

SOME STUDIES ON BOVINE VIRAL DIARRHEA IN ASSIUT GOVERNORATE, EGYPT

ZAINAB MOHAMMED AHMED YOUSSEF;
MOHAMMED MAHMOUD MOHAMMED ABDEL-BAKY;
GHADA BADR MOHAMMED MURID;
FATMA ESSAM EL-DIN ABDEL-HAMID MOHAMMED MAHRAN¹ AND
FATMA SABER MAHMOUD

Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Postal code: 71526, Egypt

Received: 23 July 2023; **Accepted:** 23 August 2023

ABSTRACT

Bovine viral diarrhoea (BVD) is a common viral disease that can affect both domesticated and wild animals. Despite their economic losses, there isn't much information available on BVD in Assiut Governorate. Therefore, the present study's objectives were to determine the clinical findings, risk factors associations with the infection rate, and molecular diagnosis of BVD virus (BVDV). The current study was conducted on 50 cattle that belonged to different villages in Assiut Governorate, Egypt. The clinical examination findings revealed fever, oral lesions, diarrhoea, respiratory symptoms, and corneal opacity. Serum samples were collected for laboratory analysis. Reverse transcriptase polymerase chain reaction (RT-PCR) assay had been employed for BVDV diagnosis. BVDV RNA was found in the serum of seven cattle. There was no significant difference ($P<0.05$) between the percentages of BVD infection and the sex, age, and breed (native and mixed breed) of molecularly tested cattle. According to the climatologic circumstances of Assiut governorate, there was a discernible variation ($P<0.05$) between the BVD infection rate and the cold and hot months. It is advisable to emphasize how crucial it is to implement efficient preventative and control measures throughout Egypt in order to reduce the prevalence of BVDV.

Keywords: BVD, Risk factors, 5'UTR, RT-PCR

INTRODUCTION

BVD is a widely spread, ubiquitous viral disease that affects domestic animals like cattle, goats, sheep, and pigs, as well as numerous wild and captive animals (Gong *et al.*, 2013; Sharawi *et al.*, 2016). BVD is one of the most serious cattle diseases in the

world and has become a global pandemic due to its widespread nature, transmission, and lack of treatment, in addition to causing significant economic losses to the livestock industry (Sharawi *et al.*, 2016 and Paixão *et al.*, 2018). BVDV is a virus of the genus *Pestivirus* and belongs to the family *Flaviviridae* (Weinstock *et al.*, 2001). Pestiviruses are enveloped viruses with a single-stranded, 11.3–13.1 kb positive-sense RNA (Porto *et al.*, 2021). Pestiviruses have been given new names, and the International Committee on Taxonomy of Viruses (ICTV)

Corresponding author: Zainab Mohammed Ahmed
E-mail address: zeinabmohammed613@aun.edu.eg
Present address: Infectious Diseases, Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University,

has classified them into 11 species (Afify *et al.*, 2022). Accordingly, they are divided into the following categories: BVDV-1 (Pestivirus A), BVDV-2 (Pestivirus B), border disease (Pestivirus D), classical swine fever (Pestivirus C), and HoBi-like (Pestivirus H) (Zambotti *et al.*, 2020 and Afify *et al.*, 2022). The BVDV genome possesses a long open reading frame (ORF) bordered at the 5' and 3' termini with a highly conserved untranslated region (UTR) for translation and replication regulations, respectively (Abd El-Hafeiz *et al.*, 2022). In the BVDV genome, the ORF encodes polyproteins that are split into viral and cellular proteases both during and after translation into four structural (C, Erns, E1, and E2) and eight nonstructural (Npro, p7, NS2-3, NS4A, NS4B, and NS5A) proteins (Abd El-Hafeiz *et al.*, 2022 and Afify *et al.*, 2022). The BVDV can be classified into cytopathic (Cp) and non-cytopathic (NCp) biotypes based on the characteristics of their in vitro cell culture and genetic variation (Kučer *et al.*, 2022). The pathogenicity and clinical symptoms of BVDV infection vary as well, depending on the strain (Weinstock *et al.*, 2001). Cattle infected with BVDV may have gastrointestinal, and respiratory problems, immunosuppression with secondary infections, thrombocytopenia, and reproductive failure (Porto *et al.*, 2021). In pregnant cows, transplacental infection with BVDV occurs with a high degree of efficiency and can result in fetal death, malformations, abortion, or the birth of a persistently infected (PI) calf in addition to the acute and chronic mucosal disease (Weinstock *et al.*, 2001 and Kučer *et al.*, 2022). The economic repercussions of BVD have led numerous endemic countries, notably Egypt, to start BVDV control or eradication programs (Afify *et al.*, 2022). Detecting PI animals, eliminating them from herds, and limiting the introduction of new PI animals through biosecurity initiatives and/or vaccination are the two main goals of the majority of control programs in many countries (Afify *et al.*, 2022). In Egypt, BVDV was discovered for the first time in a

calf with acute enteritis in 1972 (Hafez, 1972). The majority of BVDV reports from Egypt are mostly based on virus isolation and/or viral antibody identification (Soltan *et al.*, 2015). The detection of BVDV by RT-PCR has been proven to be more rapid and sensitive than virus isolation, with 100% specificity and sensitivity (Atwa *et al.*, 2019). Furthermore, unlike viral isolation, the presence of antibodies in serum samples has no impact on RT-PCR results (Atwa *et al.*, 2019). The identification of risk factors associated with BVDV infection became important as determined indicators were utilized to lead the development of preventive and control measures employed to directly lower the spreading of infection and minimize economic loss (Marques *et al.*, 2016). In Egypt, BVDV is still a concern in the cattle population in several governorates despite control measures like vaccination (El-Bagoury *et al.*, 2014). There is little information about BVD in Assiut Governorate, so the present study aimed to molecularly detect a highly conserved portion of the 5' UTR of the BVDV genome based on RT-PCR, evaluate the clinical manifestations, and study the correlation between some risk factors, such as sex, age, breed of cattle, and seasonal variation, with the infection rate of BVD in Assiut Governorate.

MATERIALS AND METHODS

1. Animals and Ethical approval

Throughout the investigation period, from March 2022 to March 2023, a total of 50 cattle of various sexes, ages, and breeds that came from various villages in Assiut Governorate were admitted to the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University. All the tested cattle were suffering from clinical signs suggesting their infection with BVDV. All cattle used in this study were handled in accordance with ethical considerations. The study was approved by the Research Ethical Committee of the Faculty of Veterinary

Medicine, Assiut University, Assiut, Egypt; the approval number was 06/2023/0085.

2. Clinical examination

The Clinical examination of the investigated diseased cattle was carried out in accordance with (Jackson and Cockcroft, 2002).

3. Sampling

Four milliliters of blood samples were collected from 50 clinically diseased cattle that suffered from fever, oral lesions (ulcers), diarrhea, respiratory symptoms like nasal discharge and cough, and corneal opacity into sterile plain vacutainer tubes without anticoagulant through the jugular vein while the animals were adequately held. Blood samples were allowed to clot and centrifuged at 3000 r/min for 20 minutes, and serum was delicately collected (Afify *et*

al., 2022) and stored in separate tubes at -20°C for subsequent RNA extraction.

4. Molecular diagnosis

4.1. Viral RNA extraction

The viral RNA was extracted from 50 serum samples using the *EasyPure*® Simple Viral DNA/RNA extraction kit (TransGen Biotech, China), according to the instructions of the manufacturer.

4.2. Primers

The specificities of the chosen primers (Metabion International AG, Germany) used in the current study for the 5'UTR of BVDV had been evaluated previously (Weinstock *et al.*, 2001). Sequences of primers and their locations in the viral genome are displayed in Table 1.

Table 1: Nucleotide sequence of used primers, locations in the 5'UTR of BVDV and size of the products produced after RT-PCR

Primer	Nucleotide sequences	Position 5' - 3'	Product size bp
Forward: 103	5'- TAG CCA TGC CCT TAG TAG GAC -3'	103 - 124	290
Reverse: 372	5'- ACT CCA TGT GCC ATG TAC AGC -3'	372 - 392	

4.3. Detection of BVDV 5'UTR by RT-PCR

Possibility of a specialized RT-PCR assay for amplifying the highly conserved region of the 5'UTR of BVDV. The extracted RNA was reverse transcribed to cDNA using the ABT 2X RT mix kit (Applied Biotechnology, Egypt) following the manufacturer's instructions and kept at -20°C until it was used. Primer sets 103 forward and 372 reverse were used to amplify cDNA fragments with a 290 bp length. In the current study, the ABT red master mix (2X) (Applied Biotechnology, Egypt) was used as a source for dNTPs and polymerase enzymes. PCR was carried out in a PCR thermocycler (Cole-Parmer, United States), with the following reagents used: A final volume of 20 µl containing 10 µl ABT

red master mix (2X), 1 µl of each primer 103 and 372 (10 pmol), 7 µl cDNA sample, and 1 µl PCR molecular grade water. In brief, thermal cycling conditions were one initial denaturation at 95°C for 5 minutes (40 cycles of denaturation at 94°C for 1 minute, 62°C for 1 minute for the annealing step, and 72°C for 2 minutes for extension), followed by 72°C for 10 minutes of final extension.

4.4. Analysis and detection of PCR products

For reaction visualization, 5 µl of amplified PCR products and 3 µl of gel loading buffer were loaded. The amplicons were analyzed by gel electrophoresis for 60 minutes at 90 V and 155 mA in a 2% agarose gel stained with ethidium bromide (10 mg/ml) and their

size was estimated with size marker DNA of 100 bp before being observed by a gel UV-transilluminator (Syngene, United Kingdom).

5. Statistical analysis

The statistical package for the social sciences (SPSS) version 16 software was used to enroll and analyze the collected epidemiology data using the Chi-square of independence (2007).

RESULTS

1. Field diagnosis of clinical cases infected with BVD

Cattle with BVD were diagnosed in the field based on clinical examination and the identification of characteristic clinical symptoms. Cattle used in this study had the typical clinical signs of BVD, such as fever, oral lesions, diarrhea, respiratory symptoms, and corneal opacity. The presence of oral lesions, like ulcers, was the most distinctive feature in 6 clinical cases of infected cattle with BVD (Table 2 and Fig.1A). Fever (40-40.2°C) was found in 5 of the tested cattle (Table 2). Diarrhea and respiratory signs like a cough and nasal discharge were detected in 3 and 2 of the investigated cattle (Table 2 and Fig.1B), respectively. One cattle suffered from bilateral corneal opacity (Table 2).

Table 2: Clinical manifestations of examined diseased cattle (No.= 50)

Clinical signs	No. of examined diseased cattle	No. of diseased cattle infected with BVD (%)
Oral lesions only	8	2 (25%)
Fever + oral lesions + Diarrhea	9	2 (22.22%)
Fever + oral lesions + Respiratory signs	14	1 (5.88%)
Fever + oral lesions + Corneal opacity	3	1 (33.33%)
Fever + Diarrhea + Respiratory signs	16	1 (6.25%)
Total	50	7 (14%)

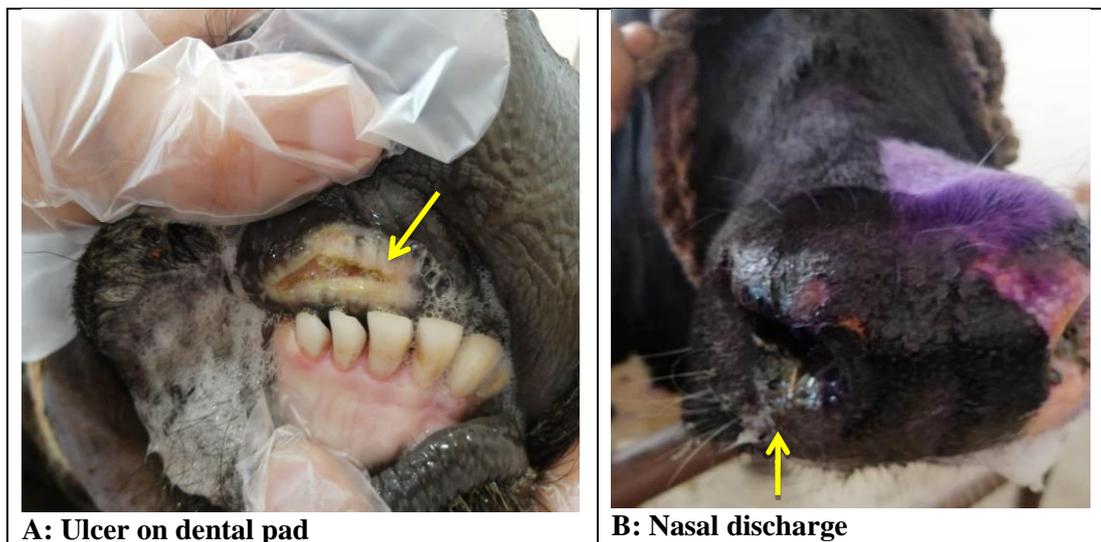


Fig. 1: Clinical findings of BVD-affected cattle

2. Detection of BVDV genome by RT-PCR

cDNA samples were examined by PCR assay to yield the necessary bands at 290 bp

as a result of the 5'UTR of BVDV (Fig. 2). Seven (14%) of the 50 serum samples had molecularly positive results.

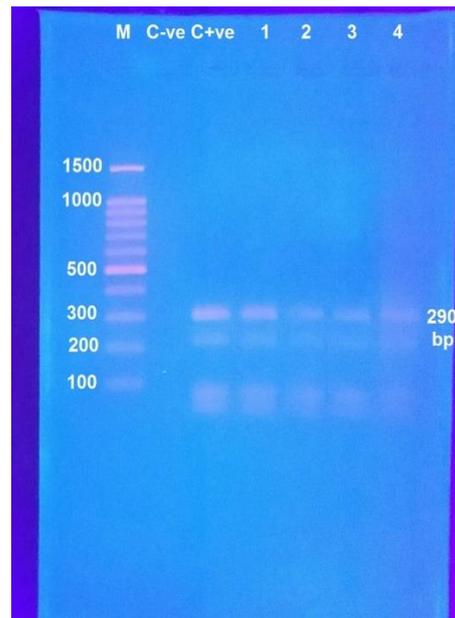


Fig. 2: Agarose gel electrophoresis of RT-PCR after amplification of 5'UTR of BVDV in diseased cattle. Line M: DNA ladder 100 bp, line C-ve: Control negative distilled water, line C+ve: Control positive sample, and lines 1,2, 3 and 4: positive serum samples of infected cattle with amplified product at 290 bp.

3. Relationship between the percentage of infection of BVD and potential risk factors

3.1. Percentage of BVD infection

The current study found that 14% (7/50) of the cattle under investigation were infected with BVD (Table 3).

3.2. Effect of sex

The analytical findings showed that there was no significant variation in BVD infection percentage between the male and female examined cattle, although a greater infection rate in males than females numerically (Table 3).

3.3. Age susceptibility

The prevalence of BVD infection was detected in cattle at the ages of 3 months-1

year, >1-3 years, and >3-5 years, with results showing that, out of 50 animals examined, 20%, 7.41%, and 25% had the infection. Age groups >3-5 years had the mathematically greatest rate of BVD infection (Table 3).

3.4. Breed susceptibility

The percentage of BVD infection in native and mixed breed cattle in the current investigation did not significantly differ, despite the higher infection rate in native breeds being more accurate numerically (Table 3).

3.5. Seasonal variation

The percentage of BVD infection was significantly higher in hot months (26.32%) than in cold months (6.45%), according to our findings (Table 3).

Table 3: Association between infection rate of BVD and potential risk factors according to RT-PCR result

Variable	No. of examined animals	No. of positive	Infection rate %	Chi-square test		
				Pearson chi-square (P-value)	Probability (p< 0.05)	
Sex	Male	30	6	20	2.243	0.134
	Female	20	1	5		
	Total	50	7	14		
Age	3 months – 1 year	15	3	20	2.227	0.328
	>1 - 3 years	27	2	7.41		
	>3 - 5 years	8	2	25		
	Total	50	7	14		
Breed	Native	28	5	17.86	0.786	0.375
	Mixed	22	2	9.09		
	Total	50	7	14		
Season	Cold months#	31	2	6.45	3.861	0.049
	Hot months##	19	5	26.32*		
	Total	50	7	14		

No significant variation at $p < 0.05$ * Significant increase at $p < 0.05$ (0.049).

#Cold months (from October to February)

##Hot months (from March to September)

DISCUSSION

It is well established that keeping animals healthy is essential for enhancing livestock productivity. For any cattle producer, BVD infection is one of several diseases that present a severe challenge, so knowing the pathogenesis of BVDV represents a potent defense against the risk of infection (Abd El-Hafeiz *et al.*, 2022). In the current study, the observed clinical signs associated with suspected clinical cases of BVD were the same as those documented in previous studies reported by Al-Rubaye and Hasso (2012); Soltan *et al.* (2015); Atwa *et al.* (2019) and Kučer *et al.* (2022). There was a previous report (Abd El-Hafeiz *et al.*, 2022) of this disease in Assiut governorate, in addition to other governorates in Egypt has been published. As a result, the present study aimed to record the percentage of BVD infection in the Assiut governorate with RT-PCR confirmation. The 5'UTR was found in 7 (14%) of the 50 serum samples of

the examined cattle that were molecularly analyzed by RT-PCR for the identification of BVD infection. When this 5'UTR was attempted to be amplified in cDNA samples of BVDV, each reaction yielded a unique single band of the expected size, which is consistent with Weinstock *et al.* (2001); Basqueira *et al.* (2017) and Zambotti *et al.* (2020). The confirmation identification was carried out using the RT-PCR method, which is still widely recognized as the best, most sensitive, specific molecular assay for detecting BVDV in clinical samples and allowing for the detection of animals that are transiently infected when the virus load is low (Weinstock *et al.*, 2001; El-Bagoury *et al.*, 2014; Soltan *et al.*, 2015 and Chang *et al.*, 2021). The primers used in this study targeted the 5'UTR region, were highly conserved, and were capable of detecting a variety of bovine *Pestivirus*es, including BVDV-1, BVDV-2, and BVDV-3 (Kosinova *et al.*, 2007 and Chang *et al.*, 2021). Our molecular result (14%) was

almost identical to the findings of the previous study (Atwa *et al.*, 2019), which discovered that 16.67% (6/36) of the investigated cattle were molecularly BVDV positive. Our result was higher than previous reports by Garoussi *et al.* (2019) and Chang *et al.* (2021) who revealed that the molecular positivity rates for BVDV in animals were 1.42% (2/140) and 7.2% (89/1234), respectively. Our finding was lower than the previous publication by Atwa *et al.* (2014) who recorded that the molecular positivity rate for the BVDV in cattle was 21.43% (3/14).

Concerning to the infection rate, the present study found that 14% of the studied cattle in Assiut Governorate, Egypt were infected with BVD. A lower percentage of BVD infection was noted by Emran *et al.* (2014); El-Bagoury *et al.* (2014); Soltan *et al.* (2015) and Mokhtar *et al.* (2021) who found that 6.7%, 8.4, 8.4%, 10.4% and 3.4%, of investigated animals had BVD infection in Fayoum, Alexandria, Qaluobia, Ismailia, and Aswan, Egypt respectively. Whereas, a higher infection rate was found by Atwa *et al.* (2014) who concluded that 23.08% of examined animals showed BVD infection in Damietta, Egypt. These discrepancies could be explained by variations in sample collection periods, sample sizes, sanitary conditions, ambient conditions, and the usage of different diagnostic techniques.

Some of the risk factors including sex, age, breed of cattle and seasonal variation were investigated for their interaction with the BVD infection rate in the current study. Despite the actual means by which these factors biologically interact with BVD infection requires further investigation, the data obtained suggests that not all these factors may play a significant impact on the BVD infection rate. Concerning sex susceptibility, there was no statistically significant variation in the rate of BVD infection between the male and female investigated cattle, despite the higher

infection rate in males than females numerically. Our result concurred with Wilson *et al.* (2016), who reported that there were no significant differences in BVD infection by sex of cattle. This finding contrasted with that of Daves *et al.* (2016), who reported that females had a higher prevalence of BVD than male cattle. Our result could imply that BVD is a non-sex-related disease and that male and female cattle are equally susceptible to BVD infection, however, both sexes of animals can be infected with BVD at the same or different periods of life, depending on a variety of environmental and management conditions. These factors include the degree of virus exposure and stress exposure. Regarding age susceptibility, age groups >3-5 years had the mathematically highest rate of BVD infection. This result corroborated those of Daves *et al.* (2016), who demonstrated a greater prevalence of BVD in adult cattle than in young calves. Our finding might be explained by the fact that animals' risk of contracting BVD increases with age (Daves *et al.*, 2016). Although there was no discernible difference in the rate of BVD infection between native and mixed breeds in the present study, native breeds mathematically had a higher rate than mixed breeds. This result differed from that of Daves *et al.* (2016), who discovered that cattle of mixed breeds were more likely to be infected with BVD. Our result would suggest that all breeds of cattle are equally susceptible to contracting BVD. Native breeds had higher numerically; since native breeds often have less robust immune systems than mixed breeds. Seasonal fluctuations and the frequency of BVD infection were studied, and found that the percentage of infection with BVD was significantly higher in hot months (26.32%) than in cold months (6.45%). This result was in contrast to that of El-Bagoury *et al.* (2014), who noted that BVD infection was most prevalent in the winter. Our finding may be attributed to the increased survivability of BVDV at warm temperatures.

CONCLUSION

The current study detected BVD-infected cattle in Assiut governorate, Egypt. These results have economic implications and highlight the necessity of implementing efficient preventative and control measures across Egypt to reduce the prevalence of BVDV. Additionally, to eliminate conditions that exhibit clinical similarities to BVD, such as infectious bovine rhinotracheitis, malignant catarrhal fever, and foot-and-mouth disease, all clinical suspicions of BVD must be confirmed by laboratory testing.

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بعض الدراسات عن الإسهال البقري الفيروسي في محافظه أسيوط ، مصر

زينب محمد أحمد يوسف ، محمد محمود محمد عبد الباقي ، غادة بدر محمد مريد ،
فاطمة عصام الدين عبد الحميد محمد مهران ، فاطمة صابر محمود

Email: zeinabmohammed613@aun.edu.eg Assiut University website: www.aun.edu.eg

الإسهال البقري الفيروسي هو مرض فيروسي شائع يمكن أن يصيب الحيوانات المستأنسة والبرية. على الرغم من خسائرهم الاقتصادية ، لا تتوفر الكثير من المعلومات حول الإسهال البقري الفيروسي في محافظة أسيوط. لذلك ، كانت أهداف الدراسة الحالية هي تحديد النتائج الاكلينيكية ، وارتباطات عوامل الخطر مع معدل العدوى، والتشخيص الجزيئي لفيروس الإسهال البقري الفيروسي. أجريت الدراسة الحالية على ٥٠ بقرة تنتمي إلى قرى مختلفة بمحافظة أسيوط، مصر. وكشفت نتائج الفحص الاكلينيكي عن وجود حمى وتقرحات بالفم وإسهال وأعراض تنفسية وعتامة القرنية. تم جمع عينات المصل للتحليل المختبري. تم استخدام مقايضة تفاعل البلمرة المتسلسل العكسي لتشخيص فيروس الإسهال البقري الفيروسي. تم العثور على حمض النووى الريبى لفيروس الإسهال البقري الفيروسي في مصل سبعة من الماشية. لم يكن هناك فرق معنوي ($P > 0.05$) بين النسب المئوية للإصابة بالإسهال البقري الفيروسي والجنس والعمر والسلالة (السلالة المحلية والمختلطة) من الأبقار المختبرة جزيئياً. وفقا للظروف المناخية لمحافظة أسيوط ، كان هناك تباين ملحوظ ($P > 0.05$) بين معدل الإصابة بالإسهال البقري الفيروسي والشهور الباردة والحارة. من المستحسن التأكيد على مدى أهمية تنفيذ تدابير وقائية ورقابية فعالة في جميع أنحاء مصر من أجل الحد من انتشار الإسهال البقري الفيروسي.