Field studies of Bovine Viral Diarrhea virus (BVDV) in El-Sharkia and El-kalubia Governorates

***Nahed, K.A.,*Eman, M. B.,* Wafaa, A.W. and** Hanaa, A. Ahmed**
*ELISA research unit and Viral Strain Bank, Animal Health Research Institute
** Biotechnology Department, Animal Health Research Institute

Abstract
In this study, a total of 154 samples (28 tissue samples , 63 buffy coats and 63 serum samples), were collected from calves (2-12 months old) suffering from respiratory manifestations, recumbency, anorexia and abdominal respiration , as well as from closely contact apparently healthy calves, from El-Sharkia and El-kalubia Governorates. The results of BVDV detection using direct immuofluroescent antibody technique (DFAT) in the tissue samples and buffy coat revealed diffuse or granular intracytoplasmic fluorescence in infected cells with a number of positive samples (6 and 12) from El-Sharkia & (10 and 8) from El-kalubia Governorates; respectively. Detection of BVD antigen using immunocapture ELISA kit (I-C ELISA) in buffy coat revealed that (30 &19) samples were positive from both Governorates; respectively. Reverse transcriptase polymerase chain reaction (RT-PCR) confirmation of BVDV in the tissue samples indicated the presence of BVDV type 1 in five samples including lungs (2), kidneys (2) and lymph node (1) with specific band at 360 bp. The results of BVDV antibodies detection in the serum samples using virus neutralization test (VNT), revealed a number of positive samples (26 & 15) while by using competitive ELISA was (33 &21) in the two Governorates; respectively. The relationship between the VNT positive samples and the reactivity in (ELISA) in the serum samples was recorded.

Key words: *BVDV , ELISA , lymph node, intracytoplasmic fluorescence .*

Introduction

Bovine Viral Diarrhea (BVD) is an important widespread disease of cattle but also reported in -pigs, other domesticated and exotic ungulates **(Løken, 1995).** BVDV is a member of Family Flaviviridae, genus Pestivirus. It is an enveloped virus with single-stranded RNA genome of approximately 12.5 kb. Two genetically distinct forms of BVDV type I and II have been identified based on genetic comparisons, BVDV type I has been subdivided into BVDV type Ia represented by reference strain **NADL** and BVDV type Ib represented by reference strain **OSLOSS**. BVDV is also separated into two biotypes, cytopathic (cp) and non-cytopathic (ncp) based on its effects in cell culture **(OIE, 2008).**

The main source of BVDV infection is the persistently infected (PI) animals that shed virus in the environment, but other infected species can also transmit it by direct contact. Transmission by indirect contact may happen through the use of contaminated equipment or through insemination with BVDV infected semen **(Fulton *et al.*; 2005)** .

BVD is an endemic disease occurs during the first year of life in calves, but reinfection can occur at any age. It causes high economic loss by which during infection, 60-85% of the cattle herds' are positive to BVDVantibody and 1-2% are (PI) **(OIE, 2008).** BVD in cattle causes various clinical syndromes in cattle including diarrhea, mucosal disease (MD), reproduction disfunctions (abortion, teratogenesis, embryonic resorption, fetal mummification and still-birth) and hemorrhagic syndrome **(Nadis, 2010).**

The diagnostic assays are in general, aimed for detection of the infectious virus or viral components. These assays include virus isolation, fluorescent antibody technique (FAT), immunohistochemistry (IHC), antigen capture ELISA (ACE), and polymerase chain reaction (PCR**) (Edmondson *et al.*; 2007).** Variety sets

of primers have been used for genotyping of BVDV mainly due to high mutation levels in some parts of its genome so the designed primers should be able to detect conserved regions of the virus genome (**Vilcek *et al.*; 2004).** Detection of virus specific antibodies by using different serological tests as virus neutralization test and enzyme linked immunosorbent assays is an important way for the virus detection **(OIE, 2008**).

In Egypt BVDV-MD complex was considered as endemic disease before 1960 **(Baz, 1992). Hafez, (1972)** isolated BVDV for first time from calf suffering from pneumoenteritis syndrome and the virus designated as IMAN-7912.The virus was also isolated from cattle have the same syndrome by **(Baz, 1975).** BVD disease becomes widely distributed in Africa, and the Middle East including Egypt **(Kahrs, 2001 and Hassanein, 2003).**

PCR was employed for the detection and diagnosis of BVDV by **(Hussein 2001; Mohamed, 2002 and Hassanein; 2003).**

Aim of this work

- Detection of BVDV in field samples using immunoflorescent antibody (FAT) and antigen detection ELISA techniques.
- Confirmation of the BVDV isolate by RT-PCR
- Detection of BVDV antibodies by virus neutralization test (VNT) and ELISA.

Material and Methods

Samples: A total of 154 samples (were collected from calves of ages ranged from(2 -12 months old) , suffering from respiratory manifestations, recumbency and anorexia, abdominal respiration, as well as from closely contact apparently healthy calves, from El-Sharkia and El-kalubia governorates. These samples included:

A-Tissue samples:

A total of 28 tissue samples ,Lung (5), spleen (5), kidney (5),lymph nods (8) and liver(5) **(Table 1),** were collected and prepared for FAT & ELISA according to (**OIE, 2008).**

B- Blood samples:

63 Blood samples were collected and divided into two parts, one with anticoagulant (Buffy coat separation) for BVDV detection and the second without anticoagulant (serum separation for BVDV antibodies detection) from the diseased and apparently healthy calves **(Table 1)**. The samples were prepared according to **(Staven and Julia, 1998).**

Cell culture:

Median Darby Bovine kidney cells (MDBK) free from non-cytopathic strains of BVDV obtained from virology department Animal Health Research Institute (AHRI), grown in Eagles MEM supplemented with 10% fetal calf serum and used for applying virus neutralization test (VNT) on the collected sera samples **(Clark *et al.*; 1984).**

Fluorescence isothiocyanate conjugate:

Anti-BVDV fluorescence isothiocyanate conjugate was supplied by Sigma immune chemicals **(USA)** and used for applying direct immunoflourescent antibody technique (DFAT).

Reference BVDV strain:

BVDV **(NADL strain),** supplied by the ELISA unit and Viral strain bank , Animal Health Research Institute and used for applying VNT on the serum samples.

Reference positive immune serum:

Standard reference positive bovine hyperimmune serum of BVDV, supplied by Sigma immune chemicals and used for applying VNT.

Direct fluorescent antibody (DFAT), technique

Tissue sample´s supernatants of (Lung, spleen, kidney, lymph nodes and liver)and buffy coats were inoculated into MDBK cells prepared on cover slip in Leighton tubes. DFAT was applied by using anti BVD Fluroescin thiocynate – conjugate 1/100 dilution according to **(Gerada *et al*; 1971).**

Antigen detection ELISA

A commercial double antibody sandwich enzymatic immuno assay kit (INGEZIM, DAS-12.BVD K2) (I-C ELISA) was used for detection of BVDV antigen in the Buffy coats, according to the instructions of the manufacturer, **(Meyling, 1984).**This kit is based on a double antibody sandwich enzymatic immuno assay

(DAS or capture ELISA). The plates are coated with a monoclonal antibody to the non structural protein p80/p125 of the BVDV. In this way when a sample is added into the wells, if it contains antigen, the antibodies will catch it and the conjugate (streptavidine peroxidase conjugate) will bind to the antigen antibody complex. The reading occurs at wave length, 450 nanometer.

Table (1). Different number of samples collected from El-Sharkia and El-kalubia Governorates

Samples Governorate	Number of samples			
	Tissue Samples (Lung, spleen, kidney , lymph nod and liver)	Buffy coats	Serum sample	Total
El-Sharkia	10	36	36	82
El-El-kalubia	18	27	27	72
Total	28	63	63	154

Reverse transcptase polymerase chain reaction (RT-PCR) genotyping

PCR of BVDV was done in the tissue samples for RNA virus detection as follow:

RNA extraction was made to cultured isolates using innuPREP RNA mini kit (Analytik Jena AG, Germany).

Nested – PCR was made according to **(Gilbert *et al.*; 1999)** using NS5B gene GenBank (accession no. AF078534 to AF078543) with the following primers and cycling condition

The external primers for primary PCR	Primer Sequence	Product size
	5\ AAG ATC CAC CCT TAT GA(A/G) GC 3\	1143 bp
	5\ AAG AAG CCA TCA TC(A/C) CCA CA 3\	
RT-PCR was carried out using Qiagen one step RT kit. Reverse transcription was done at 55°C for 30 min, followed by Hot start denaturation at 95°C for 15 min. The reactions were cycled 25 times at 94°C for 20 s, 50°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 15 min.		
The multiplex primers for secondary PCR	5\ TGG AGA TCT TTC ACA CAA TAG C3\	
	5\ GGG AAC CTA AGA ACT AAA TC 3\ (BVDV1 specific) 360 bp	
	5\ GCT GTT TCA CCC AGTT (A/G)TA CAT 3\ (BVDV2 specific) 604 bp	
The product of primary PCR was further amplified using the multiplex primers and Jena Bioscience high yield PCR master as master mix with the same cycling conditions for 40 cycles.		

The final products were electrophoresed in 2% agarose using Generuler as marker (Fermentas) and stained with ethedium bromide.

Detection of BVDV antibodies by virus neutralization test

63 cattle serum samples were heat-inactivated for 30 minutes at 56°C for non specific reaction or inhibitors. A starting of 1/5, serial two fold dilutions of the tested sera are made in a cell-culture grade flat-bottomed 96-well microtitre plate, using cell culture medium as diluent. Positive and negative control sera were used. Virus neutralization test was applied according to **(OIE, 2008).**

Detection of BVDV antibodies by ELISA technique:

A commercial ELISA kit **(INGEZIM BVD compact)** was used to detect antibodies against BVDV in 63 serum samples. The test is a competitive immune assay blocking ELISA. The plates are ready coated with BVDV antigen. Controls and serum samples were added & incubated then a specific monoclonal antibodies (MAb) was added. Positive samples will react with the coated antigen; with no reaction with (MAb) giving no color with the substrate, but

the negative samples will react with the (MAb) giving color with the substrate. The reading was done at 450 nm**. (Staven and Julia, 1998).**

Results

Detection of BVDVantigen using DFAT technique:

The results of BVDV antigen detection using (DFAT) revealed diffuse or granular intracyto-plasmic fluorescence in infected cells **(Figure 1)**. The number of positive samples were; Lung (1), spleen (1), kidnye (2), lymph nods (2) and buffy coats (12) from El-Sharkia Governorates while they were ; Lung (2), spleen (2), kidnye (4), lymph nods (2) and buffy coats (8) from El -kalubia Governorates **(Table 2)**

Table (2). Detection of BVDVantigen by using DFAT technique

Governorate	El-Sharkia			El-Kalubia			Total
Tissue samples	No. of samples	+ve	-ve	No. of samples	+ve	-ve	
Lung	2	1	1	4	2	2	6
Spleen	2	1	1	3	2	1	5
Kidney	2	2	-	4	4	-	6
Lymph Node	2	2	-	4	2	2	6
Liver	2	-	2	3	-	3	5
Buffy coats	36	12	24	27	8	19	63
Total	46	18	28	45	18	27	91

Detection of BVDV antigen using ELISA technique:

The results of BVDV antigen detection using (I -C ELISA) kit, in the buffy coats revealed (30 &19) positive samples from El-Sharkia and El-kalubia Governorates respectively.

RT-PCR confirmation:

RT-PCR was submitted on five positive samples lungs (2), kidney (2) and lymph node (1) for BVDV type 1 with the specific band at 360 bp. **Figure (2)**

Detection of BVDV antibodies in the serum samples using VNT & ELISA techniques:

The results of BVDV antibodies detection in the serum samples, using (VNT) & competitive ELISA, revealed a number of (26 & 15) and (33&21) positive samples from collected from El-Sharkia and El-kalubia Governorates respectively (**Table 3).**

Table (3). Detection of BVDV antibodies in cattle serum samples using VNT and ELISA techniques:

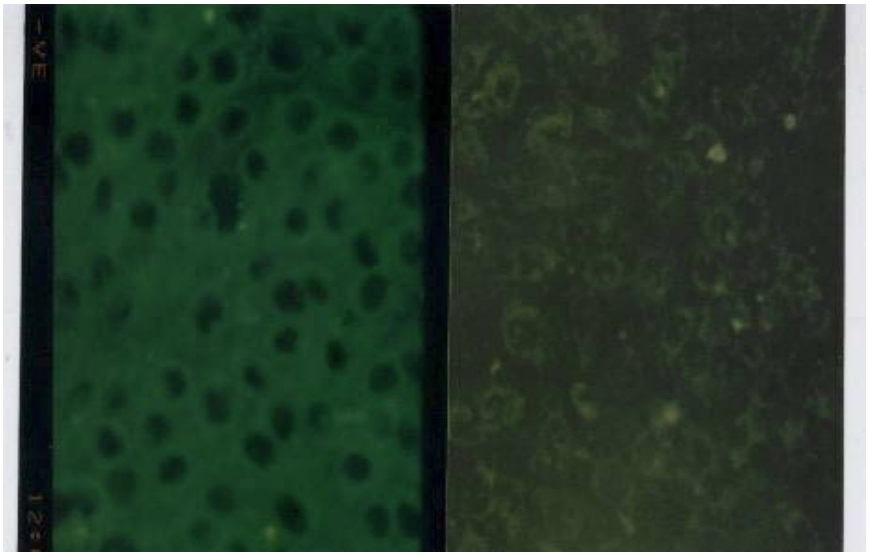
Governorate	No. of serum samples	VNT		ELISA	
		+ve	-ve	+ve	-ve
El-Sharkia	36	26	10	33	3
El- Kalubia	27	15	12	21	6
Total	63	41	22	54	9

The relationship between VNT results and the reactivity in (ELISA).

The relationship between the positive results of VNT and the reactivity of ELISA revealed that out of (36 &27) serum samples, collected from El-Sharkia and El-kalubia Governorates a number of (26 & 15) samples were positive by VNT while by ELISA the positive samples were (33& 21) in both Governorates respectively **(Table 5).**

Table (4). The relationship between results of VNT and the reactivity in (ELISA)

Governorate		Sharkia		El-kalubia	
	SNT titres	+ve SNT	+ve ELISA	+ve SNT	+ve ELISA
No. of positive samples	>5	7	14	2	8
	5	2	2	2	2
	10	3	3	3	3
	20	6	6	1	1
	40	3	3	3	3
	80	3	3	4	4
	160	2	2	--	--
	320	-	-	--	--
	640	-	-	--	--
Total		26	33	15	21



(A) **(B)**

Figure (1). (A) Control MDBK cells. (B) MDBK cells infected by BVDV local strain and stained by fluorescien conjugated anti-BVD serum showing specific diffused intracytoplasmic yellowish green fluorescent color (Mag. 400X).

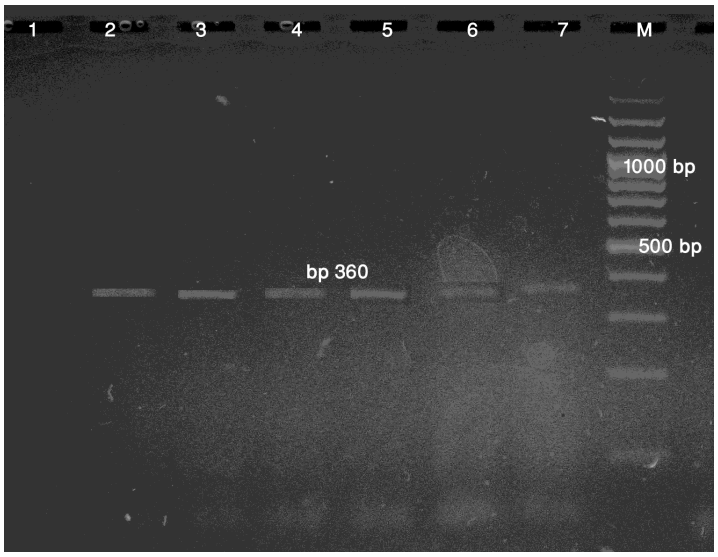


Figure (2). Electrophoretic pattern of field isolates of BVDV Type 1 with the characteristic 360 bp amplicon size

- Control negative Lane No (1)
- Control positive Reference NADL strain Lane No (2)
- Field isolate Lane No (3,4) from lung
- Field isolate Lane No (5,6) from Kidney
- Field isolate Lane No (7) from lymph node
- M Marker (Fermentas- GenRuler # SM1132)

Discussion

Bovine Viral Diarrhoea virus (BVDV) is a pathogen of worldwide importance for cattle, causing significant economic losses .The immunotolerant cattle can shed the virus via secretion and excretion into the environment for a long time, thus being a major source of BVDV infections in herds **.**The Persistently infected (PI) animals continuously shed virus and act as a major reservoir of infection for other cattle **(Hanaa, *et al.*; 2010).** BVD with these economic losses, needs accurate and sensitive diagnostic methods for rapid identification and elimination of persistent carriers in the herds. In the past, the most common method was an isolation of virus in cell cultures but it was difficult, time consuming and lengthy process that requires experienced technicians. These years several molecular methods like reverse transcriptase polymerase chain reaction (RT-PCR) are used extremely for detection of BVD viral RNA for diagnostic purposes **(Kim and Dobovi 2003).**

In our study, the results of BVDV detection using (DFAT) in the tissue samples and Buffy coats revealed diffuse or granular intracytoplasmic fluorescence in the infected cells **(Figure 1)**. The number of positive samples were; Lung (1), spleen (1), kidnye (2), lymph nods (2) and buffy coats (12) from El-Sharkia Governorates while they were ; Lung (2), spleen (2), kidnye (4), lymph nods (2) and buffy coats (8) from El-kalubia Governorates **(Table 2)** .This agreed with the studies of **(Ridpath, 2003)** who stated that FAT is the most useful test for detecting acute infections of BVDV and can detect the virus in cell cultures and in the tissues.

Antigen detection ELISA using monoclonal antibodies is specific for detection of BVD viral antigen. In our study the results of BVDV antigen detection, in the buffy coats by using (I -C ELISA) kit were (30 &19) positive samples in El-Sharkia and El-kalubia governorates respectively. These results agreed with the study of **(Mark & Gilda, 2001**) who stated that, I-C ELISA is reliable diagnostic tool, can be used for upstream detection of the virus antigen and has sensitivity & specificity reach to 100%. Another studies by (**Nahed *et al*., 2004)**, stated that, buffy coats of pneumonic calves showed high percentage of BVD antigen by using I-C ELISA. Regarding the higher positive results by I-C ELISA than FAT may be due to present of BVDV in some samples with presence of BVDV antibodies rendered it non infectious for cell culture and also improper handling or storage of the samples also affects BVDV stability, **(Baker, 1995).**

RT-PCR assays for amplification of BVDV had demonstrated its potentiality and ability to amplify strains containing considerable genetic diversity and gathered a lot of information on studying the evolution and pathogenesis of the virus **(Allam. 2000 &Vilcek *et al.*;2001).** The assay proved its usefulness in detecting BVDV

in (PI) animals even in the presence of the virus and maternal antibodies **(Tajima *et al.*; 2008).** Primers utilized in the RT-PCR assay have been previously showed its ability to detect any pestiviruses due to its location at highly conserved region of the genome **(Gilbert, 2002)** Although several researchers described PCR assays depends on utilization of primers located at Erns, E1, P54 and P80 but **Laamanen *et al.* (1997)** stated that, 5`UTR and P80 regions are considered the highly conserved regions of pestiviruses genome.

In the present study 5`UTR primers showed its ability to detect BVDV , type 1 in 5 positive (FA &ELISA) samples including, lungs (2), kidney (2) and lymph node(1) with specific band at 360 bp in both samples and the reference NADL strain with constituent and district amplification product like **(Hussein, 2001; Mohamed, 2002** and **Hassanein, 2003) (figure2)**. The amplified target revealed strong visible band (corresponding to the molecular weight marker specific bands), which confirmed primers, and fragment specificity. The PCR control showed negative result **(Deregt *et al.*; 2002 and James *et al.*; 2006).**

With respect to the results of BVDV antibodies detection in the serum samples using (VNT) & competitive ELISA, (26 & 15) and (33&21) positive samples resulted from El-Sharkia and El-kalubia Governorates respectively **(Table 3).**This agreed with the studies which stated that serological examination of serum samples for detection of BVDV antibodies is a useful tool for BVDV herd screening and also for monitoring of BVDV free herd status (**Corapi *et al.*; 2006).** The relationship between the results of VNT and ELISA **(Table 4)** can be explained as, by using VNT , it is easy to read the most laboratory ,highly cytopathogenic and laboratory-adapted strains of BVDV as 'NADL'.strain. Low levels of BVDV type 2 antibodies may not be detectable by a neutralization test that uses BVDV type 1 strain and this can lead to under reporting so ELISA test is good test because it is independent of cell cultures and viruses challenge, gives a test result within a few hours, **(Graham *et al.*; 2003** and **OIE, 2008).**

In conclusion ELISA test is recommended as a reliable diagnostic tool that can be used for upstream detection of the virus antigen and antibodies. PCR assay proved its usefulness in detecting BVDV even in P.I. animals and in more extent in the presence of the virus and maternal antibodies.

References

Allam. N. (2000). Growth behavior of BVDV. Iman strain on the level of NA synthesis and genotyping of its viral RNA". M. V. Sc. Thesis, Fac. Vet. Med.; Cairo University.

Baker, J.C. (1995). The clinical manifestations of bovine viral diarrhea infection. Veterinary Clinics of North America. Food Animal Practice 11, 425-45.

Baz, T. (1975).- Isolation, characterization and serological studies on BVD-MDvirus in Egypt Ph.D Vet. Thesesis, Microbiology, Cairo University, Cairo.

Baz, T.I. (1992). BVD-MD and Border disease in Egypt. Rev. Sci. Tech. Off Int. Epiz., 11 (3). 867-872..

Clark, M. C.; Brownlie, J. and Haward, C. J. (1984). Isolation of cytopathic and non cytopathic bovine viral diarrhea virus from tissues of infected animals. In pestivirus infections of ruminants. (J. W. Harkness. Ed). Commission of the European countries, Brussels, 3 – 12.

Corapi, W.V., French, T.W. Morgan Scott, Ole Sorensen, John T.Y. Wu, Eva Y.W. Chow, Ken Manninen, and John A. VanLeeuwen Can Vet J. (2006). Seroprevalence of Mycobacterium avium subspecies paratuberculosis, Neospora caninum, Bovine leukemia virus, and Bovine viral diarrhea virus infection among dairy cattle and herds in Alberta and agroecological risk factors associated with seropositivity October; 47(10). 981–991.

Deregt,D.; Carman,P. S. ; Clark,R. M.; Burton, K. M.; Olson,W. O. and Gilbert, S. A. (2002). A Comparison of Polymerase Chain Reaction with and without RNA Extraction and Virus Isolation for Detection of Bovine

Viral Diarrhea Virus in Young Calves. J Vet Diagn Invest 14:433–437.

Edmondson MA, Givens MD, Walz PH, Gard JA, Stringfellow DA, Carson RL. (2007). Comparison of tests for detection of bovine viral diarrhea virus in diagnostic samples. J Vet Diagn Invest 19(4).376-381

Fulton RW, Briggs RE, Ridpath JF, Saliki JT, Confer AW, Payton ME, Duff GC, Step DL, Walker DA. (2005). Transmission of bovine viral diarrhea virus 1b to susceptible and vaccinated calves by exposure to persistently infected calves. Can J Vet Res 69 (3).161-169.

Gerada, M.R. Uckerbauer: A. Girad, G.L. Bannister aand P. Boulanger (1971). Studies on Bovine viral diarrhea serum nutralization-complement fixation and immunofluorescence. Can. J. Comp. Med., July. 35 (3), 230-235.

Gilbert, S. A.; Burton, K. M.; Prins, S. E. and Deregt, D. (1999). Typing of Bovine Viral Diarrhea Viruses Directly from Blood of Persistently Infected Cattle by Multiplex PCR. J. Clin. Microbiol. 37(6). 2020–2023.

Graham, D.A., German, A., Mawhinney, K. & Goodall, E.A (2003). Antibody responses of naive cattle to two inactivated bovine viral diarrhoea virus vaccines, measured by indirect and blocking ELISAS and virus neutralisation. Veterinary Record 152, 795-800.

Hafez, S. (1972).- Preliminary studies on bovine viral diarrhea mucosal disease (BVD MD) and infectious bovine rhinotracheitis (IBR) in Egypt. Proceeding of the 10 thArab Veterinary Congress, Cairo, Egypt.

Hanaa, A. Ghoneim*, Naglaa, I. Hassan, Hanaa, A. Elhalawany and A.M.Nabih (2010). Mixed infection of bovine viral diarrhea virus, mycoplasma species and mannheimia haemolytica in calves showed chronic pneumonia with reference to the histopathological findings of the affected lungs, journal of american science;6(11) health in Norwegian dairy cows. Preventive Veterinary Medicine 43, 123-35.

Hassanein, S.A. (2003). Bovine Viral Diarrhea Virus: Molecular Diversity Of Field Strains And Isolation, Antigenic And Genomic Characterization Of Different Genotype I And Genotype II Strains In Cattle In Egypt. PH.D. Thesis, Dep. of Virology, Fac. Vet. Med., Cairo University.

Hussein, H.A. (2001).) Molecular characterization of the4 Egyptian strain (Iman) of BVDV and its RNA synthesis in cell culture." Arab. J. Biotech., 4 (1). 23-36.

James A. Kennedy,1 Robert G. Mortimer, Barbara Powers(2006). Reverse transcription-polymerase chain reaction on pooled samples to detect bovine viral diarrhea virus by using fresh ear-notch–sample supernatants , J Vet Diagn Invest 18:89–93

Kahrs, R. F. (2001)." Viral diseases of cattle" 2nd The Lowa State University Press / Ames, Iowa 89 - 106.

Kim SG, Dubovi EJ (2003). A novel simple one-step single-tube RT-duplex PCR method with an internal control for detection of bovine viral diarrhoea virus in bulk milk, blood, and follicular fluid samples. Biologicals 31:103–106.

Laamanen UI; Neuvonen EP; Yliviuhkola EM and Veijalainen PM. (1997). Comparison of RT-PCR assay and virus isolation in cell cultures for the detection of bovine viral diarrhoea virus (BVDV) in field samples. Res Vet Sci. 63(3). 199-203.

Laamanen UI; Neuvonen EP; Yliviuhkola EM and Veijalainen PM. (1997). Comparison of RT-PCR assay and virus isolation in cell cultures for the detection of bovine viral diarrhoea virus (BVDV) in field samples. Res Vet Sci. 63(3). 199-203.

Løken T. (1995). Ruminant pestivirus infections in animals other than cattle and sheep. Vet Clin North Am Food Anim Pract 11 (3).597-614. 88.

Mark Z. Plavsic and Gilda Prodafikas, (2001). Evaluation of a new sandwich enzyme-linked immunosorbent assay for detection of bovine viral diarrhea virus in unprocessed fetal bovine serum J Vet Diagn Invest 13:261–262**.**

Meyling, A. (1984). Detection of BVD virus in viremic cattle by an indirect immunoperoxi-

dase technique. In Recent advances in virus diagnosis. Haggue: Martinus Nijhoff Publishers. 37-46.

Mohamed, N.A.M. (2002): Isolation, antigenic and molecular characterization of BVDV strains from buffaloes as well their cytopathogenicity in cell culture." Ph.D. Thesis, Fac. Vet. Med., Cairo University.

NADIS (2010). NADIS livestock health bulletins, East of England Development Agency (EEDA).

Nahed, A.; Mohamed, H.; Hussein, A.; Fathia, M.; Mohamed, M. and Shalaby, M. A. (2004). "Isolation, antigenic and molecular characterization of bovine viral diarrhea virus field strains from apparently healthy buffaloer in Egypt ". Egyp. J. Virol., 1: 437 – 450.

OIE Terrestrial Manual (2008). Bovine viral diarrhoea chapter, 2 .4 .8, 698.

Ridpath, J.F. (2003). BVDV genotypes and biotypes: practical implications for diagnosis and control. Biologicals 31:127-131.

Staven R. Boulin and Julia F. Ridpath (1998). Prevalence of bovine viral diarrhea virus genotypes and antibody against those viral genotypes in faetal bovine serum. J. Vet. Diagn. Invest. 10:135-139.

Tajima M; Ohsaki T; Okazawa M; Yasutomi I.(2008). Availability of oral swab sample for the detection of bovine viral diarrhea virus (BVDV) gene from the cattle persistently infected with BVDV .Jpn J Vet Res.May; 56 (1).3-8.

Vilcek S., Durkovic B., Kolesarova M., Greiser-Wilke I., and Paton D. (2004). Genetic diversity of international bovine viral diarrhoea virus (BVDV) isolates. Vet.Res., 35: 609-615.

Vilcek S; Paton DJ; Durkovic B; Strojny L; Ibata G; Moussa A; Loitsch A; Rossmanith W; Vega S; Scicluna MT and Paifi V (2001). Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups. Arch Virol., 146(1). 99-115.

دراسات حقلية على فيروس الإسهال البقرى الفيروسى فى فى محافظتى الشرقية والقليوبية

***د./ ناهد كمال عبد الحميد,* د./ إيمان محمد بهيج,* د./ وفاء عبد الوهاب و** د./ هناء عبد القادر**

*وحدة بحوث الاليزا وبنك العترات الفيروسية- معهد بحوث صحة الحيوان

**قسم البيوتكنولوجى- معهد بحوث صحة الحيوان

الملخص العربى

تنتشر العدوى بفيروس الأسهال البقرى على مستوى العالم محدثة خسائر إقتصادية هامة .إن المرضية التى تحدث بواسطة هذا الفيروس معقدة, لأن عترة الفيروس لها نوعين (2 biotypes), نوع يحدث تغيير فى خلايا الزرع النسيجى والأخر لايحدث تغيير . من الممكن أن يحدث الفيروس عدوى دائمة وضعف مناعى فى الحيوان الذى يصاب بنوع عترة الفيروس التى لاتحدث تغيير فى خلايا الزرع النسيجى وذلك فى بداية فترة الحمل ثم يتبع ذلك إصابة بنوع عترة الفيروس التى تحدث تغيير فى خلايا الزرع النسيجى. فى هذه الدراسة تم تجميع عدد 154 عينة (28أنسجة , 63 خلايا كرات دم بيضاء و63 عينات سيرم) من عجول عمر (2 إلى 12 شهر) والتى تعانى من أعراض تنفسية , إستلقاء , فقدان شهية وتنفس من البطن, كما تم تجميع عينات من عجول سليمة ظاهريا من محافظة الشرقية والقليوبية. أظهر الكشف عن فيروس الأسهال البقرى باستخدام إختبار الفلورسنت المشع المناعى المباشرفى الأنسحة و خلايا كرات الدم بيضاء عن وحود فلورسين منتشر أو محبب داخل سيتوبلازم خلايا الزرع النسيجى المعدية بالفيروس , وكان عدد العينات الإيجابية (6 و 12) من محافظة الشرقية و (10 و 8) من محافظة القليوبية على التوالى, بينما الكشف عن أنتيجن فيروس الإسهال البقرى باستخدام إختبار الإليزا (Immuno-capture) فى عينات خلايا كرات الدم البيضاء أظهر عدد عينات إيجابية (30 و 19) فى المحافظتين على التوالى. تم التأكد من وجود الفيروسى عينات الأنسحة بإستخدام لإختبار البلمرة المتسلسل(RT-PCR) وأعطى نتيجة إيجابية لفيروس الإسهال البقرى (نوع 1) بإظهار باند غند (360 bp) فى عدد 5 عينات (2 عينات أنسجة رئوية, 2 كلة و 1 غدة ليمفاوية). الكشف عن وجود الأجسام المناعية المضادة لفيروس الإسهال البقرى باستخدام إختبار الفيروس المتعادل أظهر وجود عدد عينات إيجابية (26 و 15) بينما كان عدد عينات الإيجابية باستخدام إختبار اللإليزا (33 و 21) فى المحافظتين على التوالى . تم تسجيل العلاقة بين عدد العينات اللإيجابية باختبار الفيروس المتعادل و اختبار الإليزا .