(10)رقم البحث

DETECTION OF PERSISTENTLY INFECTED CALVES (PI) FOLLOWING BOVINE VIRAL DIARRHEA VIRUS (BVDV) DETECTION IN DAIRY FARM IN DAMIETTA GOVERNORATE

BY

Samar M. Atwa. Younis E. E and Zeidan, S.M*.

Department of internal medicine, infectious and fish disease, faculty of veterinary medicine, Mansoura University

** Chief researcher and Director of veterinary serum& vaccine research institute, Abbasia, Cairo*

ABSTRACT

A total number of 260 CattleMaster® 4 vaccinated calves were classified into 13 groups, each group contain 20 calves. Pooled blood samples were collected from each group for RT-PCR testing 21days post vaccination to detect BVDV genome. The obtained results revealed that, three groups were positive. 23 skin biopsy samples as well as, 23 sera ones of the calves from positive groups were examined by IFA and SNT respectively, then RTPCR was proceeded to confirm the persistent infection of the positive IFA calves .Three calves out of 14 (21.43%) are PI with two calves obtained the virus without seroconversion (immunotolerant) while, one obtained it with seroconversion. our study confirmed the efficiency of these diagnostic regimes in detection of PI calves.

Keyword: BVD, PI, BVD control.

INTRODUCTION

Bovine Pestivirus is one of the most widespread and important endemic virus infecting cattle throughout the world. The BVD-MD complex is considered as one of the production limiting diseases in dairy herd **(Radostits et al., 2007)**.

BVDV is RNA virus, a member of the genus Pestivirus within the family Flaviviridae **(Heinz et al., 2000)**. BVDV had two genotypes, BVDV 1and 2. BVDV- 1 subdivided into 2 subgenotypes: 1A and 1B **(Pellerin et al., 1994; Yamane et al., 2008)**, Moreover both

BVDV-1 and BVDV- 2 strains were divided according to their effect on tissue culture cells into two biotypes, cytopathic (CP) and noncytopathic (NCP) **(Goyal and Ridpath 2005).**

BVDV was manifested by respiratory troubles, immunosuppression, and decreased reproductive performance **(Brock, 2004)**. Because of the high economic losses of this virus, awareness of BVD eradication had increased substantially during the past decade **(Moening et al., 2005)**.

Effective BVDV control on dairy farms is multifaceted and includes methods for accurate detection of the virus, remove BVDV persistently infected (PI) cattle, prevent the virus introduction using comprehensive biosecurity plans, and optimize herd immunity through continuous vaccination against BVDV **(Schefers, 2009)**.

Persistently infected animals was found to act as a viral reservoir and are the main factor of the virus's continuation within herds and the primary method for the spread of BVDV and the maintenance of the virus in the cattle population **(Houe, 1999)**. Persistently infected animals should be identified and removed prior to the start of the breeding season to prevent virus transmission **(Kelling et al., 2000)**.

Although different vaccines and vaccination programs were used in Egypt since several decades, the economic losses due to clinical and subclinical BVD infection were increased, the present study was directed to identify PI animals, screening CattleMaster® 4 vaccination in different ages, detection of immunotolerant animals by SNT and detection of viral antigen by IHC and RT-PCR as 1st step for building up concrete control measures in endemic farm**.**

MATERIAL AND METHODS

1-<u>Animals and design:</u>

All calves under examination found in a private farm located in north of Nile delta in Damietta governorate and were reared under the same environmental condition and provided with adequate balanced ration and open water supply. All calves were clinically examined and revealed 260 clinically normal calves that examined for detection of BVDV persistent infection by RT- PCR on their pooled buffy coat (20 in each group) marked as (A-B-C-D-E-F-G-H-I-J-K-L-M) for presence of BVDV and retested individually by IFA on frozen ear notch skin biopsy samples and serum neutralization test (SNT) on serum samples then RT-PCR on buffy coat samples from calves with positive IFA to confirm persistent infection.

3- Vaccine and vaccination

CattleMaster® 4 (Pfizer) provides protection against pneumonic diseases. Protects against BVD (genotype 1 only), IBR, PI3 and BRSV. BVDV is killed virus, while other viruses are live. Safe for pregnant cows. U.S. Lic. No. 189. All calves were screened vaccinated with the recommended dose and route (2 ml intramuscular injection) in two doses with two weeks interval. The calves vaccinated after 2 months of age and the pregnant cows were vaccinated in the last month of pregnancy

4- Antigen and Kits

4.1. RNA extraction kit (Fermentas, #K0731).

4.2. Reverse transcription kits (Thermo fisher scientific 1454/V6 /1107 and Fermentas, # EP0451).

4.3. Diethylpyrocarbonate (DEPC) water (DEPC solution and deionizied water in a final concentration of 0.1%)

4.4. PCR reaction kit (DreamTaq™ Green PCR Master Mix 2X, Fermentas, #K1071)

4.5. Primer (from scientific matrix): based on the published sequence of BVDV gene with accession number (KC963968). To ensure primer sequence is unique for the template sequence, we checked similarity to other known sequences with BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi).

Table (1): Forward and reverse primers sequence for BVDV gene, and size of PCR production (bp), according to **Drew et al (1999):**

Forward primer	Reverse primer	Size (bp)
ATGCCCTTAGTAGGACTAGCA	CAACTCCATGTGCCATGTACAGCAG	**290**

4.6. TAE Electrophoresis Buffer (Bioshop, 1L22854)

4.7. Ethidium bromide 0.5 mg/ml

6.8. Agarose (bioshop, 1L22739), 1% (1.0 gm. agarose in 100 ml of 1X TAE buffer).

4.9. DNA loading buffer, 6x (Fermentas): (10 mMTris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol, FF60% glycerol and 60 mM EDTA).

4.10. 100 bp ladder DNA marker 0.5 µg/µl (Fermentas, #SM0321) 1kb ladder DNA marker 0.5 µg/µl (Fermentas, #SM1331)

4.11. BVD VIRUSES

Cytopathic strain of BVDV genotype-1 (Iman-strain) and cytopathic strain of BVDV genotype-2 (Strain 125) as control strain in RT-PCR and for SNT.The virus strains were kindly supplied from department of Rinderpest like Diseases, Veterinary Serum and Vaccine Research Institute, Cairo. (The virus titre was 10^5 $TCID_{50}$).

4.12. Monoclonal BVDV genotype 1 antibody

This monoclonal antibody produced in mouse. The concentration is 1.0 mg/ml in phosphate buffered saline. It can be used as a typing reagent for BVDVI using indirect Immunofluorescent (VMRD, catalog no. 210-91-CB), cell line 157, Lot No. 1570904. PO Box 502, Pullman, WA 99163 USA.

4.13. Monoclonal BVDV genotype2 antibody

This monoclonal antibody was produced in mouse. The concentration was 1.0 mg/ml in phosphate buffered saline. It could be used as a typing reagent for BVDVI using indirect immunofluorescent (VMRD, cataog no. 210-91-CB), cell line BA-2, Lot No. BA20904. PO Box 502, Pullman, WA 99163 USA.

4.14. Fluorescein-Labeled Affinity Purified Antibody to Mouse IgG (H+L): Was produced in goat, Catalog No: 02-18-06. KPL, Gaithersburg, MD, USA, 800-638-3167. It was used for IFA.

4.15. Tissue culture

Madin Darby Bovine kidney (MDBK) cell line **(Marcus and Moll., 1968)** was used for SNT. The cell line was proved to be free from non-cytopathic BVD virus.

5-Samples:

5.1- Serum:

Serum samples were separated from collected blood obtained from the 23 calves for SNT test. The samples were kept in epindorf tubes and preserved at -20°C until use.

5.2- Buffy coat:

Blood samples were collected in EDTA-containing vacutainer tubes from the jugular veins. The samples were sent immediately chilled to central biotechnology laboratory, faculty of veterinary medicine, Kafr El-sheikh University. 0.5 ml pooled in batches of 20 (from the 260 clinically normal calves) and 0.5 ml of each sample (from the 14 positive IFA calves) were thoroughly mixed and then centrifuged at 600 xg at room temperature (RT) to allow buffy coat containing white blood cells (WBC) to be aspirated and washed twice with PBS and then pelleted at 600 xg at RT for genomic RNA isolation as a step of RT-PCR technique after **Gandhi and Anand, (1982).**

5.3- Skin biopsy samples:

23 Skin biopsy samples (ear notches) were obtained from the dorsal pinna margin of each calf using individual disposable sterile blade. The ear notches were fixed in acetone then sectioned and processed. Tissue sections were cut at 5µm. according to **Cornish et al (2005)**.The samples were sectioned in the faculty of medicine, Mansoura University. It was used for IFA.

6- Reverse transcription polymerase chain reaction (RT-PCR):

6.1. RNA extraction: RNA extraction was proceeded according to the method described by **Chomczynski and Sacchi (1987).**

6.2. Reverse Transcription of RNA into cDNA: Reverse Transcription of

RNA into cDNA according to the method described by **Papavinasasundaram et al (2005).**

6.3. Polymerase chain reaction (PCR): According to **Weyant et al (1990).**

6.4. Identification of PCR product by agarose gel electrophoresis

Agarose Gel electrophoresis was used to determine the size of the PCR products using the technique adopted by **Viljoen et al (2005).**

RESULTS

I- Detection of BVDV genome in pooled buffy coat samples by RT-PCR:

BVDV was detected in three groups (23.08%) out of 13 groups, **Fig (1).**

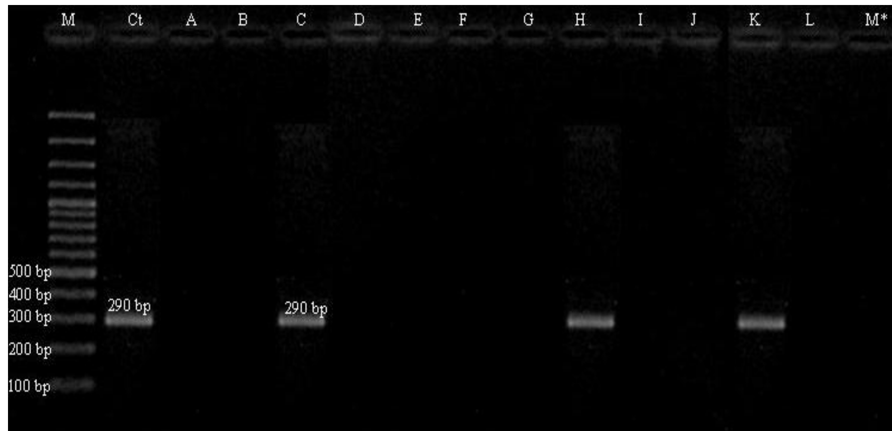

Fig. (1): Positive calf groups (C, H, and K) by RT-PCR in clinically normal calf groups.

RT-PCR product visualized after agarose gel electrophoresis. Positive result indicated at 290 bp fragments. Positive control sample is indicated. M= 100 bp ladder.

I.1- Percentage of persistent infected calves by IFA and SNT in positive calf groups.

Out of 60 calves positive to RT-PCR pooled group, only 23 calves were available to individual examination, as some calves were sold and many calves dead. 23 Skin biopsy and 23 serum samples were collected from examined calves by IFA (Fig. 2) and SNT respectively. Buffy coat samples were separated from 14 positive IFA calves and tested by RT-PCR after two months to confirm persistent infection (Table 2, Fig. 3).

Table (2): Individual results of calves in RT-PCR positive groups by IFA, SNT and RT-PCR:

Sample NO	BVDV antigen detection by IFA		BVDV antibody detection by SNT		RT-PCR* (290 bp)	Case status
	Genotyp1	Genotype 2	Genotype1	Genotype 2		
1	-	+	+	+	-	Transient viremic
2	-	+	-	-	+	PI
3	+	-	-	+	-	Transient viremic
4	+	-	+	-	-	Transient viremic
5	-	+	+	+	-	Transient viremic
6	-	-	+	+		immunocompetent
7	+	-	+	+	-	Transient viremic
8	-	+	+	+	-	Transient viremic
9	-	-	-	+		immunocompetent
10	-	+	-	-	+	PI
11	-	-	+	-		immunocompetent
12	-	-	+	+		immunocompetent
13	+	-	+	+	+	PI
14	-	+	+	+	-	Transient viremic
15	-	-	+	-		immunocompetent
16	-	+	+	+	-	Transient viremic
17	+	-	+	-	-	Transient viremic
18	-	+	+	+	-	Transient viremic
19	-	-	+	-		immunocompetent
20	-	-	+	+		immunocompetent
21	-	-	-	+		immunocompetent
22	-	+	+	-	-	Transient viremic
23	-	-	+	+		immunocompetent
Total +VE/260	5 (1.92%)	9 (3.46%)	18(6.92%)	15(5.5.77%)	3 (1.15%)	PI 3/260(1.15%) TI*11/260(4.23%)
Total -VE/260	18(6.92%)	14 (5.38%)	5 (1.92%)	8 (3.08%)	11(4.23%)	

IFA and SNT applied 21 days post vaccination. TI*Transient infection
RT-PCR*: RT-PCR applied on 14 positive IFA calves, after two months to confirm persistent infection.

Concerning to viral antigen detection by IFA in ear notch skin biopsy samples (Table 2 & Fig.3), 5 samples (21.74%) out of 23 examined samples were positive genotype 1 and 9 samples (39.13%) were positive genotype 2. While, detection of specific BVDV antibodies by SNT in sera samples revealed, 18 samples were positive genotype 1 antibodies (78.26%) out of 23 and 15 samples positive genotype 2 antibodies (65.22%). RT-PCR on 14 buffy coat samples collected from positive IFA calves revealed, Persistence of infection in 3 calves (21.43%) out of 14 examined calves (Table 2 &Fig.3).

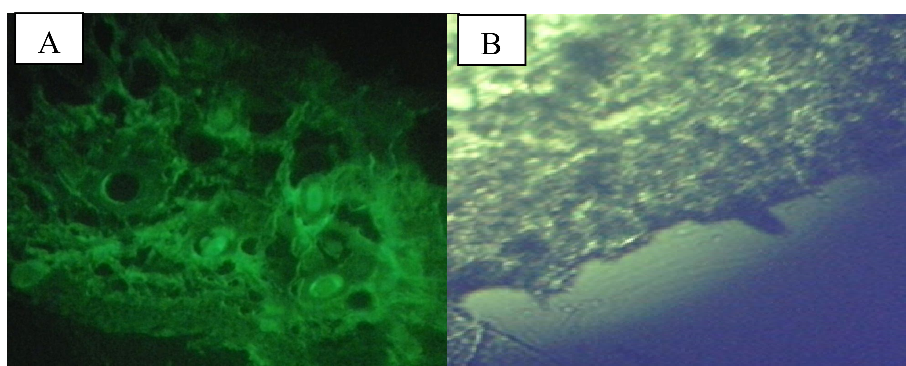

Fig. (2): Positive (A) and negative (B) IFA test for BVD genotypes identification.

Fig. (3): Positive PI calves in positive IFA calves.

RT-PCR product visualized after agarose gel electrophoresis. Positive result indicated at 290 bp fragments. Positive control sample is indicated. M= 100 bp ladder.

DISCUSSION

Bovine viral diarrhea virus (BVDV) occurs in most cattle – producing countries throughout the world, causing significant economic losses to the cattle industry. This led several countries to perform studies with the aim of planning and initiating eradication or control programs **(Houe 1999).**The PI animals are a major source of virus spread and thus, it is very important to identify and eliminate them from the cattle herd **(Kelling et al., 2000)**.

Screening the calves (N=260) by RT-PCR on Pooled blood samples collected from each of the 13 groups revealed, three positive groups (Fig.1). Our findings were supported by **Ridpath et al (1993)** who stated that RT-PCR considered as a standard method for detecting BVDV especially in pooled samples such as blood and bulk tank milk. As well as **Muñoz-Zanzi et al. (2000),** considered PCR is well suited to pooled-sample testing for the presence of BVDV PI animals because it is sensitive enough to detect minute amounts of virus. A single PI animal was detectable in pools of 200–250 negative samples. Animals contributing

to negative samples were all assumed non-PI, whereas positive pools may contain samples from PI animals or transiently viremic animals.

Out of 60 calves positive to RT-PCR pooled group only 23 calves were available for individual examination, as some male calves were sold and many calves dead although they were clinically normal, this is supported by the results of IFA (Table 2 & Fig.2), in which the most common genotype of BVDV circulating in calves was genotype 2. These results were in agreement with **Muñoz- Zanzi et al (2003)** who mentioned that the apparently normal calves have high death rate because they are predisposed to infections and have a higher risk of severe illness. This is most likely due to functional defects of the immune system causing immune suppression **(Roth et al., 1986)**. BVDV type 2 outbreaks are necessarily associated with severe disease, in which hemorrhagic syndrome is a form of severe acute BVDV that appears to be associated with type 2 strain of BVDV. Affected cattle often suffer marked thrombocytopenia, petechiation, and ecchymoses of mucosal surfaces, epistaxis, bloody diarrhea, bleeding from injection sites or trauma, fever, leukopenia, and death **(Corapi et al., 1990)**. Alternatively, it is likely that some BVDV type 1 isolates may be capable of causing severe disease **(Goyal and Ridpath 2005)**.

Individual screening of the positive three groups calves by IHC, SNT and RT-PCR (Table, 2) are used to distinguish persistent infection from transient one depending on the PI animals having the virus (by IFA) without seroconversion (by SNT) in spite of CattleMaster® 4 vaccination and the calves not recovered from the virus (persists) (by RT-PCR) with two months interval. This result agree with **Muñoz-Zanzi et al. (2000)** who revealed that when initial pool PCR was positive, the calves must be split and retested to differentiate the persistent infection from transient infection. **Bolin et al. (1994)** stated that there was an excellent correlation between IHC and RT PCR in a study of 41 cell lines for the presence of BVDV antigen or RNA. In addition to**, Goyal and Ridpath (2005) and Grooms and Keilen, (2002)** recorded that IHC of ear notch samples from calves is considered an effective method to detect PI animals. IHC is suitable for herd screening. The ear punch samples are easy to obtain from cattle of any age and are relatively stable. The test is not affected by the presence of maternal antibodies and the test is both sensitive and specific for PI cattle **(Baszler et al., 1995; Ellis et al., 1995; Thur et al., 1997**; **Njaa et al., 2000**; **Hilbe et al 2007)**.

Genotyping of the BVDV virus by IFA using the monoclonal antibody of each genotype revealed five (1.92%) having BVDV genotype 1 and 9 (3.46%) having BVDV

genotype 2. The SNT revealed 18 (6.92%) having specific antibodies against BNDV genotype 1 and 15 (5.77%) having specific antibodies against BNDV genotype 2 (Table 2). These results were supported by **Ridpath et al (2000)** who stated that the strains from the BVDV 1 and BVDV 2 genotypes may be distinguished by monoclonal antibody binding which is smaller but still significant difference among strains from the same genotype by serum neutralization test (SNT) using monoclonal antibody binding **(Ridpath et al., 1994)**.

Regarding to the 12 calves having specific antibodies against both genotypes (1&2), table (2), which may be attributed to the vaccinal strain (genotype 1) giving cross protection against both virus genotypes (genotype 1&2). These results were in agreement with many authors **(Cortese et al., 1998a; Makoschey et al., 2001 and Fairbanks et al., 2003)** who reported that genotype 1 BVDV vaccines provide some protection against type 2 BVDV, but the best protection rates obtained with the use of homologous BVDV vaccines **(Potgieter, 1995; Fulton et al., 2003)**.

Regarding to failure of seroconversion of PI animals after vaccination detected in two calves. While the third calves showed detectable level of specific antibodies (Table 2). This may attributed to the difference between the strain causing persistent infection and the strain of vaccine. These results were in agreement with results of **Goyal and Ridpath, (2005)** who stated that persistently infected cattle are immunotolerant to the infecting BVDV isolate but may mount an immune response to heterologous BVDV. As well as **Werdin et al. (1989a, b);** mentioned that if the vaccine strain is sufficiently different from the persisting strain, the PI animal may seroconvert to the vaccine strain and be declared non-PI although it still is persistently infected.

A number of 11 transiently infected calves (4.23%) identified by IFA (positive), SNT (positive) and RT-PCR (negative) after two months, table (2). These results are nearly similar to the results obtained by **Hilbe et al (2007)** who identified transiently infected animals in three cases by ELISA and RT-PCR.

This study concluded that the PI animals are the major problem in control of BVDV infection. Serological examination of calves 15 days after regular vaccination by SNT together with IFA and RT-PCR for viral detection could be considered by effective method for detection of PI calves.

REFERENCES

Baszler, T.V., Evermann, J.F., Kaylor, P.S., Byington, T.C. and Dilbeck, P.M (1995): Diagnosis of naturally occurring bovine viral diarrhea virus infections in ruminants using monoclonal antibody-based immunohistochemistry. Vet. Pathol. 32:609–618.

Bolin, S.R., Ridpath, J.F., Black, J., Macy, M. and Roblin, R (1994): Survey of cell lines in the American Type Culture Collection for bovine viral diarrhea virus. J. Virol. Methods 48:211–221.

Brock, K.V (2004): Strategies for the control and prevention of bovine viral diarrhea virus (BVDV) infections. Vet. Microbiol, 64:135-144.

Chomczynski, P and Sacchi, N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162(1), 156-159.

Corapi, W.V., Donis, R.O and Dubovi, E.J (1990): Characterization of a panel of monoclonal antibodies and their use in the study of the antigenic diversity of bovine viral diarrhea virus. Am. J. Vet. Res. 51:1388–1394.

Cornish, T.E., van Olphen, A.L., Cavender, J.L., Edwards, J.M., Jaeger, P.T., Vieyra, L.L., Woodard, L.F., Miller, D.R. and O, Toole, D (2005): Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. J. Vet. Diagn. Invest. 17:110-117.

Cortese, V.S., West, K.H., Hassard, L.E, Carman, S. and Ellis, J.A (1998): Clinical and immunologic responses of vaccinated and unvaccinated calves to infection with a virulent type-II isolate of bovine viral diarrhea virus. J. Am. Vet. Med. Assoc. 213:1312–1319.

Drew, T.W., Yapp, F and Paton, D (1999): The detection of bovine viral diarrhoea virus in bulk milk samples by the use of a single-tube RT-PCR. Vet. Microbiol. 64:145–154.

Ellis, J.A., Martin, K., Norman, G.R and Haines, D.M (1995): Comparison of detection methods for bovine viral diarrhea virus in bovine abortions and neonatal death. J. Vet. Diagn. Invest. 7:433–436.

Fairbanks, K., Schnackel, J and Chase, C.C (2003): Evaluation of a modified live virus type-1a bovine viral diarrhea virus vaccine (Singer strain) against a type-2 (Strain 890) challenge. Vet Ther 4:24–34.

Fulton, R.W., Saliki, J.T., Burge, L.J. and Payton, M.E (2003): Humoral immune response and assessment of vaccine virus shedding in calves receiving modifiedlive virus vaccines containing bovine herpesvirus-1 and bovine viral diarrhoea virus 1a. J. Vet. Med. B. 50:31.

Gandhi, K.K and Anand, S.R (1982): Regulation of glycolysis/fructolysis in buffalo spermatozoa. J.Reprod.fert. 64:145-150.

Goyal S.M. and Ridpath J.F. (2005): Bovine Viral Diarrhea Virus, Diagnosis, Management, and Control, 1st (Ed). ISBN 0-8138-0478 -7.

Grooms, D.L. and Keilen, E.D (2002): Screening of neonatal calves for persistent infection with bovine viral diarrhea virus by immunohistochemistry on skin biopsy samples. Clin. Diagn. Lab. Immunol. 9:898–900.

Heinz, F.X., Collett, M.S., Purcell, R.H., Gould, E.A., Howard, C.R., Houghton, M., Moormann, R.J.M., Rice, C.M., Theil, H. J., (2000): Family Flaviviridae. In: van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., Mcgeoch, D.J., Pringle, C.R., Wickner, R.B. (Eds.), Virus Taxonomy. Seventh Report of the International Committee on Taxonomy of viruses. Academic press, San Diego, Pp. 859-878.

Hilbe, M., Stalder, H., Peterhans, E., Haessig, M., Nussbaumer, M., Egli,C., Schelp,C., Zlinszky,K and Ehrensperger F (2007): Comparison of five diagnostic methods for detecting bovine viral diarrhea virus infection in calves. J. Vet. Diagn. Invest. 19:28–34.

Houe, H (1999): Epidemiological features and economical importance of bovine virus diarrhea virus (BVDV) infections. Vet. Microbiol. 64: 89-107.

Kelling C.L., Grotelueschen, D.M., Smith, D.R and Brodersen, B.W (2000): Testing and management strategies for effective beef and dairy herd BVDV biosecurity programs. Bov. Pract. 34:13–22..

Makoschey, B., Janssen, M.G.J., Vrijenhoek, M.P., Korsten, J.H and Marel, P (2001): An inactivated bovine virus diarrhoea virus (BVDV) type 1 vaccine affords protection against BVDV type 2. Vaccine 19:3261–3268.

Marcus, S.J and Moll, T (1968): Adaptation of bovine viral diarrhea virus to Madian Derby Bovine Kidney cell line. Am. J. Vet. Res., 29: 817-819.

Moennig, V., Houe, H. and Lindberg, A (2005): BVD control in Europe: current status and perspectives. Anim Health Res. Rev. 6, 63-74.

Muñoz-Zanzi, C.A., Hietala, S.K., Thurmond, M.C. and Johnson, W.O (2003): Quantification, risk factors, and health impact f natural congenital infection with bovine viral diarrhea virus in dairy calves. Am. J. Vet. Res. 4:358–365.

Muñoz-Zanzi, C.A., Johnson, W.O., Thurmon, M.C. and Hietala, S.K (2000): Pooled-sample testing as a herdscreening tool for detection of bovine viral diarrhea virus persistently infected cattle. J. Vet. Diagn. Invest. 12:195–203.

Njaa, B.L., Clark E.G., Janzen, E., Ellis, J.A. and Haines, D.M (2000): Diagnosis of persistent bovine viral diarrhea virus infection by immunohistochemical staining of formalin-fixed skin biopsy specimens. J. Vet. Diagn. Invest. 12:393–399.

Papavinasasundaram, K. G., Chan, B., Chung, J.H., Colston, M.J., Davis, E.O and –Gay, Y.A (2005): Deletion of the Mycobacterium tuberculosis pknH Gene Confers a Higher Bacillary Load during the Chronic Phase of Infection in BALB/c Mice. J. Bacteriol. 187, 5751-5760.

Pellerin, C., van den Hurk, J., Lecomte, J and Tussen P. (1994): Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortalities. Virology 203:260–268.

Potgieter, L.N.D. (1995): Immunology of Bovine Viral Diarrhea Virus. Vet. Clin. North Am. Food Anim. Pract. 11:501–520.

Radostitis, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D (2007): Veterinary medicine- A Textbook of the diseases of cattle, horses, sheep, pigs and goats, 10th (ed) Saunders El Sevier Printed in Spain: Pp: 1248-1276.

Ridpath, J.F. and Neill, J.D (2000): Detection and characterization of genetic recombination in cytopathic type 2 bovine viral diarrhea viruses. J. Virol. 74:8771–8774.

Ridpath, J.F., Bolin, S.R and Dubovi, E.J (1994): Segregation of bovine viral diarrhea virus into genotypes. Virology 205:66–74.

Ridpath, J.F., Bolin, S.R and Katz, J (1993): Comparison of nucleic acid hybridization and nucleic acid amplification using conserved sequences from the 5' noncoding region for detection of bovine viral diarrhea virus. J. Clin. Microbiol. 31:986–989.

Roth, J.A., Bolin, S.R and Frank, D.E (1986): Lymphocyte blastogenesis and neutrophil function in cattle persistently infected with bovine viral diarrhea virus. Am. J. Vet. Res. 47:1139–1141.

Schefers, J.M (2009): Detection, characterization and control of bovine viral diarrhea in dairy herds . Phd thesis, the faculty of graduate school of the University of Minnesota.

Thur, B., Zlinszky, K. and Ehrensperger, F (1997): Immunohistology as a reliable and efficient method for the diagnosis of BVDV infections. Schweiz Arch Tierheilkd 138:476–482.

Viljoen, G. J., Nel, L. H and Crowther, J. R (2005): Molecular Diagnostic Polymerase Chain Reaction Handbook. IAEA, Springer, Netherlands. pp. 57-62.

Werdin, R.E., Ames, T.R and Goyal, S.M (1989a): Detection and elimination of carrier animals in a dairy herd persistently infected with bovine viral diarrhea. J Vet Diag. Invest. 1:277–279.

Werdin, R.E., Ames, T.R., Goyal, S.M and DeVries, G.P (1989b): Diagnostic investigation of bovine viral diarrhea infection in a Minnesota dairy herd. J Vet Diag. Invest. 1:57–61.

Weyant, R. S., Edmonds, P and Swaminathan. B (1990): Effect of ionic and nonionic detergents on the Taq polymerase. Biotechniques 9:308-309.

Yamane, D., Kato, K., Tohya, Y and Akashi, H (2008): The relationship between the viral RNA level and upregulationof innate immunity in spleen of cattle persistently infected with bovine viral diarrhea virus. Veterinary microbiology. 129, 69-79.

الملخص العربى

تحديد الإصابة المستدامة (PI) لفيروس الإسهال المعدى البقري في العجول بعد الكشف عن المرض بإحدى المزراع الحلابة بمحافظة دمياط

سمر عطوه - عماد يونس - سيد زيدان*
قسم الأمراض الباطنه والمعديه والاسماك- كليه الطب البيطرى – جامعه المنصورة
*رئيس بحوث ومدير معهد الأمصال واللقاحات البيطرية – العباسية - القاهرة

استهدفت هذه الدراسة التعرف على الإصابة المستدامة (PI) لمرض الإسهال الفيروسي البقرى فى العجول ، حيث تم تقسيم ٢٦٠ عجلا الى ١٣ مجموعة، كل مجموعة كل منها يمثل ٢٠ عجلا . تم فصل ١٣ عينة طبقه خلايا بيضاء من مجموعات العجول للاختبار بواسطة تفاعل البلمرة المتسلسل العكسى (RT- PCR) ,والذى كشف عن وجود الفيروس في ثلاث مجموعات .تم تجميع ٢٣ عينة من جلد الأذن و ٢٣ عينة مصل للفحص بإختبارات IFA و SNT على التوالي. ثم تم فصل عينات طبقه الخلايا البيضاء من ١٤ عجلا ايجابى باختبار IFA واختبارها بواسطة تفاعل البلمره المتسلسل العكسى بعد شهرين لتأكيد الإصابة المستدامة . و كشف إختبار IFA وجود ٥ عينات (٢١,٧٤ ٪) من أصل ٢٣ إيجابية للنمط الجينى ١ و كانت ٩ عينات (٣٩,١٣ ٪) إيجابية للنمط الجينى ٢. في حين ، تم الكشف عن الأجسام المضادة للفيروس من قبل SNT في عينات الأمصال ، وكانت ١٨(٧٨,٢٦ ٪) عينة إيجابية الأجسام المضادة للفيروس من النمط الجيني ١ من أصل ٢٣ و ١٥ (٦٥,٢٢ ٪) عينه إيجابية الأجسام المضادة للفيروس من النمط الجيني ٢ . وكشف تفاعل البلمره المتسلسل العكسى عن وجود ٣ (٢١,٤٣ ٪) من أصل ١٤ عجلا لديه أستدامة فى الأصابة بعد الشهرين. وبذلك قد ُأكدت الإصابة المستدامه فى عدد ٣(١,١٥ ٪) عجول من أصل ٢٦٠. هذه الحيوانات ذات الإصابة المستدامة خطيرة و تمثل المصدر الرئيسي لمرض الإسهال الفيروسى البقرى في المزرعة ، ولذلك يجب القضاء عليها لتحقيق برنامج مكافحة فعال.