



PREVALENCE OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) IN CATTLE FROM SOME GOVERNORATES IN EGYPT.

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ABSTRACT

Diagnosis of the BVDV infection among suspected and apparently healthy cattle at Kaluobia, Giza, Menofeia and Gharbia governorates was done by detection of prevalence of BVD antibodies. A total number of 151/151(100%) and 97/151 (62.25%) of examined sera were positive for BVD antibodies using serum neutralization test (SNT) and competitive immunoenzymatic assay (cIEA), respectively. Examined sera with cIEA detected antibodies against BVDV non structural proteins P80/P125. Detection of BVDV in buffy coat samples using antigen capture ELISA showed that 22/151(14.56%) of the samples were positive for BVDV. Isolation and biotyping of suspected BVDV from buffy coat on MDBK cell line showed that 19/22 of ELISA positive buffy coat samples were cytopathogenic BVDV biotype (cpBVDV) while only 3/22 samples were CPE negative suggesting a non-cytopathogenic BVDV (ncpBVDV) biotype. Inoculated cell culture with no CPE were subjected to IFAT and IPMA using specific antisera against BVDV revealed positive results indicating presence of non-cytopathogenic strain of BVDV. It was concluded that cIEA detected antibodies against non-structural proteins P80/P125 has many advantages over SNT being for rapid diagnosis of BVDV. However, diagnosis must be confirmed with isolation, biotyping and identification of BVDV using suitable sensitive and specific methods as ELISA, IFAT and IPMA.

KEY WORDS: BVDV, ELISA, SNT.

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1. INTRODUCTION

Bovine viral diarrhea virus (BVDV) is an enveloped, single-stranded, RNA virus genus Pestivirus, family Flaviviridae [18]. BVD is characterized with depression, fever, diarrhea, drop in milk yield, loss of appetite and temporary leucopenia [14]. There are two antigenically distinct genotypes of BVDV (types 1 and 2) and each of them occurs in two forms including non-cytopathogenic and cytopathogenic according to whether/or not it produces visible change in cell cultures [21]. The 2 biotypes of BVD virus are not distinguishable serologically; however, in addition to nonstructural viral protein (p125) that is present in all BVD

virus-infected cells, cytopathic viruses produce p80 protein that not observed in cells infected with non-cytopathic BVD viruses [6]. BVDV is a world-wide distributed virus of cattle of all ages causing infection range from subclinical to highly fatal condition called mucosal disease [13].

In Egypt, the disease was continually diagnosed by [7, 15-17]. The prevalence of BVD has been mainly reported on the basis of the detection of antibody against BVDV [5]. However, diagnosis of BVDV through demonstration of BVD neutralizing antibodies needs further explanation.

Diagnosis of BVDV can be done by a variety of serological techniques as detection of BVD antibodies using Enzyme linked immuno-sorbent assay (ELISA) [11], SNT [10] or detection of viral antigen using Serum neutralization test (SNT), Immuno-fluorescent technique (IF), and Enzyme linked immuno-sorbent assay (ELISA) [17]. So, the aims of our article are screening of BVD neutralizing antibodies in cattle from different governorates in Egypt using SNT, detection of BVD antibodies against non-structural viral proteins in sera from infected animals and apparently healthy persistently infected (PI) cattle using ELISA that is confirmed with trials for isolation, biotyping and identification of the virus using indirect fluorescent assay (IFAT) and immunoperoxidase monolayer assay (IPMA).

2. MATERIAL AND METHODS

2.1. *Animals:*

A total number of 151 cattle over 6 months of age suspected for BVDV infection showing signs of respiratory disorders, mucosal ulcers in mouth and diarrhea and their apparently healthy contacts from different governorates (Kaluobia, Giza, Menofeia and Gharbia).

2.2. *Samples:*

Two blood samples were collected from the jugular vein of each examined animal. A sample set was collected in clean dry centrifuge tubes, left to clot, centrifuged at 1500x g for 20 minutes for separation of serum which kept at -20°C until used for detection of BVD antibodies using SNT and competitive ELISA; Another sample set was collected on EDTA as anticoagulant to separate buffy coat samples that used for direct detection of BVDV using antigen capture ELISA and cell culture inoculation.

2.3. *Madin-Darby Bovine Kidney (MDBK) Cell Line:*

MDBK cell line was used for isolation of the virus. It was supplied by Virology department at Animal Health Research Institute (AHRI), Dokki, Giza. Isolation of the virus was done with positive samples showed the characteristic cytopathic effect (CPE) of BVDV that denotes cytopathogenic biotype of BVDV [9].

2.4. *Reference BVDV:*

Egyptian field strain of BVDV Iman strain (cytopathic) obtained from rinder pest like disease department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. It was propagated in MDBK cell line and used for detection of BVD neutralizing antibodies using SNT.

2.5. *Reference antisera:*

Reference antisera against Egyptian field strain of BVDV used in this study was obtained from Virology department at Animal Health Research Institute (AHRI), Dokki, Giza.

2.6. *Serum neutralization test (SNT):*

It was conducted for detection of specific BVDV neutralizing antibodies in cattle serum samples [12].

2.7. *Competitive Immunoenzymatic assay (cIEA):*

It was used for the specific detection of antibodies to BVDV in cattle sera samples. BVDV P80/P125 was used as the coated antigen in addition with supplying with BVDV specific monoclonal antibodies (peroxidase conjugated) in INGEZIM BVD Compac kit.

2.8. *Antigen capture ELISA:*

A total number of 151 buffy coat samples were investigated for BVDV using antigen capture ELISA of commercial ELISA BVD/MD antigen mix screening kit from INSTITUTE POURQUIER, France, according to manufacturer description.

2.9. Indirect Fluorescent Antibody Technique (IFAT):

It was carried out on the inoculated cell cultures without CPE to detect ncp BVDV biotype [4]. Three blind passages of the tested samples were done on the inoculated cell cultures. If there was no evidence of any CPE, the third passage was conducted on MDBK tubes. Thus, tube cultures including flying cover slips were used to detect ncpBVDV by indirect IFAT technique. Anti-bovine IgG Conjugate with fluorescein isothiocyanate (developed in rabbits and supplied by Sigma) was obtained for IFAT.

2.10. Immuno-peroxidase monolayer assay (IPMA):

It was carried out on the inoculated cell cultures without CPE to detect ncpBVDV [17].

3. RESULTS

Detection of antibodies against BVDV in cattle sera using Serum neutralization test (SNT):

From all tested obtained from cattle at different localities in Kaluobia, Giza, Menofeia and Gharbia governorates (151), specific BVD antibodies were detected by SNT (100%) as shown in table (1).

Table 1 Detection of BVDV antibodies in cattle sera using SNT:

Governorate	Examined sera	Positive sera	
		Number	Percent
Kaluobia	54	54	100%
Giza	29	29	100%
Menofeia	24	24	100%
Gharbia	44	44	100%
Total	151	151	100%

Detection of antibodies against BVDV in cattle sera using Competitive Immunoenzymatic assay (cIEA):

Specific antibodies against P80/P125 non-structural protein of BVDV were detected

in 97/151(62.25%) by cIEA in tested sera from 151cattle at different localities in Kaluobia, Giza, Menofeia and Gharbia governorates, as shown in table (2).

Table 2 Detection of BVDV antibodies in cattle sera using antibody competitive ELISA:

Governorate	Examined sera	Positive sera	
		Number	Percent
Kaluobia	54	39	72.22%
Giza	29	18	62.06%
Menofeia	24	8	33.33%
Gharbia	44	32	72.73%
Total	151	97	62.25%

*cELISA detect antibodies against P80/P125 non-structural protein of BVDV in cattle sera samples.

Detection of BVDV in buffy coat Samples using antigene capture ELISA:

The total number of buffy coat samples (151) suspected for BVDV that were collected from cattle at different were identified serologically using ELISA (10 from Kaluobia, 3 from Giza, 3 from Menofeia and 6 from Gharbia,). It was found that 22 (14.56%) buffy coat samples were positive as induced change in colour of substrate by ELISA as revealed in table (3).

Table 3 Number of positive Buffy coat samples using antigen capture ELISA:

Governorate	Examined samples	Positive buffy coat samples	
		Number	Percent
Kaluobia	54	10	18.5%
Giza	29	3	10.34%
Menofeia	24	3	12.5%
Gharbia	44	6	13.63%
Total	151	22	14.56%

Isolation and biotyping of suspected BVDV from buffy coat on MDBK cell:

All of the 22 BVDV positive buffy coat samples using ELISA were inoculated in MDBK cell line. It was found that 19 samples (8 from Kaluobia, 3 from Giza, 3 from Menofeia and 5 from Gharbia) induced cytopathic effect by 3 blind serial passages in MDBK cell culture suggesting a cytopathogenic biotype of BVDV as

shown in table (4). Only 3 samples were CPE negative suggesting a non-cytopathogenic biotype of BVDV. The CPE of suspected BVDV isolates in

infected cell culture was characterized by cell rounding, cell aggregation, vacuolations followed by cellular darkness and cluster formation.

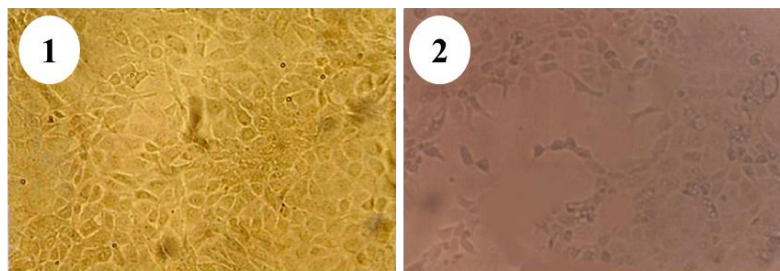
Table 4 Isolation and biotyping of BVDV from positive buffy coat samples on MDBK cell line then detection with IFAT and IPMA:

Governorate	Examined samples	Antigen capture ELISA	Positive samples			
			Isolation on MDBK cell line		IFAT	IPMA
			CPE	No CPE		
Kaluobia	54	10	8	2	8	2
Giza	29	3	3	0	3	0
Menofeia	24	3	3	0	3	0
Gharbia	44	6	5	1	5	1
Total	151	22	19	3	19	3

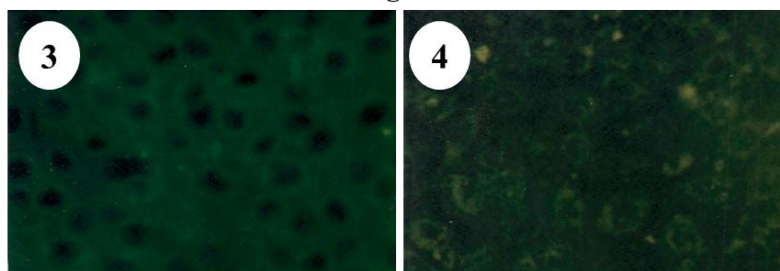
* Positive result represented by clear apple green emission on MDBK under fluorescent microscope using IFAT.

* Positive result represented by clear brownish color on MDBK using IPMA.

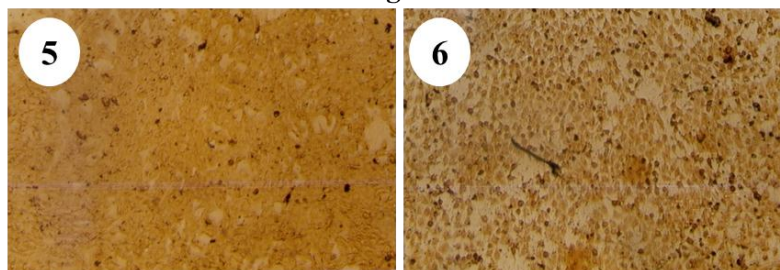
Isolation of BVDV on MDBK cell line



Identification of BVDV using IFAT



Identification of BVDV using IPMA



Identification of bovine viral diarrhea virus (BVDV) by isolation on MDBK, identification by indirect fluorescent assay (IFAT) and immunoperoxidase monolayer assay (IPMA). Photo (1) Showed normal MDBK cell line (Control) with no morphological changes examined under inverted microscope. Photo (2) Extensive cytoplasmic vacuolization as the morphological changes in response to suspected cp BVDV infection examined under inverted microscope. Photo (3) negative IFAT on inoculated MBDK cell line examined under fluorescent microscope. Photo (4) showed apple green intracytoplasmic fluorescence as positive IFAT on inoculated MBDK cell line examined under fluorescent microscope. Photo (5), negative IPMA on inoculated MBDK cell line examined under inverted microscope. Photo (6) showed clear brownish color as positive IPMA on inoculated MBDK cell line examined under inverted microscope

The CPE was clear between the 3rd and 5th day post inoculation increased gradually till 70-80 % of sheet was completely detached. These findings were demonstrated in photo (2) as compared with normal control monolayer demonstrated in photo (1).

Serological identification using Indirect Fluorescent Antibody Technique (IFAT) and Immuno Peroxidase Monolayer Assay (IPMA) in inoculated MDBK cell line for detection of BVDV.

A total of 22 suspected BVDV samples from cattle buffy coat (10 from Kaluobia, 3 from Giza, 3 from Menofeia and 6 from Gharbia) were inoculated in MDBK cell line. It was found that 3 samples 2 from Kaluobia and one from Gharbia) did not induce any cytopathic effect by 3 blind serial passages in MDBK cell culture as shown in table (4).

Inoculated cell culture not showing CPE were subjected to IFAT using specific antisera against BVDV revealed characteristic clear specific intracytoplasmic yellowish green fluorescence indicating presence of non-cytopathogenic strain of BVDV. These findings were demonstrated in photo (4) as compared with normal control monolayer demonstrated in photo (3).

The normal inoculated cell culture subjected to IPMA using specific antisera against BVDV revealed characteristic clear brownish color indicating presence of non-cytopathogenic strain of BVDV. These findings were demonstrated in photo (6) as compared with normal control monolayer demonstrated in photo (5).

4. DISCUSSION

Bovine viral diarrhoea virus (BVDV), which is the prototype of the Pestivirus genus of the Flaviviridae family, causes early embryonic death, abortion, teratogenesis, respiratory problems, chronic wasting syndrome, and immune

system dysfunction in cattle throughout the world [8].

Rapid, cheap and accurate tool for diagnosis of BVD virus and their antibodies is very important to prevent, control and eradicate the persistently infected animals, so diagnosis of BVDV in cattle and buffaloes was practiced via detection of specific anti-BVD-antibodies in sera by ELISA and SNT as well as detection of BVDV antigen by using of antigen capture ELISA commercial kits [7], FAT [17] and IPMA [19].

The results of this study showed that, out of 151 tested blood samples from cattle over 6 months of age at different localities in Kaluobia, Giza, Menofeia and Gharbia governorates; 151 (100 %) and 97 (62.25%) were positive for anti-BVD antibodies using SNT and cIEA techniques, respectively. SNT showed that all examined animals showing suspected symptoms and their apparently contact cattle were all positive for BVDV antibodies. Serum neutralizing antibody titers for positive screened sera in cattle against BVDV were ranged between 4 and 64, 122/151 serum samples (80.8 %) were positive in titer range (8-64) while 101/151 serum samples (66.8 %) were positive in a titer (4-16). These results agree to some extent with those obtained by Hanaa et al. [10].

The highest detection rate was found in cattle at Gharbia (72.73%) and Kaluobia (72.22%) governorates followed by Giza (62.06%) and Menofeia (33.33%) governorates using cIEA technique. These results were in agreement with results of [7], who found that 58.61 % and 65.5 % were positive for anti-BVD antibodies in examined animals' sera using SNT and ELISA, respectively. SNT proved to be sensitive and specific technique as well as cIEA for detection of anti-BVD antibodies, but cIEA technique was more specific test for detecting anti-BVDV antibodies against non- structural proteins from persistently infected animals.

Accurate diagnosis of BVDV infection depends upon isolating the virus from nasal swabs or blood or tissue samples from affected animals in a diagnostic laboratory [20]. Buffy coat samples from BVD suspected cattle and their apparently healthy contacts from different governorates in Egypt were subjected to antigen capture ELISA for BVDV diagnosis. From a total number of 151 only 22 (14.56%) samples were positive for BVDV. The highest detection rate was found in cattle at Kaluobia 10/54 (18.5%) and Gharbia 6/44 (13.63%) governorates followed by 3/24 Menofeia (12.5%) and Giza 3/29 (10.34%) governorates using ELISA technique. These results agreed with those obtained by previous studies [3, 10, 17] who used ELISA kits with specific monoclonal antibodies directed to NSP2-3 for detection of BVDV strains which offered sensitivity equivalent to virus isolation.

All of the 22 BVDV positive buffy coat samples using ELISA were inoculated in MDBK cell line. It was found that 19 samples (8 from Kaluobia, 3 from Giza, 3 from Menofeia and 5 from Gharbia) induced cytopathic effect by 3 blind serial passages in MDBK cell culture suggesting a cytopathogenic biotype of BVDV. This agreed with that found in earlier reports [1, 10], which found that MDBK cell line is considered the most common cell culture suitable for BVDV virus isolation. Only 3 samples gave no CPE suggesting a non-cytopathogenic biotype of BVDV. These results agreed with Aly *et al.* [2] who used the cell culture method for biotyping and then identification of both cpBVDV and ncpBVDV. Diagnosis of 3 isolates of noncytopathic BVDV was identified using IFAT and IPMA. These results agreed with previous studies [2, 10] that used immunofluorescent technique for identification of both cpBVDV and ncpBVDV biotypes after using the cell culture method and characterised by specific intracytoplasmic fluorescence as positive samples. These results also agreed

also with those of Saliki *et al.* [19] who proved that IPMA had a relative sensitivity of 85% and a relatively specificity of 100% to identify PI cattle.

It was concluded that diagnosis of BVD can be studied via detection of specific anti-BVD-antibodies in sera by SNT and ELISA, However, serological diagnosis of BVDV infections in cattle is complicated by the widespread use of BVDV vaccines, as indicated by the high SN titers found in cattle that had received multiple BVDV vaccinations and in cattle exposed to PI animals. Although cIEA has many advantages over SNT being rapid, simple, cheap and does not need ELISA reader for detection of specific anti-BVD-antibodies, diagnosis must be confirmed with isolation, biotyping and identification of BVDV using suitable sensitive and specific methods as ELISA, IFAT and IPMA.

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مدى انتشار فيروس الاسهال البقري الفيروسي فى الإبقار من بعض المحافظات فى مصر .

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الملخص العربي

تم تشخيص عدوى فيروس الاسهال البقري الفيروسي بين أبقار مصابة وأخرى مجاورة فى محافظات القليوبية والجيزة والمنوفية والغربية بدراسة مدى انتشار الاجسام المضادة للفيروس. كان العدد الكلى للأمصال المختبرة للاجسام المضادة للفيروس ايجابيا فى 100% من الحيوانات بواسطة اختبار المصل التعادلى (151/151) و 62.25% من الحيوانات (151/97) بواسطة اختبار الانزيم المناعى المتسابق. يكشف اختبار الانزيم المناعى المتسابق عن الاجسام المضادة ضد البروتينات 125/80 غير الهيكلية للفيروس فى الامصال تحت الفحص. أظهر التعرف على فيروس الاسهال البقري الفيروسي فى عينات buffy coat ان 14.56% من العينات (151/22) كان ايجابيا باستخدام اختبار الاليزا صائد الانتيجين. أظهر العزل والتعرف على النوع الحيوى لفيروس الاسهال البقري الفيروسي من عينات buffy coat الايجابية على خط خلايا MDBK ان (22/19) من العينات كان ايجابيا لفيروس الاسهال البقري الفيروسي من النوع الحيوى الممرض لخلايا الزرع النسيجي بينما (22/3) من العينات كان ايجابيا لفيروس الاسهال البقري الفيروسي وكان من النوع الحيوى غير الممرض لخلايا الزرع النسيجي والذي تم التعرف عليه من خلال اختبارات الوميض الفلوروسنتى غير المباشر و IPMA. مما سبق نستنتج أن اختبار الانزيم المناعى المتسابق الكاشف عن الاجسام المضادة ضد البروتينات 125/80 غير الهيكلية للفيروس له مميزات عديدة عن اختبار المصل التعادلى فى التشخيص السريع لعدوى فيروس الاسهال البقري الفيروسي الا أنه يجب تأكيد التشخيص بوسائل العزل والاستعراف والتصنيف الحيوى لنوع الفيروس باستخدام طرق مناسبة حساسة ومتخصصة مثل اختبارات الاليزا و الوميض الفلوروسنتى و IPMA .

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