

## Clinical forms and molecular identification of BVDV biotype 2 outbreaks in Egypt with risk assessment

O.M. Abas<sup>1</sup>, A.G. Saleh<sup>2</sup>, Y. Badr<sup>3</sup>, N. Baker<sup>3</sup>, E. Hegazy<sup>1</sup>, A. Khadr<sup>1</sup>, M.A. Donia<sup>4,5</sup>, and E.B. Ata<sup>6</sup>

<sup>1</sup>Animal Medicine Department, Faculty of Veterinary Medicine, Alexandria University, <sup>2</sup>Department of Internal Medicine, <sup>3</sup>Department of Infectious Diseases and Epidemics, Faculty of Veterinary Medicine, Damanhur University, Damanhur, El Behera, <sup>4</sup>Department of Internal Medicine, Kafr Elsheikh University, Egypt, <sup>5</sup>Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana, Champaign, USA, <sup>6</sup>Parasitology and Animal Diseases Department, Veterinary Research Institute, National Research Centre, Egypt

### Article information

#### Article history:

Received 30 June 2025

Accepted 13 September 2025

Published 15 October 2025

#### Keywords:

BVDV

Morbidity

Mortality

Molecular detections

Clinical signs

#### Correspondence:

E.B. Ata

[emadvet2003@yahoo.com](mailto:emadvet2003@yahoo.com)

### Abstract

This study aimed to investigate the occurrence of bovine viral diarrhea virus (BVDV) infection in 321 cattle heifers across 4 farms in Egypt, with risk factor assessment. Case history, clinical investigation, and blood samples were collected for molecular identification by a nested multiplex PCR using specific primers targeting the NS5B gene. While phylogenetic analysis was performed for genotyping. The obtained results indicated the presence of multiple clinical signs, with the most prominent being profuse, watery diarrhea (average 56%) and mucosal lesions in the form of erosions with varying degrees of severity and healing tissues (average 24.6%). Meanwhile, the most significant outcome was the presence of abortion in 17.7% of the infected cases. Molecular detection and genotyping of the obtained strains revealed the presence of 3 genetically related BVDV type 2 strains, with no genetic variance. The morbidity rate of BVDV infection in the investigated herds was 44/321 (13.7%). Meanwhile, the mortality rate was 6.8% (22/321). There is a positive relationship between morbidity rate and herd size in dairy herds compared to medium-sized and beef herds. This study provides valuable insights into the prevalence and genetic diversity of BVDV in the examined cattle population. These findings contribute to understanding and controlling BVDV infection in Egypt and highlight the importance of implementing effective management strategies and vaccination programs to prevent its spread.

DOI: [10.33899/ijvs.2025.162159.4380](https://doi.org/10.33899/ijvs.2025.162159.4380), ©Authors, 2025, College of Veterinary Medicine, University of Mosul.

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### Introduction

Farm animals play an essential role in maintaining global food security (1-4). They were subjected to different pathogens that affect their productivity (5-7). *Bovine viral diarrhea (BVD)* is a prevalent and widespread disease affecting cattle and other ruminant populations worldwide. It poses significant economic challenges to the animal production sector due to its detrimental impact on cattle reproduction (8) and on the overall health of other species,

including sheep, camels, and swine (9). BVD is caused by a positive-sense, single-stranded RNA virus, bovine viral diarrhea virus (BVDV), which belongs to the Flaviviridae family, the Pestivirus genus. It is classified into three species: Pestivirus A (Bovine viral diarrhea virus 1, BVDV-1), Pestivirus B (Bovine viral diarrhea virus 2, BVDV-2), and Pestivirus H (HoBi-like pestivirus). These species are further divided into sub-genotypes (10). Depending on the presence or absence of cytopathic effects (CPE) in BVDV-infected cells, the virus can be classified into two biotypes: cytopathic

(CP) and non-cytopathic (NCP), which do not necessarily correlate with pathogenicity in vivo. The NCP strains are more commonly found in natural infections and persistent infections. In contrast, CP strains, which are associated with a severe form of BVD called mucosal disease (MD), are relatively rare (11). The BVDV infection at an early stage of pregnancy can result in immunotolerance. The fetus becomes persistently infected (PI) (12), serving as a continuous source of virus dissemination horizontally through direct contact or indirectly via inhalation or ingestion of materials contaminated with the virus (13). Vertical transmission from PI-infected dams to their fetuses via the placenta may occur, and the outcomes depend on the pregnancy stage. Other mechanisms, such as contaminated semen, embryo transfer, and contaminated modified live vaccines, were also reported (14). The BVDV causes different clinical signs, including subclinical benign diarrhea, acute highly fatal diarrhea, hemorrhagic and thrombocytopenic disease, reproductive failure, fatal mucosal disease of PI animals, abortions, and malformations (15). Mucosal disease, which is characterized by severe and fatal manifestations of BVDV infection, can occur in persistently infected (PI) animals upon superinfection with cytopathic (CP) strains. The virus's immunosuppressive effects were found to contribute to the occurrence of respiratory disease in calves (16). The BVDV is present in most cattle-producing countries. BVDV-2 has been associated with severe clinical disease in adult cattle and with hemorrhagic syndrome in young animals. Subsequent studies conducted in Europe, Asia, South Africa, and Brazil have confirmed the presence of BVDV-2 beyond North America. However, its prevalence in Europe was lower than in North America (17). In Egypt, BVD has become endemic over the last decade. Both type I and type II were previously isolated (18). Detection of infected animals is the most crucial step in eradicating BVDV. Different methods, including molecular identification and sequencing, helped detect and genotype different pathogens, including BVDV (19,20). A previous meta-analysis concluded that most related studies focused mainly on BVDV seroprevalence, and there was a real gap in studies that identified the causative agent, its impact, and associated risk factors (17). Also, a local study recommended that further investigations across different localities be conducted to clarify the distribution of circulating types in Egypt (21).

Therefore, the aim of the present study was to determine the predominant clinical forms of the disease, diagnose it, and molecularly detect BVDV in imported cattle with suspected clinical signs.

## **Materials and methods**

### **Ethical approval**

All animal experiments were conducted in accordance with the Animal Welfare directives. The ethical approval for

the current study was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, Alexandria University (Approval No.: Au013071020240150).

### **Area and clinical examination**

The study was conducted on four farms located in the Beheira governorate, Egypt (30.61°N 30.43°E). A total of 321 imported heifers aged 1-2 years were examined for clinical signs associated with BVDV infection. Clinical evaluation included a thorough assessment of systemic illness indicators such as elevated body temperature ( $> 40^{\circ}\text{C}$ ), lethargy, anorexia, and characteristic gastrointestinal symptoms, including profuse, watery, and occasionally bloody diarrhea. Additional clinical signs were recorded, including severe ulceration of the buccal mucosa, respiratory manifestations (coughing, various forms of nasal discharge, dyspnea), and ocular symptoms (lacrimation, conjunctivitis, corneal opacity). Pregnant cattle were monitored for reproductive complications, including abortion. All clinical assessments followed standardized veterinary examination protocols (22).

### **Sampling**

Three hundred twenty-one whole blood samples were collected from both suspected and apparently healthy animals. Briefly, a jugular vein puncture was performed, and 5 mL of blood was collected in EDTA Vacutainer tubes. Subsequently, the samples were transferred immediately to the lab. The samples were aliquoted, labeled, and stored at  $-80^{\circ}\text{C}$  until molecular analysis was performed.

### **Ribonucleic acid (RNA) extraction**

Viral RNA from the preserved whole-blood samples was extracted using a total RNA extraction kit (Thermo Fisher Scientific)®, following the manufacturer's protocol. RNA purity and concentration were assessed using a Nanodrop Microvolume Spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) synthesis was performed using the Advantage® RT-for-PCR Kit (Takara, Japan), and the resulting cDNA was stored at  $-80^{\circ}\text{C}$  until further analysis.

### **Molecular detection**

The BVDV was identified using nested multiplex PCR targeting the NS5B gene. The first PCR reaction was performed by exploiting external NS5B primers (Table 1). Each 25  $\mu\text{L}$  reaction contained 12.5  $\mu\text{L}$  of the 2x OnePCR™ master mix (GeneDirex), 10 pmol of each external primer, 50-100ng of the cDNA, and RNase-free water up to 25  $\mu\text{L}$ . The cycling conditions included an initial denaturation for 7 min at  $94^{\circ}\text{C}$ , followed by 30 cycles of denaturation at  $94^{\circ}\text{C}$  for 20 s, annealing at  $50^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 30 s, with a final elongation for 15 min at  $72^{\circ}\text{C}$ . The reaction was then maintained at  $4^{\circ}\text{C}$ . For the second PCR reaction, an internal multiplex primer set was

used under the same thermal conditions as for the first reaction (Table 1) (16). The amplified amplicons were electrophoresed using 1.5% stained agarose gel (23,24).

### Sequencing and phylogenetic analysis

The PCR amplicons were purified from the gel using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific®), and their concentration was measured. Representative samples were sent for direct sequencing using the same internal primers. The assembly and editing of the obtained sequences were performed using the free BioEdit 7.2 tool (<https://bioedit.software.informer.com/7.2/>). The edited sequences were uploaded to the National Center for Biotechnology Information (NCBI) database under the

accession numbers (PP108739.1, PP108740.1, PP108741.1). The Basic Local Alignment Search Tool (BLAST) at NCBI was used to compare with similar sequences. The phylogenetic analysis was inferred using the minimum evolution method (25). The evolutionary distances were computed using the maximum composite likelihood method (26) in units of base substitutions per site. The Neighbor-joining algorithm was used to generate the initial tree (27). Evolutionary analyses were conducted in MEGAX (28). The study involved 28 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 248 positions in the final dataset. The confidence level of the NJ tree was assessed by bootstrapping using 1000 replicates.

Table 1: Shows the list of primer sequences used in this study for the NS5B gene (15)

name	Primer name	Primer sequence (5'-3')	Annealing °C	Amplicon size (bp)
NS5B	BVD external forward	AAGATCCACCCTTATGA(A/G)GC	50	1100 bp 360 bp (BVDV-1) 604 bp (BVDV-2)
	BVD external reverse	AAGAAGCCATCATC(A/C)CCACA		
	Internal forward 1	TGGAGATCTTCACACAATAGC		
	Internal forward 2	GGGAACCTAAGAACTAAATC		
	Internal reverse	GCTGTTCACCCAGTT(A/G)TCAT		

### Risk factor analysis

A comprehensive epidemiological assessment was conducted across the four farms to identify potential risk factors for BVDV infection. Data collection included herd characteristics, management practices, and general vaccination history of the farms to evaluate their impact on morbidity and mortality rates.

### Farm characteristics and study population

The study analyzed epidemiological parameters, including farm characteristics such as geographic location, herd size (medium vs. large), and management system (dairy vs. beef). Cattle were categorized by origin as either imported or mixed-breed populations. Health status and clinical signs, including diarrhea, mucosal lesions, fever, respiratory distress, and abortion, were documented. Additionally, the vaccination status of each farm was recorded to determine whether a BVDV vaccination program had been implemented.

### Case definition and morbidity/mortality Assessment

BVDV-positive cases were confirmed via nested multiplex PCR and clinical symptomatology. Morbidity was calculated as the percentage of infected animals per farm. At the same time, mortality was determined as the proportion of deceased cases among infected animals.

### Data collection and statistical analysis

Morbidity and mortality rates were compared across herd size, management system, and vaccination status. Farms were classified based on herd size (medium vs. large) and cattle origin (imported vs. mixed breed). The effect of dairy

vs. beef production on infection prevalence and the impact of vaccination programs on reducing infection rates were assessed. Descriptive statistics summarized morbidity and mortality rates, and statistical analyses were performed to identify significant differences based on farm type, management, and vaccination status. A  $P<0.05$  significance threshold was applied. Data analyses were conducted using GraphPad Prism 5 to ensure reproducibility.

### Results

#### Clinical findings

The investigated cases exhibited generalized symptoms of illness, including drowsiness and loss of appetite in 40.4% of the animals, and fever in 30.8%. The most prominent clinical manifestation was the GIT disturbance, including profuse, watery, and sometimes bloody diarrhea, with an average of 56% of the affected animals (Figure 1). Farm No.1 showed the highest percentage, with 70% of animals displaying these symptoms (Table 2). A more specific clinical finding was the presence of mucosal lesions, including erosions of varying severity and healing tissues, in the oral cavity and muzzle (Figure 2), which were documented in 16.9-34% of cases, with an average prevalence of 24.6%. The most significant outcome was the occurrence of abortion in 17.7% of the infected cases, with the highest incidence, 20.5% recorded in farm No. 2. Additional clinical signs included respiratory manifestations (15.8%) and nasal discharge (18.6%). Less frequently observed signs included lameness (10.2%) and corneal opacity (16.5%) (Figure 3 and Table 2).

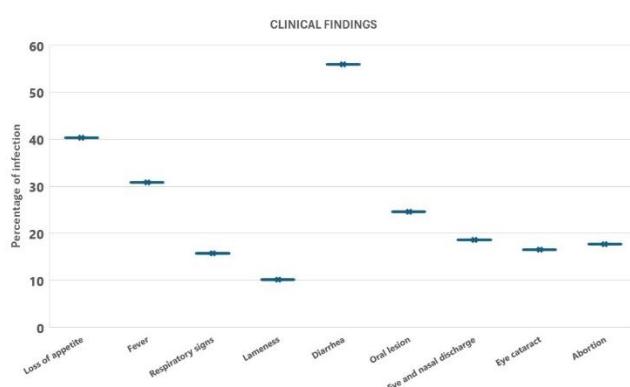


Figure 1: Shows the average percentage of the recorded clinical findings.



Figure 2: Oral lesions in the muzzle and oral cavity in the form of erosive lesions in the lips, gums, and hard palate.

Table (2): Clinical signs associated with the BVDV infection

	Farm 1			Farm 2			Farm 3			Farm 4			Average		
	Total number	Number of infected cases	%	Total number	Number of infected cases	%	Total number	Number of infected cases	%	Total number	Number of infected cases	%	Total number	Number of infected cases	%
Loss of appetite	50	28	56	112	40	35.7	95	29	30.5	64	33	51.5	321	130	40.4
Fever	50	18	36	112	35	31.2	95	21	22.1	64	25	39.0	321	99	30.8
Respiratory signs	50	6	12	112	14	12.5	95	19	20.0	64	12	18.7	321	51	15.8
Lameness	50	5	10	112	6	5.3	95	13	13.6	64	9	14.0	321	33	10.2
Diarrhea	50	35	70	112	62	55.3	95	44	46.3	64	39	60.9	321	180	56.0
Oral lesion	50	17	34	112	19	16.9	95	22	23.1	64	21	32.8	321	79	24.6
Eye and nasal discharge	50	14	28	112	12	10.7	95	19	20.0	64	15	23.4	321	60	18.6
Eye cataract	50	4	8	112	19	16.9	95	21	22.1	64	9	14.0	321	53	16.5
Abortion	50	9	18	112	23	20.5	95	14	14.7	64	11	17.1	321	57	17.7



Figure 3 shows the eye lesions in the form of corneal opacity, eye discharge, and keratitis. The last photo shows Coronitis and eruptive lesions on the skin of the interdigital cleft, which can cause lameness in some cattle.

#### Molecular detection and genotyping

Molecular analysis of BVDV was conducted using a multiplex nested PCR with external and internal primer sets specific for differentiation between types 1 and 2. The used assay successfully amplified a 603 bp fragment, confirming the presence of BVDV Type 2 (Figure 4). At the same time, no amplification was detected for BVDV Type 1, indicating its absence in the tested samples. Overall, BVDV was molecularly identified in 44 of 321 animals (13.7%), confirming the circulation of BVDV Type 2 in the examined population.

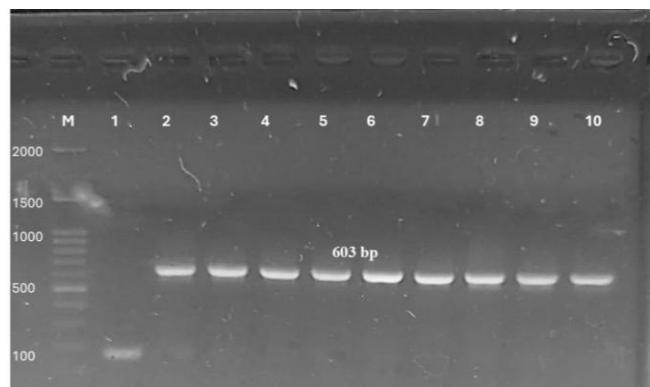


Figure 4: Electrophoresis of amplified PCR products. M: 100 bp ladder, L1: negative control, L2-L10: Amplicons of size 603 bp representing BVDV Type 2.

## Phylogenetic analysis

A phylogenetic tree was created using the maximum likelihood analysis of MEGA. A total of 28 nucleotide sequences, including the 3 isolated strains from the current study, were uploaded to the NCBI database (Accession numbers: PP108739.1, PP108740.1, PP108741.1). Analysis of the obtained results revealed the absence of considerable genetic diversity, and all strains were classified as type-2, not type-1, with a homology ranging from 99.15-100% between them. The obtained strains were closely related to the MH231134.1, which was isolated in the USA in 2018. On the same side, it is related to those isolated in Calgary, Canada (KX170651.1, KX170652.1) (Figure 5).

## Risk factors associated with the BVDV infection:

Molecular detection of BVDV revealed an overall morbidity rate of 13.7% (44/321) across the examined farms, with variations among different herd management systems. The highest morbidity rate was observed in farm No. 3, with a percentage of 15.7% (15/95). While the lowest was recorded in farms No. 2 and No. 4 (12.5%; 14/112 and 8/64, respectively). Similarly, the overall morbidity rate was 6.8% (22/321), with farm No. 3 exhibiting the highest mortality rate at was found in farm No. 3 at 10.5% (10/95), while farms No. 4 and No. 1 recorded the lowest mortality rates at 3.1% (2/64) and 4% (2/50), respectively (Table 3).

Table 3: Risk factors associated with the BVDV infection in the current study

Farm	Morbidity	Percentage	Mortality	Percentage	Breeds	Herd size	Manag system	Vaccination
1	7/50	14%	2/50	4%	Imported	Medium	Mixed	No
2	14/112	12.5%	8/112	7.1%	Imported	Large	Dairy	No
3	15/95	15.7%	10/95	10.5%	Imported	Large	Dairy	No
4	8/64	12.5%	2/64	3.1%	Mixed	Medium	Beef	No
Total	44/321	13.7%	22/321	6.8%				

Farms rearing imported cattle had higher morbidity rates than those maintaining mixed populations, though the difference was not statistically significant. However, a substantial increase in mortality rates was observed in farms with imported cattle, suggesting that these animals may be more susceptible to infection. Herd size also influenced the disease prevalence, with larger herds exhibiting higher morbidity rates than medium-sized herds. Additionally, dairy farms had higher morbidity and mortality rates than beef farms, which may be attributed to more extended animal retention periods and greater animal-to-animal contact in dairy management systems. Notably, none of the infected farms had an established BVDV-specific vaccination program, which may have contributed to the observed disease burden.

## Discussion

Bovine viral diarrhea virus (BVDV) is a highly pathogenic agent affecting multiple animal species,

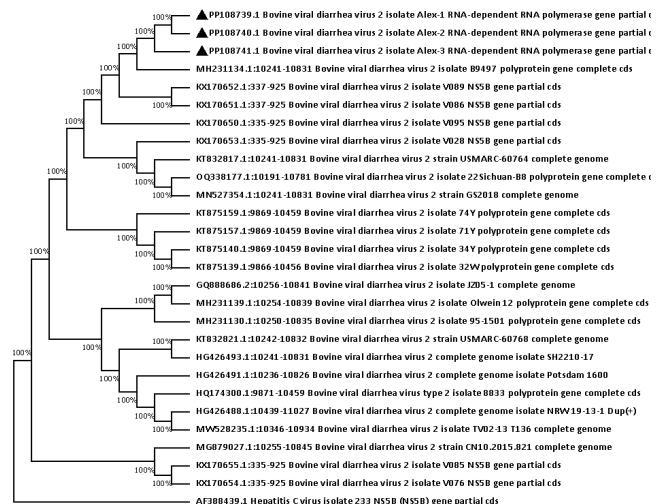


Figure 5: The phylogenetic analysis of the detected BVDV strains. A rooted cladogram was generated using MEGA7. An evolutionary history was reconstructed using the maximum-likelihood method under the Tamura-Nei model. The confidence level of the NJ tree was assessed by bootstrapping using 1000 replicates. The black triangles represent the multiple sequences obtained in the current study.

particularly the cattle industry. This virus causes severe economic losses by affecting animal health, productivity, and reproductive performance. Effective control measures, including early disease diagnosis, identification of persistently infected animals (PI), biosecurity implementation, vaccination programs, and proper herd management, are crucial to mitigating its impact (28-30).

In this study, infected cattle presented a range of systemic signs, including loss of appetite, lethargy, fever, and anorexia, with 15.8-40.4% of the tested animals affected, consistent with previous BVDV outbreaks in Egypt and globally (31). A similar ratio was previously reported in a survey of BVDV infection in cattle herds in the New Valley province, Egypt (32). It was reported that virus excretion could occur 1-2 weeks before the appearance of clinical signs. Although BVDV may be the principal cause of respiratory signs, its immunosuppressive effect could also predispose to secondary infections with other viruses and bacteria (31).

It is well known that BVDV causes a range of erosions, ulcers, and mucosal necrosis across the GIT. Accordingly, it must be differentiated from the other vesicular diseases. 24.6% of the tested cases showed mucosal lesions. A previous clinical examination of newborn infected calves failed to detect oral lesions, including mucosal erosions, tongue necrotic foci, and hyperemia of cheek papillae, in only 16 cases (18.3%) (33). Developing such skin lesions pass through different stages, including diffuse and severe parakeratotic hyperkeratosis, which thickens the stratum corneum and is associated with multifocal ballooning degeneration. These necrotic and degenerate cells tended to coalesce in the epidermis, forming numerous intraepithelial and subcorneal pustules. The ulcerative lesions of the oral mucosa and hard palate were similar and composed of multifocal areas of epithelial necrosis associated with numerous epithelial cells displaying single-cell death and a moderate inflammatory infiltrate of lymphocytes and macrophages at the mucosa-submucosa junction (34).

Although diarrhea is a very common sign of BVDV infection, a previous study reported that, in the acute infection, diarrhea and tenesmus were not recorded. The disease had a short course, as all infected calves died (35). Therefore, a high ratio 56% of examined cases suggests the presence of chronic PI or instances of PI.

One of the most common sequelae due to BVDV infection is reproductive disorders like reduced conception rates, early embryonic death, and abortion with or without congenital deformities. In the current study, the recorded abortion rate was 24.6%. It is essential to notice that the recording of this sequela was greatly varied between the different studies, as testing of livestock farms in central and Northwest Ethiopia revealed the presence of an abortion rate of 4% (36), and 11.2% in Egypt (32). In Sudan, 84.3% of the seropositive dams had a history of abortion (37).

BVDV results in corneal opacity in adult cattle. 16.5% of the infected cases exhibited unilateral corneal opacity. An earlier study showed that 6% (3/50) of cases had the same lesion (38). Exposure of dams to the BVDV during pregnancy could result in more complicated cases, including retinal atrophy, optic neuritis, and microphthalmia with retinal dysplasia. So, calves with ocular signs may be blind, and there may be ocular discharge in acute or chronic cases (39).

The complexity of this virus is also reflected in its interactions with host animals. Infections are either transient or persistent and can cause a broad spectrum of clinical signs, from no or very mild disease to severe forms, reminiscent of viral hemorrhagic fevers (40). Due to the importance of BVDV to the animal sector, classical and recent techniques were used for detection and diagnosis. It was concluded that molecular diagnostic approaches were widely used due to their high specificity and sensitivity (15). Furthermore, the development of sequencing and phylogenetic analysis enabled complete genotyping and tracing of the different

pathogens (41-43). Molecular detection of the BVDV strains obtained from the NS5B gene revealed that type 2 was detected in all investigated cases. At the national level, type I was previously detected during 2015 and 2021 (33,44). Meanwhile, the *HoBi-like Pestivirus (BVD-3)* was first detected in 2022 (45).

Phylogenetic analysis of the obtained isolates revealed no diversity among them, with a homogeneity percentage of over 99% that may have originated from a common ancestor. On the other hand, high genetic variability was detected within BVDV type 1 previously isolated in Egypt, suggesting a Middle Eastern origin (21). At the international level, subgenotypes BVDV-1a, BVDV-1b, and BVDV-2a are the most widely circulating and prevalent worldwide (46).

The morbidity rate of BVDV infection in the investigated herds was 44/321 (13.7%). The same results were recorded in Egypt in 2004, when only 9/67 tested samples (13%) were BVDV-positive by RT-PCR (47). A near result was previously obtained with type 1, as it was detected in 31/298 (10.4%) of the tested samples (33). In Western China, a lower percentage was recorded at 7.2% (89/1234) with the isolation of 13 BVDV strains of the NCP biotype (48). A much lower rate, 2.95% (9/305), was previously recorded in diarrheic calves. The differences in reported prevalence percentages could be attributed to multiple factors, such as the diagnostic technique used in each study, differences in herd management systems for the tested herds, and the locations of the investigated population (49).

The recorded mortality rate was 6.8% (22/321). The reported mortality rate of the infected calves in central and Northwest Ethiopia was 9.2% (36). And 11.6% in Ethiopian dairy farms (50). A much lower mortality rate 3.5% was recorded in Uruguay, and it was suggested that the animals were PI (51). On the other hand, a comparatively high rate of 20.7-22.3% in mixed systems of sub-Saharan African countries (52). Obtaining a high ratio of 25-30% in herds where it was hypothesized to be exposure of many cows/heifers to NCP BVDV strains during early pregnancy (53). It is worth noting that, although BVDV can infect various domestic and wild ruminants, cattle are the natural host and exhibit a high mortality rate (54).

A positive relation between herd size and BVDV prevalence was observed, with larger herds experiencing higher morbidity. This trend aligns with reports from Jordan, where high cattle density and frequent inter-farm contact facilitated viral spread (55). Larger herds are typically at greater risk due to increased animal movement, the introduction of naïve animals, and heightened exposure to infectious agents.

The analysis has shed light on the complex interplay between management practices, herd attributes, and BVDV prevalence. Certain management behaviors were identified as risk factors for increased BVDV prevalence. Notably, the introduction of new cattle into existing herds and allowing

direct contact with neighboring farms were associated with higher infection rates (56). These practices likely facilitate virus transmission between herds, underscoring the importance of biosecurity measures in BVDV control.

A higher prevalence rate was observed on dairy farms than on beef farms. Although it was reported that there was no significant difference between beef and dairy herds (56), in a previous study, the same observation was noticed as the rate was high amongst dairy cattle compared to the beef ones, with a significant difference, which could be attributed to the extended staying of dairy cows, with a high probability of contacting more pathogens. Furthermore, contact between cows and milkers could provide more opportunities for increased infection risk (57,58).

It is crucial to note that none of the studied farms had a BVDV-specific vaccination program in place. The lack of vaccination likely contributed to increased viral transmission and higher morbidity. BVDV seroprevalence in non-vaccinated herds has been reported to range between 20% and 90%, depending on regional management practices (59).

## Conclusion

It can be concluded that profuse, watery diarrhea and mucosal lesions are the most commonly reported clinical signs of BVDV infection. Genetically, 3 related strains of BVDV type 2 were determined. There is a positive correlation between morbidity rate and herd size for dairy herds compared to medium-sized and beef herds, respectively. More structured molecular surveys should be conducted primarily for farms suffering from diarrhea, mucosal lesions, and abortion.

## Competing interests

The authors declare that there are no competing interests.

## Author Contributions

NB, AK was responsible for conceptualization. OMA, AGS, YB, ENH, and EBA shared in sample collection and molecular detection; OMA, YB, and EBA shared in the phylogenetic analysis. AGS, ENH, and MAD helped in data analysis. OMA, ENH, and EBA shared the original draft in writing. MAD shared in language editing. All authors revised the final version of the manuscript and approved it for submission.

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## الأعراض السريرية والتوصيف الجزيئي لتفشي النمط الثاني من فيروس الإسهال البقرى في بعض المزارع المصرية مع تقييم المخاطر

أسامة محمد عباس<sup>١</sup>، أسماء غالب<sup>٢</sup>، ياسين بدر<sup>٣</sup>، نبيل بكر<sup>٣</sup>، عماد حجازي<sup>١</sup>، عادل خضر<sup>١</sup>، محمد دنيا<sup>١</sup>، عماد بشير عطا<sup>١</sup>

قسم طب الحيوان، كلية الطب البيطري، جامعة الإسكندرية،<sup>١</sup> قسم الأمراض الباطنة،<sup>٢</sup> قسم الأمراض المعدية والأوبئة، كلية الطب البيطري، جامعة دمنهور،<sup>٣</sup> قسم الأمراض الباطنة، كلية الطب البيطري، جامعة كفر الشيخ، مصر،<sup>١</sup> قسم علم الأمراض، كلية الطب البيطري، جامعة إلينوي، الولايات المتحدة الأمريكية،<sup>٢</sup> قسم الطفيليات وأمراض الحيوان، معهد البحوث البيطرية، المركز القومى للبحوث، مصر

### الخلاصة

هدفت هذه الدراسة إلى تشخيص عدوى فيروس الإسهال البقرى في عدد ٣٢١ من الأبقار في أربع مزارع مختلفة تقع في محافظة البحيرة بجمهورية مصر العربية مع تقييم عوامل الخطورة المرتبطة بظهور المرض. تم تسجيل التاريخ المرضي للحالات محل الدراسة والفحص السريري لها وتجمیع عینات الدم لاستخلاص الحمض النووي وإجراء تفاعل البلمرة المتسلسل العكسي. بينما تم إجراء تحليل التتابع والتشوه والتطور الجيني لتحديد النوع الجيني السادس. أظهرت النتائج وجود علامات سريرية متعددة ولكن أبرزها كان وجود إسهال مائي غزير بنسبة ٥٦% من الحالات مع وجود تقرحات بدرجات متباينة الشدة على الأنسجة الطلائية في ٤٦% من الحيوانات. بينما كان أهم عرض هو وجود حالات إجهاض بنسبة ١٧,٧% في الحالات المصابة. أظهر الكشف الجزيئي عن وجود ثلث عترات فيروسية تتنامي جينيا إلى النمط الثاني من فيروس الإسهال البقرى مع عدم وجود تباين جيني كبير بينهم. كان معدل انتشار المرض في الحيوانات هو ١٣,٧% (٣٢١/٤٤)، بينما كان معدل الوفيات ٦,٨% (٢٢١/٣٢١) مع وجود ارتباط إيجابي بين معدل انتشار المرض والقططان الكبيرة أو المنتجة للألبان مقارنة بالقططان متوسطة الحجم أو المخصصة لإنتاج اللحم على التوالي. وبناء عليه يمكن استنتاج أن هذه الدراسة توفر رؤى قيمة حول مدى انتشار فيروس الإسهال البقرى وتتنوعه الوراثي في الماشية التي تم فحصها، والتي بدورها تساهم في فهم هذه العدوى ومكافحتها في مصر وتبسيط الضوء على أهمية تنفيذ استراتيجيات فعالة وبرامج التطعيم المناسبة لمنع انتشار المرض والسيطرة عليه.