Molecular detection of Rotavirus type A in diarrheic calves of Mid-Euphrates governorates\ Iraq

Qassim Haleem Kshash[1] and Hamed Abbas Hassan[2]
[1]Department of Internal Medicine, College of Veterinary Medicine, Al-Qadissyiah University,
[2]Al-Qasim Green University, Iraq.
E-mail: Qassim.Kshash@qu.edu.iq
Received: 28/11/2019
Accepted: 16/3/2017

Summary

Four hundred fecal samples from diarrheic calves of both sexes (1-60 days old) from four Iraqi Mid-Euphrates governorates (Babylon, Al-Najaf, Karbala and Al-Qadissiyiah) were collected to investigate rotavirus type A infection by using Real-Time Polymerase Chain Reaction technique. The results showed that infection rates were (14%), while the high rate (20%) was found in age of (1-30 days), however non-significant values were related to animal gender. Moreover, a high infection was recorded at January (29.5%) and the rotraviral infection was recorded in four provinces but the higher was in Babylon (23%). The clinical examination of infected calves showed voluminous watery diarrhea, profound weakness and mild depression; the body temperature, respiration and heart rates were decreased significantly and appetite was changeable towards loss or anorectic.

Keywords: Rotavirus type A, Diarrhea, Calves, Real-Time Polymerase Chain Reaction.

Introduction

Calf diarrhea was recognized as a serious problem in dairy cattle industry for decades, which causes poorly growth and death due to dehydration and electrolytes imbalance (1). Bovine group A rotavirus is the main cause of diarrhea in neonatal calves and it is responsible for severe economic losses because of the high percentage of morbidity in newly born calves (2). The rotavirus is a double-standard RNA virus which is a member of the family Reoviridae and 11 serotypes of rotavirus have been defined, that infect human and animals; it is classified into seven groups from A to G and several sub groups based on specifity of VP6 inner shell polypeptides (3). Rotavirus is contagious and it is transmitted from animal to animal via fecal-oral route and the incubation period would last for several days –week and the virus can infect cattle but with acute and sever form in calves than in adult which mostly become asymptomatic (4). Rotavirus infection in cattle is globally distributed and recorded in Brazil (5), Turkey (6), UK (7), Sweden (8), France (9), India (10); it was recorded in Iraq in 1982 and the recent studies were carried out in Al-Mosul, in Al-Basra and in Babel governorates (11-13).

Materials and Methods

Four hundred fecal samples were collected from diarrheic calves (1-60 days old) and from both sexes within a period of seven months (November\ 2013 – May\ 2014) reflecting different areas in Mid-Euphrates of Iraq (Babylon, Al-Najaf, Karbala and Al-Qadissiyiah governorates\ Iraq) in an average of one hundred (100) samples from each governorate and the samples were collected in equal numbers (200) according to gender and two aged periods (1-30 days) and (31-60 days). The data accompanied sampling were obtained by a questionnaire paper that included owner and area name, date, animal age and sex, along with temperature, respiration and heart rates and feces description. Fecal suspension was prepared in DEPC water to 1:4 dilution then clarified at 5000 rpm\15 minutes at 4°C, and the supernatant submitted to RNA extraction according to instructions of trizol RNA extraction kit (Bioneer, Korea), then the total RNA recovered was suspended in 20 µl nuclease free water and stored at −20°C until used. Real-Time Polymerase Chain Reaction (RT-PCR) was performed for detection of bovine rotavirus by using the primers and Taq Man probe specific for glycoprotein gene of bovine rotavirus (Table, 1) and this technique was carried out according to the method

described by (14). The data were analyzed by using SPSS (version 16 software) program.

Table, 1: The primers and probe used in the study.

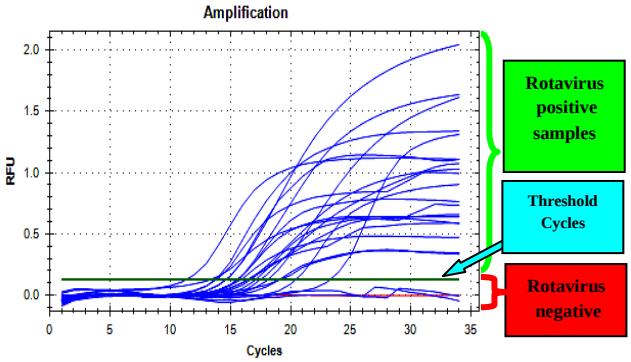
Primers	Sequence	Product size
BRV (VP6) Primers	F ACTCAGCGTCATTCACACTG R TTTCTGATCCCGCATTGAGC	84 bp
BRV (VP6) Probe	5-FAM-ACCGGCTCATGATAACTTGATGGGT-TAMRA-3	

Results and Discussion

Fifty six (56) fecal samples were found positive for bovine rotavirus (14%) screened by RT-PCR (Table, 2) (Fig. 1).

Table, 2: Rotavirus infection rate in examined diarrheic calves.

Number of examined calves	Number of positive RT-PCR samples	%
400	**56**	**14**



Figure, 1: Real-Time RT-PCR amplification plot of bovine rotavirus.

The present result of infection rate indicated that rotavirus was distributed among calves in the areas of study in Iraq in agreement with many researchers like (11) in Al-Mosul (15.5%), Al-Basrah (25%) (13) and with another resultant in the world as (33%) in China (15), (17%) in Australia (16), Japan (16.7%) (17) as well as 43% in six European countries (18) but in contrast to all above results about rotavirus infection in calves the report by (19) in Bangladesh after examining 124 calves did not record any infection.

The wide variance in rotaviral infection rate in the present study as well as another infection rates in different areas in the world depend on techniques used in these screening immune, age and nutritional status of examined animals, type of animals rearing, application of sanitary processing in their animal housing.

Table (3) summarizes the clinical signs associated with the positive rotavirus infected calves (56), temperature (38.9±0.3), respiration (24.5±0.7) and heart rate (99±09) were decreased (less than normal) respectively and 71.4% of calves were depressed and 42.8% of infected calves continued sucking, while 28.5% anorectic, as well as watery diarrhea (67%), yellowish feces (38.6%) were the predominant features of diarrhea in their examined calves. These clinical sings observed on diarrheic calves were also reported with different degrees from moderate severe dehydration as well as death of infected calves as reported by (4, 20 and 21).

The rotavirus invades the surface of epithelial cells of small intestine villi and causes loss or shortening of the villi which becomes covered by immature cells arising from the villous crypt. Immature cells with lost of epithelial surfaces dramatically reduce absorptive capacity of gut and the secretion of normal digestive enzymes so the resultant is an increase of fluidity and undigested, un absorbed food containing in gut lumen (3).

Table, 3: The main clinical signs of rotaviral diarrheic calves.

Parameter type	Clinical signs		
Temperature	M±SE	38.9 ± 0.3	
Respiration	M±SE	24.5 ± 0.7	
	quality	Shadow rapid	
Pulsation	M±SE	99± 09	
	quality	Weak\Mild	
Appetite	anorectic		28.6 %
	loss		28.5 %
	Continuous sucking		42.8 %
Faeces	consistency	Watery	67 %
		Soft	33 %
	Colour	Yellowish	38.6 %
		Milky	31.5 %
		Green-yellow	29.9%
	Mixed with blood or flackes	With	69.6%
		Without	30.4%
Nervous signs	Depression	Mild	71.4 %
		Sever	28.6 %

The increasing of microbial activity of the virus affects the villus of the small intestine, causing a loss of absorption and the osmotic

imbalance lead to draw more fluid into the intestine lumen which lead to malnutrition and malabsorption due to a compromised intestinal epithelial barrier; the subsequent diarrhea causes weakness, dehydration, acidosis, hypoglycemia and electrolytes losing that lead to generalized depression and all physiological functions may either become abnormal or stop especially in severe cases, and death may also occur (22).

Calves aged with (1-30 days) were significantly and highly infected with rotavirus type A (20 %) than older calves (31-60 days) as (8%) (Table, 4).

Table, 4: Relationship between age and rotaviral infection in examined calves.

Age period\ days	Number of calves	Number of positive infection	%	
1-30	200	40	20	a
31-60	200	16	8	b

The present result is similar to the results by (23) who reported a highly rotraviral infection in calves with age group at minimum (1day) to maximum (30 days); also it was recorded by (24-26) who recorded that newborn calves at the first two months of age were infected by rotavirus but calves at first four weeks of age were more susceptible. Also (27) postulated a hypothesis to explain neonate calves as being more susceptible to rotavirus infection because enterocytes in intestine could absorb macromolecules like S-IgA and S-IgM at first 36 hrs after calving which they provided by dams; these immunoglobulin can reach to lamina properia, blood and lymphatic streams and stimulate the local and systemic immune system against any microbe infection; then enterocytes become closed and allow passage micromolecule subsequent time as microbes like rotavirus which, if did not face any defense mechanism, could replicate and invade many intestinal cells and cause pathological processing that lead to the appearance of rotavirus infection if these calves did not intake colostrums at first day.

The Infection rate may not affected by gender of examined calves as explained in (Table, 5) which revealed infection percentage in male (14 %) and in female as (13.5 %) with slight increase in males than females but it was non-significant.

Table, 5: Relationship between gender and rotaviral infection in examined calves.

Calves gender	Numbers	Positive infection	%	
Male	200	28	14	a
Female	200	27	13.5	a

The non affection of gender of calves on rota virus infection in this study was similar to other findings by (24 and 28), as well as (19 and 29) who recorded an infection rate in females more than in male, but it was statistically non-significant. The anatomical, functional, hormonal similarities of body system of both male and female calves in early ages lead to non particular resistance against infection, but degree of contamination with virus, dose of virus, exposuring to stress factors, colostrums consumption or not as well as many environmental and magemental factors, all affect the infection occurrence and its severity in the same or different ages in both calves gender (30).

Table (6) shows the high infection rate recorded in January (29.5 %) and no infection was found in May, While infection rates was (14.5 %) and (16.4 %) were exhibited in November and December respectively, so the lowest infection was founded in April (5%).

Table, 6: The effect of months of study on rota viral infection in examined calves.

Months of year	Number of calves	Number of positive infection	%	
November	48	7	14.5	a
December	85	14	16.4	a
January	71	21	29.5	b
February	65	7	10.7	c
March	61	5	8.1	c
April	40	2	5	d
May	30	0	0	e

Many researchers found that rotraviral infection in calves was similar to present results like (31 and 32) that showed an increase of infection in winter months and raining season, but (33) showed no seasonal variation in rotraviral diarrhea occurrence in Mozambique. The high rate of rotraviral diarrhea in calves in Winter and raining season

could be due to that the climate providing a suitable micro-environments which maintains the shedding virus surviving for a long time relatively and have viability more than in warmth months, also some researchers showed that intestinal and serum immunoglobulin levels like (IgA, IgM and IgG) that have important role in preventing or at least less severity of rotavirus infection would decline in winter but increase during spring and summer seasons (34).

According to infection in the studied areas\Iraq, calves in Babylon had a high infection (23%), while a low infection rate was recorded in calves of Karbala (8 %) which is near in Al-Qadissiyiah governorate (10%), and in Al-Najaf was (15%), (Table, 7).

Table, 7: Percentages of rotraviral infection of calves in areas of study.

Area of study\ Governorates	Number of calves	Number of positive infection	%	
Babylon	100	23	23	a
Al-Najaf	100	15	15	b
Karbala	100	8	8	c
Al-Qadissiyiah	100	10	10	c

The variation in infection rates of calves in four governorates located in neighboring geographical area, with same seasonal condition as well as the same animal breeds, could be attributed to management and hygiene measurement applications and rearing system.

References

1. Reidy, N.; Lennon, G.; Fanning, S.; Power, E. and Oshea, H. (2006). Molecular characterisation and analysis of bovine rotavirus strains circulating in Ireland 2002–2004. Vet. Microbiol., 117(2-4): 242–247.
2. Badaracco, A.; Garaicoechea, L.; Rodriguez, D.; Uriarte, E.L. and Odeon, A. (2012). Bovine rotavirus strains circulating in beef and dairy herds in Argentina from 2004 to 2010. Vet. Microbiol., 158:394-399.
3. Kapikian, A.Z.; Hoshino, Y. and Chanock, R.M. (2001). Rotavirus In: Field Virology. Knipe, D.M. and Howley, P.M. (eds.) .4th editions, Lippincott Williams and Wilkins, Philadelphia, Pp:1787-1833.
4. Radostits, O.M.; Gay, C.C.; Hinchcliff, K.W. and Constable, P.D. (2007). Veterinary Medicine, Atextbook of the diseases of cattle, horses, sheep, pigs and goats. 10th ed. Saunders-Elsevier, Philadelphia, Pp:1286-1296
5. Alfieri, A.A.; Parazzi, M.E.; Takiuchi, E.; Medici, K.C. and Alfieri, A.F. (2006). Frequency of group A rotavirus in diarrheic calves in Brazilian cattle herd (1998-2002). Tropical Animal Health and Production, 38:521-526.
6. Duman, R.; Dodet, B. and Aycan, A.F. (2010). Prevalence of rotavirus infection in calves wiyh diarrhea in Konya region. J. Anim.Vet. Adv., 9(1):876-881.
7. Reynolds, D.J.; Morgan, J.H.; Chanter, N.; Jones, P.W.; Bridger, J.C.; Debney, T.G. and Bunch, K.J. (1998). Microbiology of calf diarrhea in southern Britain. The Veterinary Record .119:34-42.
8. De Verdier, K. (2006). The panorama of infection in Sweedish caves with diarrhea. SvenskVeterinartidning , 58:29-32.
9. Bendali, F.; Bichet, H.; Schelcher, F. and Sanaa, M. (1999). Pattern of diarrhea in new borne beef calves in south–west France. Vet. Res., 30:61-74.
10. Sukhija, Y. and Grover, Y.P. (2003). The application of latex agglutination test for the detection of bovine rtavirus in diarrheic fecal samples and its comparission with Elisa and RNA-PAGE. J. Immunol. Immunopathol., 5(Issue):34 -38.
11. Al-Robaiee, I.A. and Al- Farwachi, M.I. (2013). Prevalence of rotaviral infection in diarrheic neonatal calves in Mosul city, Iraq. Vet. World, Pp:538-540.
12. Hekmat, K.A. (2010). Detection of rotavirus antigens and antibodies in children and bovine. Thesis. College of Vet. Med. Basrah University. Iraq.
13. Atea, G.H. (2010). The distribution of rotavirus infection among calves of dairy cattle in Basrah city. The Iraqi J. Vet. Med., 34(2):60-68.
14. Erles, K.; Toomey, C.; Brooks, H.W. and Brownlie, J. (2003). Detection of coronavirus and rotavirus in dogs with canine infectious respiratory disease .Virol., 310:216-223.
15. Xie, L.; Zhixun, X.; Qing, F.; Jiabo, L.; Xianwen, D.; Zhiqin, X. and Mazhar, I.K.

(2012). Reverse transcription loop-mediated isothermal amplification assay for rapid detection of bovine rotavirus. BMC Vet. Res.,Virology and Prion Diseases. 8:128-133.

16. Klein, D.; Kern, A.; Lapan, G.; Benetka, V.; Mostl, K.; Hassal, A. and Baumtner, W. (2009). Evalution of rapid assays for the detection of bovine coronavirus, rotavirus A and *Cryptosporidium parvum* in fecal samples of calves.Vet. J., 182 (3):484-486.
17. Fukai, K.; Sakai, T. and Kamata, H. (1998). Distribution of G serotype and P serotype of bovine group A rotavirus isolated in Japan. Australian Vet. J., 76 (6):418-422.
18. Midgley, S.E.; Banyai, K.; Buesa, J.; Halaihel, N.; Hjulsager, C.K.; Jakab, F.; Kaplon, J.; Larsen, L.E.; Monini, M.; Polysak, M.; Pothier, P.; Ruggeri, F.M.; Steyer, A.; Koopmans, M. and Bottiger, B.(2012). Diversity and zoonotic potential of rotavirus in swine and cattle across Europe.Vet Microbiol., 156(3-4):238-245.
19. Alam, M.N.; Alam, M.M.; Nahar. A. and Kobayashi, K. (2011). Molecular epidemiological studies on rotavirus infection causing sever diarrhea in human ,animal in Bangladesh. Bangladesh J. Vet. Med., 9(2): 76-79.
20. Janice, C. and Bridger, L. (1994). A definition of bovine rotavirus virulence. J. General Virol., 75:2807-2812.
21. Chauhan, R.S.; Dhama, K. and Mahendran, M. (2008). Pathobiological of rotraviral diarrhea in calves and its diagnosis and control: A review. J. Immunol. and Immunopathol., 10(1):1-13.
22. Dhama, K.; Chauhan, R.S.; Mahendran, M. and Malik, S.V. (2009). Rotavirus diarrhea in bovine and domestic animals. Vet. Res., Common. 33(1):1-3.
23. Chinsangaram, R.; Kohara, J. and Tsunemitsu, H. (1995). Correlation between maternal serum antibodies and protection against bovine rotavirus diarrhea in calves. J. Vet. Sci., 62:219-221.
24. Nataraju, S.M.; Chattopadhyay, U.K. and Krishnan, T. (2009). A study on the possibility of zoonotic infection in rotraviral diarrhea among calves and buffalo calves in and around Kolkata, India. Eur. Rev. Med. Pharmacol. Sci .,13(1):7-11 .
25. Brooks, A.E.; Manning, K.; Wang, G.J.; Taylor, J.A.; Ge, Y.; Masell, A. and Ussher, J.E. (2013). Rotavirus NSP4 triggers secretion of proinflammatory cytokines from macrophages via Toll-Like Receptor 2. J. Virol., 87(20):11160- 11167.
26. Sharma, R.; Garrison, R.D.; Tepas, J.J.; Mollitt, D.L.; Pieper, P.; Hudak, M.L.; Bradshaw, J.A.; Stevens, G. and Premachandra, B.R. (2004). Rotavirus – associated necrotizing enterocolitis: an insight into a potentially prevalence disease. J. PedSurg., 39:453-457.
27. Huely, L.W. and Theil, K.P. (Perspectives on immunoglobulins in colostrums and milk. Nutrients, 3(4):442-474 .
28. Dash, S.K.; Tewari, A.; Krishna, K.; Goel, A. and Bhatia, K. (2011). Detection of rotavirus from diarrheic cow calves in Mathura, India. Vet. World., 4(12):554-556.
29. Hasso, S.A. and Pandey, R. (1986). Possible sex differences in the susceptibility of caves to rotavirus infection. Can. J. Vet., 50(2):287-288.
30. Haschek, B.; Klein, D.; Benetka, V.; Herra, C.; Sommerfeld, I.; VilCeK, S.; Moesh, G. and Baumgartner, K. (2006). Detection of bovine rotavirus in neonatal calf diarrhea in lower Austria and Styria\Austria. JVM series. Pp:5-12.
31. Dodet, B.; Hesseltine, E.; Mary, C. and Salion, P. (1997). Rotavirus in human and Vet. Med., SANT., 7(3):195-199.
32. Broor, S.; Ghosh, D. and Mathur, P. (2003). Molecular epidemiology of rotavirus in India. Indian J. Med. Res., 118:59-67.
33. Acha, S.J.; Kuhun, P.; Jonsson, G.; Mbazima, M. and Katouli, A. (2004). Studies on calf diarrhea in Mozambique: Prevalence of bacterial pathogens. Acta Vet. Scandinavica., 45(1-2):27-36.
34. Norheim, K.; Simensen, E. and Gjestang, K. (2005). The relationship between serum IgG level and age, weight gain in rotraviral infection in dairy calves. Nord. Vet. Med., 37:113-120.

الكشف الجزيئي عن فايروس الروتا نوع A في عينات براز عجول مصابة بالإسهال في محافظات حوض الفرات الأوسط \العراق

قاسم حليم كشاش[١] و حامد عباس حسن[٢]

فرع الطب الباطني، كلية الطب البيطري، [١]جامعة القادسية، [٢]جامعة القاسم الخضراء، العراق.

E-mail: Qassim.Kshash@qu.edu.iq

الخلاصة

تضمنت الدراسة فحص أربعمئة عينة براز جمعت من عجول مصابة بالإسهال تراوحت أعمارها مابين (١-٦٠ يوم) من أربع محافظات من الفرات الأوسط في العراق وهي (بابل، النجف، كربلاء، القادسية) خلال مدة سبعة أشهر (تشرين ثاني \٢٠١٣- نيسان \٢٠١٤) باستعمال تقنية تفاعل انزيم البلمرة المتسلسل الفائق السرعة للتحري عن فايروس الروتا البقري نوع A. أظهرت النتائج إن نسبة الخمج المئوية كانت (١٤%) وقد سجلت أعلى نسبة إصابة (٢٠%) في العجول بعمر (١-٣٠ يوم) ولم يظهر للجنس أي تأثير على نسبة الإصابة والتي سجلت بأعلى مستوياتها في شهر كانون الثاني (٢٩,٥%) وكانت الإصابة بالعجول في محافظة بابل هي الأعلى (٢٣%). أما الفحص ألسريري للعجول المصابة بفيروس الروتا البقري نوع A حيث أظهر علامات تتضمن الإسهال المائي مع الضعف والنحول مع درجات متوسطة الشدة من الخمول، في حين كانت معدلات درجات الحرارة، التنفس، النبض منخفضة معنوياً أما شهية العجول متغيرة من قلة الشهية إلى انعدامها.

الكلمات المفتاحية: فايروس الروتا نوع A، الإسهال، العجول، تفاعل انزيم البلمرة المتسلسل الفائق السرعة.