Detection and genotyping of bovine viral diarrhea virus in cattle sera

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A B S T R A C T

In the present study, we report the rapid detection and genotyping of bovine viral diarrhea virus (BVDV) in cattle sera collected from different localities at Qaluobia province, Egypt all over the four seasons of the years 2013 and 2014. A total of 250 serum samples were tested by antigen capture Enzyme linked immunosorbent assay (ELISA) and nested RT-PCR. BVDV antigen was detected in 8.4% (21/250) of examined samples with a prevalence of 18 % (9/50) in 6 month old cattle. Seasonal pattern for BVDV antigen were detected by 12.3% (16/130) during winter, 4% (1/25) during summer, 3.6% (12/56) during spring and 5.1% (2/39) during autumn. All detected BVDV by antigen capture ELISA were genotyped as BVDV type 1. In conclusion, BVDV antigen detection in cattle sera indicate presence of persistently infected (PI) animal that required culling from the herd for strict control measures.

Keywords: BVDV, ELISA, nested PCR assay, cattle sera

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1. INTRODUCTION

Rotavirus Bovine viral diarrhea virus (BVDV) is an economically important pathogen of cattle with a worldwide distribution. The BVDV can infect cattle of all ages including fetus, and has multiple target organs (Bruschke et al., 1998). Cattle infected with BVDV may display variable clinical signs from subclinical or mild to severe clinical disease (Flores et al., 2002).

Two distinct genotypes were existing, BVDV-I and BVDV-II, together with border disease virus (BDV), classical swine fever virus (CSFV) and Giraffe,they constitute the genus Pestivirus of the family Flaviviridae (Fauquet et al., 2005). However, according to cell culture behavior, BVDV occurs in two biotypes, non-cytopathic (ncp) and cytopathic (cp) (Yesilbag et al., 2008). BVDVs are genetically variable, containing a single positive-stranded RNA of approximately 12.5 kilo base (kb) in length. The viral genome contains a single large open reading frame (ORF) flanked by 5' and 3' untranslated regions (UTR). The complex epidemiology of BVDV partially lies in its ability to infect the foetus. If the infection occurs between the second and fourth month of gestation, the virus is able to cause a persistent infection of the fetus which may result in the birth of a persistently infected (PI) calf (Peterhans et al., 2010).

These PI animals are important sources of infection because they continuously shed BVDV in large quantities (Lindberg and Houe, 2005). In Egypt, despite control procedures such as vaccination, BVDV is still a problem in the cattle population at different governorates including, Behera (Abd El-Hafeiz, 2002) El-Sharquia (El-kholly et al., 2004), Cairo, Mansoura and Seuz governorates (Mohamed et al., 2004).

The target of this study was the rapid detection and genotyping of BVDV in cattle sera from a private farm at Qaluobia province during 2014.

2. MATERIALS AND METHODS

2.1. Serum samples:

A total of 250 serum samples were collected from cattle (one month up to 6 months of age) in different localities at Qaluobia province, Egypt all over the four seasons of the years 2013 and 2014 (table1). No record of BVDV vaccination in this province. These samples were stored at -20oC till used in antigen capture ELISA and nested RT-PCR.

2.2. Reference BVDV strain:

The Egyptian cytopathic BVDV Iman strain was obtained from animal health research institute, Dokki, Giza, Egypt. It was propagated in MDBK cell line and used as positive control in nested RT-PCR.

2.3. Antigen-detecting Enzyme Linked Immunosorbent assay (ELISA) based on monoclonal antibodies (MAbs) against the nonstructural BVD virus protein p125/p80:

Two hundred fifty serum samples were investigated using antigen capture ELISA of commercial ELISA BVD/MD antigen mix screening kit from INSTITUTE POURQUIER, France, according to manufacturer description.

2.4. Primers Design:

Sullivan and Akkina (1995) had designated the primers P1 5'…..AAC AAA CAT GGT TGG TGC AAC TGGT….3' (1424-1449 nts) and P2 5'… CTT ACA CAG ACA TAT TTG CCT AGG TTC CA….3' (2221-2250 nts) sequences that shared maximum homology with all ruminant pestiviruses. TS1 5'….TAT ATT ATT TGG AGA CAG TGA ATG TAG TAG CT…3' (1684-1716 nts) TS2 5'…. TGG TTA GGG AAG CAA TTA GG….3' (1802-1821 nts) and TS3 5'… GGG GGT CAC TTG TCG GAGG….3' (2027-2045 nts) sequences were type specific for BDV, BVDV genotype II and I respectively with the amplified products (P1 and P2) by using nested reverse transcription-polymerase chain reaction (RT-PCR) technique.

2.5. Polymerase Chain Reaction (PCR):

Reagents for PCR was supplied with the Kit by QIAamp® MinElute® Virus Spin kit for simultaneous purification of viral RNA from sera supplied by QIAGEN.

2.6. Genotyping the BVDV by nested RT-PCR technique:

For detection of BVDV genotypes using nested RT-PCR, 35 cycles, the first amplification of the reverse transcript RNA using the primers P1 and P2 was done while the second amplification using the primers TS1,TS2 TS3 and amplified products of the first round (P1 and P2) was carried out in 25 cycles as described by Sullivan and Akkina (1995).

3. RESULTS

3.1. Detection of BVDV by ELISA:

BVDV antigen detection in 250 serum samples of cattle by antigen capture ELISA revealed that 8.4% (21/250) of samples were positive table (2). In correlation to age, BVDV antigen was highly distributed among sera collected from cattle within 6 month old reached 18 % (9/50) than that collected from cattle with one and 3 months olds showed BVDV antigen by 6.3% (7/112), 10.7 % (5/47) respectively. Sera from 2 months olds cattle were free (table3). Seasonal pattern for BVDV antigen detection during the year 2013 and 2014 indicates prevalence of BVDV infection by 12.3% (16/130) during winter, 4% (1/25)

Table (1). Number of serum samples of cattle in relation to the age and season.

sample	Age				Total /age	season				Total/ season
	One month	2 month	3 month	6 month		Winter	Summer	Spring	Autumn	
sera	112	41	47	50	250	130	25	56	39	250

during summer, 3.6% (12/56) during spring and 5.1% (2/39) during autumn as shown in table (4).

3.2. Genotyping of BVDV in cattle sera:

twenty one samples that were positive by antigen capture ELISA were genotyped as BVDV type I (table 5) where electrophoresis of the amplified products revealed the presence of specific PCR product at the correct expected size of BVDV type I (223 bp) without significant differences between the BVDV reference strain and detected strain as represented in photo. (1).

Table (2): Number of positive BVDV antigen isolates from cattle sera using antigen capture ELISA

No of serum samples	No. of Positive	Percent Positive
250	21	8.4

Table (3): Age distribution of BVDV infection in cattle sera assessed by antigen capture ELISA.

Age/month	Total examined sera	Total Positive	Percent Positive
1	112	7	6.3
2	41	-	-
3	47	5	10.7
6	50	9	18

Table (4): Seasonal pattern of BVDV infection in cattle sera assessed by antigen capture ELISA.

season	Total examined sera	Total Positive	Percent Positive
Winter	130	16	12.3
Summer	25	1	4.0
Spring	56	2	3.6
Autumn	39	2	5.1

Table (5): Number of detected BVDV genotypes from cattle sera using nested RT-PCR

No of serum samples	No. of Positive by ELISA	No. of genotype 1
250	21	21

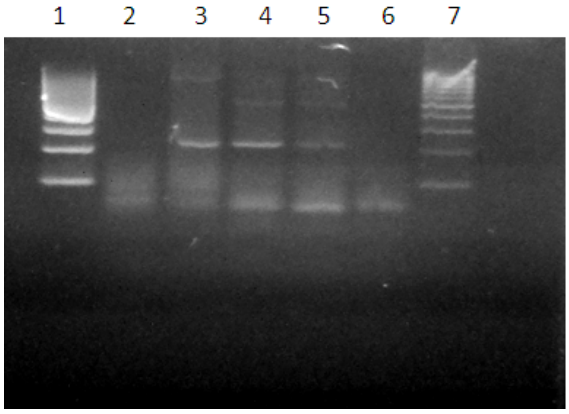

Photo (1) genotyping of BVDV by nested RT-PCR. Revealed the presence of specific PCR product at the correct expected size of BVDV type I gene (223 bp), in the same pattern with no differences between reference strain and detected one.from left to right Lane 1&7: High molecular weight nucleic acid marker Lane 2: negative control. Lane 3: Reference BVDV strain (Iman) Lane 4, 5: BVDV detected in cattle sera by antigen capture ELISA.

4. DISCUSSION

Accurate diagnosis of BVDV infection depends upon isolating the virus from nasal swabs or blood or tissue samples from affected animals (Ridpath 2005). There are three different methods of detecting the virus or viral components: virus isolation in cell culture, detection of viral antigens and detection of viral nucleic acid. In primary infected animals, BVD virus and antigen can be detected in blood from the first couple of days to around two weeks after infection whereas viral RNA could be detected even

longer (Sandvik et al., 1997; Bruschke et al., 1998; Ellis et al., 1998).

Antigen-detecting ELISA based on monoclonal antibodies (MAbs) against the nonstructural BVD virus protein p125/p80 was considered to be the test of choice for circulated infection as well as offered sensitivity equal to virus isolation if they used for calves over 6 month of age (Brinkhof et al 1996). BVDV antigen was detected in cattle sera by Antigen-captured ELISA table (2) indicating circulated infection and the role of cattle sera in establishment of infection in the herd.

Our findings showed that BVDV antigen was highly distributed among cattle aged 6 month old reached 18%. These results supported the previous results of Blood et al., (1983) who reported that infection in cattle generally ranging in age from six months to two years. The highest percentage of BVDV positive samples occurred during winter months (12.3%) this may be attributed to the herd from which sampling get collected during winter season rather than a seasonal pattern of infection.

In PI animals, BVDV antigen can be detected in sera during the whole life after maternal antibody has disappeared. Though, a positive test result for BVDV antigen is likely to originate from PI animals (Sandvik, 2005). PI animals usually die from mucosal disease before the age of 2 years (Peterhans et al., 2010) or leave the herd because their production is insufficient (Houe, 2003). Yet, PI animals can sometimes live beyond the age of 2 years (Houe, 1992)

Previously, several PCR-based assays for typing BVDV have been reported Sullivan and Akkina (1995). In our search to rapid detection and typing of detected BVDV, extracted RNA was amplified by RT-PCR using a nested set of primers complementary to sequences in the Erns of the pestivirus genome followed by nested PCR for detecting and differentiates BVDV genotypes (Sullivan and Akkina, 1995). The nested PCR product was characteristic of a bovine viral diarrhea virus (BVDV) type 1 revealed specific PCR product at the (223 bp) (photo.1). Our results come in agreement with (Ridpath, 2003) who reported that genotype 1 may exist as cytopathic or non cytopathic. Also similar results regarding the sensitivity of PCR for BVDV detection in bovine pooled serum were reported by Weinstock et al. (2001)

The present study confirms the circulation of BVDV genotype 1 in Qaluobia province and PI animal was detected through cattle sera that required culling from the herd to control BVDV in this province.

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الكشف والنوع الجينى لفيروس الاسهال البقرى الفيروسي فى امصال الابقار.

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الملخص العربي

استهدفت الدراسة الحالية سرعة الكشف وتحديد النوع الجينى لفيروس الاسهال البقرى الفيروسي فى امصال الابقار التى تم تجميعها من مختلف الاماكن لمحافظة القليوبية بمصر خلال الاربع فصول لاعوام 2013 و2014. تم اختبار اجمالى 250 عينة مصلية بواسطة الانتيجن الماسك الاليزا و الانزيم العكسي لتفاعل البلمرة المتسلسل العشى . تم الكشف عن الانتيجن لفيروس الاسهال البقرى فى 8.4% (250/21) من العينات المفحوصة وبمعدل انتشار 18% (50/9) فى الابقار ذات الستة اشهر بالعمر. وبالكشف عن فيروس الاسهال البقري خلال الاربع فصول لاعوام 2013 و2014 تبين ان انتشار الانتيجن لفيروس الاسهال البقرى ب 12.3% (130/16) اثناء الشتاء و 4% (25/1) اثناء الصيف و 3.6% (56/12) اثناء الربيع و 5.1% اثناء الخريف حيث كان الفيروس الذى تم الكشف عنه من النوع الجينى 1 و استنتاجا لذلك فان الكشف عن فيروس الاسهال البقري بامصال الابقار يشير الى وجود حيوانات دائمة العدوى مما يتطلب التخلص منها بالقطيع كاحد المقاييس للتحكم الجيد بانتشار الفيروس.

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