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## The zoonothropotic cycle of human rotavirus in rural settings and its public health burden

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Rotaviruses are one of the major causes of severe acute gastroenteritis among infants and young children throughout the world. The current study was conducted to investigate the possible role of animals in the epidemiology of human rotavirus strains to give insights about the zoonothropotic transmission cycle of such strains in rural settings. For this purpose, stool specimens were collected from 52 diarrheic children inhabiting rural settings as well as fecal samples from 38 diarrheic calves and 92 rats (88 *Rattus norvegicus* and 4 *Rattus rattus*). All human and animal samples were firstly screened for the presence of rotavirus using ELISA kit. Afterwards, all ELISA positive samples were then examined for the occurrence of human rotavirus using RT-PCR. Of 52 diarrheic children, 8 were positive for human rotavirus giving prevalence 15.4%, whereas the prevalence of human rotavirus among examined animals was 2.6% and 3.3% for calves and rats respectively. Moreover, the prevalence of human rotavirus among *R. rattus* (25%) was far higher than that of *R. norvegicus* (2.5%). Seriously, the blasting and phylogenetic analysis of randomly selected one human and one *R. rattus* sequences revealed 99% and 98% identity with human rotavirus genotype G3P[8] respectively as well as both sequences were grouped in the same clade with such genotype. In conclusion, the results of the current study provide more pieces of evidence about the circulation of human rotavirus among animals to highlight the zoonothropotic transmission cycle of human rotavirus in rural settings.

**Keywords:** human rotavirus; calves; rats; zoonothroposis.

### INTRODUCTION

The identification of rotavirus in children with severe diarrhea in 1973 is considered a breakthrough in gastroenterology. Since this date, rotavirus has a mounting impact on childhood mortality to be considered a leading cause of severe diarrheal illness among infants and young children worldwide (Dennhy 2013). In recent years, it was estimated that rotavirus accounted for 215000 deaths per year among children aged below 5 years globally, whereas about half of these deaths were located in 4 countries (India, Nigeria, Pakistan and Democratic Republic of Congo) (Tate et al., 2016). Rotavirus is a double stranded RNA virus belongs to family *Reoviridae*, genus *Rotavirus*. The virus genome comprises 11

RNA segments which encode both structural viral proteins (VP1-4, VP6, VP7) and non-structural viral proteins (NSP1-5/6). VP6 is considered a common antigen protein, which differentiates rotavirus into 8 groups designated (A-H). Almost, 90% of human cases were caused by rotavirus A (RVA). Moreover, RVA is usually classified based on the genes encode VP4 and VP7 (the most important rotavirus outer capsid surface proteins) resulting in a dual classification system of rotavirus G genotype (VP7) and P genotype (VP4) (Matthijssens et al., 2011; Gautam and Bowen 2016). Till now, at least 32 G and 47 P genotypes were identified nonetheless only few ones were commonly circulated among humans worldwide, including G1P[8], G2P[4], G3P[8], G4P[8], G9P[8]

and G12P[8] (Komoto et al., 2018). Humans usually acquired rotavirus through consumption of contaminated food and water or via fecal-oral route (Chen et al., 2012). The disease in young children begins with fever and vomiting, then profuse watery diarrhea, which may lead to dehydration and death (Parashar et al., 2013). On the other hand, rotavirus has emerged in animals since 1960s and now the virus has a wide spectrum of animal reservoirs, including cattle, swine, small ruminants, cats, dogs, birds and even zoo animals but it may cause significant losses among calves, piglets and foals (Martella et al., 2010). Consequently, several studies have proposed the zoonotic potential of rotavirus through the detection of animal genotypes among human cases to highlight the zoonotic transmission of animal rotaviruses to humans (Wu et al., 2012; Mukherjee et al., 2013; Papp et al., 2013; Dóro et al., 2015). Conversely, little is known about the role of animal in transmission of human rotavirus genotypes. However, few recent papers underscore that animals may be implicated in the transmission of human rotavirus genotypes a matter which exerts a great public health concern (Choudhary et al., 2017; Kumar et al., 2018). Therefore, the current study was carried out to investigate the occurrence of human rotavirus among cattle and rats in rural settings to explore the potential zoonotic transmission cycle of human rotavirus.

## MATERIALS AND METHODS

### Samples:

A total of 92 rats (88 *Rattus norvegicus* (Norway rat) and 4 *Rattus rattus* (black rat)) were enrolled in the current study. Rats were trapped from the rural districts of Giza governorate, Cairo, Egypt. Upon arrival in the laboratory, rats were anesthetized and humanely killed by cervical dislocation (Noroozi et al., 2014). Fecal samples were collected from the recta of the examined rats and kept at -80 °C until further processing. In addition, fecal samples were collected from the recta of diarrheic calves that reared in private farms in the same locality where rats were caught. Fecal samples from calves were sent to the laboratory in an icebox to be stored at -80 °C until further processing. On the other hand, stool specimens were gathered from 52 diarrheic young children aged below 3 years who admitted to

private medical laboratories for stool analysis. Human samples were kindly supplied by parents through voluntary contribution after the aim of the study was verbally explained and gave their consent. Stool specimens were sent to the laboratory and stored as previously mentioned.

### Detection of rotavirus using Enzyme linked Immunosorbent assay (ELISA):

All stool specimens and animal fecal samples were screened for the presence of rotavirus using ELISA. The test was conducted using RIDASCREEN-Rotavirus (R-Biopharm, Germany) which is a monoclonal antibodies based sandwich ELISA kit against the product of the VP6 gene of rotaviruses infecting humans. The procedure was done according to the directions of the kit insert.

### Molecular detection of human rotavirus A:

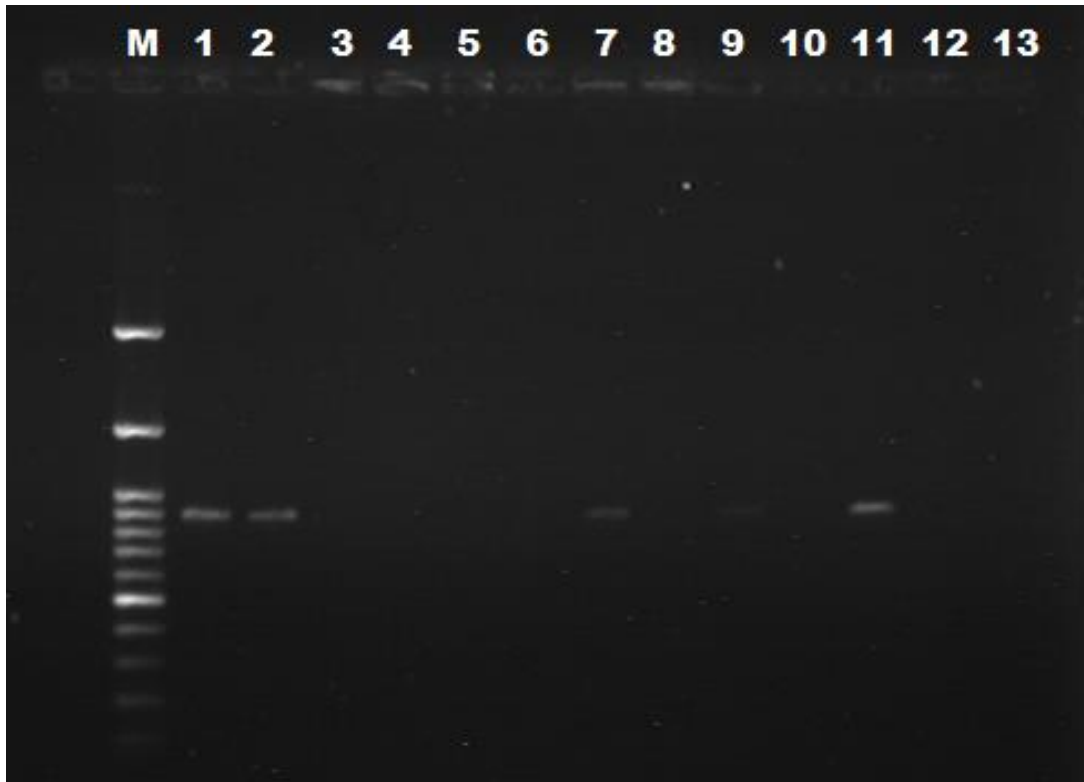
All ELISA positive samples were tested by reverse transcription-polymerase chain reaction (RT-PCR) for the presence of human RVA.

### RNA extraction:

RNAs were extracted from all positive ELISA samples using QIAamp Viral RNA Mini kit (Qiagen, Germany). The test was carried out according to the instructions of the manufacturer. All extracted RNAs were stored at -80 °C till tested.

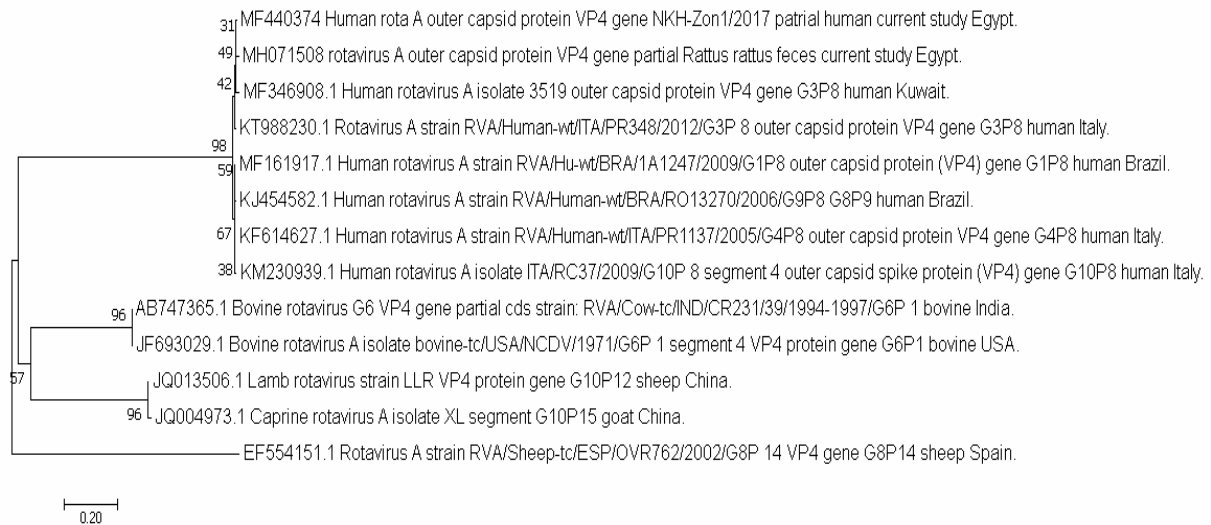
### RT-PCR step:

One step RT-PCR test was conducted using a pair of primers: forward primer con 3: (5'TGGCTTCGCCATTTTATAGACA 3') and reverse one con 2: (5' ATTTCCGACCATTATAACC 3'); which amplify a highly conserved region of VP4 protein encoding gene of human RVA genotypes. Primers were synthesized by Metabion (Germany) and according to Gentsch et al. (1992). The reaction was performed using OneStep RT-PCR Kit (Qiagen, Germany) with the following conditions: 50 °C for 30 min; 95 °C for 15 min followed by 45 cycles of 94 °C for 30 s; 48 °C for 30 s; 72 °C for 60 s; then final extension at 72 °C for 10 min. Afterwards, Amplicons were entered gel electrophoresis step and positive samples showed specific bands at 876 bp (Figure 1). Moreover, VP7 gene of human RVA was also detected using one step RT-PCR test and according to Gouvea et al., (1990).



**Figure 1: Molecular detection of human rotavirus among examined human and animal samples**  
Lane M: DNA ladder 100bp; lanes 1, 2, 7, 9, 11 positive samples with specific bands at 876 bp; lanes 3-6, 8, 10, 12 negative samples; lane 13 negative control.

**Figure 2: Phylogenetic tree infers the evolutionary history of the obtained human rotavirus sequences from human and black rat as well as sequences retrieved from Genbank. The analysis was carried out with neighbor-joining method using MEGA7 software version 7.0.26 and based on the partial sequence of human rotavirus VP4 gene.**



#### VP4 gene sequencing and phylogenetic analysis:

PCR products from 2 positive samples (one human and one *Rattus rattus*) were entered the sequencing step. The amplicons were purified using a Qiaquick PCR purification kit (Qiagen, Germany), then sequencing was performed using Big Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems). The obtained sequences were blasted in GenBank to determine their identity. Furthermore, both human and rat sequences were aligned against the most similar sequences selected from different human RVA genotypes retrieved from GenBank as well as sequences of bovine, caprine and ovine RVAs available at NCBI database were also included. Multiple alignments were carried out using Clustal W, BioEdit software version (7.0.9) whereas phylogenetic tree was constructed through the neighbor-joining method using MEGA7 software version 7.0.26 (Figure 2).

GenBank accession numbers:

Both human and rat sequences obtained in this study were deposited in the GenBank database under the following accession numbers:

MF440374 for human sequence.

MH071508 for *Rattus rattus* sequence.

#### RESULTS

8 out of 52 diarrheic children were positive with a prevalence 15.4%. Regarding to animal samples, only one calf was positive for RVA with a prevalence 2.6%, but 3 out of 92 rats were positive giving a percentage 3.3%. Of 3 positive rats, 2 were Norway rat 2.3% and one was black rat 25% (Table 1). The human sequence obtained in the current study was highly similar (100% query cover and 99% identity) to human RVA G3P[8] strains which circulated in Italy and Kuwait. In a similar vein, the black rat sequence shows high homology to human RVA G3P[8] strains from the same countries with 99% query cover and 98% identity (Table 2).

**Table 1: The prevalence of human rotavirus A among examined humans and animals**

Host	Number examined	Numbers positive	Percentage %
Human	52	8	15.4
Calves	38	1	2.6
<i>Rattus norvegicus</i>	88	2	2.3
<i>Rattus rattus</i>	4	1	25
<b>Total rats</b>	92	3	3.3

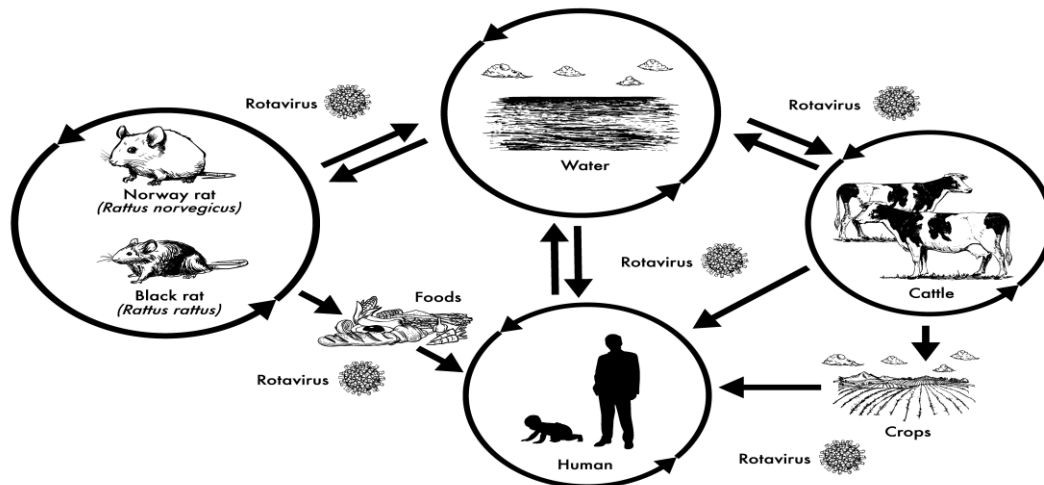
**Table 2: The identity of the obtained human and *Rattus rattus* VP4 gene sequences compared with the most similar ones available at GenBank.**

Obtained sequence	Query cover %	Identity %	Genotype	Host	Accession number	Country
Human sequence MF440374	100	99	G3 P8	Human	KT988241	Italy
	100	99	G3 P8	Human	KT988230	Italy
	100	99	G3 P8	Human	MF346921	Kuwait
<i>Rattus rattus</i> sequence MH071508	99	98	G3 P8	Human	KT988241	Italy
	99	98	G3 P8	Human	KT988230	Italy
	99	98	G3 P8	Human	MF346925	Kuwait

## DISCUSSION

Group A rotaviruses have a great public health burden throughout the world because of such viruses stand behind million cases of severe gastroenteritis among young children resulting in 2 million of hospitalizations annually (Zhang et al. 2016). The results of the present study revealed that the prevalence of human RVA among examined diarrheic children was 15.4%. Such result is higher than those obtained by El-Shabrawi et al., (2015) and Wierzba et al., (2006) who recorded prevalence rates 11% and 10% among Egyptian children respectively, whereas it is lower than that obtained by Shoeib et al., (2015) among outpatients 29.9%; El-Senousy et al., (2015) 31.8% in Egypt. On the other side, the prevalence of human RVA among examined diarrheic calves was 2.6%, which is far lower than those obtained by Otto et al., (2015) in Germany who recorded prevalence 85.2% among diarrheic

bovines and Midgley et al., (2012) 43% in across Europe study a matter which may be owed to that the used primers in our study target only human RVA genotypes rather than bovine ones. Despite our result revealed low prevalence of human RVA among diarrheic calves, it has a great public health implication as the diseased calves shed huge numbers of human RVA which may contaminate soil, crops and could pass through run-off water to small water streams where children in rural settings usually swim regarding RVA could survive for several days in water and contaminated environment (Rzezutka and Cook 2004; Julian 2016) and thereby gives a lot of opportunities for human RVA to reach human guts through human-environment interaction to draw the zoonothroponotic transmission cycle of human rotavirus (Figure 3).



**Figure 3: The zoonothroponotic transmission cycle of human rotavirus in rural settings, highlighting the potential role of infected cattle and rats**

Rats are important reservoirs for many emerging pathogens which cause severe human illness (Himsworth et al., 2013). The prevalence of human RVA among examined rats in the current study was 3.3% with marked species wise distribution, 25% among black rats versus 2.3% in Norway rats. Few studies have investigated the occurrence of RVA among rats; Ianiro et al., (2017) detected RVA in only one rat out of 40 examined black rats giving a prevalence 2.5% which conflicts with our result in the same rat species 25%. However, such high prevalence of human RVA in our study may be attributed to few numbers of examined black rats. Noteworthy, both human and black rat sequences give high identity (99% and 98%, respectively) with human RVA

genotype G3P[8] which circulated in 2 different countries (Italy and Kuwait) after blasting analysis using GenBank database. Moreover, both human and black rat sequences were grouped in the same clade with human RVA genotype G3P[8] whereas other human RVA genotypes occupied another clade. Therefore, based on sequences blasting results and phylogenetic analysis, both human and black rat sequences could be classified as human RVA genotype G3P[8] considering such genotype is the most prevalent human RVA genotype circulated among children in Cairo, Egypt (Shoeib et al., 2015). Remarkably, the phylogenetic analysis infers the evolutionary history of black rat sequence to provide a concrete evidence about its human origin as the

phylogenetic tree comprises two clusters, one of them includes all human RVA genotypes as well as black rat sequence obtained in the current study, whereas the other cluster includes rotaviruses of animal origin likewise bovine, ovine and caprine rotaviruses. The occurrence of RVA G3P[8] genotype among black rats has a great public health burden due to RVA G3P[8] genotype is a strict human genotype and can be easily disseminated among humans on contrary zoonotic RVA genotypes have limited transmission potential among humans (McDonald et al., 2009; Medici et al., 2016; De Graaf et al., 2017). Moreover, the circulation of RVA G3P[8] genotype among rats gives a chance for minor mutations to be taking place, resulting in new strains which may be well adapted to humans, but able to escape immunity evoked by vaccination and thus may lead to outbreaks or even epidemics. Additionally, black rat is a domestic rat, it is also referred as roof rat or house rat accordingly, such rat able to contaminate households, kitchens, restaurants and human foodstuffs with human RVA and accordingly expanding the spectrum of human RVA dissemination in human vicinity resulting in more human infections (Figure 3).

### CONCLUSION

The present study underscores the circulation of human RVA among animals to shed more light on the zoonthropotic transmission cycle of human RVA with especial concern to the rats in this scenario to find out the overlooked transmission pathway of such dangerous virus in rural settings where human-animal-environment interface.

### CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

### AUTHOR CONTRIBUTIONS

NHG and KAA designed the work, data analysis, wrote and reviewed the manuscript. HS samples collection performed the experiment and also, wrote the manuscript. All authors read and approved the final version.

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