CLINICAL AND LABORATORY INVESTIGATION IN AN OUTBREAK OF ACUTE BOVINE VIRAL DIARRHEA VIRUS INFECTION (BVDV) MIXED WITH BOVINE HERPESVIRUS 1 (BHV-1) IN SALHEA FARM AT SHARKIA PROVINCE.

Abdel Samie, M. M* ,Mahmoud. M.M.* , Ghalley, H. M.** , Abd-Eldaim, M. M. *, Hassan, S. A, El-Hayeg, M. M * Salem, M. A.*.***

** Suez Canal University, Faculty of Veterinary Medicine.*

*** Serum and Vaccine Research Institute ,Abbassia ,Egypt, Department of Rinderpest like Diseases.*

**** Animal Health Research Institute, Dokki, Giza.*

ABSTRACT

During March 2009, an outbreak of acute bovine viral diarrhea virus (BVDV) mixed with bovine herpesvirus 1 (BHV-1) occurred in Salhea farm (Sharkia, Egypt). During this outbreak clinical findings were noticed only in newlyborn calves less than two months of age. Morbidity, mortality and case fatality rates were 89.7%, 17.9% and 20%, respectively. The most clinical signs of outbreak were fever (39-41.5°C), anorexia, muzzle lesions which appeared as redness, ulceration and crust formation, conjunctivitis and corneal opacity. Abnormal nasal discharges (serous, seromucoid, mucoid and mucopurulent), lung auscultation revealed harsh loud breath sound and wheezes, oral lesions in tongue, lower gum and cheek papillae appeared as ulceration, necrosis and hyperemia, dry cough and dyspnea in some cases. Diarrhea wasn't prominant sign of outbreak. Nasal swabs, ocular swabs and buffy coat samples were collected from affected calves for viral isolation. Cytopathic BVDV was isolated and identified by direct immunofluoresence from buffy coat samples, while BHV-1 was isolated and identified by indirect immunofluoresence and PCR from nasal swabs.

INTRODUCTION:

Bovine viral diarrhoea virus (BVDV) and bovine herpesvirus type 1 (BHV-1) are well known, important pathogens of cattle that give rise to substantial economic losses due to reproductive failures and increased calf mortality, as well as enteric and respiratory disorders.

These pathogens have a worldwide distribution and tend to be endemic in most populations. **(Kahrs, 2001 and Lindberg, 2003).**

Bovine viral diarrhoea virus is a heterogeneous group of viruses of the family Flaviviridae grouped in the genus Pestivirus together with closely related to classical swine fever virus (CFSV) and border disease virus (BDV) **(Donis 1995).**

On the basis of their ability to induce a cytopathic effect on cell cultures, BVDV strains are divided into a cytopathogenic (cp) biotype and a non cytopathogenic (ncp) biotype. The majority of acute infe-ctions are caused by the ncp bio-type, while the cp biotype is comm.-only isolated, together with the ncp biotype, in animals suffering from mucosal disease. **(Moennig et al., 1990).**

The bovine herpesvirus-1 (BHV-1) is a double-stranded deoxynucleic acid virus. It is a member of the genus Varicellovirus within the subfamily Alphaherpesvirinae, which belongs to the family Herpesviridae **(Schwyzer & Ackermann 1996).** BHV-1 is an economically important pathogen. Infectious rhinotracheitis is one of the most common clinical signs of BHV-1 in cattle **(Fray et al., 2000).**

BVDV induces immunosuppresion **(Abd El-Rahim 1996)**, which allows for secondary infection of the respiratory tract, and causes significant losses as a result of its interaction with other pathogens (**Fray et al., 2000)**

The purposes of this study were to:

1-Investigate clinical findings in outbreak of bovine viral diarrhea virus infection mixed with bovine herpesvirus 1 in Salhea farm at Sharkia province.

2-Trials for isolation and identification of causative viruses.

MATERIALS & METHODS:

I- Case history and data collection:

Case history and data were collected from farm owner. The collected data including: vaccination program in the farm, housing system, feeding colostrum regime and previous treatment for affected calves.

II- Clinical examination:

A total number of 39 newlyborn calves under two months of age were examined. Each calf was clinically examined by body temperature measurement, examination of respiratory system, examination of eyes and examination of oral cavity according to **Radostits et al., (2000).**

III- Virological investigation:

1- Samples collection and processing:

A total number of 35 nasal swabs, 10 ocular swabs and 28 buffy coats were collected from affected calves. The swabs were vigorously rubbed against the mucosal surface, and then suspended in 5 ml of Minimal essential media (MEM) containing 2% Fetal Calf Serum (FCS) and antibiotics (800µg streptomycine and 800 IU penicillin / ml). Samples were cooled at 4°C and rapidly submitted to the laboratory. After arrival to the labo-ratory, swabs were agitated in the transport medium to elute virus. The specimens were clarified by centrifugation at 1500 g for 10 minutes and the supernatant of these specimens were used for virus isolation. The EDTA blood samples were centrifuged at 1000 Xg for 10 minutes. The plasma was removed and the buffy coat (leukocytic fraction) was aspirated with a sterile Pasteur pipette into a sterile tube and 5 ml of MEM containing 2% FCS was added. The mixture was left at room temperature for 1 hour and then frozen at – 70C until used.

2- Virus isolation:

One hundred micro liter (µl) volumes of processed samples were inoculated into Madian derby bovine kidney (MDBK) cell line that was grown in 24 wells plastic plate. After one hour adsorption period, the cultures were rinsed and maintenance medium containing 2% FCS was added. The cell cultures were observed daily for CPE and if no CPE after 7 days has appeared, a blind passage should be made. The cell culture was frozen and thawed, clarified by centrifugation and the supernatant was used for inoculation of fresh monolayer. Three blind passages were done for each sample.

3- Identification of isolated viruses by immuno fluorescence antibody technique: according to **Potgieter and Aldridge (1977):**

A- Fixation of infected cells:

One hundred µl of isolated viruses were inoculated into 25 cm^2 tissue culture flask containing monolayer of MDBK cell line. The flasks were observed daily for CPE. At 50% CPE, the cells were trypsinized and collected in 1.5 ml microfuge tubes. The cells were washed three times with PBS and suspended in appropriate volume of Phosphate buffer saline (PBS). The suspended cells were then spread to glass slides and left for dryness. The fixation was done by immersing the glass slides in acetone for 30 minutes.

B- Direct immunofluoresence for detection of BVDV:

Fifteen µl of goat anti-BVDV conjugated by Fluorescin isothiocynate (FITC) put on the fixed slides and incubated at 37°C for 30 minutes in humid atmosphere. Then washing was done for 3 times using PBS.

A final washing was done using distilled water and the slides were air dried at room temperature. PBS

mixed with glycerin (1:1) was added and slides were covered by cover slip then examined by inverted fluorescence microscope.

C- Indirect immunofluoresence for detection of BHV1:

Fifteen micro liter goat anti-BHV1 antibodies put on the fixed tissue culture slides and incubated at 37°C for 30 minutes in humid atomsphere, then washing was done for 3 times using PBS. A final washing was done using distilled water and the slides were air dried at room temperature. Anti-goat conjugated by FITC was added to slides then incubated at 37°C for 30 minutes in humid atmosphere. The washing was done for 3 times using PBS. A final washing was done using distilled water and the slides were air dried at room temperature. PBS mixed with glycerin (1:1) was added and slides were covered by cover slip then examined by inverted fluorescence microscope.

4- Identification of isolated BHV-1 from nasal swabs by PCR:

All isolated viruses were examined by PCR for detection of BHV-1.

A- DNA extraction:

DNA was extracted from 200 µl of tissue culture supernatant by Wizard® SV Genomic DNA Purification System, Promega, INC. according to manufacturer.

B- Primer selection:

PCR primers used in detection of BHV-1 were shown in table (1).

Table (1): List of primers used in PCR.

Name of Oligo-nucleotide	Sequence	Product length (bp)	Reference
gB1 (F)	5'-TAC GAC TCG TTC GCG CTC TC-3'	478	Fuchs et al.,(1999)
gB2 (R)	5'-GGT ACG TCT CCA AGC TGC CC-3'		Engelenburg
gC1(F)	5'- CTG CTG TTC GTA GCC CAC AAC G-3'	173	et al., (1993)
gC2(R)	5'- TGT GAC TTG GTG CCC ATG TCG C -3'		Moore et al.,(2000)
Tk1(F)	5'- TGG TAC GGA CGC CTT AAG TGG -3'	298	
TK2(R)	5'- GTT GAT CTC GCG GAG GCA GTA -3'		

C- PCR procedure:

PCR was performed in a total volume of 25µl containing 1.5 unit dream taq polymerase, 200µM deoxynucleoside triphosphates, 1X dream taq buffer, 20 pmole from each primer, 5% DMSO, 80 ng of DNA template and sterile distilled water up to 25µl. samples were amplified in techne progene thermal cycler under the following conditions, initial denaturation at 95°C for 5 min., then 30 cycles of 95°C for 30 seconds, 56°C for 30 seconds (for gB and gC primers) or 63°C for 30 seconds (for TK primers), 72°C fro 30 seconds,

and final extention at 72°C for 10 minutes.

D- Visualization of PCR products by agarose gel- electrophoresis:

To confirm the targeted PCR amplification, 7 μl of the PCR product from each tube was mixed with 2 μl of 6X gel loading buffer and electrophoresed along with 100bp DNA molecular weight marker (Gene Ruler, MBI Fermentas) on 2.0% agarose gel containing ethidium bromide (at the rate of 0.5μg/ml) at constant 80V for 30min in 1X TAE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by Sony video camera.

RESULTS:

1- Case history data collection:

All newly born calves were separated from their dams just after parturition, they were housed in a single pen until one month of age then transferred to open yards. No vaccination program against BVDV and BHV-1 were applied.

Newly born calves fed colostrum directly from their dams just after parturition by bottle. All calves were treated previously with antibi-otics as oxytetracycline and enrof-loxacin (10mg/kg b.wt.) with antip-yretics without response.

Morbidity, mortality and case fatality rates were 89.7%, 17.9% and 20%, respectively.

2- Clinical examination:

Out of clinically examined 39 newlyborn calves under 2 months of age, 35 calves were suffering from respiratory affections. Clinical examination revealed that: fever (71.5%), abnormal nasal discharges ranging from serous (5.7%), seromucoid (57.1%), mucoid (17.1%) and mucopurulent (20%), lung auscultation revealed presence of harsh loud breath sound (57.1%) and wheezes (17.1%), muzzle examination revealed redness (11.4%), ulceration (22.9%) and crust formation (51.4%), dry cough (77.1%), moist cough (5.7%), labored breathing (11%), conjunctivitis (25.7%), corneal opacity (2.9%) and oral lesions in tongue, lower gum and cheek papillae appeared as hyperemia (11.4)%, ulceration (22.9%) and necrosis (34.3%).(Photo 1 to 7).

3- Virus isolation:

Out of 35 nasal swabs, 10 ocular swabs and 28 buffy coat samples inoculated in MDBK cells.,only 4 cytopathic viruses were isolated from nasal swabs and 28 from buffy coat samples.

4- Identification of isolated viruses by immunofluoresence:

All 4 isolated viruses from nasal swabs were identified as BHV-1 by indirect immunofluoresence, while only 22 isolates from buffy coat were identified as BVDV by direct immunofluoresence.(Photo 7 and 8).

5- Detection of BHV-1 in isolated viruses by PCR:

All isolated viruses were examined by PCR for detection of BHV-1, only 4 isolates from nasal swabs were positive. (Photos 9, 10 and 11).

DISCUSSION:

The BVDV infection in cattle has been reported throughout the world, and the wide spectrum of clinical syndromes associated with this positive-stranded and enveloped RNA virus makes it one of the most important viral pathogens of cattle **(Corapi et al., 1989).** The BVDV spreads widely among cattle herds in the world **(Shimizu 1990)**. The BVDV was isolated for the first time in Egypt in 1972 from a calf suffering from severe enteritis **(Hafez 1975a)**. The present investtigation reported an outbreak of acute BVDV infection mixed with BHV-1 infection in Salhea farm at Sharkia province. Isolation and identification of the causative viruses indicated that the main cause of outbreak was acute bovine viral diarrhea mixed with BHV-1.The BVDV was widespread throughout the country as determined by the detection of neutralising BVD antibodies in 99 (37.6%) out of 263 sheep sera in 24 different localities throughout all the provinces of Egypt **(Hafez 1975b)**. Field outbreaks of bovine viral diarrhoea virus (BVDV) infection, either alone or mixed with bovine herpe-svirus-1 (BHV-1) and/or parainflue-nza-3 virus (PI-3V) were reported in Egypt by **(Aly., et al 2003)**.The BVDV seems to be transmitted directly from either acutely or persistently infected cattle (PI) to susceptible one. Persistently infected cattle play a more significant role in the transmission and maintenance of BVDV than acutely infected cattle **(Brownlie J. and Clarke M.C. 1990).** Persistently infected-calves usually have poor viability and suffer early disease and death with or without signs of diarrhoea **(Radostits & Littlejohns 1988)**.

This study suggested that presence of persistence infected animals in Salhea farm is considered the main source of infection for all acutely infected cases. Clinically, fever, anorexia, muzzle lesions appeared as redness, ulceration and crust formation, conjunctivitis and corneal opacity, abnormal nasal discharges (serous, seromucoid, mucoid and mucopurulent), harsh loud breath sound and wheezes, oral lesions in tongue, lower gum and cheek papillae appeared as ulceration, necrosis and hyperemia and dry cough were the most observable signs of the outbreak. **Evermann and barrings-tom (2005)** stated that oral erosions and ulcers, oral papillae blunting and hemorrhage were present in acute BVDV infection. Muzzle lesions and Conjunctivitis in infected calves may be attri-

buted to BHV-1 infection, these findings agreed with **Curtis et al., (1966); Janzen et al., (1980); Radostits et al., (2007).**

No cases of diarrhea were recorded in this outbreak, these finding agreed with **Baule et al., (2001)** who experimentally infected seronegative immunocompetent calves by new genetic cluster of BVDV type I, and they found that lack of diarrhea in the infected calves indicates that infection of gastrointestinal tissue did not result in significant impairment of gastrointestinal functions. This is consistent with the fact that gastrointestinal lesions were mild or absent in these experiments.

Newly born calves under 2 months of age were more susceptible to the infection and showed severe clinical signs, these findings may be due to absence of vaccineation program in farm that made newly born calves more susceptible to infection by BVDV and BHV-1.

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 (**Baker J.C. 1987; Donis R.O. 1988),** In this study, laboratory investigations revealed isolation and identification of cpBVDV biotype from buffy coat samples and BHV-1 from nasal swabs using the cell culture method and the immunofluorescent technique for identification. **Fulton et al., (2000)** found that only ncp strains of BVDV were detected in sera samples and both CP and NCP strains were found in buffy coat samples. Inasmuch as CP in the blood, the buffy coat sample would be the preferred sample for isolation. Isolation of cytopathic BVDV from acute non mucosal disease BVDV cases were disagreed with **Bendfeldt et al., 2003 and Schweizer et al., 2006,** who stated that only cytopathic biotypes have only been isolated in connection with outbreaks of mucosal disease (MD). The ncp biotype is comm.-only found in nature and causes persistent infection in animals. This finding was in agreement with **Baule et al., (2001)** who studied the pathogenesis of infection induced by cytopathogenic isolates from the newly identified genetic cluster Id of bovine viral diarrhea virus (BVDV) type I in two experimental infections of previously seronegative, immunocompetent calves. All infected calves developed respiretory signs and seroconverted to BVDV positivity.

The current study suggested that the immunosuppressive effect of BVDV facilitated the infection of the diseased calves with other viral agents such as BHV-1. This suggestion coincides with the findings reported by **Potgieter et al., (1984).** The concurrent infection with the BVDV and BHV-1 worsened the

clinical disease and the economic losses in affected farm.

CONCLUSION:

The present study leads us to conclude that:

Bovine viral diarrhea virus and Bovine herpesvirus 1 infection are endemic diseases in Egypt so proper control measures such as elimination of persistently infected calves for BVDV infection and application of vaccination programs for BVDV and BHV-1 control is permanently indicated.

Photo (1): Infected newlyborn calf showing bilateral seromucoid nasal discharges, crusted muzzle, labored breathing and necrosis in lateral aspect of tongue.

Photo (2): Infected newlyborn calf showing redness in muzzle.

Photo(3): Infected newlyborn calf showing crusted muzzle.

Photo (4): Infected newlyborn calf showing conjunctivitis.

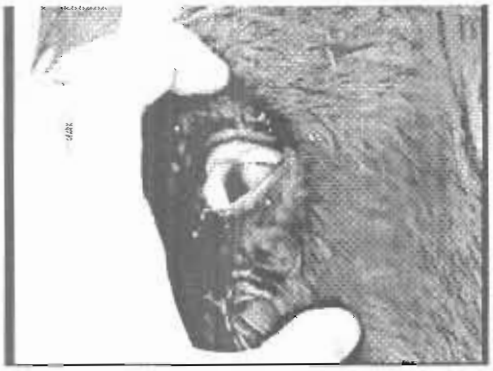

Photo (5):Infected newlyborn calf showing corneal opacity.

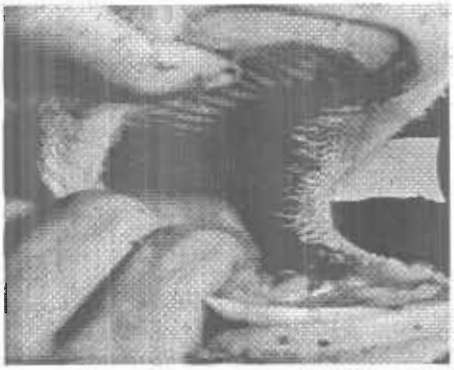

Photo (6): Infected newlyborn calf showing hyperemia in cheek papillae.

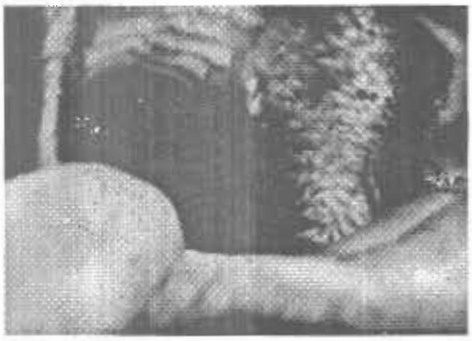

Photo (7): Infected newlyborn calf showing ulce-ration in hard palate.

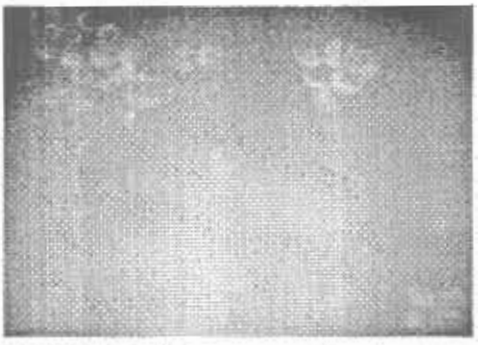

Photo (8): No fluorescence illumination (Negative control).

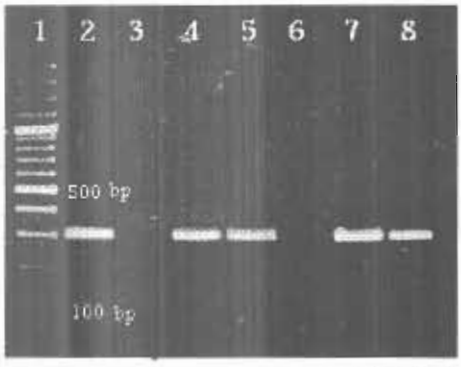

Photo (9): Fluorescence illumination of infected MDBK cells by BVDV.

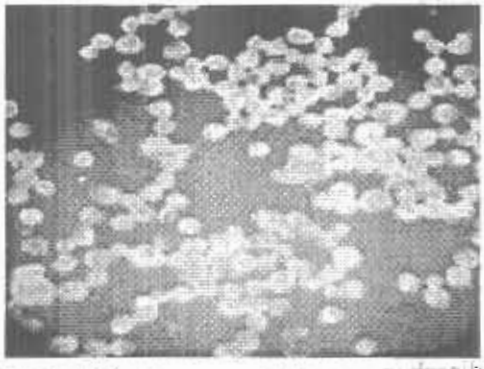

Photo (10): Agarose gel electrophoresis pattern of BHV-1 TK gene 298 bp specific PCR product.
Lane 1 & 6: 100 bp DNA ladder.
Lane 2: Positive control (Modified live BHV-1 from nasalgen vaccine).
Lane 3 & 6: Negative control.
Lane 4, 5, 7 & 8: positive samples.

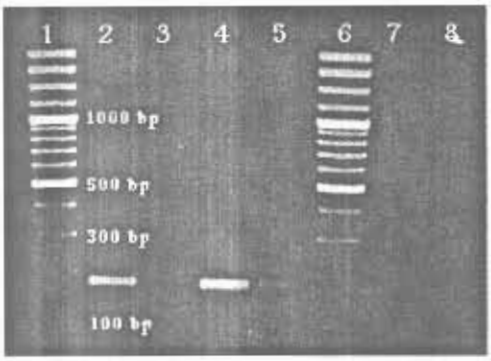

Photo (11): Agarose gel electrophoresis pattern of BHV-1 gC gene 173 bp specific PCR product.
Lane 1 & 6: 100 bp DNA ladder.
Lane 2: Positive control (Modified live BHV-1 from nasalgen vaccine).
Lane 3: Negative control.
Lane 4, 5, 7 & 8: positive samples.

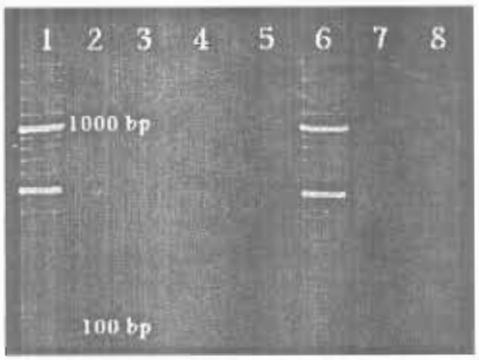

Photo (12): Agarose gel electrophoresis pattern of BHV-1 gB gene 478 bp specific PCR product.
Lane 1 & 6: 100 bp DNA ladder.
Lane 2: Positive control (Modified live BHV-1 from nasalgen vaccine).
Lane 3: Negative control.
Lane 4, 5, 7 & 8: positive samples.

REFERENCES:

Abd El-Rahim I.H.A. (1996): Clinical and laboratory studies on mucosal disease in cattle. PhD thesis (infectious diseases). Faculty of Veterinary Medicine, Assiut University, Egypt, 87-98.

Aly.N.M., G.G. Shehab & I.H.A. Abd El-Rahim.(2003): Bovine viral diarrhoea, bovine herpesvirus and parainfluenza-3 virus infection in three cattle herds in Egypt in 2000. Rev. sci. tech. Off. int. Epiz., 2003, 22 (3), 879-892

Baker J.C. (1987): Bovine viral diarrhea virus: a review. *J. Am. vet. med. Assoc.*, **190**, 1449-1458.

Baule. C., G. Kulcsar, K. Belak, M. Albert ,c. Mittelhalzer ,T. Soo´ S, L. Kucsera, and S. Belak. (2001): Pathogenesis of Primary Respiratory Disease Induced by Isolates from a New Genetic Cluster of Bovine Viral Diarrhea Virus TypeI. Journal of clinical micro-biology, Jan. 2001, p. 146–153.

Bendfeldt, S., Grummer, B. and Greiser-Wilke, I. (2003): No caspase activation but overexpression of Bcl-2 in bovine cells infected with noncytopathic bovine virus diarrhoea virus. *Veterinary Microbiology 96*, 313-26.

Brownlie J. and Clarke M.C. (1990): Bovine virus diarrhea virus: speculation and observations on current concepts (J. Brownlie & M.C. Clarke, eds). *Rev. sci. tech. Off. int. Epiz.*, **9** (1), 223-230.

Corapi W.V., French T.W. & Dubovi E.J. (1989): Severe thrombocytopenia in young calves experimentally infected with noncy-

topathic bovine viral diarrhea virus. *J. Virol.*, **63** (9), 3934-3943.

Curtis R. A, Vandreumel A. A, and Ditchfield J. (1966): Infectious bovine rhinotracheitis – clinical, pathological, and virological aspects. Can. Vet. J.1966; 7: 161-168.

Donis R.O. (1988): Bovine viral diarrhea: the unraveling of a complex of clinical presentations. *Bovine Proc.*, **20**, 16-22.

Donis, R.O. (1995): Molecular biology of bovine viral diarrhea virus and its interactions with the host.*Veterinary Clinics of North America. Food Animal Practice 11*, 393-423.

Engelenburg, F. A. C. van; Maes, R. K.; Oirschot, J. T. van and Rijsewijk, F. A. M. (1993): Development of a rapid and sensitive polymerase chain reaction assay for detection of bovine herpesvirus type 1 in bovine semen. J. Clin. Microbiol. **31**: 3129-3135.

Evermann JF and Barrington BM. (2005): Clinical features of bovine virus diarrhea virus. In: *Bovine viral diarrhea virus, diagnosis, management and control*, 1st Ed. Ed. Gayal SM, Ridpath JF, pp: 105-119

Fray M.D., Supple E.A., Morrison W.I. and Charleston B.(2000): Germinal centre localization of bovine viral diarrhoea virus in persistently infected animals. *J. gen. Virol.*, **81**, 1669-1673.

Fuchs, M.; Peter H., Jan Detterer, and Hanns Joachim R. (1999): Detection of Bovine Herpesvirus Type 1 in Blood from Naturally Infected Cattle by Using a Sensitive PCR That Discriminates between Wild-Type Virus and Virus Lacking Glycoprotein E. J. Clin. Microbiol. **37**: 2498-2507.

Fulton, R W., Saliki J. T., Confer A. W., Lurinda J. Burge, d'Offay J. M., Helman R. G., Bolin S. R., Ridpath J. F. and. Payton M E., (2000): biotypes and type 1 and 2 genotypes in diagnostic laboratory accessions: clinical and necropsy samples from cattle J Vet Diagn Invest 12:33–38 (2000).

Janzen E.D, Smart J.N and Nicholson H.H (1980): Obser-vations on an Outbreak of Infectious Bovine Rhinotracheitis in a Bull Test Station.Can. vet. J. 1980; 21: 24-27

Hafez S.M. (1975a): Isolation and identification of bovine viral diarrhea-mucosal disease virus in Egypt *J. Egypt. Vet. Med. Assoc.*, **35**, 1-9.

Hafez S.M. (1975b): Geographical distribution of bovine viral diarrhea-mucosal disease in Egypt determined by the detection of neutrallizing antibodies in sheep sera. *J. Egypt. Vet. Med. Assoc.*, **34**, 1-12.

Kahrs RF: (2001): Infectious bovine rhinotracheitis. In: Viral diseases of cattle. 2nd ed., Iowa state university. pp. 159-170.

Lindberg AL(2003): Bovine viral diarrhoea virus infections and its

control. A review. .Vet. Q. 2003, *25 (1),* 1-16.

Moennig, V., H.-R. Frey, E. Liebler, J. Pohlenz, and B. Liess. (1990): Reproduction of mucosal disease with cytopathogenic bovine viral diarrhoea virus selected in vitro. Vet. Rec. **127**:200–203.

Moore S, Gunn M, Walls D. (2000): A rapid and sensitive PCR-based diagnostic assay to detect bovine herpesvirus 1 in routine diagnostic submissions. Vet Microbiol. 2000 Jul 31;75(2):145-53.

Potgieter .L.and Aldridge .P. (1977): Use of the indirect fluorescent antibody test in the detection of bovine respiratory syncytial virus antibodies in bovine serum. Am. J. Vet. Res. **38** (1977), pp. 1341–1343.

Potgieter L.N.D., McCracken M.D., Hopkins F.M., Walker R.D. & Guy J.S. (1984): Experimental production of bovine respiratory tract disease with bovine viral diarrhea virus. *Am. J. vet. Res.*, **45** (8), 1582-1585.

Radostits O.M. & Littlejohns I.R. (1988): New concepts in the pathogenesis, diagnosis and control of diseases caused by the bovine viral diarrhea virus. *Can. vet. J.*, **29**, 513-528.

Radosits OM, Mayhew IG, Houston DM. (2000): Veterinary clinical examination and diagnosis. Edited by: Radostits OM, Mayhew IG, Houston DM. London: W. B. Saunders; 2000

Radostits OM, Gay CC, Hinchcliff KW, Constable PD, Done SH.(2007): Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Saunders, Philadelphia Canadian Veterinary Journal 29, 513-528.

Schwyzer M. and Ackermann M. (1996): Molecular virology of ruminant herpesviruses. *Vet. Microbiol.*, **53** (1-2), 17-29.

Schweizer, M., Matzener, P., Pfaffen, G., Stalder, H. and Peterhans, E. (2006): "Self" and "Non-self" Manipulation of Interferon Defense during Persistent Infection: Bovine Viral Diarrhea Virus Resists Alpha/Beta Interferon without Blocking Antiviral Activity against Unrelated Viruses Replicating in Its Host Cells. *Journal of Virology 80,* 6926-35.

Shimizu M. (1990): Current situation of bovine virus diarrhoea-mucosal disease (BVD-MD) virus infections and their antigenic diversity in Hokkaido, Japan. *Rev. sci. tech. Off. int. Epiz.*, **9** (1), 181-194.

الملخص العربي

دراسة أكلينكية ومعملية علي وباء الاسهال الفيروسي المعدي الحاد مختلطا مع فيروس الهربيس البقري 1 في مزرعة الصالحية بمحافظة الشرقية

حدث وباء من مرض الاسهال الفيروسي المعدي الحاد مختلطا مع الاصابة بفيروس الهربس البقري في مزرعة الصالحية (محافظة الشرقية). هذا الوباء أصاب العجول الرضيعة تحت عمر شهرين فقط، وكان معدل الاصابة والنفوق يتراوح بين 89.7%و 17.9% و 20%. كانت الاعراض الاكلينكية تتمثل في أرتفاع في درجات حرارة الجسم (39-41.5 م°) وفقدان الشهية واصابات في المخصم مثل احمرار وتقرحات وقشور وكذلك التهاب الملتحمة وارتشاحات أنفية مختلفة وبسماع أصوات الرئتين وجدت أصوات تنفسية مرتفعة وكذلك يوجد علامات باثولوجية علي اللسان واللثة السفلية وحلمات الفك مثل التقرحات واحمرار ويوجد ايضا كحة جافة في معظم العجول وصعوبة في التنفس. وبأخذ عينات من الانف والعين وخلايا الدم البيضاء لعزل المسبب وجد أن المسبب الاساسي هو الفيروس البقري المعدي وذلك بواسطة العزل والتصنيف بواسطة أختبار الفلورسينتي المباشر بينما عزل أيضا فيروس الهربس البقري 1 وتم تصنيفه بواسطة أختبار الفلورسينتي الغير مباشر وأختبار البلمرة المتسلسل.