رقم البحث (18)

RESPIRATORY INFECTION OF BOVINE VIRAL DIARRHEA (BVD) IN CALVES IN SHARKIA AND ISAMAILIA GOVERNORATES

By

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ABSTRACT

This study was applied to reported the effect of respiratory form of bovine viral diarrhea (BVD) virus on calves in Srarkia and Isamailia Governorates . 158 nasal samples, 139 lung tissues and 158 serum samples were collected from diseased and slaughtered animals were used in this study. The results of this study revealed that bovine viral diarrhea (BVD) virus was isolated from 51 (32.3%) nasal swabs and 45 (32.4%) lung tissues,, Identification of BVD virus by direct fluorescent antibody test was carried out and revealed that 66 (41.8%) nasal swabs and 55 (39.6 %) lung tissues were giving positive results. While 76 (48.1%) serum samples were giving positive results for presence of antibodies against BVD virus by using ELISA test.

INTRODUCTION

Bovine viral diarrhea (BVD) was originally described in 1946 in New York state as acute, rarely fetal highly contagious disease with fever, diarrhea, mucosal lesions , and leucopenia ***(Olafson, et al. 1946)***. Although initial reports described a clinically severe disease, acute BVD has since been considered a mild or subclinical disease of a few days duration with negligible mortality at any age ***(Radostits, and littlejohns 1988)*** .Occasionally, scattered outbreaks of severe disease do occur***(Barker, et al. 1993)***. The emergence of virulent viral strains in the USA ***(Corapi , et al. 1989)***, and reports of severe acute BVD in Great Britain in 1992 ***(David, et al. 1994)*** preceded the occurrence of widespread outbreaks of severe acute BVD in both Quebec and Ontario (Canada) in 1993 ***(Carman, et al. 1994)***.

Bovine viral diarrhea (BVD) virus cause infection and disease in cattle and involve 1 or more organ systems ***(Fulton. et al. 2000)***. Bovine viral diarrhea virus has been isolated from several clinical forms of disease and from necropsy samples, including cattle with signs and or lesions of bovine respiratory disease (BRD) ***(Fulton. et al. 2000)***.

Bovine viral diarrhea viruses are classified by biotype and genotype ***(Baker, 1995)***. The biotypes, cytopathic (CP) and noncytopathic (NCP) are based on the presence or absence of visible cytopathic effect (CPE) in the infected cell culture. BVDV genotypes (1&2) are detected by polymerase chain reaction (PCR) for nucleotide differences and antigenic differences ***(Pellerin, et al. 1994)***. Bovine viral diarrhea genotypes have been associated with particular disease forms. In one study , BVDV NCP biotypes were isolated more frequently than CP biotypes and BVDV1 NCP biotypes were isolated more frequently than BVDV2 genotype from cattle with history of BRD ***(Fulton. et al. 2000)***. Bovine viral diarrhea virus 1 genotypes were isolated more frequently than BVDV2 genotypes from necropsy cases of calves with fibrinous pneumonia ***(Fulton. et al. 2000)***. BVDV have been associated with BRD in calves of mixed source and those under observation for BRD signs and lesions ***(Fulton, et al. 2002)***. The BVDV isolations and seroconversions have occurred along with parainfluenza 3 virus (PI3V), Bovine respiratory syncytial virus (BRSV). Bovine adenovirus 7 (BAV7), and Bovine herpesvirus 1 (BHV1). Persistently infected (PI) cattle are a major source of exposure for susceptible cattle. The PI infected calves are the result of fetal infection with NCP starin occurred between 42-125 days of gestation ***(McClurkin, et al. 1984)***. The PI calves shed the virus in all secretions especially in the nasal secretions throughout their lives ***(Fulton, et al. 2002)***.

Although many researches was done in BVD in Egypt ***(Youssef, 2006 & Aly. Et al. 2003)***, it still one of the greatest problems that threaten livestock and causes heavy losses among susceptible animals.

The purpose of this study was directed to trails for isolation of BVD virus from the nasal swabs collected from the clinically affected animals and also from lung tissues collected after PM finding from slaughtered animals with respiratory signs and identification of the isolated virus by direct florescent antibody technique. Moreover, detection of antibodies against BVD virus in the collected serum samples from affected animals by using of ELISA test.

MATERIAL AND METHODS

Material:

1-Animals.

A total of 158 claves (87 from Sharkia Governorate , and 71 Ismailia Governorate) were tested in this research at the period from March to December 2008. These calves are aged from 6-18 menths. These calves are suffering from respiratory signs in the from of nasal discharge, coughing, sneezing, and the body temperature of these animals are ranged from 39.5- 41^{o}C.

2- Samples:

a- Nasal swabs:

158 nasal swabs were collected . Sterile swab was inserted in nasal opening as far as possible to avoid the contamination with mucous discharge. Each swab was immersed in sterilized tube containing phosphate buffer saline (PH 7.2) , the collected swabs were used for isolation and identification of the causative virus.

b- lung tissues:

About 139 lung tissue samples were collected from slaughtered animals (88 from sharkia Governorate abattoirs and 51 from Ismailia governor orate abattoirs). The collected lung tissue samples were showing some pathological lesions (as swilling and congestion). After slaughtering, the affected lung samples were collected in sterile plastic bags and transported as early as possible to the laboratory under cooling condition. The lung tissue was homogenized in 2ml of phosphate buffer saline and centrifuged at 3000 rpm/15 minutes to obtain the supernatant. The supernatant was aspirated to small cryovials, labeled and kept at – 70^{o}C until used.

C- Serum samples:

A total of 158 serum samples were collected from the same tested animals, blood samples were collected from jugular vein using a sterile needle for each animal in a sterile labeled venoject tubes. All tubes were left for clotting then

transported as early as possible on an ice packed thermos to the laboratory where it centrifuged at 2000 rpm for 10 minutes to obtain clear serum samples. The sera were separated in a sterile capped vials and inactivated at – 56C for 30 minutes to remove non specific inhebitors and preserved at – 70^{o}C tell used.

3- Phosphate buffer saline:

This solution was prepared ***(Pollard, and Walker. 1997)*** and autoclaved at 15 lb pressure for 15 minutes and used in preparation of collected nasal swabs and lung tissue samples.

4-Cell culture:

A continuous cell line of Madian Darby Bovine kidney (MDBK) cells were Supplied by Rinderpest like Disease Department, Veterinary Serum and Vaccine Research Institute, Abassia – Cairo. The cells were grown and propagated using modified Egle's minimum essential medium (EMEM). The MDBK cells were used for virus isolation and identification.

5- Reference BVD virus and BVD antiserum conjugated with fluorescin isothiocyanate (FITC):

It were originally obtained from Oklahoma Animal Disease Diagnostic Laboratory (OADDL), USA, and maintained in Virology Department, Animal Health Research Institute, Dokki – Giza.

6- ELISA Kits:

ELISA Kits for bovine viral diarrhea (BVD) virus antibody detection were supplied by IDEXX Laboratories Inc. One IDEXX Drive , Westbrook, Maine 04092, USA, and was used for detection of antibodies against BVDV in the collected serum samples .

Methods:

1- Trails For virus isolation:

Trails for virus isolation was carried according to the described protocol ***(Duffell, and Harknen. 1995)***. Isolation attempts were done on 158 nasal swabs and 139 lung

tissue samples in trails for isolation of BVD virus on MDBK cells . MDBK cells were distributed in plastic 96 wells tissue culture plate for 70% confluence, the growth media was discarded and 50 µl from each sample were inoculated into triplicate wells. For each plate, cell control and virus control were included. The plates were incubated for one hour for adsorption, the virus inoculum and the plates were washed using 200µl 0f EMEM added to each well, then incubated at 37°C for 2-7 days with daily examination for recording the development of the cytopathic changes. After 2-7 days the virus harvested and used for subsequent passages. After the 3rd passage, the cells were harvested and kept at – 70°C for virus identification.

2- Identification of the isolated virus:

The identification of the isolated virus was carried out by using direct fluorescent antibody test ***(Plamer, et al. 1982)***. Two ml of MDBK cells were seeded and grown in each tube with growth medium till the monolayer confluent sheet was formed. Growth medium from each isolate was used as inoculum to infect the MDBK cells. The tubes were incubated at 37°C with 5% CO2 for 24 hours. Using maintenance medium, the lighten tubes were filled inverted on its concave surface and the cover slides was picked up by clean sterile forcipes and washed by PBS then dried for 10 minutes. Fixation was done by immersing the cover slips into Petri dish containing 100% acetone for 10 minutes then dried at room temperature and fix on glass slides. The cover slips were placed into humidified chamber and stained with BVD antiserum conjugated with FITC. After 60 minutes of incubation at 37°C, the cover slips were rensed and washed with PBS 3 times each for 5 minutes at room temperature. The slides were then drained, dried and mounted with 50% buffered glycerol and examined under fluorescent microscope. Uninfected control cells and reference positive virus control were included in each assay .

3- Detection of antibodies against BVD virus:

The detection of antibodies against BVD virus in the collected serum samples was done by using ELISA test according to the method descried by IDEXX Laboratories Inc. One IDEXX Drive , Westbrook, Maine 04092, USA.

RESULTS

1-Virus isolation:

The virus isolation was carried out on 158 collected nasal swabs from affected calves and 139 lung tissues collected from slaughtered animals. The results revealed that 51 (32.3%) nasal swabs and 45 (32.4%) lung tissues giving positive results (Table 1).

Virus isolation was applied by injection of prepared MDBK tissue culture cell lines, and with daily observation of the injected tissue culture for 2-7 days. The positive samples were showed characteristic cytopathic effect (CPE), the CPE characterized by enlarged, rounded, retractile cells in grape – like clusters with formation of vacuoles. (Fig 1)

2- Identification of BVD virus:

Identification of the isolated BVD virus was carried out by direct fluorescent antibody test on 158 collected nasal swabs and 139 lung tissues. The results revealed that 66 (41.8%) nasal swabs and 55 (39.6%) lung tissues giving positive results. (Table 2).

The infected cells showed bright greenish yellow fluorescence after 24 hours post inoculation (Fig 2)

3- Detection of antibodies against BVD virus:

The detection of antibodies against BVD virus in 158 collected serum samples from affected animals by using ELISA test. The results revealed that 76 (48.1%) serum samples were giving positive results. (Table 3)

Table (1): Results of isolation of BVD virus from the collected nasal swabs and lung tissues from the affected animals.

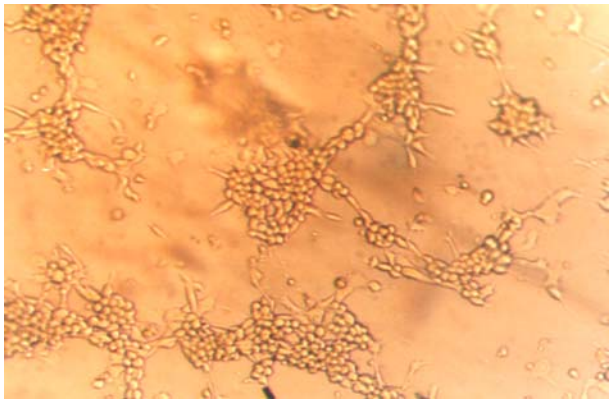
locality		Nasal swabs			Lung tissues		
		No of samples	+ ve samples	%	No of samples	+ ve samples	%
Sharkia	El Slaihia El Gededa	31	8	25.8	19	7	36.8
	Abou Kebier	17	6	25.3	23	5	21.7
	Abou Hammad	14	6	42.9	16	7	43.8
	Belbies	25	7	28	30	9	30
Ismailia	El Tall El Kebier	24	10	41.7	18	4	22.2
	El Kasasine	30	7	23.3	22	11	50
	Abou Sowier	17	7	41.2	11	2	18
Total		158	51	32.3	139	45	32.4

Table (2): Results of identification of the isolated BVD by direct fluorescent antibody test.

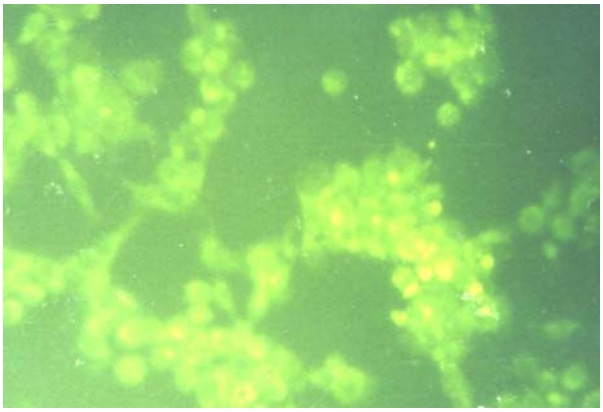
locality		Nasal swabs			Lung tissues		
		No of samples	+ ve samples	%	No of samples	+ ve samples	%
Sharkia	El Slaihia El Gededa	31	11	35.5	19	8	42.1
	Abou Kebier	17	8	47.1	23	8	34.8
	Abou Hammad	14	6	42.9	16	7	43.8
	Belbies	25	10	40	30	12	40
Ismailia	El Tall El Kebier	24	12	50	18	5	27.8
	El Kasasine	30	9	30	22	12	54.5
	Abou Sowier	17	10	58.8	11	3	27.2
Total		158	66	41.8	139	55	39.6

Table (3) Results of ELISA test for detection of antibodies against BVDV in the collected serum samples.

locality		Serum samples		
		No of samples	**+ ve samples**	**%**
Sharkia	El Slaihia El Gededa	31	13	41.9
	Abou Kebier	17	10	58.8
	Abou Hammad	14	7	50
	Belbies	25	11	44
Ismailia	El Tall El Kebier	24	12	50
	El Kasasine	30	12	40
	Abou Sowier	17	11	64.7
Total		158	76	48.1



(Fig 1) infected MDBK cells with BVD virus showing grape-like clusters.



(Fig 2) positive FA test for the presence of BVD virus showing green yellowish colour**.**

DISCUSSION

Bovine viral diarrhea virus (BVDV) is an important pathogen of dairy and beef cattle populations and is associated with many clinical forms and the role of BVDV in bovine respiratory disease has been subjected ***(Grooms, 1988)***. The virus is capable of primary infection of bovine lungs, also BVDV may act in combination with other agents such as *Pasteurella haemolytica*, bovine herpesvirus 1 (BHV1), and bovine respiratory syncytial virus (BRSV) ***(Potgeiter, et al. 1984)***.

The present study include trails for isolation and identification of bovine viral diarrhea virus from 158 nasal swabs collected from affected calves and 139 lung samples collected from slaughtered animals, in addition to detection of antibodies against bovine viral diarrhea virus in the collected serum samples from 158 calves suffering from respiratory signs by using ELISA test.

The results of virus isolation on MDBK cells revealed that 51 (32.3%) out of 158 nasal swabs and 45 (32.4 %) out of 139 collected pneumonic lungs are positive for the presence of cytopathic strain of bovine viral diarrhea virus after 3 passages. the isolated virus was identified as bovine viral diarrhea virus by using direct fluorescent antibody test (Tables1& 2)

The parcntages of isolated BVD virus in the present study was in agreement with isolation of BVD virus on other studies, on one study the BVD virus was isolated form 25 (20 %) out of 105 nasal swabs collected from affected calves with reparatory signs ***(17)***, on the other study, BVDV was isolated from 45 (22.3 %) out of 202 calves exposed to respiratory infection ***(Potgeiter, et al. 1984)***.

Higher percentages were recorded on other following studies that revealed that BVDV was isolated from 13 (68.4%) out of 19 cases , 22 (62.9%) out of 35 cases and from 10 (62.5%) out of 16 cased of pneumonic calves ***(Fulton, et al. 2002, Fulton, et al. 2005 & McGowan, et al. 1993)***.

Failure of isolation of BVDV from the collected nasal swabs and lung samples was recorded in another study ***(Walz, et al. 2001)***.

Results of identification of the isolated BVD virus by using direct fluorescent antibody test revealed that 66 (41.8 %) out of 158 collected nasal swabs and 55 (39.6 %) out of 139 lung tissues are positive for the presence of bovine viral diarrhea virus (Table 2)

The percentages of identified BVD virus in the present study were in agreement with identification of BVD virus by FA test in other study in which the virus was identified in 98 (45.8%) out of 214 isolated viruses from nasal swabs and lung tissues collected from calves suffering from respiratory symptoms ***(Antonis, et al. 2004)*** .

Higher percentages were recorded with an other studies who identified isolated BVD virus by FA test in 88 (86.3%) out of 102 isolated viruses and in 238 (75 %) out of 316 isolated viruses from affected calves respectively ***(Graham, et al. 1998 & El- Kholy, et al. 1998)***.

Lower percentage was recorded in an other study who identified BVD virus in 15 (4.8%) out of 312 samples collected from calves and tested by FA test ***(Vickers, and Minocha. 1990)***.

It is noticed that the results which recorded by FA test is more than the results which recorded in virus isolation. This difference between the recorded results attributed to fluorescent antibody test is more rapid and sensitive over virus isolation in the diagnosis of bovine viral diarrhea virus ***(El- Kholy, et al. 1998)***.

It is noticed that the results of identification of the isolated virus by fluorescent antibody test were higher that that results of virus isolation on tissue culture. This difference was attributed to BVD virus was found in two biotypes (cytopathic and noncytopathic) strains ***(Pellerin, et al. 1994)***.

Results of detection of antibodies against BVD virus in the collected serum samples by ELTSA test revealed that 76 (48.1%) out of 158 collected samples from affected calves giving positive results (Table 3).

The obtained result in the present study was in agreement with other findings who detected antibody against BVD virus in 102 (47.7%) out of 214 serum samples and in

39 (42 %) out of 93 serum samples collected from affected calves respectively and tested by ELISA test ***(Walz, et al. 2001 & Kabongo, and Van Vuuren. 2004)***.

Higher percentages were recorded in an other studies in which antibodies against BVD virus were detected by ELISA test in 143 (59.6%) out of 240 tested samples, 97 (68.79%) out of 141 tested sera samples and in 374 (79.2%) out of 472 tested sera samples respectively ***(Muvavaririva, et al. 1995, Youssef, 2006 & Talebkhan Garoussi, et al. 2008)***.

Negative result was recorded in other study who fail to detect antibodies against BVD in 130 collected serum samples and tested by ELISA test ***(Kampa, et al. 2007)***.

I**n conclusion**, bovine viral diarrhea represent a major problem on dairy and beef calves because it play a role in secondary bacterial invasion resulting in lobar pneumonia in which leading to a great economic losses due to death and retardated growth of the affected calves. So vaccination against this virus is necessary to control and eradicate this disease.

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الملخص العربي

الإصابات التنفسية بمرض الإسهال الفيروس المعدي للإبقار في العجول في محافظتي الشرقية والإسماعيليه

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***قسم الفيرولوجيا – معهد بحوث صحة الحيوان- الدقي**

أجريت هذه الدراسة لبيان تأثير الطور التنفسي لمرض الإسهال الفيروسي المعدي للأبقار على العجول بمحافظتي الشرقية الإسماعيلية. ١٥٨ مسحات أنفية ، ١٣٩ من أنسجة رئات مصابة و ١٥٨ عينه من مصل الدم تم تجميعها من الحيوانات المريضة والمذبوحة بمحافظتي الشرقية والإسماعيلية وتم استخدامها في هذه الدراسة وقد تم عزل فيروس الإسهال الفيروسي المعدي للأبقار من عدد ٥١ (٣٢,٣ %) من مسحات الأنف و ٤٥ (٣٢,٤ %) من أنسجة الرئات المصابة ، و تم التعرف على وجود الفيروس باستخدام اختبار الفلوروسين للأجسام المضادة في ٦٦ (٤١,٨%) من المسحات الأنفية و ٥٥ (٣٩,٦ %) من أنسجة الرئات المصابة ، بينما تم الكشف عند وجود أجسام مناعية في ٧٦ (٤٨,١ %) من أمصال الحيوانات المصابة باستخدام اختبار الإليزا.