The differences in sensitivity and specificity between three different kits for the detection Rotavirus

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Summary

Rotavirus is the common key etiologic agents of attained diarrhea in infant, young children and neonatal calves globally. It is very important to early diagnose the disease in purpose of effective patient treatment. This study was conducted by using three different kits for detecting Rotavirus in calves in five Iraqi governorates (Al-qadissiya, Babel, Kerbala, Missan, Wassit). A total of 125 stool specimens were examined, they were collected from calves in the (period from November 2015 to February 2016). The ages ranged from 1 to 16 weeks. Stool samples were collected and examined using Chromatographic Immunoassay, enzyme-linked immunosorbent assay and Polymerase-chain reaction. The results obtained by chromatographic immunoassay were 44% positive, ELISA 42% positive, and 38% Polymerase-chain reaction positive. Chromatographic immunoassay was easy, simple, economic, and rapid and showed high sensitivity with accepted specificity while ELISA permit quantitative estimation of Rotavirus antigens. These results indicate that ELISA is as sensitive and specific assay as the chromatographic immunoassay, and it could be applied on a large scale for screening stool specimens in suspected rotavirus diarrhea. Conventional Polymerase-chain reaction demonstrated more sensitivity and highest specificity.

Keywords: Rotavirus, Enzyme-linked immunosorbent assay, Chromatographic Immunoassay.

Introduction

Rotaviruses were identified as a significant enteric virus and essential reason of acute diarrhea in infants and young children in many mammalian species particularly human and calves (1). Enteritis linked to rotavirus is a main trouble in domestic animals, particularly in young calves (2). Rotaviruses, belong to the *Reoviridae* family - subdivided into the sub-families of the Sedoreovirinae and the Spinareovirinae in which Rotavirus is one of 15 genera (3) and have a genome including eleven segments of double-stranded RNA enclosed in capsid of three layers (4). They classified into G and P types based on differences in the outer capsid proteins VP7 (a glycoprotein) and VP4 (a protease-sensitive protein), respectively (5). But, depending on antigenic relationships of VP6 which is one structural virus protein of other six, the authors classified the Rotaviruses into eight groups (3) whereas they number these eight groups from (A) to (H) as A, B, and C have been found in humans, but D, E, F, and G were in animals (1). H group worldwide spread in dogs and pigs, the outer layer is formed by two proteins, VP7 and VP4, which elicit neutralizing antibody responses and form the basis of the current dual classification system in G (VP7) and P (VP4) types (4). As well, the eight groups are distinguished depending on the serological reactivity and genetic variability of VP6 (3). Several techniques have been developed for diagnosing rotavirus in feces. The detection of the viral agent was performed by electronic microscopy, polyacrylamide gel electrophoresis (PAGE), immunofluorescence, radioimmune assay, reverse passive hemagglutination, enzyme immunoassays (EIA), chromatographic immunoassay, and more recently by reverse transcriptase with polymerase chain reaction. Among these assays, chromatographic immunoassay was reported as a being easy to perform in a short time, for diagnosis and control of the disease caused by rotavirus in humans (6). PCR methods have supplanted other diagnostic tests with the feature of higher analytical sensitivity and specificity; such tests are conducted in commercial or diagnostic research facilities and need high expensive tools and specialized abilities, causing higher expenditures of the test, which minimize the utilization of lab tests to reinforce diagnostic examinations (6).

This study aims to compare the test of Rotavirus chromatographic immunoassay, a

rapid slide agglutination test in which particles coated with rotavirus-specific antibodies agglutinate in the presence of rotavirus antigens, against ELISA and PCR for detecting rotavirus in stool specimens of diarrheic calves in middle and south of Iraq.

Materials and Methods

One hundred and twenty five stool samples were collected in a clean, container, free of detergent from calves with mean age ranging from 1 to 16 weeks, including 62 male and 63 female with acute diarrhea. The study subjects were calves in Iraqi farms at five governorates (Alqadissiya, Babel, Kerbala, Missan, Wassit). Eeach sample was divided into three parts kept at -20˚C until they underwent further testing by the additional assays.

The samples were divided into groups according to the sex and age variables. The qualitative Rotavirus assay was performed with Rat rotavirus (RV) antigen (Ag) ELISA kit from (Cusabio Biotech Co., Ltd, China), the chromatographic Rotagen from (ABON Biopharm Co., Ltd., Hangzhou, China) was evaluated for rotavirus detection in fecal samples of calves while the conventional PCR was evaluated by using (*AccuPower* PCR Premix of Bioneer Corporation) from Republic of Korea. All assays were performed according to the manufacturers' instructions.

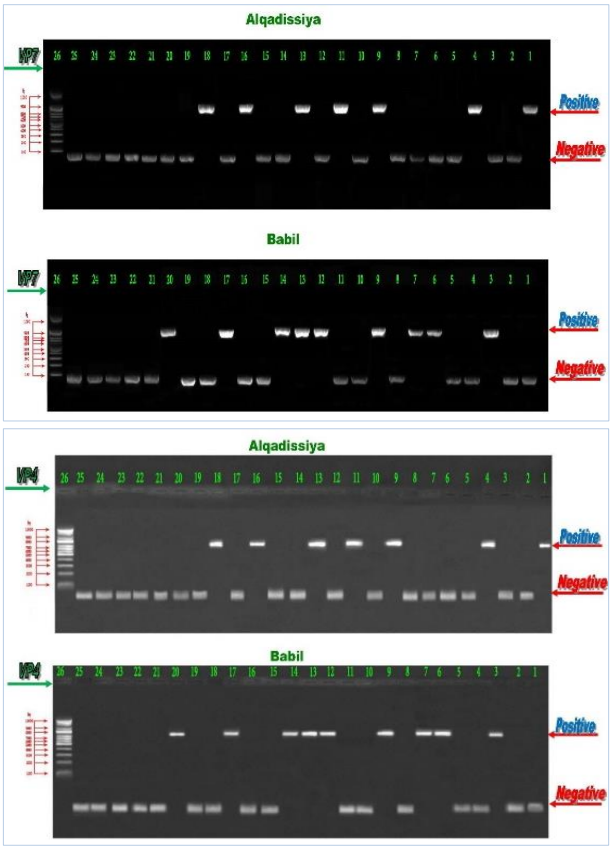
Results and Discussion

In this study, we found that, of 125 collected stool specimens and submitted for diagnostic testing, 53 (42%) samples were confirmed as positive using ELISA method but ٥٥ (44%) samples using chromatographic method were positive and 47 (٣٨%) samples were positive according to PCR test. While 72 (58%) samples were recorded as negative using ELISA, 70 (56%) using chromatographic, 78 (٦٢%) were detected as negative by using PCR test. The (Tables, ١-٥) show the agreement between the three tests was approximately 100%, whereas the PCR demonstrated more sensitivity and greater specificity.

The laboratory diagnosis of gastroenteritis is currently founded on applying of different tests, each one with variable sensitivity and specificity and typically able to detect limited number of pathogens at a time (7 and 8).

Table, ١: The percentage for three testing methods for Rotavirus in 125 fecal samples.

Site	ELISA (%)	Chromato g-raphic Immunoa-ssay (%)	PCR
Alqadissiya			
Positive	٥ (4%)	٧ (6%)	7 (6%)
Negative	٢٠ (16%)	١٨ (14%)	18 (14%)
Total	٢٥ (20%)	٢٥ (20%)	٢٥ (20%)
Babel			
Positive	١١ (9%)	١١(9%)	9 (7%)
Negative	١٤ (11%)	١٤ (11%)	١٦ (13%)
Total	٢٥ (20%)	٢٥ (20%)	٢٥ (20%)
Kerbala			
Positive	٩ (7%)	٩ (7%)	8 (6%)
Negative	١٦ (13%)	١٦ (13%)	17 (14%)
Total	٢٥ (20%)	٢٥ (20%)	٢٥ (20%)
Missan			
Positive	١٥ (12%)	١٥ (12%)	12 (10%)
Negative	١٠ (8%)	١٠ (8%)	13 (10%)
Total	٢٥ (20%)	٢٥ (20%)	٢٥ (20%)
Wassit			
Positive	١٣ (10%)	١٣ (10%)	11 (9%)
Negative	١٢ (10%)	١٢ (10%)	14 (11%)
Total	٢٥ (20%)	٢٥ (20%)	٢٥ (20%)



Figure, 1: The results have been obtained by using PCR method for Rotavirus in 125 fecal samples, which detected VP4 and VP7 (the figure show in two governates Al-qadissiya and Babil).

Rotavirus is a major agent associated with acute diarrhea in human and animal species species (9) for this reason a rapid, simple, sensitive, and specific diagnostic technique for the detection of viral agents causing gastroenteritis is needed to facilitate timely treatment of the disease (10).

Chromatographic Immunoassay and ELISA are the simple, good standard methods for detection of rotavirus and require low cost equipment and simple experience. Some researchers for detecting rotavirus infection have used the ELISA and Conventional PCR, these methods are expensive and require long time. The results suggested that chromatographic immunoassay could be used for screening stool specimens in suspected rotavirus diarrhea. The cost of ELISA was about double of chromatographic immunoassay cost; the test can be achieved within 5 min. for a single specimen compared with one hour to ELISA.

A study in United States, used chromatographic immunoassay and ELISA test for identifying bovine rotavirus antigen. Sixty-three fecal samples from calves infected with diarrhea were collected and examined by chromatographic immunoassay and Rotazyme ELISA, 37 were positive and 26 were negative by chromatographic immunoassay, by ELISA 36 were positive and 27 negative (10).

Table, 2: Comparison of results between three testing methods for Rotavirus in 125 fecal samples.

Site	SEX	Chromatographic Immunoassay		ELISA		PCR	
		(+)	(-)	(+)	(-)	(+)	(-)
Alqadissiya	Male	3	10	2	11	4	13
	Female	4	8	3	9	3	5
	Total	7	18	5	20	7	18
Babel	Male	7	7	7	7	6	9
	Female	4	7	4	7	3	7
	Total	11	14	11	14	9	16
Kerbala	Male	5	8	5	8	3	10
	Female	4	8	4	8	5	7
	Total	9	16	9	16	8	17
Missan	Male	6	5	6	5	5	5
	Female	9	5	9	5	7	8
	Total	15	10	15	10	12	13
Wassit	Male	5	6	5	6	7	8
	Female	8	6	8	6	4	6
	Total	13	12	13	12	11	14

Table, 3: The results obtained by using Chromatographic Immunoassay method for Rotavirus in 125 fecal samples according to age of tested calves.

Chromatographic Immunoassay Method											
SITE	AGE										
	1 wk.	2 wk.s	3 wk.s	4 wk.s	5 wk.s	6 wk.s	7 wk.s	8 wk.s	12 wk.s	16 wk.s	Total
Alqadissiya											
Positive	0	1	1	1	2	0	2	0	0	0	7
Negative	1	4	2	4	1	1	0	2	3	0	18
Total	1	5	3	5	3	1	2	2	3	0	25
Babel											
Positive	0	0	0	4	0	1	0	4	2	0	11
Negative	1	2	2	4	1	0	0	3	0	1	14
Total	1	2	2	8	1	1	0	7	2	1	25
Kerbala											
Positive	0	3	1	0	2	2	0	1	0	0	9
Negative	0	1	2	1	2	1	1	5	3	0	16
Total	0	4	3	1	4	3	1	6	3	0	25
Missan											
Positive	0	2	2	4	3	1	0	5	0	0	15
Negative	0	0	1	0	2	1	0	2	4	0	10
Total	0	2	3	4	5	2	0	7	4	0	25
Wassit											
Positive	3	7	0	0	0	0	0	2	1	0	13
Negative	0	0	3	0	0	2	0	6	1	0	12
Total	3	7	3	0	0	2	0	8	2	0	25

wk. = week

In Iran, ELISA and chromatographic immunoassay methods had been used to test 94 fecal samples from infant with severe gastroenteritis to diagnose the rotavirus. The results demonstrated 38 samples were positive by using the chromatographic immunoassay and 56 were negative, but by ELISA 39 were positive and 55 were negative (11).

In Iraq, ELISA results indicated that 17 out of 110 from diarrheic calves (15.5 %) and five out of 110 for non-diarrheic animals (4.5%) were found to be positive for rotavirus infection (12). By using Chromatographic immunoassay the result of rapid test showed that 18 out of 50 fecal samples of diarrheic calves were positive into rotavirus at percentage (36%), while 32 out of 50 were negative (64%) and 45.4% of rotavirus infection was found in calves less than 30 days of age which was significantly higher than 28.5% calves (30-60) days old, which male and female (37.5%, 40% respectively) were seen infected without any significant differences (13).

Table, 4: The results obtained by using ELISA method for Rotavirus in 125 fecal samples according to age of tested calves.

ELISA Method											
SITE	AGE										
	1 wk.	2 wk.s	3 wk.s	4 wk.s	5 wk.s	6 wk.s	7 wk.s	8 wk.s	12 wk.s	16 wk.s	Total
Alqadissiya											
Positive	0	0	1	1	1	0	2	0	0	0	5
Negative	1	5	2	4	2	1	0	2	3	0	20
Total	1	5	3	5	3	1	2	2	3	0	25
Babel											
Positive	0	0	0	4	0	1	0	4	2	0	11
Negative	1	2	2	4	1	0	0	3	0	1	14
Total	1	2	2	8	1	1	0	7	2	1	25
Kerbala											
Positive	0	3	1	0	2	2	0	1	0	0	9
Negative	0	1	2	1	2	1	1	5	3	0	16
Total	0	4	3	1	4	3	1	6	3	0	25
Missin											
Positive	0	2	2	2	3	1	0	5	0	0	15
Negative	0	0	1	0	2	1	0	2	4	0	10
Total	0	2	3	2	5	2	0	7	4	0	25
Wassit											
Positive	3	7	0	0	0	0	0	2	1	0	13
Negative	0	0	0	3	0	2	0	6	1	0	12
Total	3	7	0	3	0	2	0	8	2	0	25

wk. = week

Our study is considered as the first study in Iraq for rotavirus detection by Conventional PCR. The results of the present study indicate that the ELISA method illustrates approximately similar positive results to chromatographic method, which is probably responsible to the reported superior accuracy of the method. Rotavirus were detected in ٥٥ (44%) samples with chromatographic immuno-assay method, ELISA revealed 53 (42%) positives and Conventional PCR detected ٤٧ (٣٨%) and 26 male samples (21%) were positive while 29 female sample (23%) were positive in Chromatographic immunoassay test. The highest percent in chromatographic immunoassay in Missan 15 samples (12%) and the lowest was in Alqadissiya 7 samples (6%), while in ELISA the highest percent was also in Missan 15 samples (12%), where the lowest percent was in Alqadissiya 5 samples (4%), in PCR 12 samples (10%) positive in Missan while the lowest percent were in Alqadissiya 7 samples (6%) positive. Two samples were positive in Chromatographic while there were negative in ELISA test, 8 samples were positive in Chromatographic but were negative in Conventional PCR. In addition, 6 samples were given positive result in ELISA but they had given negative result in Conventional PCR. According to the results of the present study, the chromatographic immunoassay method possesses low sensitivity compared to ELISA and the results appear to be reliable. Specificity was highest for the kits of *AccuPower* PCR Premix of Bioneer Corporation,whereas observed specificities for the remaining methods were approximately 100%.

Table, 5: The results obtained by using Conventional PCR method for Rotavirus in 125 fecal samples according to age of tested calves.

PCR Method											
SITE	AGE										
	1 wk.	2 wk.s	3 wk.s	4 wk.s	5 wk.s	6 wk.s	7 wk.s	8 wk.s	12 wk.s	16 wk.s	Total
Alqadissiya											
Positive	2	1	1	1	1	0	0	0	0	1	7
Negative	2	3	1	3	4	2	0	0	0	3	18
Total	4	4	2	4	5	2	0	0	0	4	25
Babel											
Positive	3	1	1	1	0	0	1	1	1	0	9
Negative	2	1	0	2	1	1	2	1	4	2	16
Total	5	2	1	3	1	1	3	2	5	2	25
Kerbala											
Positive	2	1	1	1	0	0	0	1	1	1	8
Negative	3	4	1	2	1	0	0	2	1	3	17
Total	5	5	2	3	1	0	0	3	2	4	25
Missan											
Positive	2	1	1	1	2	1	1	1	1	1	12
Negative	0	2	1	2	5	0	0	1	0	2	13
Total	2	3	2	3	7	1	1	2	0	3	25
Wassit											
Positive	2	1	2	0	1	0	2	1	1	1	11
Negative	1	3	4	0	1	0	3	2	0	0	14
Total	3	4	6	0	2	0	5	3	1	1	25

wk. = week.

Our findings illustrated no significant difference in the results of the three experimented methods. Chromatographic immunoassay method showed higher positives than ELISA and Conventional PCR.

In conclusion, our study showed that the chromatographic immunoassay is clearly a dependable and quick method for identifying the rotavirus while ELISA and PCR are more sensitive than the chromatographic immunoassay. Chromatographic immunoassay is suitable for screening of rotavirus gastroenteritis and be easily implemented in the hospitals and clinics. This method is non-expensive, rapid and simple to perform as compared with ELISA and Conventional PCR, which is high, expensive but it can recognize infection shedding of low-level from infection weeks prior to the present disease. The ELISA and Conventional PCR tests analyzed in the current study were presented generally to have comparable achievement for rotavirus identification in fecal samples. The Conventional PCR test is excellent method for diagnostic laboratories and working in a portable PCR machine is highly sensitive and specific and offers guarantee for on-farm molecular detection of rotavirus.

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الفروقات في الحساسية والخصوصية بين ثلاث مجموعات كشف عن الفَيروسَات العَجَلِيَّة (الروتا)

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الخلاصة

الفَيروسَات العَجَلِيَّة (الروتا) هي إحدى العوامل الرئيسة الأكثر شيوعاً المسببة للإسهال في الرضع والأطفال الصغار والعجول حديثي الولادة على مستوى العالم. من المهم جداً تشخيص المرض مبكراً لغرض علاج المرضى المصابين بشكل فعّال. لقد أجريت هذه الدراسة باستعمال ثلاث طرائق مختلفة للكشف عن فيروس الروتا في خمسة محافظات عراقية (القادسية، بابل، كربلاء، ميسان، واسط). جُمع و فُحِصَ ما مجموعه ١٢٥ عينة من براز عجول تراوحت أعمارها ما بين ١ أسبوع إلى ١٦ أسبوع في المدة من تشرين الثاني ٢٠١٥ إلى شباط ٢٠١٦، باستعمال المُقايَسَةُ المَناعِيَّة الكروماتوغرافيّة ومُقَايَسَةُ المُمْتَزّ المَناعِيّ المُرْتَبِطِ بالإنْزِيْم (الألايزا) واختبار تَفاعُلُ البلمرة التسلْسِلِيّ. كانت النتائج التي حُصلَ عليها باستعمال المُقايَسَةُ المَناعِيَّة الكروماتوغرافيّة 44% ومُقَايَسَةُ المُمْتَزّ المَناعِيّ المُرْتَبِطِ بالإنْزِيْم ٤٢% وتَفاعُلُ البلمرة التسلْسِلِيّ 38% حيث كانت الأولى سهلة، بسيطة وسريعة وأظهرت حساسية عالية مع خصوصية مقبولة في حين سمحت مُقَايَسَةُ المُمْتَزّ المَناعِيّ المُرْتَبِطِ بالإنْزِيْم بتقدير كمي لمستضدات الفيروس العجلي بينما أظهر اختبار تَفاعُلُ البلمرة التسلْسِلِيّ حساسية أكثر وخصوصية عالية جداً. وتشير هذه النتائج إلى أن الالايزا حساس ومحدد مثل المُقايَسَةُ المَناعِيَّة الكروماتوغرافيّة، وأنه يمكن تطبيقهما على نطاق واسع لفحص عينات البراز في الحالات التي يشتبه اصابتها بالإسهال الناجم عن الفَيروسَةُ العَجَلِيَّة. وكانت الفحوصات الثلاثة موثوقة عموما للكشف عن فيروس الروتا، وبالتالي، يمكن تطبيقهما بنجاح للتشخيص الروتيني.

الكلمات المفتاحية: روتا فايرس، مُقَايَسَةُ المُمْتَزّ المَناعِيّ المُرْتَبِطِ بالإنْزِيْم، المُقايَسَةُ المَناعِيَّة الكروماتوغرافيّة.