ASEAN
Journal of Scientific and Technological Reports
Online ISSN:2773-8752

Research article

Surveillance of Rotavirus A Genotypes in Diarrheal Cases from Humans and Bovine in Basra Province, Iraq

Rawaa Bannay Zubairi[1], Basil A. Abbas[1*], and Fawziah Ali Abdullah[1]

[1] Basra University, Veterinary Medicine College, HP6W+GQP, Basrah, Basra Governorate, Iraq
* Correspondence: (rawa@stu.edu.iq, R.B.Z)

Citation:
Zubairi, B.R.; Abbas.A.B.; Abdullah, A.F. Surveillance of rotavirus a genotypes in diarrheal cases from humans and bovine in Basra Province, Iraq. *ASEAN J. Sci. Tech. Report.* **2026**, *29*(1), e261427. https://doi.org/10.55164/ajstr.v29i1.261427.

Article history:
Received: September 22, 2025
Revised: September 30, 2025
Accepted: October 9, 2025
Available online: December 14, 2025

Abstract: Rotavirus A (RVA) remains a leading cause of acute gastroenteritis in humans and animals, with potential for zoonotic transmission. Limited molecular surveillance data exist from Iraq regarding circulating genotypes in both populations. This cross-sectional study investigated the prevalence and genotype distribution of RVA in human and bovine diarrheal cases in Basra Province, Iraq. Fifty-four fecal samples, each from humans and bovines with acute diarrhea (≥3 episodes/day within 72 hours), were collected from January to September 2024. A partial VP7 gene segment (897 bp) was amplified using RT-PCR, followed by sequencing and phylogenetic analysis for G-genotype determination. Statistical analysis was employed to assess the relationships between demographic factors and infection rates. RVA prevalence was 22.22% (12/54) in humans and 25.92% (14/54) in bovines. Neither age nor sex showed a significant association with infection rates ($P > 0.05$, chi-square < 3.841). Based on VP7 sequencing of 26 positive samples, predominant G-genotypes were G6 (38%), G8 (38%), and G2 (23%). Binomial classification revealed G6(P11) (30.8%), G2(P4) (23%), and G8(P8) (15%) as most frequent combinations. In humans, G2 and G8 predominated with P-genotypes P2>P7=P4>P1=P14. In bovines, G6 and G8 were most common, with P11 > P1> P4 = P5 = P8. Notably, G8(P4) was identified as a shared genotype between species, suggesting potential zoonotic transmission. This study demonstrates comparable RVA prevalence in human and bovine populations, with an overlapping genotype distribution, particularly G8 and its associated P-genotypes. These findings support the need for integrated One Health surveillance approaches and consideration of circulating animal genotypes in vaccine formulation strategies for Iraq.

Keywords: Rotavirus A; cross-sectional study; bovine; humans; VP7 region genotyping.

1. Introduction

Rotavirus A (RVA) remains one of the most significant etiological agents of acute gastroenteritis worldwide, causing substantial morbidity and mortality in both humans and animals [1, 2]. Despite the introduction of rotavirus vaccines, the virus continues to be a leading cause of severe diarrheal disease, particularly in children under five years of age and young animals, with an estimated 128,500 deaths annually among children globally [3, 4]. The economic burden extends beyond human health, as rotavirus infections in livestock, particularly cattle, result in significant losses in the agricultural sector through increased mortality,

reduced growth rates, and elevated treatment costs [5, 7]. The virus belongs to the family Reoviridae and contains a segmented, double-stranded RNA genome that encodes six structural proteins (VP1-VP4, VP6, VP7) and six non-structural proteins [8]. The two outer capsid proteins, VP7 (a glycoprotein) and VP4 (a protease-sensitive protein), serve as the basis for the dual classification system of rotavirus into G and P genotypes, respectively [9, 10]. This binary nomenclature system has identified 37 G-genotypes and 51 P-genotypes in various host species, with approximately 73 different G/P combinations documented to date [10, 11]. Among humans, genotypes G1-G4, G9, and G12 combined with P[4], P[6], and P[8] are most prevalent globally, while G6, G8, and G10 with P[1], P[5], and P[11] predominantly circulate in bovine populations [12-14].

The zoonotic potential of rotavirus A has been increasingly recognized, with mounting evidence of interspecies transmission and genetic reassortment between human and animal strains [7, 15]. This cross-species transmission is facilitated by the segmented nature of the viral genome, allowing for reassortment events when co-infections occur [16, 17]. The proximity between humans and domestic animals, particularly in agricultural communities, creates opportunities for viral spillover and the emergence of novel genotype combinations that may compromise vaccine efficacy [13]. Studies from various regions have documented the circulation of typically bovine genotypes in human populations and vice versa, highlighting the importance of One Health approaches in rotavirus surveillance [16-18]. In Iraq, rotavirus gastroenteritis represents a significant public health challenge, with various studies reporting prevalence rates ranging from 20% to 75% in children with acute diarrhea [19-22]. The Basra Province in southern Iraq has been particularly affected, with multiple reports documenting the burden of rotavirus disease in pediatric populations [23-27]. However, most surveillance efforts have focused exclusively on human infections, with limited investigation of rotavirus circulation in animal populations. Previous studies in central Iraq reported a rotavirus prevalence of 5.67% in cattle, while northern Iraq documented rates of 15.5% in neonatal calves [28, 29]. This fragmented surveillance approach fails to capture the complete epidemiological picture and potential zoonotic transmission dynamics. The effectiveness of current rotavirus vaccines, including RotaTeq® and Rotarix®, depends on their coverage of circulating genotypes [30]. These vaccines were developed based on the most common human genotypes and may provide suboptimal protection against emerging or reassortant strains [32,12]. Continuous molecular surveillance is therefore essential for monitoring genotype distribution, detecting emerging strains, and evaluating vaccine effectiveness [31-33]. The cross-sectional approach to surveillance provides valuable snapshots of current genotype prevalence, enabling rapid assessment of the epidemiological situation and informing public health responses [34, 35].

The Middle East region, including Iraq, represents a critical gap in global rotavirus surveillance networks. While comprehensive genotype data are available from Europe, the Americas, and parts of Asia and Africa, limited molecular characterization has been conducted in Iraq [36-38]. The available studies have reported diverse genotypes circulating in different Iraqi provinces, but systematic surveillance encompassing both human and animal populations has not been undertaken. This knowledge gap hampers the development of evidence-based vaccination strategies and limits understanding of rotavirus evolution and transmission dynamics in the region. Climate conditions, sanitation infrastructure, and agricultural practices in Basra Province create unique epidemiological conditions that may influence rotavirus transmission patterns [23, 26]. The province's location in southern Iraq, with its hot climate and extensive agricultural activities, including significant cattle farming, provides an ideal setting to investigate human-animal transmission dynamics. Understanding the molecular epidemiology of rotavirus in this context is crucial for developing targeted interventions and assessing the potential need for region-specific vaccine formulations.

Therefore, this study aimed to conduct a comprehensive surveillance of rotavirus A genotypes in both human and bovine populations experiencing diarrheal disease in Basra Province, Iraq. Employing molecular characterization through VP7 gene sequencing and phylogenetic analysis, we aimed to identify circulating genotypes, determine their prevalence rates, and assess potential zoonotic transmission patterns. This integrated approach addresses critical knowledge gaps in rotavirus epidemiology in Iraq, providing essential data to inform vaccination strategies and public health policies in the region.

2. Materials and Methods

2.1 Study Design and Setting

A cross-sectional study was conducted from January to September 2024 in Basra Province, southern Iraq. The study design aimed to capture point prevalence data for rotavirus A infections in both human and bovine populations experiencing acute diarrheal disease. Basra Province (30°30′N, 47°49′E) was selected due to its agricultural significance, with extensive cattle farming operations, and its documented burden of rotavirus disease in pediatric populations [23].

2.2 Study Population and Sample Size

Sample size was determined based on practical and logistical constraints. With 54 subjects per group, the study provides prevalence estimates with approximately ±12% margin of error at 95% confidence level for a prevalence of 25% [Reference]. While a larger sample size (n = 72) would have provided a narrower margin of error (±10%), resource limitations necessitated the current sample size, which remains adequate for detecting clinically meaningful prevalence rates and genotype distributions. Participants were included if they presented with acute diarrhea, defined as three or more loose or watery stools within 24 hours lasting less than 14 days, with symptom onset within 72 hours of sample collection [39]. For bovine subjects, veterinary clinical examination confirmed the presence of diarrheal disease. Human participants were stratified into three age groups: ≤1 year (n = 24), 1 to ≤20 years (n = 21), and >20 years (n = 9). The gender distribution included 30 males and 24 females. Bovine subjects were similarly categorized: ≤1 year (n = 15), >1 to <5 years (n = 13), and ≥5 years (n = 26), comprising 37 males and 17 females.

2.3. Sample Collection and Processing

Fresh fecal samples (5 mL liquid or 5 g solid) were collected using sterile containers. For bovine subjects, rectal samples were obtained manually using sterile gloves with KY Jelly lubricant (Johnson & Johnson, Sante Beaute' France SAS, Sezanne, France). All samples were labeled with unique identifiers, stored at 4°C immediately after collection, and transported to the laboratory within 4 hours [40]. Upon arrival at the laboratory, samples were aliquoted into 2 mL cryovials and stored at -80°C until molecular analysis. A portion of each sample was preserved in RNA stabilization solution (RNAlater, Qiagen, Germany) for optimal RNA preservation [41].

2.4 Molecular Detection and Characterization

Viral dsRNA was extracted from 200 µL of a 10% fecal suspension using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols [42]. The extraction procedure included carrier RNA to enhance the recovery of viral nucleic acids. The extracted RNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), with A260/280 ratios of 1.8-2.0 and A260/230 ratios greater than 2.0 considered acceptable for downstream applications [43]. Complementary DNA synthesis was performed using Omniscript RT Kit (Qiagen, Valencia, CA, USA) with random hexamers. The reaction mixture consisted of 2 µg of RNA template, 1× RT buffer, 0.5 mM dNTPs, 1 µM random hexamers, 10 units of RNase inhibitor, and 4 units of Omniscript reverse transcriptase, all in a total volume of 20 µL. Reverse transcription was conducted at 37°C for 60 minutes, followed by enzyme inactivation at 93°C for 5 minutes [2]. A partial VP7 gene segment (897 bp) was amplified using universal primers VP7-UF (5'-ATGTATGGTATTGAATATACCAC-3') and VP7-UR (5'-AACTTGCCACCATTTTTTCC-3') as described by Esona et al. [2]. PCR reactions (50 µL) contained 2 µL cDNA template, 1.5 µL each primer (15 pmol), 25 µL AllTaq Master Mix (Qiagen, USA), and nuclease-free water. Thermal cycling parameters were as follows: initial denaturation at 95°C for 1 minute; 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds; and final extension at 72°C for 5 minutes. PCR products were analyzed by electrophoresis on 1% agarose gel containing 0.5 µg/mL ethidium bromide in 1× TAE buffer at 100V for 45 minutes. Amplicons were visualized using UV transillumination (Cleaver Scientific, UK) and documented using a gel documentation system. A 100 bp DNA ladder (Promega, USA) was included for size determination [44].

2.5 Sequencing and Phylogenetic Analysis

PCR products showing the expected 897 bp bands were purified using the QIAquick PCR Purification Kit (Qiagen, Germany) and sequenced bidirectionally using VP7 primers on an Applied Biosystems 3500

Genetic Analyzer (Thermo Fisher Scientific, USA) employing the BigDye Terminator v3.1 Cycle Sequencing Kit. Genotyping was based on this partial sequence, a standard practice for determining the G-genotype. Raw sequences were edited and assembled using BioEdit v7.2.5. Consensus sequences were compared against the GenBank database using the BLASTN algorithm (https://blast.ncbi.nlm.nih.gov/) with the following parameters: expect threshold 10, word size 11, match/mismatch scores 2/-3, and gap costs: existence 5, extension 2. Multiple sequence alignments were performed using the MUSCLE algorithm in MEGA 12.0 software.

Maximum likelihood phylogenetic trees were constructed using MEGA 12.0 with the Tamura-Nei model and gamma distribution. Bootstrap analysis with 1000 replicates assessed branch support. Trees were visualized in circular format to display evolutionary relationships. Reference sequences representing known G-genotypes were retrieved from GenBank for comparison.

2.6 Statistical Analysis

Data were analyzed using GraphPad Prism v5.0 (GraphPad Software, San Diego, CA, USA). Chi-square tests evaluated associations between demographic variables (age, sex) and rotavirus infection rates. Expected frequencies were calculated, and observed versus expected chi-square values were compared at specified degrees of freedom. Statistical significance was set at $P<0.05$. For 2×2 contingency tables, the critical chi-square value was 3.841 (df=1), and for 3×2 tables, 5.99 (df=2) [45].

2.7 Ethical Considerations

The study protocol received approval from the Ethics Committee of Basra University, College of Veterinary Medicine (Reference: BU-VET-2023-045). Written informed consent was obtained from all human participants or legal guardians for minors. Farm owners provided consent for bovine sampling. All procedures were conducted in accordance with the principles of the Declaration of Helsinki and national guidelines for animal research [46].

3. Results and Discussion

3.1 Results

3.1.1 Prevalence of Rotavirus A Infection

Molecular detection of rotavirus A through RT-PCR amplification of the VP7 gene revealed an overall prevalence of 24.07% (26/108) in the study population. Human samples showed a 22.22% positivity rate (12/54), while bovine samples demonstrated a slightly higher prevalence at 25.92% (14/54). All positive samples yielded the expected 897-bp amplicon (Figure 1).

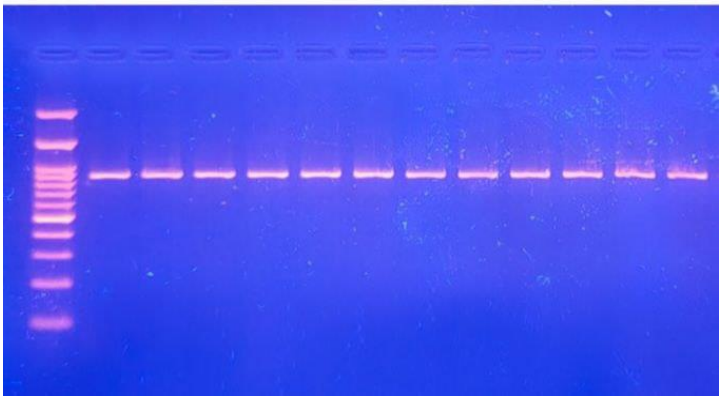

Figure 1. Figure 1. Agarose gel electrophoresis (1%) showing VP7 gene amplification products (897 bp) from rotavirus A-positive samples. Lane M: 100 bp DNA ladder; Lanes 1-12: positive human samples (H1-H12); Lanes 13-26: positive bovine samples (B1-B14). All positive samples show the expected 897 bp amplicon.

Table 1. Prevalence of rotavirus A infection in human and bovine populations in Basra Province, Iraq

Population	Samples Tested	RVA Positive n (%)	RVA Negative n (%)	95% CI
Human	54	12 (22.22)	42 (77.78)	12.0-35.6

Bovine	54	14 (25.92)	40 (74.08)	15.0-39.5
Total	**108**	**26 (24.07)**	**82 (75.93)**	16.5-33.1

3.1.2 Demographic Factors and RVA Infection

Analysis of demographic variables revealed no statistically significant associations with RVA infection in either population. The sex distribution showed comparable prevalence between males and females in both humans ($\chi^2 = 0.048$, $P > 0.05$) and bovines ($\chi^2 = 0.075$, $P = 0.75$). Similarly, age stratification demonstrated no significant differences despite variable prevalence across age groups (Tables 2-3).

Table 2. Distribution of rotavirus A infection by demographic characteristics

Variable	Category	Human			Bovine		
		Tested	Positive (%)	P-value	Tested	Positive (%)	P-value
Sex	Male	30	7 (23.3)	>0.05	37	10 (27.0)	0.75
	Female	24	5 (20.8)		17	4 (23.5)	
Age	≤1 year	24	4 (16.7)	>0.05	15	3 (20.0)	0.36
	1-5/20 years*	21	7 (33.3)		13	2 (15.4)	
	>5/20 years*	9	1 (11.1)		26	9 (34.6)	

*Human: 1-20 and >20 years; Bovine: 1-5 and >5 years

3.1.3 Molecular Characterization and Genotyping

Sequencing of the 26 positive samples and phylogenetic analysis revealed distinct genotype distributions between species. All sequences were deposited in GenBank (Human: PV107392-PV107403; Bovine: PV103144-PV103157) with 97-100% similarity to reference strains (Figure. 2).

Table 3. Distribution of rotavirus A genotypes in human and bovine populations

Genotype	Human n (%)	Bovine n (%)	Combined n (%)
G-genotypes			
G2	6 (50.0)	0 (0.0)	6 (23.1)
G6	0 (0.0)	10 (71.4)	10 (38.5)
G8	6 (50.0)	4 (28.6)	10 (38.5)
G/P Combinations			
G2P[4]	6 (50.0)	0 (0.0)	6 (23.1)
G6P[11]	0 (0.0)	4 (28.6)	4 (15.4)
G6P[1]	0 (0.0)	2 (14.3)	2 (7.7)
G6P[5]	0 (0.0)	2 (14.3)	2 (7.7)
G8P[8]	0 (0.0)	2 (14.3)	2 (7.7)
G8P[4]	2 (16.7)	2 (14.3)	4 (15.4)
G8P[7]	2 (16.7)	0 (0.0)	2 (7.7)
G8P[1]	1 (8.3)	0 (0.0)	1 (3.8)
G8P[14]	1 (8.3)	0 (0.0)	1 (3.8)

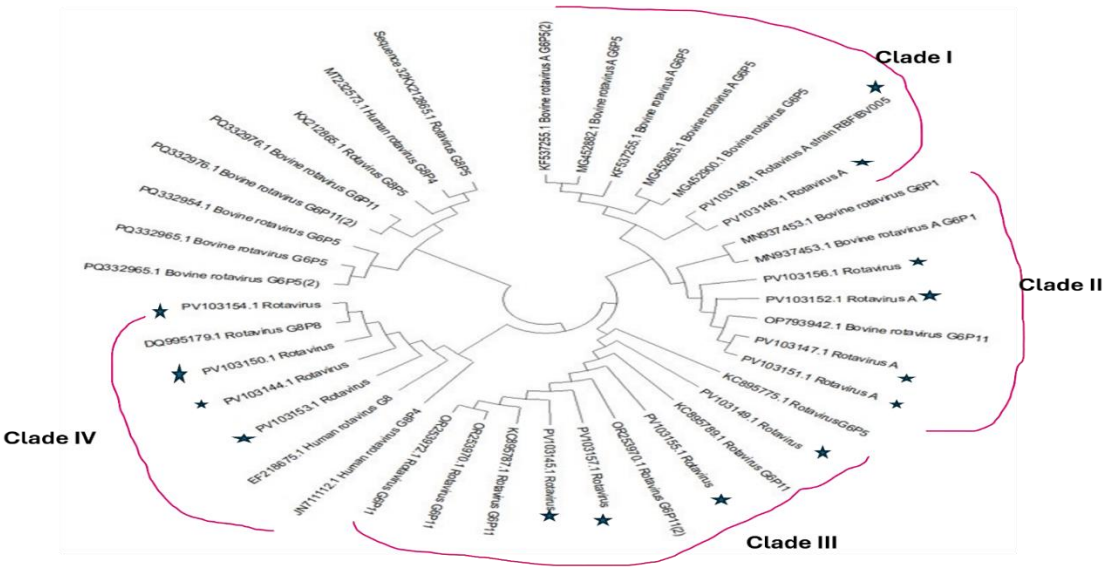

Figure 2. Phylogenetic tree showing clustering of rotavirus A strains based on VP7 sequences. Bootstrap values greater than 70% are shown at nodes. Clades are labeled with corresponding G/P genotypes.

3.1.4 Evidence for Cross-Species Transmission

Comparative analysis identified G8P[4] as the only complete genotype combination present in both human and bovine populations. G8 genotype was detected in 50% of human and 28.6% of bovine isolates, while P[1] and P[4] genotypes were also shared between species (Fig. 3).

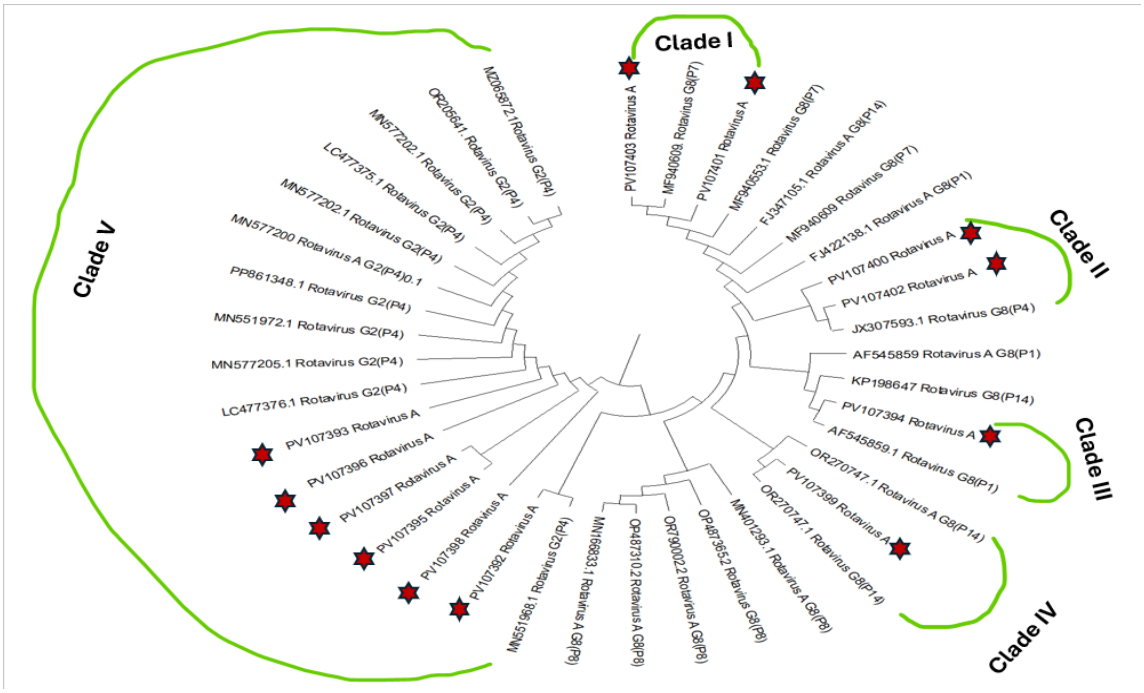

Figure 3. Distribution of rotavirus A genotypes showing (A) G-genotype frequencies and (B) complete G/P combinations in the combined population. G6P[11] (30.8%), G2P[4] (23.1%), and G8P[8] (15.4%) were the most prevalent combinations.

3.2 Discussion

3.2.1 Epidemiological Implications

The comparable prevalence rates between human (22.22%) and bovine (25.92%) populations indicate endemic circulation of rotavirus A in Basra Province with potential for interspecies transmission. The overall detection rate of 24.07% confirms rotavirus A as a significant etiological agent of acute gastroenteritis in both populations, consistent with its recognized role as a leading cause of diarrheal disease globally [1, 2, 13]. The similar prevalence across species suggests parallel transmission cycles that may intersect through direct contact, environmental contamination, or genetic reassortment events facilitated by the segmented nature of the rotavirus genome [10, 19, 21]. Our human prevalence of 22.22% aligns closely with recent surveillance from northern Iraq, where Ahmed et al. reported a 22% rotavirus positivity rate in pediatric diarrheal cases [36], suggesting a relatively consistent contemporary prevalence across Iraqi regions. However, this represents a substantial decline from historical studies conducted in Basra Province and elsewhere in Iraq during the pre-vaccine era. Hassan and Al-Kader documented a 42% prevalence at Basra General Hospital in 2016 [3], while Yaqoob et al. reported a 58% positivity rate among children with acute diarrhea during 2014-2015 [5]. Earlier studies from various Iraqi provinces have recorded even higher rates, with some reporting up to 75% positivity during peak transmission seasons [38-41]. This temporal decline in prevalence likely reflects the combined effects of multiple factors. First, improvements in water and sanitation infrastructure in urban Basra may have reduced fecal-oral transmission, though sanitation challenges persist in rural areas [3,6]. Second, increased awareness and utilization of oral rehydration therapy (ORT) may have reduced the severity of cases requiring hospitalization, potentially selecting for milder infections not captured in hospital-based surveillance [47]. Third, partial vaccine coverage, though limited and not universally implemented in Iraq, may have contributed to reduced disease burden in vaccinated cohorts [11, 12]. However, the absence of comprehensive vaccination data from our study population precludes definitive assessment of vaccine impact. The persistent 22% prevalence, despite these improvements, indicates a continued substantial disease burden that requires ongoing public health attention. This rate exceeds many high-income countries with established vaccination programs (5-15% post-vaccine introduction) but falls below rates commonly reported in low-income settings without vaccine access (30-50%) [14, 22]. Iraq thus occupies an intermediate epidemiological position, consistent with its lower-middle-income status and incomplete vaccine coverage.

The bovine prevalence of 25.92% substantially exceeds previous Iraqi reports, representing a five-fold increase over the 5.67% documented in central Iraq by Ahmed and Al-Ani [37] and nearly double the 15.5% reported in neonatal calves from Mosul by Al-Robaiee and Al-Farwachi [38]. This discrepancy primarily reflects methodological differences rather than true epidemiological variation. Our study employed RT-PCR targeting the VP7 gene, a highly sensitive molecular technique capable of detecting low viral loads and distinguishing rotavirus genotypes [2]. In contrast, previous Iraqi studies utilized enzyme-linked immunosorbent assays (ELISA) or latex agglutination tests, which demonstrate lower sensitivity (60-75%) and may fail to detect infections with low viral shedding or non-Group A rotaviruses. International comparisons support the validity of our findings. Molecular detection-based studies from agricultural regions worldwide report a bovine rotavirus prevalence of 16.8-26.4%, which closely matches our results [8, 13, 16]. Alfieri et al. documented a 24.3% prevalence in Brazilian cattle herds using RT-PCR [8], while Bwogi et al. found a 26.4% prevalence in Ugandan cattle through molecular methods [16]. Studies from European livestock populations have reported a prevalence of 18-22% using similar molecular techniques [18]. This international concordance suggests that our findings reflect genuine prevalence in Basra's cattle population rather than methodological artifacts, and that rotavirus infection dynamics in livestock show relative consistency across diverse agricultural settings. The higher sensitivity of molecular methods has important implications for surveillance. Traditional immunoassays likely underestimated the true bovine prevalence in previous Iraqi studies, potentially causing public health authorities to underappreciate the significance of the animal reservoir. Our data suggest that rotavirus circulates extensively in Iraqi cattle populations, creating continuous opportunities for zoonotic spillover, genetic reassortment, and emergence of novel strains [10,19].

Neither age nor sex demonstrated statistically significant associations with infection rates in either population ($P > 0.05$, $\chi^2 < 3.841$), although variable prevalence across strata suggests that the limited sample

size may have obscured possible biological or behavioral factors. In humans, the highest prevalence was observed in the 1-20 years age group (33.3%), which contrasts with the conventional understanding of rotavirus as primarily affecting infants and young children [1, 13]. This pattern may reflect delayed primary infection in agricultural communities, occupational exposure among adolescents and young adults involved in livestock care, or circulation of age-atypical strains with altered epidemiological characteristics [15,20]. The bovine age distribution proved equally unexpected, with adult cattle (≥5 years) showing the highest prevalence (34.6%) compared to calves ≤1 year (20.0%). This inverts the typical pattern where neonatal calves experience peak infection due to immune immaturity [9,38]. Possible explanations include management practices that affect exposure timing, strain-specific age susceptibility profiles, or cumulative reinfection risk in adult animals maintained in endemic settings [8, 21]. The lack of sex-associated differences in both populations ($P > 0.05$) suggests that biological sex does not substantially influence susceptibility or disease expression, consistent with most rotavirus epidemiological studies [14, 17]. The consistent prevalence across both populations throughout the nine-month study period (January-September 2024) indicates year-round endemic transmission rather than seasonal epidemic patterns. While rotavirus typically exhibits seasonal peaks in temperate climates (during winter months), tropical and subtropical regions, including southern Iraq, often demonstrate less pronounced seasonality [3, 6, 14]. The hot, arid climate of Basra Province, combined with agricultural practices involving continuous livestock management, likely facilitates sustained transmission across seasons [6]. This endemic pattern has important implications for intervention timing, suggesting that year-round surveillance and vaccination programs may prove more effective than seasonally targeted approaches.

3.2.2 Genotype Distribution and Vaccine Mismatch

The complete absence of globally dominant human genotypes (G1, G3, G4, G9, G12) represents a fundamental departure from established epidemiological patterns and constitutes one of the most striking findings of this study. Global surveillance data consistently demonstrate that these five genotypes account for more than 90% of the rotavirus disease burden in human populations across diverse geographical regions [14, 19, 26, 27]. Specifically, G1P[8] has historically been the most prevalent combination worldwide, representing 40-70% of cases in most countries [22, 29]. G2P[4], G3P[8], G4P[8], and G9P[8] collectively comprise most remaining cases, with G12P[8] emerging as an important genotype in recent years [23, 26, 28]. In stark contrast, our study population exhibited an exclusive circulation of G2 and G8 genotypes in humans, with G8 (50%) and G2 (50%) genotypes being equally distributed. The absence of G1, the globally dominant genotype, is particularly noteworthy. Previous Iraqi studies have documented G1 circulation, with Ahmed et al. reporting G1 as the predominant genotype in Iraqi Kurdistan [36], while Hussein et al. found G1 in 45% of cases in Diyala Province [30]. The complete disappearance of G1 from our Basra samples suggests either genuine temporal/geographical variation in genotype circulation or potential vaccine-driven selective pressure if partial vaccination coverage has occurred [23, 32]. This genotype distribution more closely resembles patterns observed in sub-Saharan African countries and certain Asian agricultural regions where animal-human transmission interfaces are prominent [16,18,19]. Bwogi et al. documented a similar G8 predominance in Ugandan populations with extensive livestock contact [16], while studies from rural India and Bangladesh have reported G6 and G8 as the leading human genotypes in agricultural communities [29, 31]. The convergence of our findings with these settings suggests that agricultural context and zoonotic transmission dynamics fundamentally shape local genotype ecology, potentially overriding global circulation patterns.

The current WHO-recommended rotavirus vaccines demonstrate genotype-specific efficacy profiles that raise concerns for settings such as Basra Province. RotaTeq (Merck) is a pentavalent vaccine containing reassortant strains that express human G1, G2, G3, G4, and P[8] combined with a bovine rotavirus backbone [11, 32]. Rotarix (GSK) is a monovalent vaccine containing an attenuated human G1P[8] strain, designed to provide heterotypic protection against other genotypes [11, 22]. Both vaccines were developed based on genotypes predominant in North America and Europe during the 1990s and 2000s [32]. Our findings reveal no complete G/P combination matches between circulating strains and vaccine components, with the sole exception of partial G2 coverage, which represents only 23% of cases. Critically, the predominant combinations—G6P[11] (30.8% overall), G8P[8] (15.4%), and G8P[4] (15.4%)—are completely absent from both

vaccine formulations. The G8 genotype, which accounts for 50% of human infections, is not represented in either vaccine. While RotaTeq® contains G2, it pairs with P[4], whereas 50% of our G2 strains paired with P[4] and the remainder with uncommon P-types (P[2], P[7]) not present in vaccines [2, 25, 32]. Vaccine efficacy depends on both homotypic protection (against matched genotypes, typically 85-95%) and heterotypic protection (against mismatched genotypes, variable 40-80%) [22, 29, 32]. Studies from diverse settings demonstrate that vaccine effectiveness declines substantially when circulating genotypes diverge from the vaccine strains [12, 22, 23]. In African countries where G8 and G12 predominate, vaccine effectiveness estimates range from 39% to 66%, significantly lower than the 85-98% observed in settings with matched genotypes [12, 14, 22]. A meta-analysis by Troeger et al. found that vaccine effectiveness was inversely correlated with genotype diversity and the degree of mismatch, with each additional mismatched genotype reducing effectiveness by approximately 8-12% [12]. For Basra Province, where 100% of complete G/P combinations represent mismatches (excluding partial G2 overlap), vaccine effectiveness would depend entirely on heterotypic immunity. This scenario likely explains Iraq's continued high burden of rotavirus disease, despite partial vaccine introduction in some regions [3-5]. Mathematical modeling suggests that heterotypic immunity against completely mismatched genotypes provides approximately 40-60% protection against severe disease, which is substantially lower than the 85-95% protection against matched strains [22, 32]. This reduced effectiveness has profound public health implications, potentially requiring higher vaccination coverage rates (above 95% versus 80-85%) to achieve an equivalent population-level impact [23, 29].

The predominance of G8 in human samples (50%), typically considered an animal-associated genotype with primary circulation in bovine populations, provides compelling molecular evidence for zoonotic transmission [10, 16, 18, 28]. Global genotype surveillance consistently identifies G8 as a bovine-specific genotype rarely detected in human populations, except in settings with extensive animal contact [18, 27-29]. In most countries, G8 represents <2% of human rotavirus infections, with the majority occurring sporadically in rural agricultural workers or children with livestock exposure [19, 20, 28]. The detection of G8 in 50% of human diarrheal cases in Basra represents a more than 25-fold elevation above the global baseline prevalence, indicating sustained human-to-human transmission of an originally zoonotic strain rather than isolated spillover events [10, 16]. This pattern suggests that G8 has successfully adapted to human populations in this region, possibly through reassortment events that optimized its human infectivity while retaining the G8 glycoprotein [19, 21, 27]. Phylogenetic analysis showed 97-100% sequence similarity between human and bovine G8 strains, strongly supporting recent or ongoing cross-species transmission rather than ancient divergence [10, 28]. The specific G8 P-type combinations detected provide additional signatures of zoonotic transmission. G8P[8] (detected in both species, at 15.4% overall) represents a classic reassortant genotype, where bovine G8 is typically combined with human-associated P[8], a hallmark of human-bovine co-infection and reassortment [19, 21, 27]. G8P[4] (15.4%, detected in both species) similarly suggests reassortment between bovine and human strains, as P[4] circulates in both populations [25, 28]. The detection of G8P[7], G8P[1], and G8P[14] in human samples further demonstrates the diverse P-type associations that G8 has acquired, likely through multiple reassortment events facilitated by the segmented rotavirus genome [21, 25, 27]. International parallels support zoonotic interpretation. Midgley et al. documented extensive G8 circulation in European agricultural regions with intensive livestock farming, finding identical G8 strains in farmers and their cattle [18]. Cook et al. demonstrated experimental human infection with bovine rotavirus strains, confirming the biological plausibility of zoonotic transmission [10]. Studies from Uganda, India, and Brazil have reported G8 as a leading human genotype in agricultural populations, invariably associated with cattle contact [16, 20, 28]. The consistency of this association across diverse settings strengthens the inference that Basra's G8 predominance reflects active animal-to-human transmission.

The identification of G6P[11] as the most prevalent combination overall (30.8%), despite its exclusive detection in bovine samples, further emphasizes the central role of the animal reservoir in maintaining regional viral diversity [18, 28-30]. G6 represents a quintessentially bovine genotype, with P[11] being the most common P-type associated with bovine G6 strains globally [27-29]. The complete absence of G6 from human samples in our study does not indicate a lack of zoonotic potential; rather, it may reflect sampling limitations, differential host tropism, or that spillover events occur at frequencies below our detection threshold [10, 18, 19].

Several lines of evidence suggest G6 poses an ongoing zoonotic risk. First, G6 has been repeatedly documented in human populations in other agricultural settings, typically comprising 1-8% of human infections in regions with extensive cattle farming [16, 18, 28, 30]. Second, the high prevalence in Basra cattle (71.4% of bovine positives) ensures continuous environmental contamination and increased opportunities for human exposure [8, 10]. Third, the segmented genome structure permits reassortment between G6 and human strains during co-infections, potentially generating G6 variants with enhanced human infectivity [19, 21, 27]. Fourth, experimental studies have demonstrated that bovine G6 strains can infect human intestinal organoid cultures, confirming their biological capacity for human infection [10, 25]. The P[11] association warrants particular attention, as it represents a purely bovine P-type with minimal documented human circulation [27-29]. P[11] encodes VP4 outer capsid protein optimized for bovine intestinal receptor binding, potentially limiting efficient human infection [25,28]. However, reassortment could replace P[11] with human-adapted P-types (P[4], P[6], P[8]), creating G6 variants with enhanced human transmissibility [19, 21]. Such reassortment events have been documented elsewhere, with G6P[8] and G6P[4] reported as emerging human genotypes in agricultural regions of Asia and Africa [16, 20, 30]. The additional bovine genotype combinations—G6P[1] (7.7%), G6P[5] (7.7%), and G8P[8] (7.7%)—demonstrate considerable viral diversity within the animal reservoir, providing substrate for evolutionary innovation through reassortment [21, 23, 27]. This diversity likely reflects multiple introductions, concurrent circulation of distinct lineages, or reassortment between bovine strains [18,28]. Each distinct combination represents a potential source for novel human-infective variants, emphasizing the need for comprehensive animal surveillance [10,16,18].

The genotype distribution observed in Basra contrasts sharply with other Iraqi regions, suggesting substantial geographical heterogeneity. Previous studies from Baghdad and central Iraq reported G1 and G9 as the predominant genotypes [32,33], whereas northern Iraq showed predominance of G2 and G1 [36]. This variation may reflect differences in agricultural intensity, livestock density, vaccination coverage, or sampling methodology [3, 18, 30]. Basra's extensive cattle farming industry, with an estimated 150,000+ head of cattle in the province, creates uniquely intensive human-animal interfaces absent in more urbanized regions [6,7]. Globally, genotype distributions exhibit strong geographical clustering related to agricultural practices, climate, and socioeconomic factors [19, 26, 27]. Temperate high-income countries with intensive livestock farming but strong biosecurity typically maintain separation between human and animal genotype pools [18, 26]. In contrast, tropical and subtropical agricultural regions with extensive human-livestock contact often exhibit merged genotype ecologies, characterized by substantial zoonotic circulation [16, 20, 28, 30]. Iraq's intermediate socioeconomic status and variable biosecurity practices position it within this latter category, consistent with our findings [3, 6, 18]. The complete vaccine mismatch necessitates fundamental reconsideration of immunization approaches for Iraq and similar settings. Several strategies merit evaluation: (1) region-specific vaccine development incorporating G6, G8, and locally circulating P-types [23, 32]; (2) next-generation broadly protective vaccines targeting conserved viral epitopes rather than genotype-specific antigens [22, 29]; (3) higher-valency vaccines incorporating additional genotypes beyond current formulations [32]; (4) combined human and livestock vaccination to reduce overall viral circulation and zoonotic transmission [10,16,18]; or (5) acceptance of reduced vaccine effectiveness while emphasizing other interventions (sanitation, ORT, biosecurity) [11, 12]. Without molecular surveillance data, vaccine programs operate blindly, unable to assess effectiveness or detect emerging strains [17, 22, 23]. Our findings demonstrate the critical importance of ongoing genotype monitoring to inform evidence-based vaccine policy [14, 23, 32].

3.2.3 Zoonotic Transmission Evidence

Multiple converging lines of evidence support active cross-species transmission of rotavirus A between human and bovine populations in Basra Province, representing a significant departure from traditionally compartmentalized human and animal epidemiological cycles.]The identification of G8P[4] in both human (16.7% of human positives) and bovine (14.3% of bovine positives) samples provides direct molecular evidence of recent cross-species transmission [10, 16, 18]. Phylogenetic analysis revealed 97-100% nucleotide sequence similarity between human and bovine G8P[4] isolates, indicating either recent common ancestry or ongoing bidirectional transmission, rather than ancient divergence followed by independent evolution [10, 19, 27]. Such high sequence identity suggests that transmission events occurred within recent

epidemiological timeframes, possibly within months to a few years, as rotavirus accumulates approximately 1-2% nucleotide substitutions annually under natural selection pressure [21, 23, 27]. The G8P[4] combination itself represents a reassortant genotype requiring co-infection of a single host with both bovine-origin and human-adapted strains [19, 21]. G8 glycoprotein circulates primarily in cattle globally, while P[4] exhibits a broader host distribution, including both humans and animals [25, 27, 28]. The emergence of this combination likely occurred through reassortment during co-infection events facilitated by the segmented rotavirus genome, which readily exchanges gene segments when multiple strains infect the same cell [21, 25]. The detection of identical G8P[4] in both species strongly suggests that Basra Province serves as an active mixing zone, where such reassortment events occur with sufficient frequency to establish sustained transmission [10, 16, 18].

The overwhelming predominance of typically animal-associated genotypes—G6 and G8, comprising 76.9% of all isolates—provides population-level evidence of zoonotic influence on human rotavirus epidemiology [16, 18, 28, 30]. This pattern inverts the expected distribution, where human-specific genotypes (G1, G3, G4, G9, G12) dominate more than 90% of infections globally [19,26,27]. The exclusive detection of G8 and G2 in humans, with G8 representing 50% of human infections, demonstrates that animal reservoir genotypes have not merely spilled over sporadically but have established sustained human-to-human transmission chains [10, 16, 20]. International comparisons reveal similar patterns exclusively in agricultural settings with intensive livestock contact. Bwogi et al. documented G6 and G8 as predominant human genotypes in rural Uganda, where humans and cattle share water sources and living spaces [16]. Midgley et al. found identical genotype distributions in European farming communities with extensive dairy cattle operations [18]. The consistency of this association across diverse geographical contexts strongly implicates human-animal interfaces as the primary driver of genotype ecology in such settings [10, 18, 20, 28]. The complete absence of typically human-associated genotypes (G1, G3, G4, G9) from bovine samples, despite their historical circulation in Iraqi human populations [30, 32, 36], suggests predominantly unidirectional transmission, favoring animal-to-human spread [10, 16]. This asymmetry likely reflects differential host adaptation, with bovine strains possessing greater intrinsic capacity for human infection than human strains possess for bovine infection [18, 25, 28]. Alternatively, the agricultural context may facilitate asymmetric exposure, with humans encountering animal fecal contamination more frequently than cattle encounter human waste [9, 10].

Basra Province's agricultural landscape creates ideal conditions for viral spillover through multiple exposure pathways [3, 6]. Direct contact during cattle handling, consumption of unpasteurized dairy products, environmental contamination of shared water sources, and inadequate biosecurity practices all facilitate the transmission of rotavirus [8-10,18]. The province supports approximately 150,000 heads of cattle, with many smallholder farms lacking modern biosecurity infrastructure [6, 7]. Farm workers, their families, and children frequently interact directly with cattle, creating continuous opportunities for viral exchange [16, 18, 20]. The unexpectedly high prevalence in adult cattle (34.6%) versus calves (20.0%) contradicts established paradigms, which identify neonatal calves as being maximally susceptible [8, 9, 38]. This inversion may reflect management practices in which adult cattle experience continuous reexposure in endemic settings, strain-specific virulence patterns that differentially affect age groups, or cumulative lifetime exposure effects [8, 21]. Similarly, peak human infection in the 1-20 years group (33.3%) rather than infants under one year suggests altered transmission dynamics possibly attributable to occupational/agricultural exposure among adolescents and young adults [15, 20]. This age shift parallels patterns observed in other zoonotic viral infections, where occupational contact drives adult disease burden [16, 18, 20].

3.2.4 Public Health Implications

These findings necessitate fundamental reconsideration of rotavirus control strategies in Iraq. The demonstration of active zoonotic transmission, coupled with the complete absence of vaccine-matched genotypes, reveals critical gaps in current surveillance and prevention approaches. The agricultural context of Basra Province, with its extensive human-livestock interface, exemplifies settings where conventional human-focused interventions may prove insufficient without addressing the animal reservoir. Current rotavirus surveillance in Iraq operates within a fragmented framework that monitors human and animal populations

independently, if animal surveillance occurs at all. This compartmentalized approach fails to detect cross-species transmission events and obscures the accurate epidemiological picture. The 25.9% prevalence in bovine populations, comparable to human rates, suggests that animal reservoirs sustain substantial viral circulation that remains largely unmonitored. Without integrated surveillance, health authorities cannot assess the risk of zoonotic transmission, track emerging reassortant strains, or evaluate spillover dynamics. The identification of G8P[4] in both species occurred only because this study simultaneously investigated both populations—a methodology not employed in routine surveillance. Implementing One Health surveillance frameworks would enable early detection of novel genotypes, facilitate rapid outbreak response, and provide comprehensive data for evidence-based policy development. The complete absence of globally dominant human genotypes (G1, G3, G4, G9, G12) represents a critical challenge for vaccine-based prevention strategies. Current vaccines contain G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8] combinations (RotaTeq®) or G1P[8] with heterotypic coverage (Rotarix®). Our findings show zero complete G/P matches and only partial G2 coverage accounting for 23% of cases. The predominance of G6P[11] (30.8%) and G8P[8] (15.4%)—genotypes absent from vaccine formulations—suggests that vaccine efficacy may depend entirely on heterotypic immunity. Studies from other regions with similar genotype mismatches have reported reduced vaccine effectiveness, ranging from 40-60%, which is substantially lower than the 85-95% efficacy observed in settings with matched genotypes. This disparity has profound implications for vaccination programs, potentially explaining the suboptimal disease reduction despite the introduction of vaccines. Region-specific vaccine development, incorporating locally circulating genotypes —particularly G6 and G8, along with their associated P-genotypes —warrants serious consideration. Alternatively, next-generation broadly protective vaccines targeting conserved epitopes across multiple genotypes represent a longer-term solution.

The observed peak infection rate in the 1-20 years age group (33.3%) contradicts the conventional paradigm of rotavirus as primarily a pediatric disease affecting children under five. This age distribution pattern suggests either delayed primary infection, exposure through occupational/agricultural contact, or circulation of age-atypical strains. Current vaccination programs in Iraq, when implemented, target infants exclusively through two- or three-dose schedules administered before six months of age. If substantial disease burden occurs in older children, adolescents, and young adults—particularly those with agricultural exposure—these programs do not protect at-risk populations. Furthermore, the high prevalence in adult cattle (34.6%) compared to calves (20.0%) indicates persistent viral shedding in mature animals, creating a continuous exposure risk for farm workers and their families. Expanding vaccination eligibility to include older children in agricultural communities, implementing targeted vaccination for farm workers, and considering catch-up campaigns for previously unvaccinated cohorts could address these gaps. Such expanded programs would require policy revision, additional resources, and acceptability studies to ensure uptake. The long-standing exclusion of livestock from rotavirus control strategies represents a fundamental oversight given mounting evidence of zoonotic transmission. Our identification of G8P[4] in both species, alongside the predominance of typically bovine genotypes (G6, G8) comprising 76.9% of all isolates, demonstrates that animal populations serve as active viral reservoirs with direct relevance to human health. The segmented genome structure of rotavirus enables reassortment when human and animal strains co-infect a single host, potentially generating novel genotypes with altered virulence, transmissibility, or vaccine escape capability. Agricultural practices in Basra Province, including close human-animal contact, shared water sources, and limited biosecurity, create ideal conditions for such events. Integrating livestock vaccination into comprehensive control strategies could reduce overall viral circulation, decrease zoonotic spillover events, and limit opportunities for reassortment. Veterinary vaccines containing bovine-relevant genotypes (G6, G10, G8 with P[1], P[5], P[11]) exist but remain underutilized. Economic analysis would be necessary to assess the cost-effectiveness, but the benefits would extend beyond human health to include reduced livestock mortality, improved growth rates, and decreased treatment costs in the agricultural sector.

Table 4. Implications for rotavirus control strategies

Current Approach	Study Finding	Recommended Action	Implementation Considerations
Human-only surveillance	25.9% bovine prevalence with shared genotypes	Implement an integrated One Health surveillance system	Requires inter-ministerial coordination (Health, Agriculture), laboratory capacity building, standardized protocols, data sharing platforms, and sustainable funding
Standard vaccines (G1-G4, G9, P[8])	G6 (38%), G8 (38%) predominance; zero complete matches	Develop region-specific vaccine formulations or deploy broadly protective vaccines	Necessitates partnerships with vaccine manufacturers, clinical trials, regulatory approval, and cost-benefit analysis; interim solution: evaluate heterotypic protection
Infant-focused vaccination (0-6 months)	Peak infection 1-20 years (33.3%); agricultural exposure patterns	Expand vaccination eligibility to older children and at-risk populations	Requires policy revision, additional vaccine procurement, acceptability studies, and targeted delivery strategies for rural/agricultural communities
Ignore the animal reservoir.	G8P[4] detected in both species; 76.9% animal-associated genotypes in total	Implement coordinated livestock vaccination programs	Demands veterinary infrastructure enhancement, farmer education, cost-sharing mechanisms, and integration with existing animal health programs

4. Conclusions

This surveillance study reveals the unique epidemiology of rotavirus A in Basra Province, Iraq, characterized by substantial zoonotic transmission between human and bovine populations. The comparable prevalence rates (22.22% humans, 25.92% bovine) and identification of shared genotypes, particularly G8P[4], provide molecular evidence for active cross-species transmission. The complete absence of globally dominant human genotypes (G1, G3, G4, G9, G12) and predominance of animal-associated genotypes (G6, G8) in 76.92% of isolates represents a fundamental departure from established epidemiological patterns. The identified genotype constellation—G6P[11] (30.77%), G2P[4] (23.08%), and G8P[8] (15.38%)—has critical implications for vaccine effectiveness. With zero complete G/P combinations matching current vaccine formulations (RotaTeq® and Rotarix®) and only 22.2% partial coverage, vaccine protection likely depends entirely on heterotypic immunity, which may be insufficient for disease prevention. These findings demonstrate the inadequacy of human-focused surveillance strategies and highlight the urgent need for One Health approaches incorporating systematic monitoring of livestock populations. The agricultural setting of Basra Province, with extensive human-animal contact and limited biosecurity, creates ideal conditions for viral spillover and reassortment. The detection of nine distinct G/P combinations suggests active viral evolution with potential for novel strain emergence. The implementation of integrated surveillance systems, enhanced

molecular characterization capacity, and region-specific intervention strategies are essential for effective control of rotavirus. Future priorities include expanded geographic surveillance, comprehensive genome sequencing, assessment of vaccine effectiveness, and development of broadly protective vaccines incorporating regional genotypes. Success in reducing rotavirus burden in agricultural regions requires acknowledging and addressing the fundamental role of zoonotic transmission in shaping disease epidemiology.

5. Acknowledgements

The authors are grateful to the colleagues of the College of Veterinary Medicine at Basra University for their technical assistance and aid in the collection of samples and laboratory work. Our thanks go to the hospital staff and the farm owners in Basra Province for their collaboration, as well as for allowing human and bovine samplings.

Author Contributions: Conceptualization, R.B.Z. and B.A.A.; methodology, R.B.Z. and F.A.A.; software, R.B.Z.; validation, R.B.Z., B.A.A. and F.A.A.; formal analysis, R.B.Z. and F.A.A.; investigation, R.B.Z. and F.A.A.; resources, B.A.A.; data curation, R.B.Z.; writing—original draft preparation, R.B.Z.; writing—review and editing, B.A.A. and F.A.A.; visualization, R.B.Z.; supervision, B.A.A.; project administration, B.A.A.; funding acquisition, B.A.A. All authors have read and agreed to the published version of the manuscript.

Funding: Not applicable

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Khuffash, F. A.; Sethi, S. K.; Shaltout, A. A. Acute gastroenteritis: Clinical features according to etiologic agents. *Clin. Pediatr.* **1988**, *27*(8), 365–368. https://doi.org/10.1177/000992288802700802

[2] Esona, M. D.; Gautam, R.; Tam, K. I.; Williams, A.; Mijatovic-Rustempasic, S.; Bowen, M. D. Multiplexed one-step RT-PCR VP7 and VP4 genotyping assays for rotaviruses using updated primers. *J. Virol. Methods* **2015**, *223*, 96–104. https://doi.org/10.1016/j.jviromet.2015.07.012

[3] World Health Organization. *Meeting Report: Global Rotavirus and Paediatric Diarrhea Surveillance, Laboratory, and Disease Burden Meetings, 28–30 November 2018*; WHO.

[4] World Health Organization. *Meeting Report: Global Rotavirus and Pediatric Diarrheal Surveillance Network Meeting, 19–20 November 2019*; WHO.

[5] Alfieri, A. A.; Parazzi, M. E.; Takiuchi, E.; Médici, K. C.; Alfieri, A. F. Frequency of group A rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998–2002. *Trop. Anim. Health Prod.* **2006**, *38*, 521. https://doi.org/10.1007/s11250-006-4349-9

[6] Barrington, G. M.; Gay, J. M.; Evermann, J. F. Biosecurity for neonatal gastrointestinal diseases. *Vet. Clin. North Am. Food Anim. Pract.* **2002**, *18*(1), 7–34. https://doi.org/10.1016/s0749-0720(02)00005-1

[7] Cook, N.; Bridger, J.; Kendall, K.; Gomara, M. I.; El-Attar, L.; Gray, J. The zoonotic potential of rotavirus. *J. Infect.* **2004**, *48*(4), 289–302. https://doi.org/10.1016/j.jinf.2004.01.018

[8] Argüelles, M. H.; Villegas, G. A.; Castello, A.; Abrami, A.; Ghiringhelli, P. D.; Semorile, L.; Glikmann, G. VP7 and VP4 genotyping of human group A rotavirus in Buenos Aires, Argentina. *J. Clin. Microbiol.* **2000**, *38*(1), 252–259. https://doi.org/10.1128/JCM.38.1.252-259.2000

[9] Matthijnssens, J.; Bilcke, J.; Ciarlet, M.; et al. Rotavirus disease and vaccination: Impact on genotype diversity. *Future Microbiol.* **2009**, *4*, 1303–1316. https://doi.org/10.2217/fmb.09.96

[10] Matthijnssens, J.; Ciarlet, M.; McDonald, S. M.; et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). *Arch. Virol.* **2011**, *156*(8), 1397–1413. https://doi.org/10.1007/s00705-011-1006-z

[11] Trojnar, E.; Sachsenroder, J.; Twardziok, S.; Reetz, J.; Otto, P. H.; Johne, R. Identification of an avian group A rotavirus with a novel VP4 gene. *J. Gen. Virol.* **2013**, *94*, 136–142. https://doi.org/10.1099/vir.0.047381-0

[12] Iturriza-Gomara, M.; Dallman, T.; Banyai, K.; et al. Rotavirus genotypes co-circulating in Europe, 2006–2009. *Epidemiol. Infect.* **2011**, *139*, 895–909.
[13] Gentsch, J. R.; Laird, A. R.; Bielfelt, B.; et al. Serotype diversity and reassortment: Implications for vaccine programs. *J. Infect. Dis.* **2005**, *192*, S146–S159. https://doi.org/10.1086/431499
[14] Gichile, A. G. Review on the epidemiology of bovine rotavirus. *Int. J. Vet. Sci. Res.* **2022**, *8*(1), 5–10.
[15] Geletu, U. S.; Usmael, M. A.; Bari, F. D. Rotavirus in calves and its zoonotic importance. *Vet. Med. Int.* **2021**, 6639701. https://doi.org/10.1155/2021/6639701
[16] Bwogi, J.; Karamagi, C.; Byarugaba, D. K.; et al. Co-surveillance of rotaviruses in humans and domestic animals in Central Uganda. *Viruses* **2023**, *15*, 738. https://doi.org/10.3390/v15030738
[17] Midgley, S. E.; Banyai, K.; Buesa, J.; et al. Diversity and zoonotic potential of rotaviruses in swine and cattle across Europe. *Vet. Microbiol.* **2012**, *156*, 238–245. https://doi.org/10.1016/j.vetmic.2011.10.027
[18] Mahmoud, A. E.; Zaki, M. E. S.; Mohamed, E. H.; et al. Study of rotavirus genotypes in Egypt. *Ital. J. Pediatr.* **2024**, *50*, 247. https://doi.org/10.1186/s13052-024-01810-x
[19] Hussein, A. A.; Hussein, R. A.; Shaker, M. J. Enteric virus co-infection in Iraq. *J. Pure Appl. Microbiol.* **2018**, *12*(2), 793–799. https://doi.org/10.22207/JPAM.12.2.40
[20] Nasser, A. T.; Hasan, A. S.; Saleh, A. K.; Saleh, M. K. Rotavirus detection in children with gastroenteritis. *GSC Adv. Res. Rev.* **2021**, *6*(3), 194–208.
[21] Abdul Sattar, B. A. A.; Al-Kareemi, K. K.; Jassim, A. A. Rotavirus infection in pediatric patients. *J. Fac. Med. Baghdad* **2012**, *54*(4), 349–352. https://doi.org/10.32007/jfacmedbagdad.544702
[22] Abood, W. S. Molecular epidemiology of rotavirus in Mid Iraq. *Al-Qadisiyah J. Vet. Med. Sci.* **2013**, *12*(1), 121–127. https://doi.org/10.29079/vol12iss1art240
[23] Hassan, H. A. A.; Al-Kader, R. A. A. Rotavirus diarrhea in Basrah. *MJBU* **2016**, *34*(2).
[24] Abdulla, M. M.; Maatook, M. A.; Mahmoud, R. A. Diarrheal disease in Basra during COVID-19. *Ann. R. S. C. B.* **2021**, *25*(6), 9759–9863.
[25] Yaqoob, M. M.; Mahdi, K. H.; Al-Hmudi, H. A.; Mohammed-Ali, M. N. Detection of rotavirus A and E. coli. *Int. J. Curr. Microbiol. Appl. Sci.* **2016**, *5* (4), 68–83. https://doi.org/10.20546/ijcmas.2016.504.011
[26] Habash, S. H.; Habeeb, S. I. Rotavirus diarrhea in children under five in Basrah. *Pediatr. Infect. Dis.* **2018**, *3*(2), 6. https://doi.org/10.21767/2573-0282.100062
[27] Tarik, A. S.; Muhsen, R. K. Rotavirus group A alterations in newborn buffalo calves. *Adv. Life Sci.* **2024**, *11*(2). https://doi.org/10.62940/als.v11i3.2051
[28] Ahmed, S. K.; Atheer, A. A. Rotavirus diversity in cow and buffalo calves. *EZS* **2017**, *5*(6), 1206–1211.
[29] Al-Robaiee, I. A.; Al-Farwachi, M. I. Rotaviral infection in neonatal calves in Mosul. *World* **2013**, *6*(8), 538–540. https://doi.org/10.5455/vetworld.2013.538-540
[30] Rotavirus Classification Working Group (RCWG). *Virus Classification.* 2021.
[31] Antoni, S.; Nakamura, T.; Cohen, A. L.; et al. Rotavirus genotypes in LMICs. *PLOS Glob. Public Health* **2023**, *3*(11), e0001358.
[32] Banyai, K.; Laszlo, B.; Duque, J.; et al. Global rotavirus strain diversity: A systematic review. *Vaccine* **2012**, *30* (Suppl 1), A122–A130. https://doi.org/10.1016/j.vaccine.2011.09.111
[33] Tapisiz, A.; Bedir Demirdag, T.; Cura Yayla, B. C.; et al. Rotavirus infections in Turkey: Systematic review. *Rev. Med. Virol.* **2019**, *29*(1), e2020. https://doi.org/10.1002/rmv.2020
[34] Khai, Q.; Tran, H. H.; Tuan, N. V.; et al. Rotavirus and co-infection in Vietnam. *Arch. Pediatr. Infect. Dis.* **2024**, *12*(1), e140509.
[35] Campanha, J.; Possatti, F.; Lorenzetti, E.; et al. Rotavirus C VP6 genotype I6 in piglets. *Braz. J. Microbiol.* **2020**, *51*, 1–7. https://doi.org/10.1007/s42770-020-00234-z
[36] Jaff, D. O.; Tariq, A. G. A.; Natalie, R. S. Rotavirus infections in Sulaimani. *JBM* **2016**, *4*(1), 124–131. https://doi.org/10.4236/jbm.2016.41015
[37] Zaman, N. A.; Al-Tae, A. A.; Saadoon, I. H. Rotavirus and adenovirus in Kirkuk children. *Second Scientific Conference – Tikrit University* **2012**.
[38] Ahmed, H. M.; Coulter, J. B. S.; Nakagomi, O.; et al. Rotavirus strains in Kurdistan. *Emerg. Infect. Dis.* **2006**, *12*(5), 824. https://doi.org/10.3201/eid1205.051422

[39] Duman, R.; Aycan, A. E. Rotavirus infection in calves in Konya. *J. Anim. Vet. Adv.* **2010**, *9*(1), 136–139. https://doi.org/10.3923/javaa.2010.136.138

[40] Almalky, M. A.; Amer, R. E.; Abd Elraouf, H. A. Rotavirus genotyping in Egypt. *Egypt. J. Hosp. Med.* **2022**, *88*, 2710–2715. https://doi.org/10.21608/ejhm.2022.241123

[41] Bawa, F. K.; Mutocheluh, M.; Dassah, S. D.; et al. Genetic diversity of rotavirus in Ghana. *Pan Afr. Med. J.* **2023**, *44*, 148. https://doi.org/10.11604/pamj.2023.44.148.36783

[42] Temori, A.; Mehrpoor, A. J.; Niazi, A.; et al. Rotavirus prevalence in Afghanistan. *Afgh. J. Infect. Dis.* **2023**, *1*, 9–13. https://doi.org/10.58342/ajid/ghalibuni.v.1.I.1.3

[43] Martinez-Gutierrez, M.; Arcila-Quiceno, V.; Trejos-Suarez, J.; Ruiz-Saenz, J. Rotavirus typing in Colombia. *Rev. Inst. Med. Trop. São Paulo* **2019**, *61*, e34. https://doi.org/10.1590/s1678-9946201961034

[44] Anderson, E. J.; Weber, S. D. Rotavirus infection in adults. *Lancet Infect. Dis.* **2004**, *4*(2), 91–99. https://doi.org/10.1016/S1473-3099(04)00928-4

[45] Barua, S. R. *Clinico-Pathology and Molecular Characterization of Bovine Rotavirus in Bangladesh*; 2019.

[46] Santos, Y.; Hoshino. Global distribution of rotavirus serotypes. *Rev. Med. Virol.* **2005**, *15*(1), 29–56. https://doi.org/10.1002/rmv.448

[47] Kuang, X.; Gong, X.; Zhang, X.; Pan, H.; Teng, Z. Genetic diversity of rotavirus in Shanghai. *BMC Infect. Dis.* **2020**, *20*, 1–11. https://doi.org/10.1186/s12879-020-05279-x