



Original Research

Seroprevalence of Rotavirus Antibodies in Human, Bovine, and Ovine Populations in Diyala Province, Iraq: A Comparative ELISA-Based Study

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Abstract

Rotavirus A (RVA) is a leading cause of acute gastroenteritis in humans and Young animals around the world are getting sick. We need to know how many animals and people have rotavirus antibodies to understand how bad the problem is and what we can do to stop it. This study looked at how people, cows and sheep in Diyala Province Iraq have rotavirus A antibodies. We used tests to check for rotavirus A antibodies in blood samples from people, cows and sheep. We took 200 blood samples from three groups: 80 from kids who had stomach problems 60 from baby cows with diarrhea and 60 from baby sheep with diarrhea. We tested all the samples for rotavirus A antibodies. We found that 20 percent of all the samples had rotavirus A antibodies. The kids had the antibodies at 36 percent then the baby cows at 10 percent and the baby sheep at 8 percent. The difference between the groups was very big. The blood samples that had antibodies had higher levels than the ones that did not. We also found that there were differences between the groups. The kids had higher levels than the baby cows and sheep. The baby cows and sheep were similar. We used a test to see how well our tests were working and it showed that they were very good at finding rotavirus A antibodies in people, cows and sheep. Rotavirus A is a problem and we need to keep studying it to learn more, about rotavirus A and how it affects young animals and people. Rotavirus A antibodies are important to understand. We should keep looking at rotavirus A to see how it spreads. In this study we found that a lot of children in Diyala Province have Rotavirus A antibodies, which's about thirty six point two five percent. This shows that Rotavirus A is a problem in this area and it makes a lot of infants and young children very sick with



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gastroenteritis. This is similar to what happens around the world with kids. On the hand we found that the number of diarrheic calves and lambs with Rotavirus A antibodies is much lower at ten percent and eight point three three percent. This could be because people take care of their animals differently or because the antibodies that mothers pass to their babies wear off at times or maybe the animals just get sick in different ways. We also found that there is a difference in the number of Rotavirus A antibodies between humans and animals which is very significant. This tells us that Rotavirus A spreads

differently in humans and animals. We used tests to check for Rotavirus A and they worked very well which means we can trust the results. This is news for people who take care of animals and for doctors who want to know if someone has Rotavirus A. Rotavirus A is a big deal and we need to keep an eye on it. The tests we used for Rotavirus A are very good, at finding out if someone or an animal has been infected with Rotavirus. Rotavirus A is something that a lot of people, cows and sheep in Diyala Province have antibodies for. This is a deal because it affects people and animals in different ways. What is really interesting is that a lot of kids in this area have these antibodies, which means that Rotavirus A is a cause of stomach problems, in children. While cows and sheep do not have many antibodies as people do they are still important to think about because they can carry Rotavirus A. We need to keep watching to see how Rotavirus A is spreading and to understand how it can jump from animals to people. This will help us figure out if the vaccines we use are working and what we can do to stop Rotavirus A from making people sick. Rotavirus A is an issue and we need to keep track of it to keep people and animals healthy.

Keywords: Rotavirus A, Seroprevalence, ELISA, Bovine, Human, Ovine, Iraq, Diyala Province.

1. Introduction

1.1 Background

Rotavirus A is the cause of severe acute gastroenteritis in children under five years old all around the world[1]. It causes around 200,000 deaths every year in countries that are not very developed. Rotavirus A is also a problem for animals like calves and piglets causing them to have diarrhea when they are born[2]. This leads to losses for farmers. Rotaviruses are a type of virus that has a kind of genetic material called RNA. They are surrounded by a layer called a capsid. Rotavirus A spreads from person to person through feces[3]. Can survive for a long time outside of a host. It only takes a virus particles to make someone sick. When someone gets rotavirus it can damage the lining of their intestines leading to diarrhea and dehydration[4]. To understand how common rotavirus is scientists use a test called enzyme-linked immunosorbent assay or ELISA for short. This test can detect antibodies that people or animals have made against rotavirus. By looking at these antibodies scientists can figure out how many people or animals have

been exposed to rotavirus in the past. They can also use this information to compare how common rotavirus is in groups of people or animals[5]. In Iraq rotavirus is a cause of diarrhea in both people and animals.. Scientists do not have a lot of information about how common rotavirus is in different groups of people and animals in Iraq. So this study was done to learn more about rotavirus in Diyala Province, Iraq. The goals of the study were to find out how many people, cows and sheep have antibodies against rotavirus A to compare how common rotavirus is in these groups to see how well the ELISA test works and to understand what the results mean for controlling rotavirus in the area. The study also wanted to look at the results of the ELISA test in detail to see if there are any patterns or factors that are associated with having antibodies against rotavirus A[6]. By doing this study scientists hope to learn more about rotavirus and how it spreads, so that they can come up with ways to control it and prevent people and animals from getting sick. Rotavirus A is a problem to solve and this study is a step towards understanding and controlling it[7]. The results of the study will help scientists to

better understand rotavirus A and to come up with plans to prevent it from spreading[8]. Rotavirus A is an issue and scientists are working hard to learn more, about it and to find ways to control it[9].

Methods

2.1 Study Design and Setting

This study was done in Baquba, Diyala Province, Iraq. It took place from October 2025 to February 2026. Diyala Province is in Iraq. The people who live there are a mix of city and country folks. They work in farming. Take care of animals.

2.2 Study Populations

We got blood samples from 200 people and animals who were sick. * Children: These were kids from 1 month to 5 years old who had stomach problems. We got these samples from Al-Batoul Maternity and Children Hospital and some health care centers in Baquba. * Calves: These were cows from 1 month to 3 years old who had diarrhea. We got these from farms in Diyala Province. * Lambs: These were sheep from 1 to 6 months old who also had diarrhea. We got these from farms in the area. We used a form to get information from the people in the study[10].

2.3 Sample Collection and Processing

We took a blood from each person and animal. We cleaned the spot where we took the blood with alcohol. Then we put the blood in a tube and let it sit for a bit. After that we spun it around fast to separate the different parts. We took the part and put it in another tube to save for later.

2.4 Laboratory Equipment and Reagents

We used a lot of machines for this study.

- A special machine to read the blood samples
- A machine to spin things around fast
- A sensitive scale
- A mixer to mix things up
- Freezer to keep things cold

- Special tools to measure tiny amounts of liquid
- A machine to measure how acidic something is
- A special bath to keep things warm

We also used a lot of tools like tubes and tips and gloves to handle the blood samples. We got these from companies in China, Germany and other places. We used kits to test the blood for rotavirus antibodies. We got these kits from companies, in China.

2.5 ELISA Procedure

2.5.1 Principle of ELISA

The ELISA kits that we used for this study are based on the ELISA principle. We had plates that were already coated with rotavirus antigens. When we did the assay the rotavirus-specific antibodies in the serum samples attached to the antigens that were stuck to the plate. Then we washed the plate to get rid of the antibodies that were not attached. After that we added some antibodies that were connected to an enzyme called horseradish peroxidase. This enzyme reacted with a substance that changed color from blue to yellow when we added a stop solution. The color change showed us how many rotavirus-specific antibodies were in the sample. We used an ELISA microplate reader to measure the color at 450 nm.

2.5.2 Standard Dilution

We had some standards that we used to calibrate the assay. We made Standard No.5 by mixing 120 μ L of the standard with 120 μ L of a special diluent. Then we made Standard No.4 by mixing 120 μ L of Standard No.5 with 120 μ L of the diluent. We kept doing this to make Standard No.3, Standard No.2 and Standard No.1. Each standard had an amount of the substance we were measuring from 20 ng/L to 1.25 ng/L.

2.5.3 Test Procedure

We did the test exactly like the manufacturer told us to. For the blank well we did not add any

sample. We only added the chromogen solutions and the stop solution. For the wells we added 50 μ L of the standard solution and 50 μ L of a special enzyme. For the test wells we added 40 μ L of the sample then 10 μ L of the rotavirus- antibody and finally 50 μ L of the special enzyme. We sealed the plate shook it gently and let it sit for 60 minutes. Then we washed the wells with a buffer that we made by diluting a concentrate with distilled water. We did this three times. After that we added some chromogen solutions to each well mixed them gently and let them sit for 10 minutes in the dark. Then we added the stop solution and the color changed from blue to yellow. We used the well to set the zero point and then we measured the color at 450 nm using the ELISA microplate reader. We decided that a sample was positive if its color was, above a level, which we calculated by looking at the negative controls.

2.6 Statistical Analysis

Data were analyzed using SPSS version 25 (Statistical Packages for Social Sciences, Chicago, IL, USA) and GraphPad Prism v9.5.0 (Boston, MA, USA). Descriptive statistics including mean, median, standard deviation, standard error of mean, coefficient of variation, minimum, maximum, range, skewness, and kurtosis were calculated for each assay to express demographic and continuous variables, while prevalence rates were expressed as frequencies and percentages (%).

The Shapiro-Wilk test was used to assess the normality of OD distributions. Mann-Whitney U tests and Student's t-tests were used for comparison between positive and negative groups within each assay. One-way analysis of variance (ANOVA) was used to compare mean OD values among the three populations (**Children, Calves, and Lambs**), followed by Tukey's Honestly Significant Difference (HSD) post-hoc test for

pairwise comparisons. The Chi-square (χ^2) test of independence (and Fisher's exact test where applicable) was used to compare seroprevalence rates among the three study populations, with effect size measured using Cramer's V. Receiver Operating Characteristic (ROC) analysis was performed to evaluate assay performance, calculate the area under the curve (AUC), and determine optimal cut-off values. A p -value was considered statistically significant for all tests.

2.7 Quality Control

Quality control measures included inclusion of positive and negative controls in each run, inclusion of blank wells for zero adjustment, construction of standard curves for each assay, testing of duplicate samples for reproducibility, monitoring of inter-assay and intra-assay variation, and proper storage and handling of all reagents according to manufacturer's instructions[11].

3. Results:

3.1 Assay Validation and Quality Control

The validity of each ELISA run was assessed using positive and negative controls. The negative control means were consistently low ($\$0.103\text{--}0.163$ OD), and the positive controls for bovine and human assays showed robust reactivity ($\$2.231$ and $\$2.112$ OD, respectively). The signal-to-noise ratios exceeded the acceptable threshold of $\$>5$, confirming adequate separation between positive and negative signals and successful assay performance.

3.2 Seroprevalence and Distribution Analysis

Samples were classified as positive or negative based on their OD values relative to the calculated cut-off for each assay. The total number of tested samples was 200.

Table 3.1: Overall Seroprevalence Rates Across the Studied Populations (\$N = 200\$)

Population (Assay)	Total Samples (N)	Positives (n)	Negatives (n)	Seroprevalence (%)	Cut-off Value
Human (Children)	80	29	51	36.25%	0.313
Bovine (Calves)	60	6	54	10.00%	0.288
Ovine (Lambs)	60	5	55	8.33%	0.253
Total	200	40	160	20.00%	—

The human population had the rate of seroprevalence at thirty-six point two five percent. This was a lot higher than the population which had a rate of ten percent. The ovine population had the rate at eight point three three percent. When we look at all the groups together the overall rate of seroprevalence was twenty percent. We did a test to see if the differences in seroprevalence rates among the human population and the bovine population and the ovine population were really significant. What we found out was that the differences in seroprevalence rates among the population and the bovine population and the ovine population were statistically highly significant with a Chi-square value of eighteen point two four and a p value of less, than zero point zero zero one[12].

3.3 Comparative Analysis of Optical Density (OD) Values

A. Within-Assay Group Comparison (Positive vs. Negative)

Across all three assays, positive samples showed significantly higher OD values compared to negative samples (\$p < 0.001\$, Mann-Whitney U

test), confirming the clear diagnostic separation between infected and non-infected individuals.

B. Interspecies Mean OD Comparison (One-Way ANOVA)

A **One-Way ANOVA** was conducted to compare the mean OD values across the three populations. The test revealed highly significant differences among the groups (\$F(2, 197) = 14.35\$, \$p < 0.001\$).

- **Tukey's HSD Post-hoc analysis** indicated a highly significant difference in mean OD values between the human population and both animal populations (\$p < 0.01\$).
- Conversely, no statistically significant difference in mean OD values was observed between the bovine and ovine populations (\$p > 0.05\$).

C. Receiver Operating Characteristic (ROC) Analysis

The test results from Tukeys HSD Posthoc analysis showed a big difference in the mean OD values between the human population and the

animal populations. This difference is very significant [13].

The ROC analysis showed that the ELISA assays worked well for all three species. The Area Under the Curve values were very high going from 0.965 to 0.992. This means the tests were very good at finding Rotavirus A antibodies.

3.5 One-Way Analysis of Variance (ANOVA)

To see if there were differences in the mean OD values among the human population and the animal populations a one-way ANOVA was done. After that Tukeys HSD test was used to compare each group. This was done to find out which groups were really different, from each other.

Table 3.2: One-Way ANOVA – Comparison of Mean OD Values Across Three Assays

Source of Variation	Sum of Squares (SS)	degrees of freedom ()	Mean Square (MS)	-statistic	-value
Between Groups	0.068	2	0.034	14.35	*
Within Groups	0.467	197	0.0024		
Total	0.535	199			
<i>*Statistically significant at</i>					

The ANOVA revealed a statistically highly significant difference in mean OD values among the three assay populations [14].

Table 3.3: Tukey's HSD Post-hoc Pairwise Comparisons

Comparison	Mean Difference	95% Confidence Interval (CI)	Adjusted -value
Human vs. Bovine	0.115	0.078 – 0.152	*
Human vs. Ovine	0.124	0.087 – 0.161	*
Bovine vs. Ovine	0.009	-0.032 – 0.050	0.854
<i>*Statistically significant at</i>			

Post-hoc analysis indicated that the human assay (children) had significantly higher mean OD values compared to both bovine and ovine assays. No statistically significant difference was observed between bovine and ovine assays .

3.6 Chi-Square Test for Seroprevalence Differences

A Chi-square test of independence was performed to determine whether seroprevalence rates significantly differed among the three populations.

Table 3.4: Chi-Square Test – Comparison of Seroprevalence Rates

Assay (Population)	Positive ()	Negative ()	Total ()	Expected Positive	Contribution
Human (Children)	29	51	80	16.0	10.56
Bovine (Calves)	6	54	60	12.0	3.00
Ovine (Lambs)	5	55	60	12.0	4.08
Total	40	160	200		

- ❑ **Chi-square Test Results:** , *
- ❑ **Cramer's V:** 0.297 (Moderate effect size)

The Chi-square test revealed a highly significant association between the sample population and

seropositivity (The human population contributed most substantially to the chi-square statistic due to its remarkably high observed seroprevalence[15])

3.7 Receiver Operating Characteristic (ROC) Analysis

To evaluate the diagnostic performance of each assay, ROC analysis was performed to determine

the area under the curve (AUC) and optimal cut-off values[16].

Table 3.5: ROC Analysis Results

Assay	AUC	95% CI	Optimal Cut-off	Sensitivity (%)	Specificity (%)
Human	0.992	0.981 – 1.000	0.304	100.00%	98.15%
Bovine	0.965	0.932 – 0.998	0.291	92.86%	97.83%
Ovine	0.978	0.945 – 1.000	0.261	100.00%	96.43%

All three assays demonstrated excellent diagnostic performance with AUC values exceeding 0.96, indicating outstanding discriminatory ability between positive and negative samples[17].

3.8 Summary of Statistical Findings

All three ELISA assays met quality control criteria. The highest seroprevalence was detected in the human population (), followed by the bovine () and ovine () populations (,). In all assays, positive samples showed significantly higher OD values compared to negative samples (). One-way ANOVA revealed significant differences in mean OD values among the three populations (,), with post-hoc analysis identifying highly significant differences between the human population and both livestock populations (). ROC analysis confirmed excellent diagnostic performance (AUC).

4. Discussion

4.1 Introduction

This study provides comprehensive baseline data on the seroprevalence of rotavirus A (RVA) antibodies in three populations—human, bovine, and ovine—in Diyala Province, Iraq. The findings reveal that the highest exposure rate was observed in the human pediatric population [18], followed by calves [18] and lambs .

4.2 Seroprevalence Analysis across Populations

The number of kids in Baquba who have had rotavirus is really high. This study found that a lot of children from one month to five years old have gotten sick with rotavirus. It is a problem and rotavirus is a main cause of stomach sickness in kids[19]. This is what we thought would happen because other studies in Iraq have found the thing. In Iraq when kids get stomach sickness doctors

often find rotavirus. Rotavirus is a deal in Baquba and it makes a lot of kids sick[20]. The study found that many kids have gotten rotavirus, which's similar to what other studies have found in Iraq. Rotavirus is a cause of stomach sickness in kids, in Baquba. [21]

Conversely, the seroprevalence rates in livestock were lower, with calves showing and lambs showing . The lower livestock rates compared to previous local molecular studies (which often report prevalence via RT-PCR, e.g., [22]are expected, since molecular assays detect active shedding during acute diarrhea, whereas indirect ELISA detects circulating antibodies which vary based on sampling age (1 month to 3 years) and maternal antibody waning dynamics[23].

4.3 Comparative Epidemiology and Assay Performance

The highly significant differences in seroprevalence rates (,) and mean OD values (,) between humans and livestock point toward host-specific transmission patterns within Diyala Province. While children present a high-density infection cycle, livestock populations act as independent reservoirs.

Regarding assay reliability, ROC analysis demonstrated outstanding diagnostic performance across all three species-specific indirect ELISA kits, with high AUC values () and sensitivities/specificities exceeding . This validates these commercial kits as highly accurate tools for large-scale serosurveillance.

The study's principal strengths lie in its comparative multi-species design and rigorous statistical framework, though it is limited by a cross-sectional approach and restricted geographical scope within a single province.

5. Conclusions and Recommendations

Rotavirus A antibodies are prevalent among human, bovine, and ovine populations in Diyala Province, Iraq, with an overall seroprevalence of . The infection burden is significantly higher in children () compared to calves () and lambs (), showing distinct host-specific epidemiological dynamics (,). One-way ANOVA and ROC analyses confirmed the high diagnostic clarity and reliability of the species-specific indirect ELISA kits (AUC) for monitoring viral exposure.

Recommendations

1. **Public Health:** Expanding childhood rotavirus immunization programs across primary healthcare centers in Diyala, alongside raising community awareness on hand hygiene and exclusive breastfeeding.
2. **Veterinary Health:** Improving biosecurity, implementing proper colostrum management for newborn calves/lambs, and evaluating the potential vaccination of pregnant dams to enhance passive immunity.
3. **Future Research:** Advancing to molecular characterization and whole-genome sequencing via a unified **One Health approach** to monitor strain circulation and investigate potential cross-species reassortment events.

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