

SEROLOGICAL DETECTION OF ROTAVIRUS INFECTION IN BOVINE AND HUMAN

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ABSTRACT

Rotaviruses, causing acute gastroenteritis, that infect humans and animals around the world. There are many assays had been developed for the detection of rotavirus or the viral antigens. The present study was done on 79 samples of stool collected from pediatric patients with acute watery diarrhea aged from one months to 5 years admitted to Basrah Maternity and children hospital in Basrah province, during the period from October 2014 to February 2015. Ninety diarrheic fecal bovine samples were included in this study. All samples were used for the investigation and detection of rotavirus antigen by Enzyme-Linked Immunosorbent assay (ELISA). According to ELISA results, 10 out of 79(12.7%) pediatric stool samples rotavirus antigens were detected in children. Percentage (20.7%) of positive rotavirus antigen were detected in the patients at second age group (>6 months). Followed by 8% of patients at first age group (<6 months) these differences were not significant ($P>0.05$). The percentage of rotavirus antigen was higher in males patients (16.7%) compared to females ($P>0.05$) and also the differences were not significant differences ($P>0.05$). These results of rotavirus antigen detection in 90 diarrheic bovine fecal samples showed that this antigen was excreted by 56.7% of diarrheic calves. Additionally the higher non-significant ($P>0.05$) excretion percentage according to age was observed in 63.4 % of calves > 1 year old and the lower percentage(51.1%) was observed in the first age group(< 1year) calves old. The differences in sex were not significant ($P>0.05$) in the percentage of rotavirus antigen detection were also detected as 63.5% of male fecal samples show positive rotavirus antigen excretion whereas only 47.4% of female fecal samples were positive.

INTRODUCTION

Group A rotaviruses, members of the genus *Rotavirus* within the family Reoviridae, are common cause of acute infectious diarrheal illness in infants and the young of a wide variety of mammalian and avian species. Bovine rotaviruses are important causative agents of neonatal calf diarrhea throughout the world, and rotavirus infection is a significant cause of economic loss in the cattle industry [1].

Gastrointestinal illnesses resulting from rotavirus infections among young children contribute greatly to morbidity and mortality rates in many countries in Iraq and other parts of the world. Human rotaviruses are one of the major contributors of severe infantile diarrhea, responsible for more than 600,000 deaths annually [2]. Furthermore, rotavirus infections are an important cause of hospitalization, causing considerable economic impact on poor countries [2,3]. In Iraq, the death rate in children <5 years of age was reported to be 130/1,000 for boys and 120/1,000 for girls in 2003 [4]. Diarrhea is a major cause of illness and death in Iraqi children; however, little information exists about the origin of childhood diarrhea. Only a single study showed that rotavirus accounted for 24% of acute diarrhea in hospitalized children in Basrah [5].

Several rotavirus genotypes, believed to be of animal origin have been detected in low numbers in children with acute gastroenteritis [6,7]. Rotaviruses are most commonly transmitted *via* fecal / oral route; there is evidence that they can be transmitted in respiratory tract [8]. So the aim of the study is Detection of rotavirus antigen in pediatric stool and bovine fecal samples and the determination of its relationship with age, sex and months of the study.

MATERIALS AND METHODS

Pediatric Stool Samples:

Stool samples were collected from 79 infants and children aged from one month to 5 years who presented with acute gastroenteritis during the period from October 2014 to February 2015 to Basrah Maternity and children hospital in Basrah province. These samples were varied in amount from 0.2gm. or 200µl per patient and collected in sterile disposable closed plastic containers. All samples were transported

under cold conditions to the laboratory where the necessary tests were performed or stored frozen at -20 C° until use.

Bovine Fecal Samples:

During the winter months of 2014-2015, 90 fecal samples were collected from Basra province household diarrheic calves, with age range of three months to two years.. For collection of faeces, rectal stimulation was made for the calves then the feces were collected directly into disposable plastic containers, transported under sterile cold conditions to the laboratory where the required tests were done or stored at -20 C° until use.

Immunological detection of Rotavirus

Sandwich Enzyme Linked Immunosorbent Assay (ELISA) kits were used for detection of rotavirus antigens in both children and bovine fecal samples. These kits were Human Rotavirus antigen ELISA Kit and Bovine Rotavirus Antigen ELISA Kit from. Qiagen, Germany

Detection of Rotavirus antigens

Fecal suspension Preparation:

Fecal suspension (20%) was prepared using 1X of 0.01M PBS (pH 7.2-7.4). 200µl of liquid sample or 200mg of solid sample was suspended in 800µl and 1000µl of PBS respectively in a 1.5ml Eppendorf tube and vortexed for 40 seconds. The contents were centrifuged at 10,000rpm for 10 minutes at -4°C. the supernatant was transferred to a new Eppendorf tube without disturbing the pellet and discard the pellet. The fecal suspensions was stored at -20°C for further analysis. All stool samples conducted with permission from parents of the children and from local veterinary authorities and in accord with accepted standards of animal care.

Human and Bovine Rotavirus antigen (RV Ag) ELISA

The kit uses a double-antigen sandwich enzyme-linked immunosorbent assay (ELISA) to test the level of human or bovine rotavirus antigen(RV Ag) in samples . Human or bovine rotavirus antigen(RV Ag) were added to pre-coated human or bovine hepatitis B virus core antigen microelisa well, incubation; washing. HRP were added that tagged human or bovine hepatitis B virus core antigen. After another incubation and washing, the unbound enzyme was moved, then Chromogen solution

A, B, added, the color of the liquid change into the blue, and at the effect of acid the color finally become yellow. Using the micro plate reader of 450nm wavelength to measure the absorbency, and comparing with the critical value to determine the existence of the Human Rotavirus antigen(RV Ag) of the sample. The first well of first vertical row of the microtiter plate was left empty for blanking. Diluted standard (50 μ l) was added to standard well; Sample dilution 40 μ l was added to testing sample wells which on Assay plate, then the testing sample (10 μ l) was added and mixed with gentle shaking. The plate was covered with cover seal. and incubated for 30minutes at 37 C $^{\circ}$. Then the liquid was discarded, each well was dried to be flooded with 30-times diluted washing liquid, oscillated for 30 seconds, the washing liquid was discarded with absorbent paper. Repeat five times. Then HRP-conjugate reagent (50 μ l) was added to each well, except the blank well, mixed with gentle shaking and incubated for 30 minutes at 37 C $^{\circ}$. The liquid was discarded , each well was dried to be flooded with 30-times diluted washing liquid, oscillated for 30 seconds, the washing liquid was discarded with absorbent paper. Repeat five times dry. Repeat five times, Pat dry. Chromogen solution A(50 μ l) and chromogen solution B(50 μ l) were added to each well, gently mixed and incubated for 10 min at 37 C $^{\circ}$. The reaction was stopped by addition of stop solution (50 μ l) to each well (the blue color change to yellow color Immediately). The blank well was taken as zero , the optical density (OD) was measured at 450 nm after adding stop solution and within 15min

The validity of the experiment: the mean of the masculine comparative hole ≥ 1.00 ;the mean of the feminine comparative hole ≤ 0.15 ; The critical value calculation: the critical value=the mean of the feminine comparative hole+0.15; Feminine determinant: if the sample OD value<the critical value, the Human Rotavirus antigen(RV Ag) is feminine; Masculine determinant: if the sample ODvalue \geq the critical value, the human Rotavirus antigen(RV Ag) is masculine. spiratory droplets [8].

RESULTS

Detection of rotavirus antigen

Diarrheic patient stool samples

Among a total of 79 stool samples rotavirus antigens was detected in 10(12.7%) diarrheic infants. The frequency of rotavirus antigen varied with age but that

difference was non significant ($P>0.05$), however high percentage of positive rotavirus antigen based ELISA results were observed in the infants at second age group (>6 months) (20.7%). Compare to those at first age group (<6 months) was (8 %) (Table 3). The effect of sex on the frequency of rotavirus antigen detection was also considered to be not statistically significant ($P>0.05$). Nevertheless it was higher in males infants 16.7% compared to female 9.3% (Table 4)

Table (1): ELISA based distribution of rotavirus antigen in Pediatric stool samples according age of diarrheic infants.

Age group (Month)	Tested n.(%)	Rotaviral antigen Positive		Rotaviral –antigen Negative	
		no.	%	no.	%
<6	50(63.3)	4	8	46	92
>6	29(36.7)	6	20.7	23	79.3
Total	79(100)	10	12.7	69	87.3

$P > 0.05$

Table (2): ELISA based distribution of rotavirus antigen in Pediatric stool samples according to sex of diarrheic infants.

Sex	Tested no.(%)	Rotaviral –antigen Positive		Rotaviral –antigen Negative	
		no.	%	no.	%
Male	36(45.6)	6	16.7	30	83.3
Female	43(54.4)	4	9.3	39	90.7
Total	79(100)	10	12.7	69	87.3

$P > 0.05$

Bovine fecal samples

The rotaviral antigen based ELISA results in the 90 diarrheic bovine fecal samples revealed that rotavirus antigen was detected in 51 (56.7%) of diarrheic calves. The higher percentage of rotavirus antigen detection was observed in 63.4 % of calves at the second age group (> 1 year) and the lower percentage (51.1%) was observed in the first age group (< 1 year) calves (Table 5). Sex differences in the

frequency of rotavirus antigen detection were also observed. Of 51 bovine diarrheic bovine fecal samples that showed positive ELISA results, 63.5% of the samples from male were positive for rotavirus antigens whereas 47.4% of the samples from female calves were positive (Table 6). The effect of age and sex of diarrheic calves on rotavirus antigen detection by ELISA test was considered to be not statistically significant ($P > 0.05$).

Table (3): ELISA based distribution of rotavirus antigen in bovine fecal samples according to age

Age groups (years)	Tested no.(%)	Rotaviral –antigen Positive		Rotaviral –antigen Negative	
		no.	%	no.	%
<1	49(54.4)	25	51.1	24	48.9
>1	41(45.6)	26	63.4	15	36.6
Total	90(100)	51	56.7	39	43.3

$P > 0.05$

Table (4): ELISA based distribution of rotavirus antigen in bovine fecal samples according to sex

Sex	Tested No.(%)	Rotaviral –antigen Positive		Rotaviral –antigen Negative	
		no.	%	no.	%
Male	52(57.8)	33	63.5	19	36.5
Female	38(42.2)	18	47.4	20	52.6
Total	90(100)	51	56.7	39	43.3

$P > 0.05$

DISCUSSION

There was an increasing tendency in clinical practice to include antigen detection assays in rotavirus diarrheal disease evaluation protocols. Enzyme immunoassays (EIAs) have replaced electron microscopy (EM) as the standard method for the detection of rotaviruses in stool samples in the 1980s [9,10,11]. Additionally and in order to understand the burden of rotavirus gastroenteritis in Basra province, Iraq, the state of rotavirus infection in children under five years of

age was investigated by ELISA testing of 79 stool samples. The present findings showed that rotavirus is present as gastroenteritis etiologic agent, being responsible for 10/79(12.7%) of admissions for acute gastroenteritis. This proportion was lower than that 40.5% (81/200) and 24.6% (34/138), observed in Basrah children by, [12 and 13] respectively . Also the current results were agreed in parte and disagreed with previous studies from other parts of Iraq which had revealed varying rate of rotavirus excretion in pediatric stool ranged from as low as 11.8% to 43.3 % [14,15,16,17,18,19,20,21]. And because of the rotaviruses have been associated with diarrhea of calves in Iraq and many other countries. The ELISA test had been used to detect rotavirus antigens in diarrheic fecal samples collected at random from 90 calves The rotaviral antigen based ELISA results revealed that rotavirus antigen was detected in 56.7% of diarrheic calves which was in contrast to other studies from Iraq, [13] who reported that rotavirus was excreted in 6.5% of tested bovine fecal. On the other hand the current results were agreed in parte and disagreed with previous studies from Iraq and other countries wherein varying rates of rotavirus excretion ranged from as low as 7.1% to 80% [22,23,24,25,26,27,28,29,30,31,32,33]. The variation in the rate of rotavirus excretion among the previous studies and the present study might suggest that non-viral or other viral causes are more common in studied cattle farm or due only to methodological differences or due to the variation in samples size.

الكشف المصلي والجزئي لاصابات الروتافايروس في الابقار والانسان

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الخلاصة

فيروسات الروتا، تسبب التهاب المعدة والأمعاء الحاد، التي تصيب الإنسان والحيوان في جميع أنحاء العالم. هناك العديد من الاختبارات التي وضعت للكشف عن فيروس الروتا أو المستضدات الفيروسية. أجريت هذه الدراسة على 79 عينة من البراز، والتي تم جمعها من الأطفال المصابين بالإسهال المائي الحاد الذين تتراوح أعمارهم بين بضعة أيام إلى 5 سنوات من مستشفى البصرة للولادة و الأطفال في مدينة البصرة. خلال الفترة من أكتوبر 2014 إلى فبراير 2015. تضمنت الدراسة تسعون عينة براز إسهالي من العجول. وفقا لنتائج اختبار ELISA فقد تم الكشف عن مستضدات فيروس الروتا في 10 عينات (12.7%) من اصل 79 عينة من براز الأطفال المصابين بالإسهال. وكانت النسب العالية (20.7%) والموجبة لمستضد فيروس الروتا قد سجلت

في مرضى الفئة العمرية الثانية (< 6 أشهر) والتي لم تظهر اختلافات معنوية ($P > 0.05$). تليها نسبة 8% من المرضى في الفئة العمرية الأولى (> 6 أشهر). بينما كانت نسبة مستضد فيروس الروتا اعتماداً على اختبار ELISA أعلى في المرضى الذكور (16.7%) مقارنة بنسبة (9.3%) في الإناث وبدون فروق معنوية ($P > 0.05$)، أيضاً أظهرت نتائج فحص ELISA لمستضدات فيروس الروتا في 90 عينة من براز العجول المصابة بالإسهال أن هذا المستضد تم إفرازه بنسبة 56.7% من قبل العجول المصابة. بينما لم تلاحظ أي فروق معنوية ($P > 0.05$) في النسب العالية لإفراز مستضد فيروس الروتا وفقاً للسن في 63.4% من العجول > 1 سنة من العمر والنسبة الأقل (51.1%) لوحظت في الفئة العمرية الأولى (< 1 سنة) للعجول. بالإضافة إلى ذلك لم تلاحظ أي فروق معنوية ($P > 0.05$) على أساس الجنس حيث أظهرت 63.5% من عينات البراز للذكور نتيجة موجبة لمستضد فيروس الروتا بينما أظهرت 47.4% فقط من عينات البراز للإناث نتيجة موجبة لمستضد فيروس الروتا.

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