1, NSP2, NSP3, NSP4, NSP5 also resulted in high homology with

horse rotavirus from Argentina and bats from China [8]. These studies show that these Asian atypical human strains are also closely related to rotavirus in bats from Bulgaria [8]. (Figure 2).

In Serbia, after capturing *Miniopterus schreibersii* bats, stool samples were extracted and analyzed for rotavirus detection. When sequencing they were found to be very different from others previously reported. These sequence reads were distributed among several rotavirus species (including RVB, RVG, RVH, and RVI). To place the new bat-borne strain, BO4351/Ms/2014, in the latest rotavirus taxonomic framework (Matthijnssens et al., 2012; http://www.ictvonline.org), researchers selected additional sequences from the VP6 gene of the GenBank to represent a broader spectrum of genetic diversity for various rotavirus species [28]. The new strain of rotavirus transmitted by bats represents a new species tentatively named Rotavirus J (RVJ); therefore, the reference strain was designated as RVJ/Batwt/SRB/BO4351/Ms/2014/G1P1 [28]. It was found distributed among the same species of bats that lived in the same colony. JVRs have been detected exclusively in birds and mammals. The data suggest that bats may be a true reservoir of JVRs, although the researchers consider that more studies are required to confirm this hypothesis and that it is also important to study the pathogenicity, prevalence and the effect of the virus on bats colonies [28]. The new strain was closely related to representative strains of HRV, as the researchers suggested that these rotaviruses had diverged from a common ancestor [28].

AFRICA

In Kenya, Esona MD et al., [7] in 2007 took feces samples from the bat species *Eidolon helvum*, where genetic analysis identified the presence of rotavirus were 7 genes were unique and 2 were similar to the rotavirus genotypes described [7]. The researchers detected and genetically characterized a strain of rotavirus associated with bats: Bat/KE4852/07 [7]. Although the sequences of the NSP1 and VP3 genes remain undefined; for researchers, this genomic sequence provides comprehensive information on the diversity, evolution, classification, and ecology of rotaviruses [7]. The RCWG has classified the bat strain Bat/KE4852/07 as G25-P[6]-I15-R8(provisional)-C8-Mx-Ax-N8-T11-E2-H10 [7]. The finding that the Bat/KE4852/07 VP4 gene was nearly identical to human P [6] strains suggests that the investigators arose as a result of a reassortment event between human and bat rotaviruses [7]. There are two P6 identified in Brazil (HST435 and HST369) and in South Korea (KMR720) that have high homology with this strain identified in *E. helvum* (KE4852/07) [7]. Investigators consider the VP4 and NSP4 gene segments to have original bat rotavirus genes, which have evolved and become ubiquitous among human rotaviruses for several years [7]. Another study conducted in Kenya: Two rotaviruses specifically related to group A rotaviruses are detected in two different species of bats from two geographically distant populations, specifically 2980/BatRVA-R. aegyptiacus in Meru and 322/BatRVA-*Taphozous mauritianus* bat in the Kwale region [29]. Phylogenetic analysis based on the partial sequence of the VP6 gene revealed that 2980/BatRVA was most closely related to a human strain of RVA (G3P[2]) isolated from Kenya in 1997, while 322/BatRVA was closely related to a strain RVA human (G6P[14]) isolated in Belgium in 1997 [29]. This confirms the reassortment of human and bat virus strains that supports previous research [29].

In Zambia, they identified a new strain of group A rotavirus in the intestinal contents of *Rhinolophus spp* [30]. Whole genome sequencing revealed that the identified virus, named RVA/Bat-wt/ZMB/LUS12-14/2012/G3P[3], possessed the constellation of genotypes G3-P[3]-I3-R2-C2-M3 -A9-N2-T3-E2-H3. Several similar genome segments of the Zambian bat rotavirus

strain LUS12-14 were closely matched to those of group A rotaviruses identified in humans, cattle, and antelope, indicating interspecies transmission of rotavirus between bats and other mammals suggesting possible multiple genomic rearrangement events [30]. Another investigation in Zambia, in the year 2014-15 after collecting bat feces samples, genotypes N21 for NSP2 and E27 for NSP4 were identified previously in the previously identified strain ZFB14–126 of *R. aegyptiacus* rotavirus [31]. In this investigation, all detected genome segments were divergent from those of other mammalian RVAs that are tentatively considered bat-specific [31]. In Cameroon, 2013-14, the RVA/Bat-wt/CMR/BatLy03/2014/G25P[43] strain was identified in bats, which has the constellation of genotypes G25-P[43]-I15-R16-C8-M15- A26-N8-T11-E23-H10, and also that it shares six genotypes with those of the Kenya bat rotavirus strain KE4852 [7]. To better understand the spread and diversity of rotavirus in bats, researchers achieved a rotavirus detection in Cameroonian bats, after trapping bats near human habitations in the southwestern region of Cameroon; using an unbiased viral metagenomics approach, 5 new divergent bat strains were identified, 4 of which were genetically similar to each other [32]. The high genetic divergence and partial relatedness of most segments of the different RVA bat strains and those identified in the study indicate the high frequency of rearrangement events in the general bat population [32]. In another study, also carried out in Cameroon, fecal samples of two species of bats (E.helvum and E.gambianus) were collected from three locations in the southwestern region of Cameroon: Limbe, Lysoka and Muyuka; where rotavirus H readings were identified in two different groups of bats, including a nearly complete genome [33]. Based on genetic relatedness, the recovered bat HRV segments were distantly related to those of human and porcine HRV strains [33]. Similarly, based on phylogenetic analysis, bats, pigs, and human HRV strains formed three distinct, distantly related subgroups. These Korean bat HRV strains were only distantly related to human bat HRV strains, pigs, and Cameroonians, suggesting a wide genetic diversity of bat HRV strains [33]. In addition, the researchers showed that the phylogeny of HRV is distinct from human, pig, and bat; and they consider that interspecies transmission of this virus did not occur in the recent past [33]. In Nigeria, a hidden phylogenetic analysis study found a wide range of RVs present in Nigerian bats, some grouping with human RVs and some representing new species. Bat RVA sequences from Nigeria are placed in four groups on the phylogenetic tree, the two nearly identical RVA/Bat-wt/NGA/B113/2011 and RVA/Bat-wt/NGA/B114/2011 sequences from *C. pumilus* grouped together with human rotavirus group A (HM627553), two nearly identical sequences of RVA/Bat-wt/NGA/B51/2011 from Nycteris sp. and H. ruber RVA/Bat-wt/NGA/59/2011 clustered with the Gabon strain (KM214473) also identified in bats [34]. An *E. helvum* bat (RVA/Bat-wt/NGA/B192/2010) had high identity with bat RV A from an *E. helvum* bat from Cameroon (KX268776); the RVA/Bat-wt/NGA/B82/2011 sequence detected from H. ruber shares 98% sequence identity with a chicken RV A virus (FJ169853) [34]. Clusters of five identical Nigerian bat RVA viruses obtained from *E. helvum* were similarly related to *E. helvum* RVA from Cameroon; the RVA/Bat-wt/NGA/B8/2011 sequence from *E. gambianus* had 91% identity to porcine RV C (MK379289), and the RVA/Bat-wt/NGA/B38/2011 sequence from L. frons shared 87% nucleotide identity with chicken RV F (JN596591) [34]. The two identical sequences RVA/Bat-wt/NGA/B68/2010 and RVA/Bat-wt/NGA/B131/2010 from *E. helvum* bats were related to bat RV H (MG693157) identified from an *E. helvum* bat from Cameroon with 98% identity [34]. RVA/Bat-wt/NGA/67/2010 VP6 is the genotype I22 with 97.2% identity to bat RV A (CMR/BatLi09/2014/G30P42, KX268758) [34]. In other studies where samples were collected in Gabon and Ghana, the following bats were sampled: *Hipposideros caffer*, *Macronycteris gigas*, *Eidolon helvum* [8]. The Gabonese strains were similar to each other, with certain genotypes shared with the Bulgarian strains (G3 (VP7), P[3] (VP4), C3(VP2), M3(VP3), N3(NSP2), T3(NSP3) and

E3(NSP4) [8]; however, they do not cluster phylogenetically very close together. For researchers, this indicates that they are not recent rearrangement events. A sample with a different genotype G30 P[47] (GKS-934) is detected , which shares homology with the VP4 P[47] strain from Costa Rica (KCR10-93) [8]. Three rotavirus samples from Gabon were found to possess more than one strain that has not been identified in bats; from bats in Gabon share (VP1, VP2, VP3 and NSP5) with human rotavirus strains in Kenya (G3P[2]) isolated in 1987 [8]. Also, other samples from Gabon share (VP2, VP3 and NSP5), with simian rotavirus ZAF/SA11-H96 (G3 P[2]) and human rotavirus in China ZTR-5 (G3P[2]) [8]. In Ghana, the bat rotavirus strain RVA/Bat-wt/GHA/K212/2009/G30P47 was reported.This strain reported high homology with all as the bat rotavirus proteins detected in Cameroon, with the exception of the G31 (VP7) genotype detected in a strain in that country (BatLi08/2014/G31P[42]). In addition, it shares homology for VP6 proteins with Zambian bat rotavirus strains (I22) and NSP3 (T17) [8].

ASIA

In China, metagenomic analysis, RT-PCR on bat samples and in vitro virus isolation in cell cultures, confirmed the presence of a new RVA, named RVA/Bat-tc/MYAS33/2013/G3P[10], in one of Stoliczka's trident bats [35]. The VP7 gene of this MYAS33 strain was related to that of an equine RVA strain from Argentina (Figure 2) and the nucleotide sequence similarity was 93%; while its VP4 gene was a rare P[10] type. The study highlights the potential role of bats as RVA reservoirs [35]. Another study in China, based again on RT-PCR viral metagenomics and viral isolation in cell cultures, confirmed the presence of a new RVA strain, named RVA/Bat-tc/MSLH14/2012/G3P[3] in horseshoe bats minors. Whole genome sequencing analysis showed that MSLH14 possessed the genotype constellation G3-P[3]-I8-R3-C3-M3-A9-N3-T3-E3-H6, which is similar to human and animal rotaviruses, believed to be feline/canine in origin [36]. Phylogenetic analysis excluded that VP7 was more closely related to bovine strains of RVA from India, while VP4 was more closely related to an unusual human strain of RVA, CMH222, with animal characteristics isolated in Thailand [36]. The researchers believe that this virus is not closely related to any known RVA strain and speculate that it is a true bat RVA strain and not a virus transmitted between species [36]. An additional study conducted to the isolation of another bat RVA strain RVA/Bat-tc/CHN/MYAS33/2013/G3P[10] from a stoliczka trident bat (*A. stoliczkanus*) in Yunnan, China [37]. The sequences of its RNA segments encoding VP7, VP4, and NSP5 (KF649186–KF649188) were determined, and the analysis based on VP7 and VP4 indicates that MYAS33 possessed the rare combination of G3P[10] genotypes [37]. The results showed that MYAS33 possessed the genotype constellation G3-P[10]-I8-R3-C3-M3-A9-N3-T3-E3-H6 and shared the same genotype constellation with the bat RVA strain MSLH14 except by VP4 (strain MYAS33 contained P[10] instead of P[3]) [37]. Phylogenetically, MYAS33 and MSLH14 are related to each other for the VP3, NSP1, NSP2, and NSP5 gene segments (94–97%) [37]. Despite belonging to the same genotype, the VP7, VP6, VP1, VP2, NSP3, and NSP4 gene segments were more distantly related. Interestingly, the MYAS33 strain had 9 of 11 genotypes in common (except VP4 and VP6) with the unusual strain, equine RVA E3198 [37]. (Figure 2). Of special interest are the RVA VP6 and P[10] genotypes I8 and VP4, respectively, also the P[10] genotype is very rare, and apart from MYAS33; it has only been identified in a few humans as rare strains identified in the India (37). The identification of this genotype in bats may indicate that P[10] could be a species between hosts transmitted between bats and humans [37]. Another investigation in China collected bat samples between August 2011 in four cities: Huizhou, Guangzhou, Yunfu and Haikou in South China [38]. Compared to RVA G3 strains from humans, bats, cats, dogs, and apes, the identified bat RVAs show high sequence homologies to that of a simian RVA strain (RVA-simian-tc-USA-RRV-

n1975-G3P[3]) [38]. The VP7 sequences of four *Scotophilus kuhlii* rotavirus strains (SK/13YF128/129/200/212) were determined; These segments were most closely related to RVA G3 strains from simians and dogs (RVA-simian-tc-USA-RRV-1975-G3P[3], RVA-dog-tc-ITA-RV52-96-1996-G3P[3]) [38]. In another study in China, they detected from swabs of *Scotophilus kuhlii* in Beihai (strain YSSK5), *Hipposideros pomona* in Luzhai (strain LZHP2), and *Taphozous melanopogon* in Baise, Guangxi (strain BSTM70) [39]. The fourth strain was identified from the intestinal tissue of a Rousettus *leschenaultii* in Luoding, Guangdong (strain GLRL1). Thus, only five RVA strains have been identified in insectivorous bats in China: MSLH14 and MYAS33 in Yunnan and BSTM70, YSSK5 and LZHP2 in Guangxi [39]. Although all of these 5 strains were identified at least 300 km apart, they shared a similar genotype constellation, G3-P[3]-I8-R3-C3-M3-A9-N3-T3-E3-H6, to MSLH14, suggesting to researchers that MSLH14-like RVAs are true bat viruses widely distributed in insectivorous bat populations in this region [39]. One of the most surprising findings of this study is that the LZHP2 strain, identified from an insectivorous bat sampled in a cave very close to a village, had the same genotype as the human strain M2-102, sharing unique nucleotides identities in several of its segments in the genome [39]. M2-102 was previously identified as the pathogen causing diarrhea in a 3-year-old boy in 2014 in Sanjiang, Guangxi, which is only 120 km from the place (Luzhai) where LZHP2 was identified. Because the M2-102 strain was so different from all known human RVA strains, the data concluded that this strain was the result of interspecies transmission of an MSLH14 bat RVA to the child; this finding has developed a list of bats for which their RVA strains have been shown to be capable of infecting humans [39]. It is also very interesting that the same constellation of genotypes was found in the Argentine horse strain E3198, which, at the time, is probably distantly related to feline and canine RVA strains [39]. Bat RVA strains BSTM70 also possessed the constellation of genotypes similar to MSLH14, with the sole exception of a novel A29 genotype for NSP1, further increasing the genetic diversity of bat RVA strains in China [39].

Another study conducted in China pulled out the P[3] genotype that has been identified in many species, including humans, apes, dogs, and bats [40]. Several glycans, including the resistant group sialic acid (HBGA), are rotavirus-binding factors [40]. The glycan binding specificity of different genotypes of P[3]VP8*s was investigated [40]. The results obtained were that human HCR3A and dog P[3] RV VP8*s recognized terminal sialic acid glycans and hemagglutinated red blood cells, while bat P[3] VP8* did not show binding to glycans. glycans or hemagglutination [40]. However, the bat P[3]VP8* mutant of C189Y gained the ability to hemagglutinate red blood cells, whereas the human P[3]HCR3A/M2-102 mutants of Y189C lost the ability [40]. Structural overlay showed that the P[3] VP8* bat model was quite different from the P[3] simian Rhesus rotavirus (RRV) P[3] VP8* genotype, indicating that the P[3] rotavirus genotype from bats is relatively distinct and partially contributes to the binding of the analyzed glycans [40]. These results promote understanding of P[3] VP8*/glycan interactions and possible transmission of the bat/human P[3] genotype, providing more information on rotavirus infection and prevalence [40].

In Singapore, urine and feces samples were collected from a colony of nectar-eating cave bats (*Eonycteris spelaea*) in Singapore during the year 2013-14. When analyzing the VP1 gene of rotavirus-positive samples from *Eonycteris*, they were very similar to the rotavirus detected in a *Rousettus leschenaultii* from China in 2005 (KX814935) [41]. The *Eonycteris* and *Rousettus* (MK603148) sequences had 99% homology [41]. In contrast, the Rotavirus VP7 gene sequence from *Eonycteris* (MK603149) formed homology with other rotavirus sequences from a wide range of hosts, including humans, pigs, cattle, and horses [41]. In Korea, viruses in fecal samples of bats were investigated in 2015. The main bat species at collection sites were *Rhinolophus*

ferrumequinum, Myotis macrodactylus, Myotis aurascens Kuzyakin, Myotis petax, and *Miniopterus schreibersii*. Most Korean bats are believed to be insectivorous, as no fruit bats have been found in Korea to date [42]. Rotavirus-related sequences were found to belong to rotavirus group H, which is a recently proposed group of rotaviruses that includes strains ADRV-N and B219, which infect adult humans [42, 43, 44, 45]. Another study conducted in Korea required two sets of metagenomic data from a picornavirus-positive bat feces sample, where partial rotavirus A (RVA) VP2, VP3, and VP7 genomes were identified, showing high nucleotide similarity with VP3 of RVA/Bat/CHN/ZFC23 (MK292705) [46]. For VP2 it shared high sequence similarity with the VP2 genomes of RVA/Human-tc/JPN/K8 (JQ713646) and RVA/Rabbit-wt/KOR/rab1404 (MK751433), followed by RAV/Bat/KEN/322/2015 (MH285827) [46]. The VP7 genome sequence showed the highest similarity to the VP7 genome sequence of RVA/Bat/CHN/LZH and RVA/Bat/CHN/MYAS33 discovered in China, and high similarity to RVA/Human-weight/CHN/M2-102. /2014 [46]. In Bangladesh, rotavirus was identified in *P. medius* and *R. leschenaultii.* The G1 and G8 genotypes were identified but the P genotype was unsuccessful. The sequence of bat genotypes G1 and G8 had high similarity to human RVA strains [47]. The bat G8 genotype also had high similarity to bovine strains from India and pig strains from South Korea [47]. The identified sequences were MK674285 and MK674286 ; researchers suggest that identification of genotypes related to human strains of RVA possibly prevent through anthropozoonotic transmission that may be occurring between people and bats because G1 has rarely been detected in animal species worldwide [48], although it was previously identified on Indian cattle [47]. This G1 strain has not previously been identified in a bat species and, although it is phylogenetically distinct from other G1 strains in humans, they are 98–99% identical [47]. *Pteropus* bats perch near people, which may require them to share food and water resources; bats have been reported to drink from surface water reservoirs [47, 49]. The G8 genotype is commonly detected in people in Africa [50]; It has been detected in people in Hungary within a zoonotic strain of RVA that spreads from sheep and goats to people. G8 has also been previously identified in cattle [51, 52], poultry [47].

MOLECULAR CHARACTERIZATION OF HUMAN ROTAVIRUS STRAINS WITH GENES RELATED TO BAT ROTAVIRUS

Two studies conducted in Thailand in rotavirus-positive children with gastroenteritis revealed high homology with group A rotavirus genes of bat origin [53]. In the first study, an unusual strain of rotavirus with the genotype G3P[10] (RVA/Human-wt/THA/MS2015-1-0001/2015/G3P[10]) was identified in a stool sample from a hospitalized 11 months old child with severe gastroenteritis. After sequencing, the strain was identified as MS2015-1-0001 [53]. In the complete genomic analysis, the configuration G3-P[10]-I8-R3-C3-M3-A9-N3-T3-E3-H6 was obtained, which is identical or closely related to those of bats in its 11 genes and bat-like rotavirus strains (MYAS33) [53]. In the second study involving several hospitalized children with gastroenteritis, a rare RVA G3P[10] genotype was detected, with VP7 and VP4 genes similar to those of bats [53]. In Japan, after performing the detection and molecular characterization of two rare strains of rotavirus that were collected from children with acute gastroenteritis G8P[14] (strain 12 597) and one G3P[3] (strain 12 638) [54]. When characterizing them, the genes NSP1 to NSP3 shared higher identities with those of a bat rotavirus (strain RVA/Bat-wt/ZMB/LUS12-14/2012/G3P[3]) [54]. In China in 2014, an unusual human G3P[3] group A rotavirus (RVA) strain M2-102 was identified in a stool

sample collected from a child with diarrhea in Guangxi province, China: this G3P genotype [3] is a common identifier in feline and canine RVA (55). However, preliminary phylogenetic analysis of the VP7 and VP4 genes of strain M2-102, indicated that these two genes were related to the bat RVA strain MYAS33 [55]. Epidemiological data indicated that the child infected with M2-102 came from a rural village, mountainous forested area, which could provide a natural setting for rearrangement events occurring between animals and humans [55]. In Australia, a rotavirus strain of genotype G3P[14] was identified in a 12-year-old boy with diarrhea; this strain has previously been identified in rabbits in Japan, China, USA [56]. Complete genome sequence analysis of RVA/Human-wt/AUS/RCH272/2012/G3P[14] (RCH272) revealed that the strain contained a genome characterization G3-P[14]-I2-R3-C3- M3-A9-N2-T6-E2-H3 [56]. Phylogenetic analysis revealed that the genes were distantly related to a variety of animal strains, including bats [56]. In Brazil, during the national surveillance of rotavirus group A (RVA) in 2009, five unusual strains of the human genotype G8P[6] were detected in indigenous Brazilian children with acute gastroenteritis; The identified strain obtained a close genetic relationship with the RVA strain KE4852/07 of the African fruit bat *Eidolon helvum* detected in Kenya in 2007 [57]. Research carried out in the Dominican Republic revealed possible genetic rearrangements in children, 3 of the 15 sequenced strains had genes that encoded VP7, very similar to those of bat rotaviruses of the G3 genotype detected in Bulgaria in 2008. These VP7 sequences were more distantly related to other G3 rotaviruses found in bats and other animals [58]. A bat-like rotavirus VP4 sequence with the P[6] genotype. In addition, 4 of 10 available VP6 sequences, including all 3 from G3 bat-like strains, showed high nucleotide identity with VP6 from rotavirus genotype I2 detected in Kenya in 2015 [58].

IS THERE TRANSMISSION OF ROTAVIRUSES FROM BATS TO HUMANS?

In this review novel findings were identified, of the few investigations carried out globally, most of the rotaviruses identified in bats were identified in group A; however, there are reports such as group H rotaviruses identified in Switzerland [25], Denmark [27], Cameroon [33], Korea [42], Nigeria [34]; the novel finding of rotavirus (HbrV) in Germany [26] and rotavirus (J) in Serbia [28].

It is important to note that in this review the most frequent G3P[3] combination was identified for VP7(G) and VP4 (P) in bats in countries such as: Brazil, Bulgaria, Zambia, Gabon and China [8]; Interestingly, this same combination of this genotype has also been reported in humans; where these investigations demonstrate the presence of rearrangement with rotavirus genes of bat origin in the countries of Japan and China [8].

The prediction of epidemics of viral zoonosis has become an important public health problem [4,6]. An in-depth understanding of the key animal species that act as reservoirs represents an important step in achieving this goal. Bats harbor a variety of viruses, some of which are of particular concern because they cause serious human diseases [4,6]. However, little is known about the diversity of the global population of viruses found in bats [4,6]. This is of particular interest, given that research has shown that many viruses transmitted by bats can cause serious illnesses in humans. The researchers consider it important to study whether the new strains of rotavirus detected in bats pose any occupational risk to professional chiropterologists or people that comes into contact with bats and their feces [28]. Studies based on RT-PCR detection followed by classical sequencing approaches have shown that bats naturally harbor many viruses and have provided evidence that viral epidemics can result from interspecies transmission of viruses from this reservoir to humans or other animals

[4,6]. The crossing of the species barrier by viruses is also facilitated by the expansion of the human population and the destruction of the natural habitats of bats, bringing bats into even closer contact with humans and other animals [5]. The development of genome sequencing methods and their metabolomics application, applied to samples taken from bats, such as guano and saliva in particular, has led to the discovery of considerable viral diversity in bat species [59].

In some Asian and Latin American countries where sanitary conditions are poor, it happens that some bats consume water from areas where this water is shared with animals and humans. *E. helvum* bats have been observed skimming bodies of water in Africa, probably collecting water to drink [47, 49]. In Asia and Africa, human excrement is sometimes used as fertilizer on farmland [47]. Viable human rotaviruses have been detected in surface water, reservoirs, sewage, and drinking water [60, 61]. "Contact with human feces during drinking or feeding provides a mechanism by which fruit bats can likely ingest human rotaviruses, serving as a source of rotavirus genetic rearrangement. Hence the probable explanation of why rotavirus strains of human origin were identified in the feces of bats in Bangladesh" [47] and "in other countries, as is the case of the G1 genotype; It has also been detected in environmental water samples" [62] and bivalve shellfish samples [63], supporting our hypothesis that human RVA strains may contaminate local water sources [61]. Additional research on the actual mechanisms and frequency of rotavirus transmission between humans and bats is needed as there are strong studies of genetic rearrangements between bats, other animals, and humans [38, 39]. Livestock feces are also often disposed of in water reservoirs, which can also be contaminated if rainwater washes fecal material into the reservoir. Researchers have detected the G8 genotype in children from Mato do Sul in Brazil, detecting homology of this genotype with the G8 that has been detected in cattle [20]; In addition, this genotype is detected in association with the P genotype [6] represented by the BRA898/07 sequence, closely related to porcine [20]. There are P [6] in Brazil (HST435 and HST369) and in South Korea (KMR720) which has homology with the African fruit bat (Eidolom helvum) (KE4852/07) detected in Kenya in 2007 [20].

In this review I consider the current identification of rotavirus A, together with the detected genetic rearrangements that share high homology with bats, other animals, humans, as well as the identification of rotavirus H in both humans [64], as well as pigs [65, 66] in bats [25, 27, 33, 34, 42], the identification of rotavirus J in Serbia [28], HbrV in Germany [26] in bats, opens new perspectives on the evolutionary origin and history of this pathogen, requiring further in vivo and extensive molecular epidemiological studies to fully understand their genetic diversity and geographic distribution. Importantly, after conducting this global review the researchers did not observe diarrhea or other obvious signs of disease in bats, which may suggest that bats may experience active virus replication and excretion without overt clinical signs, which could potentially increase exposure to humans.

I consider it necessary to carry out more studies regarding the migrations of bats [67] for example, the case of *Carollia perspicillata* , where rotaviruses have been identified, in Orotina in Costa Rica in which a unique sequence of rotavirus KCR10-93 was discovered [8] that shares high homology to the VP4 protein (P[47]) with the strain detected in Ghana (K212) [8], Cameroon (BatLy17) [8]; It also shares homology for other genes with the Ecu53428 strain identified in humans in Ecuador [17]. It is closely related to sequences from bats from Brazil [18] and Suriname [16], bat strains from Bulgaria (BGR/BB89-15) [8], China (CHN/LZHP2, MSLH14, BSTM70, MYAS33) [39], Brazil (BRA/4754) [21], share high homology with strains of human rotavirus identified in Japan (JPN/AU-1) [54], China (E2451, L621), and horse rotavirus from Argentina (E3198) [8].

Additionally, genomic comparisons of RVA strains from Chinese bats and humans partially from Thailand (CMH079 and CMH222) [36] and India (69 M, 57 M and mcs60) [36], are very similar. The identification of the M2-102 strain that was identified as the cause of diarrhea in a 3-year-old boy who lived 120 km from the Luzhai site in China [39] where LZHP2 was identified, resulted in the M2-102 strain being different from the rest of the already identified human strains. For all the above, it can be speculated that RVA from Asian, Latin American and African bats have crossed the host species barrier to humans producing roles of interspecies transmission and genetic rearrangement on a large number of occasions to generate the rotavirus genetic diversity.

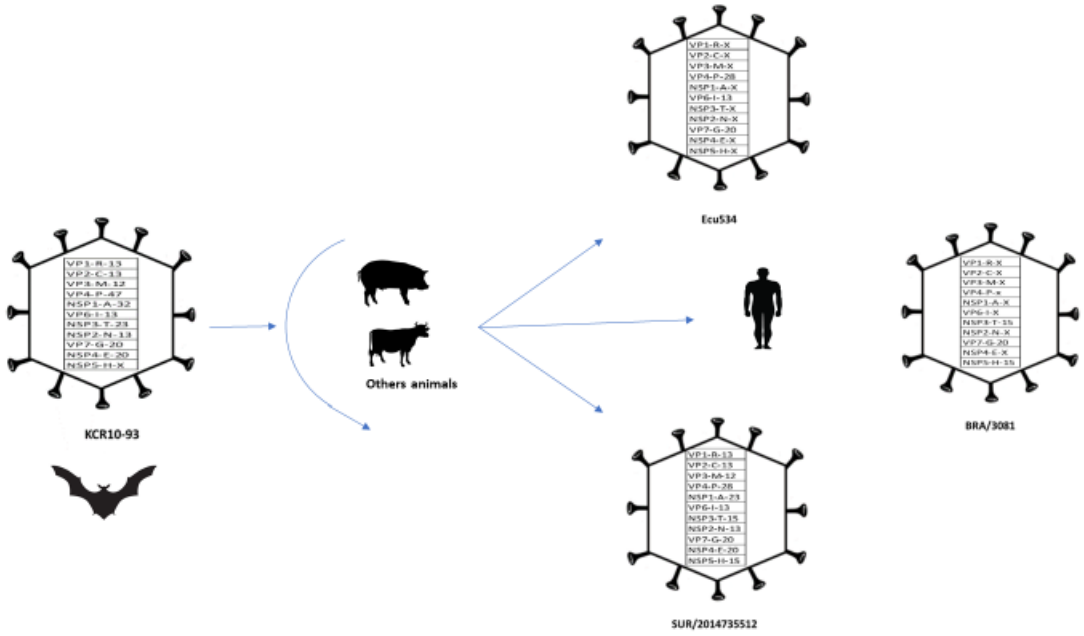
CONCLUSION

This revision reinforces the initiative to improve rotavirus surveillance in domestic, farmed, and wildlife animals, not only to investigate the genetic need for rotaviruses, but also to understand the origin of unusual strains being detected in humans, which would contribute to improving the design of current vaccines.

Studies also show that each of these rotavirus strains, isolated from a variety of host species, is atypical for that particular host species; and therefore, may also be examples of bat RV strains transmitting between host species. However, further molecular characterization of bat RV strains globally would be necessary to confirm or refute this hypothesis.

The identification of rotavirus in bats and the genetic analysis of this virus provide new insight into the ecology and evolution of rotaviruses. The different findings not only reinforce the potential role of bats as reservoirs of zoonotic viruses that are threats to human health, but also suggest that humans may serve as reservoirs of viruses, which can result in anthropozoonotic rotaviruses transmission. More research is needed to establish the precise role of bats in the natural history of rotavirus.

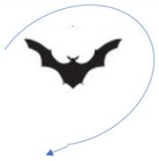
Figure 1. Proposal of Possible Transmission and Genetic Rearrangement Identified in Latin America of the Genotype VP7 (G20) Bat Rotavirus (KCR10-93) to Animals and from there to Man.



Possibly the bat rotavirus strain identified in *Carollia perspicillata* in Costa Rica (KCR10-93) has been transmitted to animals, with rearrangement events occurring that have led to the rotavirus strains identified in Ecuador (Ecu534), Suriname (SOUTH /2014735512), Brazil (BRA/3081) and in humans.

Figure 2. Possible Interspecies Transmission and Genetic Rearrangement of Rotavirus

Strains	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Bat/BGR/BB89-15	G3	P[3]	I3	R3	C3	M3	A9	N3	T3	E3	H6
Bat/CHN/LZHP2	G3	P[3]	I3	R3	C3	M3	A9	N3	T3	E3	H6
Bat/BRA/4754	G3	P[3]	I-X	R-X	C-X	M-X	A-X	N-X	T3	E3	H6
Bat/CHN/MSLH14	G3	P[3]	I8	R3	C3	M3	A9	N3	T3	E3	H6
Bat/CHN/BSTM70	G3	P[3]	I8	R3	C3	M3	A29	N3	T3	E3	H6
Bat/CHN/MYAS33	G3	P[10]	I8	R3	C3	M3	A9	N3	T3	E3	H6



Strains	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
Human/JPN/AU-1	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
Human/CHN/E2451	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
Human/CHN/L621	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H6
Horse/ARG/E3198	G3	P[3]	I3	R3	C3	M3	A9	N3	T3	E3	H6

Possible interspecies transmission of bat rotavirus strains from Bulgaria (BGR/BB89-15), China (CHN/ LZHP2, MSLH14, BSTM70, MYAS33) and, Brazil (4754) that may have produced genetic rearrangement to give rise to the human strains detected in China (CHN/E2451, CHN/L621), Japan (JPN/AU-1) and horse from Argentina (ARG/E3198).

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