

Prevalence of Bovine viral diarrhea virus in cattle herds from Basrah and Nassirya Provinces by direct and indirect Elisa and Real time qPCR

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Abstract

The current study was conducted to investigate the prevalence of BVD virus in Basrah and Nassirya city by using ELISA and RT-PCR. Two hundreds and eighty two samples of non vaccinated cattle sera samples collected from two regions of Iraq (188 samples from Nassirya city and 92 samples from Basrah city). Samples tested by Enzyme Linked Immunosorbent Assay (ELISA) antigen capture. Positive results were 20 samples (8 sample in Thi-Qar and 12 positive samples from Basrah). All samples submitted to indirect ELISA(IDEXX HerdCheck ELISA)for detect BVDV antibodies .Genotyping of all 20 positive samples to antigen detection were tested by Real time PCR, using Cador BVDV ½ kit, after extraction of virus RNA by QIAamp mini kit. The results revealed that there were 20 positive sample according to direct ELISA(Ag detection), while 66 sample were positive to indirect ELISA, as well as, the result of RT-PCR showed that there were two sample positive to BVDV type-1 (one sample form each city).

Key words: BVDV, Genotype, ELISA, Iraq, Real time PCR.

Introduction

Bovine viral diarrhea virus (BVDV) is an important pathogen of cattle which causes significant economic losses to the livestock industry around the world (1). BVDV is a small enveloped RNA virus belonging to the Flaviviridafamily, genus *Pestivirus*, along with classical swine fever virus and border disease virus of sheep (1,2). BVDV has many different manifestations in a herd, depending on the herd's immune and reproductive status. Transient diarrhoea, mixed respiratory infection, infertility or abortion and mucosal disease are the most common clinical signs of the disease and can be seen simultaneously in a herd. The BVDV genome is a linear, single-stranded, positive sense RNA molecule of approximately 12.5 kb in length (3).The genome contains a single large open reading frame (ORF) encoding a polyprotein of about 3900 amino acids. The ORF is flanked by a 5' 381-386-nucleotide long untranslated region (5'UTR) and by a 3' 229-nucleotide secondary structure believed to act as an

internal ribosomal entry site to direct the translation of the ORF upon internalization of the viral genome into the host cell (4). Translation of the ORF produces a long polyprotein, which is co- and post-translationally processed by viral and cellular proteases giving rise to 11 to 12 mature viral proteins (3,5). The structural proteins (the exception is N^{pro}, a non-structural protein) are encoded by the 5' third of the genome as follows: N^{pro}, C, E^{ms}, E1, E2, and p7. Non-structural proteins are encoded downstream: NS2-3, NS4A, NS4B, NS5A, and NS5B (3,5,6). The vast majority of BVDV field isolates do not induce cytopathology in cell culture and are called non-cytopathic (ncp); cytopathic (cp) isolates are found almost exclusively in cattle suffering from mucosal disease (6,7).Many antigen-capture ELISA's have been developed for detection of BVDV antigen. The most commonly used antigen capture ELISA uses Mab directed against a conserved antigenic domain of a non-

structural protein (NS2/3) of pestiviruses. Serum is a good sample for the detection of infected animals by antigen-capture ELISA [8]. These antigen-capture ELISA have been found to yield results comparable to those of virus isolation [8, 9]. Several molecular diagnostic tests (RT-PCR) have been described for the detection of BVDV in serum, tissues, and isolates. Most of these tests are based on the detection of 5'UTR region or E2 gene [8]. A number of tests have developed for identification of BVDV and differentiation of BVDV1, BVDV2, and BDV [10, 11, 12, 13]. Both BVDV genotypes

have been reported in North America [14, 15], South America (Brazilian) [16], Europe (Germany; Belgium; Slovakia; United Kingdom, Austria, Italy) [17, 18, 19, 20, 21, 22, 23], Asia (Japan) [24]. In republic of Iraq, very little studies about BVDV, but some research revealed that first demonstrated of disease in year 1977 (25). Identified BVDV antibodies in cattle from different areas in Iraq. In year 2004 demonstrated cytopathogenic strain in cultured cells in vitro (26). Another study detected the virus in buffalos and cow herds, also demonstrated persistent infection (27).

Material and methods

Two hundred eighty blood samples collected from non-vaccinated cattle herds in Nassirya city (188 samples) and Basrah city (92 samples) south of Iraq. Samples were collected in 10 ml. vacuum tubes without anticoagulant and centrifuged at 2500 rpm for 20 minutes in order to obtain sera samples. The sera samples were refrigerated and tested as soon as possible.

1-ELISA:

After obtained serum from blood samples. They are testing by using a commercial kit IDEXX HerdCheck ELISA BVDV Antigen Test. The test was realized as manufacturer's instructions. Results were read by spectrophotometer by 450 nm. Wave length. All samples submitted to direct and indirect Elisa (IDEXX Herd Check ELISA) for detect BVDV antibodies.

2-Real time PCR:

In this method were included two steps firstly by extraction RNA from each sample by using QIAamp RNA Mini Kit (supplied by Qiagen company). After extraction of RNA followed manual procedure supplied with kit. Second step was amplification of extracted RNA in real time quantitative-PCR system (7300 Real time PCR Biotechnol. System) using QIAamp Cador BVDV1/2 kit (supply by Qiagen company) which is differentiated of three different viruses in same kit include BVDV 1, BVDV2 and Border disease. Under the manual book supplied with kit steps and after fifty cycles, read the qualified product by the same procedure of manufacturer's. The method was done under restricted sterile conditions to prevent contamination of samples and amplification RNA.

Results

Results of the first step of the study revealed that 20 positive samples according to direct Elisa (Ag detection) test (8 in samples from Nassirya city while 12 samples

were positive in Basrah city), while 66 samples were positive to indirect Elisa (Ab detection) Table(1).

Table(1): show results of direct and indirect Elisa test in cows serum samples

Elisa test	Positive samples	%	Negative samples	Total
Direct Elisa (Ag)	20	7.14	260	280
Indirect Elisa (Ab)	66	23.57	214	

The results of real time-qPCR, after extraction RNA from samples exhibited that only two positive samples detected from the

20 samples as BVDV type one .as following in figures.

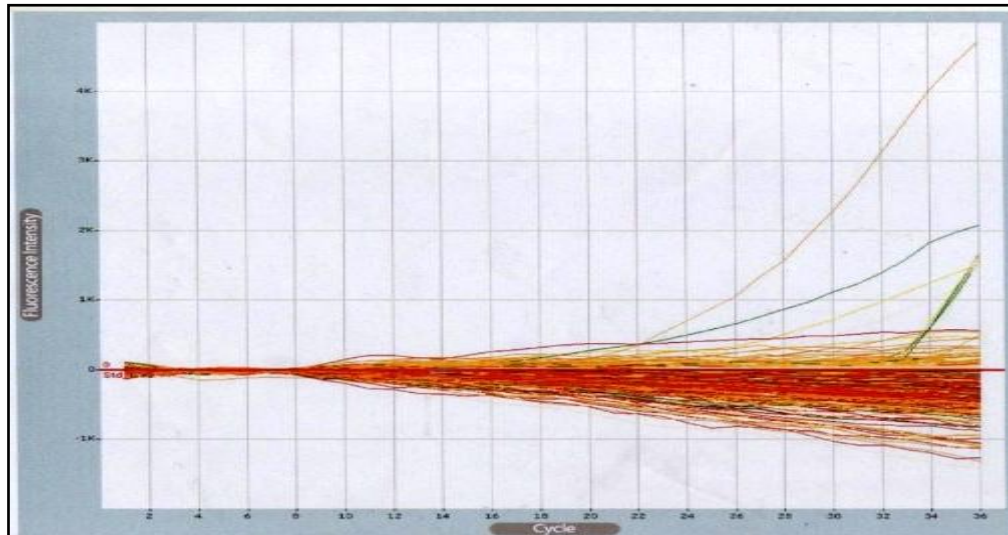


Fig.(1):positive tow samples of BVDV1 yellow lines, while blue line represent positive control of BVDV1.



Fig.(1): negative results to BVDV type 2, green curved line represent positive control to BVDV2

Discussion

The results revealed that 20(7.14%) samples were positive to direct Elisa(Ag detection) test (8 in samples from Nassirya city and 12 samples in Basrah city), while 66 (23.57%) samples were positive to indirect Elisa(Ab detection). Our result, are in accordance with other studies in Iraq by using same test, but are different when compared with 89.80% in Konya , 72.25% in Mashhad(28) that's may be attributed to animals density. RT-qPCR give better results than the other techniques, it is give as true positive results if keep restricted from contamination and follow procedure well. The RT-PCR method had the advantage of ascertaining BVDV nucleic acid sequences in samples in which the virus had been inactivated, e.g. during transport or storage or due to the presence of neutralizing antibodies (29). And that give the answer why ELISA test was not 100% positive results, also the presence of antibodies will interfere with the test. As a result, it should not be used with animals less than three months of age (30). In cattle populations BVDV-1 strains are predominant in most part of the world, whereas BVDV-2 is recognized as the cause of severe acute haemorrhagic disease in North America being recently reported in Europe and Asia with low virulence (31). In our study there are wide range between ELISA results and PCR and this may be due to that RNA viruses are have low stability. Our result of RT.PCR show 2 positive samples (10%) out of 20 samples are near of similar study results (11.1%) done in Iran-Mashhad in 2007 by

tank using bulk milk samples(32). The selection of herds in this study was based on randomization and we can expect to generalize this prevalence from our samples for two cities (nassirya- basrah) investigation of the influence of herd on BVDV distribution revealed that only one herd from each city were infected by BVDV, These results show that BVDV can be found in its minimum ratio, but a recent or an ongoing viremic most likely occur due to presence of Persisting Infection cattle which can distribute the BVD virus in other herds and regions(32) . Although from the low ratio of the PCR test result, a positive result would provide useful information. Positive PCR results would be weighted more heavily than negative results. The use of this assay may be most beneficial as a method of focusing on or justifying BVDV-positive herds for development of control strategies and not as a definitive test to ensure BVDV negative herd. It can be concluded that BVDV infection is an important disease in cattle of some region in south of Iraq. These herds include presence of PI animals which play an important role in spreading infection in the herd, because they act as life-long producers of the virus and do not induce the production of BVDV-specific antibodies (33). These results indicate that RT-PCR analysis of blood samples may provide a rapid and sensitive screening method for the detection of BVDV infections in non-vaccinated cattle herds. They also indicate that BVDV may be highly prevalent in cattle herds in the south of Iraq.

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انتشار فيروس الإسهال البقري الحمي في قطعان الماشية من محافظات البصرة والناصرية باستخدام اختباري المقايضة المناعية المرتبطة بالانزيم (المباشر وغير المباشر) وتفاعل سلسلة البلمرة نوع (RT-PCR)

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الخلاصة

أجريت الدراسة الحالية للاستقصاء عن مدى انتشار حميات مرض الإسهال البقري الحمي عن طريق استخدام اختباري المقايضة المناعية المرتبطة بالانزيم (ELISA) وكذلك اختبار تفاعل سلسلة البلمرة نوع (RT-PCR). ومانتين واثان وثمانون عينة تم جمعها من الأبقار غير الملقحة ضد مرض الإسهال الفيروسي من محافظتي البصرة والناصرية (188 عينة من مدينة الناصرية و 92 عينة من مدينة البصرة). فحصت العينات بفحص المقايضة المناعية المرتبطة بالانزيم المباشرة (IDEXX ELISA Herd Check) للكشف عن المستضد وكانت النتائج إيجابية في 20 عينة (8 عينة في ذي قار و 12 عينة في البصرة) وبنسبة إصابة بلغت 7.14%. كما خضعت جميع العينات الى فحص المقايضة المناعية المرتبطة بالانزيم غير المباشرة (IDEXX ELISA Herd Check) للكشف عن الأجسام المضادة لفيروس BVDV وكانت نسبة الإصابة 23.57%. واجري التتميط الجيني لجميع العينات الإيجابية لفحص المستضد باستخدام اختبار PCR Real time، باستخدام Cadov BVDV 1/2 kit بعد استخلاص الحمض النووي الريبي للفيروس باستخدام QIAamp mini kit. أظهرت النتائج لفحص المقايضة المناعية المرتبطة بالانزيم وجود 20 عينة موجبة للاختبار المباشر و 66 عينة موجبة للاختبار غير المباشر، بينما أظهرت نتائج فحص تفاعل سلسلة البلمرة نوع (RT-PCR) وجود عینتين موجبة للنوع 1 من حميات مرض الإسهال البقري الحمي (نموذج واحد لكل مدينة).