

**SURVEY ON THE DISTRIBUTION OF CYTOPATHIC AND  
NON CYTOPATHIC STRAINS OF BOVINE VIRAL  
DIARRHEA VIRUS IN CATTLE FARM**

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### **1-Abstract**

Bovine viral diarrhea virus disease is a common disease in cattle population world wide causing severe economic losses. The virus exists in two biotypes, cytopathic (CP) and noncytopathic (NCP). In this study, the prevalence of both biotypes in cattle farms in Sharkia governorate was investigated. The study included virus isolation, serological studies for detection of viral antigen and antibodies by IP and IF. The result showed that the virus was isolated from 8 animals out of 192 animals with percent of 4.16%. Detection of virus antigen by IP showed that 26 animals were positive out of 192 animals with percent of 13.54%. Detection of virus antibodies, by IP and IF, showed that 27 animals out of 192 animals were positive, with percent of 14.06%. The study showed that both BVDV biotypes, CP and NCP, excite in cattle and buffaloes farms in Sharkia governorate, however NCP were more prevailed.

### **2-Introduction**

Bovine viral diarrhea virus (BVDV) is an important cattle pathogen of a worldwide distribution. It can cause severe, if not fatal, infections and create devastating economic losses (**Brownlie 1991**). Although BVDV has two biotypes that occur naturally, namely the non-cytopathic (NCP) and the cytopathic (CP), only the NCP BVDV biotype crosses the placenta to establish an infection in the fetus (**Brownlie et al., 1989**). Investigation the differences in cytopathogenesis between both biotypes were studied by many works (**Elsheikh, 2002**).

Bovine Viral diarrhea virus is capable of producing severe illness and death in cattle that acquire the virus before birth (mucosal disease) but the most frequent form of infection is sub clinical or mild disease in cattle that are first exposed to the virus after birth (**Torgerson et al.,1989**). Evidence for immunosuppression by the virus comes from studies on animals affected with mucosal disease and on animals mildly affected by BVDV after experimental inoculation (**Edwards et al., 1987**).

In Egypt, several studies were done on BVDV. The studies included viral isolation (**Baz, 1990**), the virus growth curve (**El -Sanousi et al., 1990**) and the immune response against the virus (**Shalaby, 1992**) and (**Khafagi et al., 1998**). Furthermore, (**Hassan, 1993**) detected the virus in bovine turbinate cells by PCR. The aims of this work were directed to isolation and differentiation of field strains of BVDV from samples collected from symptomatic affected cattle and buffaloes in Sharkia governorate and detection of BVDV antigen and antibodies by indirect immunoperoxidase (IP) and immunofluorescent tests.

### **3-Material and methods**

**Samples:** Different samples were collected From 192 symptomatic diseased animals suffering from fever, respiratory disorder and diarrhea distributed in many places in Sharkia Governorate. The collected samples were blood samples with anticoagulant, rectal swabs, nasal swabs and sera from each animal.

**Reference BVDV and Polyclonal anti- BVDV antibodies:** The virus and antibodies used in this study are originally obtained from Animal Diagnostic Research Lab., SDSU, USA. The viral strains are NADL (CP), titer  $1 \times 10^{5.6}$  and NY-1(NCP), titer  $1 \times 10^6$ .

**Isolation of BVDV on MDBK:** Propagation of field samples on Madin-Darby bovine kidney cells (MDBK) was carried out according to (**Jewett et al., 1990**). A continuous cell line of MDBK, BVDV free was used for trials of isolation and differentiation of local strains of BVDV from collected samples. The cells were provided by virology department, faculty of veterinary medicine, Zagazig university. It was grown with modified Eagle's minimum essential medium (EMEM) with Earl's balanced salt solution and L. glutamine without sodium bicarbonate. The medium was supplemented with 2% new born calf serum and antibiotic mixture and antimycotic. The collected nasal swabs, buffy coat and rectal swabs from 192 animals (121 from cattle and 71 from buffaloes) were inoculated to MDBK and propagated for three blind passages. The inoculated plates were examined daily for recording the cytopathic changes in the cells.

**Identification of isolates by indirect immunoperoxidase:** The technique was applied on BVDV isolates according to **Dubovi 1990**. After the third passage, the medium was decanted and cells were washed with PB and the plates were dried. The cells were fixed with 20% acetone for 1 hour. The plates were dried overnight at room temperature. The antiserum of BVDV was diluted in PBS 0.05% tween and added by 50 ul /well, then was incubated 1 hr at 37°C. After incubation, the antiserum was decanted and the plates were washed three times with PBS 0.05% tween (200 ul/ well). The conjugate (rabbit anti-bovine peroxidase) diluted to 1/300 was added by 50 ul/ well and incubated 1 hr at 37°C.

The plates were washed three times with PBS tween 0.05% and IP substrate (3-amino-9-ethyl-carbazole and 0.2% urea hydrogen peroxide in 95% ethyl alcohol) was added by 50 ul/ well. The plates were incubated 10 minutes at room temperature, washed by double distilled water then dried at room temperature and the result was read under the inverted microscope.

#### **Identification of BVD antibodies in the collected serum by indirect IP:**

The test was carried out according to **Ward and Kaeberle 1984**. MDBK cells were prepared as 80% confluent in tissue culture plate, then inoculated by 10 ul BVDV NY-1 strain in each well and incubated for 24 hours. Fixation was done by 100 ul/ well of PBS acetone 20%, incubated 1 hour at room temperature. The collected serum was investigated by indirect IP on this plates as described above.

**Detection of BVDV antibodies in collected sera by indirect fluorescent antibody technique:** The technique was done according to **Coria and Mc Clurkin (1981)**. The MDBK cells were trypsinized and incubated at 37°C for 10 minutes to obtain separated individual cells. The growth media was added to the cell suspension and cells were distributed into sterile lieghton tubes containing sterile coverslips by 2 ml of MDBK cells suspension per tube. The tubes were closed and incubated at 37°C for 24 hr. After formation of 60 – 80 % cells confluence on the tubes and coverslips, the growth media was discarded and the cells were inoculated with 30ul BVDV NY-1 strain in each lieghton tube. The inoculated tubes were incubated at 37°C with maintenance medium for 24 hours. By using wormed maintenance medium, the lieghton 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 acetone 100 % for 10 minutes then dried at room temp. The collected serum samples were added to the cover slips, and incubated at 37 °C for 30 minutes in humidified atmosphere. After incubation, the cover slips were washed three times with PBS for 5 minutes. The rabbit anti-bovine conjugate with FITC (Sigma) was diluted at 1:200 with PBS and added to the cover slips and incubated at 37 °C for 60 minutes in humidified atmosphere. After washing as above, slips were examined under fluorescence microscope after mounted in a pre-warmed mounting buffer.

## 4-Results

### Results of BVDV isolation from collected samples on MDBK cell line:

The virus induces cells rounding, aggregation in scattered area of the monolayer followed by cellular darkness and clusters formation. The CPE was clear between the 3<sup>rd</sup> and 5<sup>th</sup> days post inoculation, especially in 3<sup>rd</sup> passages. Different samples (rectal swab, nasal swab, and buffy coat) of the same animal showed positive results (clear CPE). The percentage of positive results after 3<sup>rd</sup> passage were, out of 121 examined animals 6 animals showed clear CPE with percent of 4.96% (Table: 1). In buffaloes, out of 71 examined animals, 2 animals showed clear CPE with percent of 2.81% (Table 2). (Fig 1 and 2) represent the negative control of MDBK cells and positive CPE on MDBK cells.

### Results of IP test for detection of BVDV antigen:

Out of 192 tested animals, 26 animals (17 from cattle and 9 from buffaloes) showed positive results with percent of 13.54%. The positive animals included 8 animals that showed CPE with percent of 4.16% and 18 animals that did not show CPE with percent of 9.37%. Table (3) summarized the result of IP as a screening test for detection of BVDV antigen in the samples collected from animals.

### Results of IP test for detection of BVDV antibodies:

Out of 192 tested serum samples, 27 samples (18 from cattle and 9 from buffaloes) showed positive results (intracytoplasmic brown precipitate) with percent of 14.06%. The positive samples included 8 samples that showed CPE with percent of 4.16% and 19 samples that did not show CPE with percent of 9.89%. Table (4) summarized results of IP screening test for detection of BVDV antibodies in the samples collected from cattle and buffaloes.

### The result of IFA technique for detection of BVDV antibodies:

The results were similar to IP test, in which out of 192 tested serum samples, 27 samples (18 from cattle and 9 from buffaloes) showed positive results with percent of 14.06%. The positive samples include 8 samples that showed CPE with percent of 4.16% and 19 samples that did not show CPE with percent of 9.89%. Fig (3) shows green yellowish color which represents the positive result of IFA. The results of viral isolation, IP and IF showed that the prevalence of CP strains in Sharkia cattle were 4.16% and the prevalence of NCP strains was 9.37% (Table 5).

## 5- Discussion

BVDV is a common disease in cattle populations worldwide and provoking a considerable economic losses (**Duffell and Harkness 1985**). The virus causes a variety of clinical symptoms, including pyrexia, anorexia, diarrhoea and respiratory distress

(**Malmquist, 1968**). The aims of the present study were to isolate and differentiation BVDV circulating in cattle and buffalo population using tissue culture and serological techniques.

MDBK cells free of BVDV latent infection were used in the first approach for the virus isolation and propagation of field samples for three successive blind passages. Out of 121 cattle samples (nasal swabs, rectal swabs and buffy coats), 6 cattle samples revealed a clear CPE on the cells with percent of 4.96% and out of 71 buffaloes samples, 2 buffaloes samples revealed a clear CPE on cells with percent of 2.81%. Only one calf sample revealed clear CPE from 78 samples collected from cattle and buffaloes calves. These results agree with (**Tsuboi and Imada 1998**) who isolated the virus with 7% from persist infected animals.

The second approach in the current study was identification of BVDV antigen in the collected samples by using IP. The results showed that out of 121 cattle samples, 17 cattle samples were positive with percent of 14.05%. Also, out of 71 buffaloes samples 9 buffaloes samples were positive with percent of 12.67%. Identification of BVDV antibodies in the collected serum samples by IP showed that out of 121 collected serum samples from cattle, 18 samples were positive by IP, with percent of 14.87% and out of 71 collected sera sample from buffaloes 9 samples were positive with percent of 12.67%. These results agree with (**Bechmann, 1997**) who detected BVDV antigen by IP with 14.01% and BVDV antibodies with 15%. The results showed only one serum sample, collected from cattle calf, gave positive result by IP for antibodies detection while antigen detection was negative. Our explanation, it may be due to presence of maternal antibodies and not due to infection that was accepted by (**Endsley et al., 2002**) who showed that antibodies in newly born calves without virus isolate is maternal origin.

The third approach in the current study was identification of BVDV antibodies in collected serum samples by indirect fluorescent antibody technique. The results were similar to that obtained by IP. Out of 121 collected serum sample from cattle, 18 samples were positive with percent of 14.87%. Also, out of 71 sera sample collected from buffaloes 9 samples were positive with percent of 12.67%. The result accepted by (**Frey et al., 1991**) who recorded positive percent 16.2% in samples detected by IF for BVDV antibodies identification. Analysis of the results of all tests showed that the prevalence of CP strains in Sharkia cattle was 4.16% and the prevalence of NCP strains was 9.37%. These results agree with (**Baker and Houe 1995**) who recorded that the prevalence of NCP strains in clinical infected animals is more than the prevalence of CP strains.

**Fig 1: MDBK cell represent the negative control**

Confluent monolayer cells (X 100).

**Fig 2: MDBK cells showed CPE.****Fig (3): Positive result of IFA test.**

MDBK cell infected with BVDV showed green yellowish color by fluorescence microscope (X 400).

**Table (1): The cattle samples showed a clear CPE on MDBK cells after the third passage:**

Cattle		Nasal swabs			Rectal swabs			Buffy coat		
Breed	Age	No.	+ve	%	No.	+ve	%	No.	+ve	%
Native	Calves<6m	25	0	0	25	0	0	25	0	0
	Calves>6m	34	2	5.88 *(1.65)	34	2	5.8 *(1.65)	34	2	5.88 *(1.65)
Holisatin	Calves< 6m	32	1	3.12 *(0.83)	32	1	3.12 *(0.83)	32	1	3.12 *(0.83)
	Calves>6m	30	3	10 *(2.48)	30	3	10 *(2.48)	30	3	10 *(2.48)
<b>Total</b>		121	6	4.96	121	6	4.96	4.96	6	4.96

**M** : month. **NO.** : number of the animals. **+ve** : positive result.  
**%** : percent. \* Percent of positive out of total (121)

**Table (2): The buffaloes samples showed a clear CPE on MDBK cells after the third passage:**

Buffaloes	Nasal swabs			Rectal swabs			Buffy coat		
Buffaloes	No.	+ve	%	No.	+ve	%	No.	+ve	%
calves< 6m	21	0	0	21	0	0	21	0	0
calves>6m	50	2	4	50	2	4	50	2	4
<b>Total</b>	71	2	2.81	71	2	2.81	71	2	2.81

**M** : month. **No** : number of buffaloes.  
**+ve** : positive result. **%** : percent.

**Table (3): The result of IP test for detection of BVDV antigen in the samples collected from cattle and buffaloes:**

Animal		Nasal swabs			Rectal swabs			Buffy coat		
Breed	Age	No.	+ve	%	No.	+ve	%	No.	+ve	%
Native cattle	Calves < 6m	25	3	12 *(1.56)	25	3	12 *(1.56)	25	3	12 *(1.56)
	Calves > 6m	34	4	11.76 *(2.08)	34	4	11.76 *(2.08)	34	4	11.76 *(2.08)
Holistin cattle	Calves < 6m	32	5	15.62 *(2.6)	32	5	15.62 *(2.6)	32	5	15.62 *(2.6)
	Calves > 6m	30	5	16.66 *(2.08)	30	5	16.66 *(2.08)	30	5	16.66 *(2.08)
Buffaloe	Calves < 6m	21	4	19.04 *(2.08)	21	4	19.04 *(2.08)	21	4	19.04 *(2.08)
	Calves > 6m	50	5	10 *(2.6)	50	5	10 *(2.6)	50	5	10 *(2.6)
<b>Total</b>		192	26	13.54	192	26	13.54	192	26	13.54

m. : month. No. : number of animals. +v : Positive % : Percent \* : Percent out of total (192)

**Table (4) Results of IP screening test for detection of BVDV antibodies in cattle and buffaloes:**

Animal species	Age	NO	+ve	%
Native cattle	Calves < 6m	25	3	12 *(1.56)
	Calves > 6m	34	4	11.76 *(2.08)
Holistin cattle	Calves < 6m	32	6	18.75 *(3.13)
	Calves > 6m	30	5	16.66 *(2.60)
Buffaloes	Calves < 6m	21	4	19.05 *(2.08)
	Calves > 6m	50	5	10 *(2.60)
<b>Total</b>		192	27	14.06

M : month. No : Number of animals +ve : Positive % : Percent \* : Percent out of total

**Table 5: Cytopathic and non cythaopathic cases in the animals under investigation**

Animal			cytopathic		noncythaopathi c		Total	Total%
Breed	Age	No.	+ve	%	+ve	%		
Native cattle	Calves < 6m	25	0	0	3	12	3	1.56
	Calves >6m	34	2	5.88	2	5.88	4	2.08
Holistin cattle	Calves < 6m	32	1	3.12	4	12.5	5	2.60
	Calves >6m	30	3	10	2	6.66	5	2.60
Buffaloes	Calves < 6m	21	0	0	4	19.04	4	2.08
	Calves >6m	50	2	4	3	6	5	2.60
<b>Total</b>		192	8	4.16	18	9.37	26	13.53

M : month. No : Number of animals +ve : Positive % : Percent

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## الملخص العربي

يعتبر مرض الإسهال البقري الفيروسي من الأمراض الشائعة في مزارع الأبقار على مستوى العالم ويسبب هذا المرض خسائر اقتصادية فادحة . ويتم تصنيف الفيروس المسبب للمرض من حيث تأثيره على الخلايا المصابة إلي نوعين ، نوع قاتل للخلايا المصابة ونوع غير قاتل للخلايا المصابة . وفي الدراسة المقدمة كان الهدف استكشاف نوعي الفيروس في مزارع الأبقار في محافظة الشرقية فتم تناول الموضوع في جانبين هما عزل الفيروس على خلايا نسيجية والاختبارات السيرولوجية لاكتشاف الفيروس والأجسام المناعية المضادة للفيروس من خلال الاختيار المناعي الأنزيمي الغير مباشر والاختيار المناعي الفلورنستي الغير مباشر . وكانت النتيجة تم عزل الفيروس من ٨ حالات من إجمالي ١٩٢ حالة بنسبة مئوية ٤,١٦ % . وفي اختيار انتيجينات الفيروس بواسطة الاختيار المناعي الأنزيمي الغير مباشر تم اكتشاف تواجد الفيروس في ٢٦ حالة من إجمالي ١٩٢ حالة بنسبة مئوية ١٣,٥٤ % بينما في اختيار الأجسام المناعية عن طريق الاختيار المناعي الأنزيمي الغير مباشر تم اكتشاف ٢٧ حالة إيجابية من إجمالي ١٩٢ عينة بنسبة مئوية ١٤,٠٦ % أيضا في اختيار الأجسام المناعية عن طريق الاختيار المناعي الفلورنستي الغير مباشر تم اكتشاف ٢٧ حالة إيجابية من إجمالي ١٩٢ حالة بنسبة مئوية ١٤,٠٦ % . أظهرت الدراسة المقدمة تواجد مرض الإسهال البقري الفيروسي بنوعيه العترة القاتلة للخلايا المصابة و العترة غير قاتلة للخلايا المصابة في مزارع الأبقار في محافظة الشرقية وان كان النوع الغير قاتل للخلايا أكثر انتشارا .