Detection of bovine rotavirus in diarrheic calves by using rapid test in some Mid-Euphrates provinces

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Abstract

The study was aimed to determine the Rota viral infection rates in diarrheic calves and effectiveness of some epidemiological factors on that rate in some provinces in Mid–Euphrates region in Iraq (Babylon, Al-Najaf, Karbala, Al-Qadisyia provinces). A total of 530 calves aged between 1-60 day were examined and fifty fecal (50) samples were collected from diarrheic calves only within six months period from November-2012 to April -2013. The fecal samples were examined for the presence of rotavirus by rapid rotavirus test (screening test), as well as the clinical examination for these diarrheic calves were done. The rapid test demonstrated that the rotavirus infection rate was 18/50 (36%). A 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. Male and female (37.5%, 40% respectively) were seen infected without any significant differences. The main clinical signs observed on rotavirus diarrheic calves were include, watery voluminous diarrhea with profound weakness and mild depression, so as temperature, respiration, heart rates could be decreased and appetite was changeable towards loss or anorectic. In Babylon, the highest rotavirus infection rate was recorded 60%, and less rates in Karbala 20% and 40%, 33% in AL-Najaf and AL-Qadisyia provinces respectively. The rotavirus infection rate in diarrheic calves was affected by months significantly, which it was higher at January 75%, so as in November 50%, December 40% and February 33.3%, but not recorded in March and April.
Key words: Rota virus, diarrhea, calf, rapid test.

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الكشف عن فايروس الروتا في العجول المصابة بالإسهال باستخدام الاختبار المسحي السريع في بعض محافظات الفرات الأوسط

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الخلاصة

تضمنت هذه الدراسة تحديد معدلات الإصابة بفايروس الروتا في العجول المصابة بالإسهال وتأثير بعض العوامل الوبائية على معدلات الإصابة في محافظات الفرات الاوسط (بابل، النجف، كربلاء والقادسية) من مجموع 530 عجل و التي تتراوح اعمارها بين (1- 60) يوم تم فحصها ، جمعت خمسون (50)عينة براز من عجول مصابه بالإسهال وخلال ستة اشهر من شهر تشرين الثاني 2012 الى شهر نيسان 2013 .فحصت العينات لتشخيص فايروس الروتا بطريقة الاختبار السريع (الاختبار المسحي)،فاظهر الاختبار السريع 18 حالة موجبة (50/18) وبنسبة (36%). كذلك بينت الدراسة إن نسبة الإصابة في الأعمار اقل من 30 يوم هي 45,4 % والعجول التي بعمر (30 – 60) يوم هي 28,5%بالنسبة للإصابة في الذكور والاناث فلا توجد اختلافات معنويه حيث كانت (3705، 40%) على التوالي. العلامات السريرية الواضحة والمميزة للعجول المصابة بفايروس الروتا هي (اسهال بدرجات مختلفة ، ضعف عام، خمول ، ارتفاع في درجة الحرارة رغم وجود الإسهال ، زيادة في معدلات التنفس و ضربات القلب، الشهية متغيره بين الفقدان وتصل إلى الفقدان الكامل للشهية. سجلت اعلى معدلات للإصابة بالفايروس في محافظة بابل وهي 60% وبمعدل اقل في كربلاء (20%)و(40%، 33%) في النجف والقادسية على التوالي .اما نسب الإصابة وخلال الأشهر التي جمعت بها العينات فقد سجلت اعلى نسبه في شهر كانون الثاني بنسبة 75% وكذلك شهر تشرين الثاني 50% وكانون الأول 40% وشباط 3303% ،أما شهر آذار و نيسان فلم تسجل إصابات تذكر.
الكلمات المفتاحية : فايروس الروتا ، الاسهال ، العجول ، الاختبار المسحي السريع .

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Introduction

Neonatal diarrhea is a worldwide problem and its seen as one of the biggest challenges for both the beef and dairy industry (1). Many infectious or non infectious agents play role in newborn calves with diarrhea ,there are many infectious agents e.g., rotavirus, coronavirus, astrovirus, calicvirus, as well as *E-coli, salmonella spp, shigella* responsible for newborn calves with diarrhea in tie –sall barns (2). Mainly rotavirus lead to loss in many species of newborn ,human including too (3). Many studies have been showed 50% of newborn deaths due to diarrhea in calves rotavirus infections constitute the basic causes of economic loss owing to grow delay ,birth of weak calves and high mortality levels in herds (2). Rotaviruses are now recognized as the most important cause of severe viral gastroenteritis in humans (4), and are also major infectious cause of diarrhea in calves (5). Rotavirus infection in cattle is worldwide distribution which obtained by (6) in Brazil as19.4%, in Turkey 10.5% (7), in Sweden between 1993 and 2006 as 24-47% (8),also in UK the 42% (9) , in Costa Rica 7% (10), in calves in Ohio 16% (11) and 47% in south-west France (12), 27% in India by (13) ,16.8% in Southern Italy (14). Also in Iraq was reported in AL-Mosul 15.5% and in AL-Basrah 25% (15), (16). Conventional techniques like rapid bovine rotavirus test, electron microscopy (EM), isolation in MA-104 cell line, electropherotyping, and various serological tests as well as PCR technique are used for diagnosis of rotavirus (17). To investigate e about rotavirus as one of many important causative agent of neonate diarrhea in calves, the present study was designed in some Mid-Euphrates regions in Iraq.

Materials and methods

Materials:

One Step Rotavirus Rapid Test device:

One Step Rotavirus Rapid Test device kit was used in this study was manufactured by Acon company /Germany.

Principles:

The Antigen Rapid test (Rota Ag Test) Kit is a chromatographic immunoassay for the qualitative detection of Rota virus, group A antigen in porcine, bovine and canine feces with high degree of accuracy. The specially selected Rota virus antibodies are used in test band as both capture and detector materials.

Procedure of the test :

Collect the samples from calves feces using the swab, then insert the swab into the specimen tube containing 1ml of assay diluents and mix the swab samples with assay diluents to extract well, after that remove the test device from the foil pouch, and place it on a flat and dry surface ,then using the disposable dropper provided, take the samples from extracted and mixed specimens in the tube and add four (4) drops into the sample hole using the disposable dropper, drop by drop, then as the test begins to work, you will see purple color move across the result window in the center of the test device. If the migration has not appeared after 1minute, add one more drop of the mixed assay diluents to the sample well. For best result, test 5 diarrhea feces in the farm. Interpret test results at 5 ~ 10 minutes. Do not decide after 20 minutes.

Interpretation of the test:

1) A color band will appear in the left section of the result window. This means that the test is working properly. This band is the control band. The right section of the result window indicates the test results. If another color band appears in the right section of the result window, it means that the specimens have the Rota virus antigens (Fig.1 positive, and Fig. 2 negative).

Figure(1):Two color bands show positive result of rotavirus to rapid test.
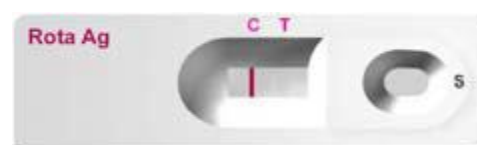
Figure(2): One color band show negative result of rotavirus to rapid test.

2) If the purple color band is not visible within the result window after performing the

test, the result is considered invalid. The directions may not have been followed correctly or the test kit may have deteriorated. It is recommended that the specimen be re-tested.

Methods

Clinical study

Five hundred and thirty newly calves were examined and 50 fecal samples were collected from acutely diarrheic calves with age grouped between 1- 60 day by aseptic methods (per rectally using gloved finger) taking care of all precautions so as to avoid contamination of samples ,which kept in sterilized plastic containers , transported under cold (ice) conditions and stored at -20 c (deep freezing) till further processing. Samples were collected over a period of six months duration from November 2012 to April 2013 from different farms and animal sale lands in many provinces (Babylon 17, AL-Najaf 10, Karbalaa 12, and AL- Qadisyia 11). The data was obtained by questionnaire paper which designed and included animal owners name, date , area, breed, sex, age, of calves, also temperature , respiratory and heart rates and other apparent clinical signs, consistency and color of feces and any note may be observed.

Statistical analysis

The data was analyzed by using statistical package for the social sciences(SPSS)version 16 software program (2007).

Results

Out of 530 calves were examined there were only 50 calves suffering from various types of diarrhea , therefore 19.43% of examined calves in present study were diarrheic as well as there was no significant effect of calves sex on diarrhea percentage as 10.04% in male and 9.03% in female, table (1). The results of rapid test showed that 18 out of 50 fecal samples of diarrheic calves were positive into rotavirus at percentage (36%), table (2). The main clinical signs observed on rotavirus diarrheic calves were include, watery voluminous yellowish or bloody diarrhea with profound weakness and mild depression, so as temperature, respiration, heart rates could be decreased and appetite was changeable towards loss or

anorectic. The result showed there was non-significant effect of sex rates of calves on infection rates by BRV which 37.5% in male and 40% in female, table (3). The results of infection rate according to age groups (1-29 days,30 -60 days) were (45.4%, 28.5%,) respectively, the result showed that the highest rate of infection 45.4% were in age group (1 -29 days), while age group (30 - 60 days) had taken the lowest infection rate 28.5%. Statically, there was significant effect (p< 0.05) age groups 1-29 days on BRV infection rate, table (4). The effect of infection rates according to the months of the year were (50%, 40%, 75%, 33.3%, 0% , and 0%) at (November, December, February, January, March, and April) respectively. The highest significant percentage rates of infection shared, January 75%, while in February had record the lowest infection rate. But infection not recommended in March and April, table (5). Rotavirus infection rates in the different provinces (Babylon, AL-Najaf, Karbala, and AL-Qadisyia) were 60%, 40%, 20%, and 33.3%, respectively. The highest rates were in Babylon 60% while the lowest one was in Karbala 20 %. Statically, There was significant differences in BRV infection at (p< 0.05) between Babylon with AL-Najaf and Karbala . The significant differences at (p< 0.05) between Karbala with AL-Najaf and AL-Qadisyia , and significant differences on (p< 0.05) between Karbala and AL-Qadisyia. No significant differences on (p< 0.05) between Babylon and AL-Qadisyia, table (6).

Table (1): Percentage of diarrhea among examined young calves.

calves sex	Number of Examined calves	Number of Diarrheic calves	Diarrhea rate (%)
Male	209	21	10.04a
Female	321	29	9.03a
Total	530	50	19.43

Similar letters refers to the non-significant differences(p> 0.05).

Table(2): Results of rapid rotavirus test.

No. of examined samples	No. of positive samples	Infection rate %
50	18	36

Table(3):Bovine rotavirus in calves, the rate of infection in relation to the sex.

Sex	No. of samples	positive	%
Male	8	3	37.5a
Female	10	4	40a
Total	18	7	38.8

Similar letters refers to the non-significant differences between sex at (p> 0.05).

Table(4):Relationship between age and bovine rotavirus infection rates.

Ages	N0. of samples	positive	%
1-29 days	11	5	45.4a
30 -60 days	7	2	28.5b
Total	18	7	38.8

Different letters refers to significant differences at (p< 0.05)

Table(5): The effect of the months on infection rate in calves by rotavirus.

Months	No. of samples	positive	%
November	2	1	50a
December	5	2	40a
January	4	3	75b
February	3	1	33.3ac
March	3	0	0
April	1	0	0
Total	18	7	38.8

Similar letters refers to the non-significant differences among months while different letters refers to significant differences at (p< 0.05).

Table(6): The results of Rota viral infection rate in relation to study regions.

The region	No. of samples	positive	%
babylon	5	3	60a
AL-Najaf	5	2	40b
Karblaa	5	1	20c
AL-Qadisyia	3	1	33.3b
Total	18	7	38.8

Similar letters refers to the non-significant differences among region while different letters refers to significant differences at (p< 0.05)

Discussion

Our result about rate of diarrhea (19.43%) is closely related to report by (7) in Hungary which showed diarrhea incidence in newly calves (10.5%) but diarrhea percentage in calves in Southern Italy reach to 16.8% (14) and the researcher expect this rate able to increase like 19.4% by (6) in Czech Republic ,because occurrence of diarrhea in calves is a result of complex interactions of three sets of factors; the calf , their dam and the calf's environment including management and infectious agents. While , (18) showed diarrheic newly calves (5%) in Mozambique which that attributed to strict measurement applied on dairy herd by separate pregnant dam from other herds three days before and three day after calving and feeding new borne calf on colostrum freely during three days. The present result of Rota viral infection in diarrheic examined calves (36%) is closely similar to results obtained by (19) in Brazil as (36.1%), but it higher than other reports like 17.7% by (20) and 19.4% by (6) in Belgium , as well as 27% in India by (13) and 24% in Sweden (8), but in Swiss, (21) showed rotavirus infection (59%) by rapid test, also (22) recorded rotavirus infection (43%) in Germany. The different rotavirus infection rates in diarrheic calves

which established in the present study and by the above other researchers is in agreement with the scientific fact by that rotaviruses a worldwide distribution and able to infect human and domesticated animals. The result from this study show that the rapid test is available tool in diagnosis of rotavirus infection, this assay has the number of advantage including its simple format , rapidity and low cost , and it can be performed without need of trained personnel or expensive equipment also it can read with naked eye, making it easy to perform in every laboratory. Temperature, respiration and heart rate were decline (38.9,24.5,/min 99 beat/min) respectively and 71.3% of calves could be depressed and 42.8% of infected calves continued sucking, while 28.5% anorectic, also watery, yellowish faces contain indigestible food particles with blood were predominant features of diarrhea in their calves. These clinical signs that showed on diarrheic calves was reported with different degree from moderate till to sever dehydration as well as death of infection calves which reported by (23); (24) and (25). The virus invades the surface epithelial cells of small intestinal villi ,and loss of epithelial cell result in shortened

villi ,which become covered by immature cells arising from the villous crypt. the immature cells and the loss of surface area dramatically reduce the absorptive capacity of the gut and the secretion of normal digestive enzymes. The result is dramatic increase in fluid ,containing undigested and unabsorbed nutrients in gut lumen. The increasing of microbial activity and the osmotic imbalance lead to draw more water into the intestine , the subsequent diarrhea causes weakness, dehydration also acidosis and hypoglycemia, electrolytes losing that lead to respiration and heart work could be affected ,depression and all physiological function may be either alteration or stop in severe cases lead to death. The resultant of effectiveness of sex on infection rate in present study which slightly higher in male than female but non-significant is similar to other finding by (26) whom recorded infection rate (20.37%)in male and (12.76%) in female, also (27) that found in male higher than in female but without remarkable significant variance. But (28) and (29) showed high rotavirus infection in female than in male but also in non-significant differences. The anatomical ,functional ,hormonal similarities of body systems of male and female calves in early ages lead to non-particular resistance against infection ,but degree of contamination with virus, dose of viruses, exposing to stress factors ,consumption colostrum or non and another many environmental and management factors, all effect on infection rate and severity in both sex of calves in same or different periodic age (30). The present result is in agreement with other resultant by(31) whom had reported highly infection rates at age group as minimum 1 day to maximum of 30 days ,as well as the observations by other group of workers like (27), (24) and (32) which them exhibit that peak rotavirus infection in calves at age 5-15 days .As well as result by (33) indicated that newborn calves of first two months of age were infected with rotavirus but calves more susceptible at first 4 week of age. Animal studies have consistently indicated antibody in lumen of small intestine was of major importance in resistance of rotavirus diseases ,and the pivotal role for local intestine IgA antibody which are in first day after birth depend on S- IgA from dam colostrum and the level of S-IgA may be increasing with age(34).While, (35) were postulated a hypothesis to explain neonate calves are more susceptible to rotavirus because the enterocyte could be not absorb macro-molecules (closure) like S-IgA and S-IgM 36hrs after birth but micro-molecules can absorbed and enter like rotavirus which replicate and caused pathological processing finally lead to signs of rotavirus infection early. The highest rotavirus infection rate at January in our study is agreement with (36) reported that rotavirus diarrhea had higher prevalence rate in calves during winter months, also in similar with resultant by (37) in which increase Rota viral diarrhea in raining season. Also the present result is agreement with finding by (38) that a major peak of rotavirus infection in neonate calves occur during coldest dried months of year. But (18) in Mozambique showed no seasonal variation in Rota viral diarrhea in occurrence. So (39) explained high rate of Rota viral diarrhea in coldest months due to the suitable climate for still shedding virus a live in micro-environment for a long time relatively and have viability more than in warm months. also he and his team was showing that mean intestinal ,serum immunoglobulin level like IgA, IgM, IgG (which responsible to prevent or less severity of rotavirus infection) were lowest in winter and increasing during spring and early Summer. As well as above many reasons, in Iraq most calving occur in end of autumn and firstly month of winter in which these ages are more susceptible for Rota viral infection ,that Summarized high Rota viral infection incidence in autumn and winter month. Incidence or Rota viral diarrhea was established in four provinces with significant increasable in Babylon in similar to previous studies some Iraq provinces like in Mosul 15.5% (15), (16) in Basrah province 25% ,and the variation between rate infection in these provinces which in same of geographical ,seasonal condition as well as the same animals breed and method of breeding , therefore these variance could be

attributed to management measurement at animal herd, also number of randomly fecal samples that collected , previously Rota viral infection finding. Babylon province has a large animals market which visiting by large number of peoples to sale or purchase different animals from all Iraq area at each Friday in AL-Qassim city, that explain Babylon became source of infection ,then distributed infection to neighboring provinces.

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