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Seroprevalence and Epidemiological Studies on Bovine Viral Diarrhea in Cattle in New Valley Province, Egypt

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ABSTRACT: Bovine viral diarrhea (BVD) is one of the most imperative worldwide diseases in ruminants leading to substantial damage as well as extensive economic losses for the cattle industry. To our knowledge, there have been no reports about the disease's situation in New Valley province, so the goal of this study was to estimate the level of bovine viral diarrhea virus (BVDV) infection in cattle herds through serodiagnosis and determining factors influencing the course of infection (current epidemiological situation) which will aid in establishment of control strategy. In this study a total of 1400 cattle of different ages, sexes and breeds (not previously vaccinated with BVD vaccines) were screened clinically for symptoms of BVD infection. 260 cattle out of totally 1400 screened cattle (18.6%) showing clinical signs arousing suspicion of BVD (49 cattle (18.8%) exhibit respiratory manifestation, 63 (24.2%) cattle suffered from diarrhea, 37 (14.2%) with mixed enteric and respiratory signs , 43 (16.5%) with retarded growth, oral lesion was detected in only 6 cattle (2.3%) , While the congenital anomalies were recorded in 18 (6.9%) , abortion occurred in 29 female cattle (11.1%) and only 15 cattle (5.8%) suffered from infertility trouble) . Totally 260 clinically diseased cattle undergo Serum Neutralization Test (SNT) for detection of BVDV infection. The results revealed that 83 cattle out of 260 (31.9%) were detected as seropositive (positive for BVDV antibodies), showed clinical signs of BVD infection represented in 17 cattle showed respiratory manifestation (nasal discharge), 22 cattle suffered from diarrhea, 12 with mixed enteric and respiratory signs , 6 with retarded growth, oral lesion was detected in Only 2 cattle , While the congenital anomalies were recorded in 5 , abortion occurred in 15 female cattle and only 4 cattle suffered from Infertility trouble According to age, the seroprevalence of BVDV for antibodies was higher in in cattle aged (>6M to 2 year) (37.50%) and the lowest rate was (21.73%) in cattle aged (1day: 2month). Rendering to sex and breed, female cattle (37.80%) had a greater infection rate than male (21.87%) and infection was higher in Frisian bread (34.05%) than native bread (29.50%). Concerning to season, the highest prevalence of infection was (40.24%) in Winter and the lowest rate was (16.66%) in Autumn. Regarding to locality, the highest prevalence of infection was (45.45%) in EL-Farfara, and the lowest rate was (31%) in Balat. This prevalence of BVDV in cattle of new valley province, Egypt reveals the necessity of further studying BVDV infection in the area

KEYWORDS: Snt, Bvd , serum

1. Introduction

BVD is a common infection of cattle worldwide, the broad nature of the disease, transmittance, and lack of treatment have made it a globally enzootic, Bovine viral diarrhea virus (BVDV) is a small, enveloped single-stranded positive sense RNA virus, about 12.5 kb, is a member of the genus Pestivirus, family Flaviviridae [1, 2] , causing multiple disease and clinical syndromes including embryonic mortalities, abortion, fetal mummification, stillbirths, congenital deformities, respiratory disease [3] BVD disease becomes widely distributed in Africa, and the Middle East including Egypt [4, 5]. The first Egyptian strain were isolated from breeding farm of Iman village; north Tahreer province and designed as Iman-7912 that from pneumoenteric calve [6] , Moreover, the second Egyptian strain of BVD-MD Pestivirus (Kenna strain) was isolated from pneumoenteric cattle herd [7]. BVDV can be divided into two genotypes (BVDV-1 and BVDV-2) on the basis of antigenic and genetic differences, with each genotype containing both the CP and NCP forms. Both genotypes are divided into subtypes [8]. The main transmission route in infected herds is direct contact with a PI animal. The horizontal transmission of BVDV may be direct or indirect via inhalation or ingestion of virus contaminated materials [9]. If a cow is PI, its fetus will become infected. The virus has the ability to cause transplacental infection resulting in different outcomes depending on the stage of gestation at which the acute infection takes place, leading to fetal death, malformations, acute syndromes of the neonate, immune tolerance and lifelong viral persistence [10]. There

are two forms of infection associated with BVDV: acute or transient infection and persistent infection (PI). Acute infection is post-natal infection in an immunocompetent host. In contrast, PI only occur in utero infection of the developing fetus with ncp-BVDV prior to the development of immunocompetence [11]. BVD with these economic losses, needs accurate and sensitive diagnostic methods for rapid identification and elimination of persistent carriers in the herds. In the past, the most common method was an isolation of virus in cell cultures but it was difficult, time consuming and lengthy process that requires experienced technicians [12]. Detection of virus specific antibodies by using different serological tests as serum neutralization test and enzyme linked immunosorbent assays is an important way for the virus detection[13] . All control programs which are in use in many countries of the world, mainly depend upon the detection and removal of PI animals, [14] . In Egypt, the BVDV control program is based on mass vaccination by commercially available inactivated vaccines. There are no surveillance programs or measures for detection and elimination of PI calves from farms [15]

2. Material and methods

Eithical Approval

Samples were collected by standard sample collection method without any stress to animals.

2.1. Study area and animals

This study was comprised of one year during the period from March 2021 to February 2022 in New valley province (coordinates: 24◦ 32, 44, N, 27◦ 10, 24" E) in the South-Western part of Egypt from different localities (EL-Dakhla, EL-Kharga, EL- Farafra, Balat and Paris). A total of 1400 cattle of different ages (ranged between one day to five year), sexes (420 males and 980 female) and breeds (355 native and 1045 Frisian) were screened clinically for symptoms of BVD infection according to [16, 17] and the data of each examined animal were recorded (complete data on case history, owners complain, clinical examination). No recorded BVD Vaccination in this province

2.2. Sampling

2.2.1. Serum Samples

5 ml Blood was collected from each clinically diseased animals by jugular vein puncture on vacutainer tubes without EDTA kept to clot, and serum was separated by centrifuging at 3000 rpm for 10 minutes and stored at -20º C until used for Serum neutralization test (SNT) to determine the antibody status of herds against BVDV

Serum neutralization test

The absence of any vaccination programs against this disease suggests that the neutralizing antibodies to BVD virus in the farm animals had arisen as a consequence of natural infection with BVD virus. serum neutralizing titer of 1:8 or higher is considered to be positive

Virus used in serum neutralization test (SNT)

Local Iman strain of Bovine viral diarrhea virus (BVDV) was supplied by the Virology Laboratory (BVD-Unit) of the VET. Serum and Vaccine Research Institute, Abbasia. It was adapted on Madin Darby Bovine Kidney (MDBK) cells. It gave a titer of 105 TClDso/ml, the virus was preserved at ˜196c

Serum neutralization test

To determine the antibody status of herds against BVDV, serum samples were subjected to SNT which carried out according to [18]. All sera were inactivated in water bath at 56 ◦C for 30 minutes and serially diluted in microtiter plates starting with dilution 4. An equal volume of BVDV (100 TCID50/50 µL)were added to each well and incubated for one hour at 37◦C. Each well in the plate received 0.1 ml of MDBK cell suspension containing 15000 cells. The plates were incubated for 7 days at 37◦ C and microscopically examined daily for the development of cytopathic effect. The endpoint was expressed as the highest dilution of serum, which neutralized the virus. The Serum Neutralizing Antibody titers of the tested serum samples were expressed as log 10 of the The starting dilutions of the serum from 1:4 to 1:5120 in the minimal essential medium (MEM) were added to the 96-well polystyrene plates. Then, each of the diluted samples received a 50 µL stock solution containing 100 TCID50 of the NADL

cytopathogenic strain of BVDV. The plates were incubated for 1 h at 37∘C with 5% CO2. Subsequently, 50 µL of Madin-Darby bovine kidney (MDBK) cell suspension was added to each of the wells at a concentration of 3 × 105 cells/mL. The plates were again incubated at 37∘C with 5% CO2 for 4-5 days. The infectivity was measured on the basis of the cytopathic effect (CPE) of the BVDV infection visible in the monolayer cell of the plates, under an inverted microscope. The antibody titer was expressed as the highest dilution of the serum that completely inhibited the infectivity CPE, in both the wells of each dilution, according to the [19].

3. RESULTS

3.1. Results of Serum Neutralization Test (SNT)

Results of Serum Neutralization Test (SNT) The results revealed that 83 cattle out of 260 (31.9%) were detected as seropositive (positive for BVDV antibodies) as shown in Table 1

3.2. Clinical findings

3.2.1. clinical findings in clinically diseased cattle

From total clinically examined cattle (1400), the results showed that 260 out of 1400 showed clinical signs arousing suspicion of BVD infection ,the results showed that 49 (18.8%) cattle exhibit respiratory manifestation (nasal discharge), 63 (24.2%) cattle suffered from diarrhea, 37 (14.2%) with mixed enteric and respiratory signs , 43 (16.5%) with retarded growth, oral lesion was detected in Only 6 cattle (2.3%) , While the congenital anomalies were recorded in 18 (7 %), abortion occurred in 29 female cattle (11.2) and only 15 cattle (5.8%) suffered from Infertility trouble as shown in, Fig. 5

3.2.2. Clinical findings in BVD seropositive cattle

From total clinically diseased cattle (260), The results revealed that 83 cattle out of 260 (31.9%) were detected as seropositive , showed clinical signs of BVD infection represented in 17 cattle showed respiratory manifestation (nasal discharge), 22 cattle suffered from diarrhea, 12 with mixed enteric and respiratory signs , 6 with retarded growth, oral lesion was detected in Only 2 cattle , While the congenital anomalies were recorded in 5 , abortion occurred in 15 female cattle and only 4 cattle suffered from Infertility trouble as shown in Table 1

3.3. Relation of prevalence of BVD cattle infection by SNT with some risk factors (Age, season, sex, breed and locality):

3.3.1. Relation between prevalence of BVD cattle infection and age:

According to age, the highest prevalence of infection was 37.50%, in group 3 (>6 month – 2years) followed by 34.84% in group 4 (>2years – 5years) 25.33%, in group 2 (> 2months –6month) and the lowest rate was 21.73%. in group 1 1day: 2month) as shown in Table 4 and Fig. 1 .

3.3.2. Relation among prevalence of BVD cattle infection and sex and breed.

Rendering to sex and breed, the prevalence of infection in female cattle (37.80%) had a greater infection rate than male (21.87%) and prevalence of infection was higher in Frisian breed (34.05%) than native breed (29.50%) as shown in Table 3 and Fig. 2.

3.3.3. Relation between prevalence of BVD cattle infection and season:

Concerning to season, the highest prevalence of infection was (40.24%) in Winter, followed by (32.87%) in Summer, then (28.98 %) in Spring and the lowest rate was (16.66%) in Autumn as shown in Table 5 and Fig. 3.

3.3.4. Relation between prevalence of BVD infection in cattle and locality

Regarding to locality, the highest prevalence of infection was (39.51%) in EL-Dakhla, followed by (33.33%) in El-Farfara and (26.47%) in Paris, then (18.18%) in El-Kharga , and the lowest rate was (13.79%) in Balat as in Table 6 and Fig. 4.

Table 1: Result of seroprevalence of BVD in cattle using SNT

Examined Sera	SNT Titer	+VE	Positive %
260	1/8	37	(14.23)
	1/16	32	(12.30)
	1/32	11	(4.23)
	1/64	3	(1.15)
Total		**83**	**(31.9%)**

Table 2: Clinical findings in clinically diseased and BVD seropositive cattle

Clinical finding	Clinically Diseased	Seropositive BVD
	NO.	NO
Respiratory manifestation (nasal discharge)	49	17
Diarrhea	63	22
Mixed Enteric Respiratory	37	12
Retarded Growth	43	6
Oral lesion	6	2
Abortion	29	15
Congenital anomalies	18	5
Infertility trouble	15	4
Total	**260**	**83**

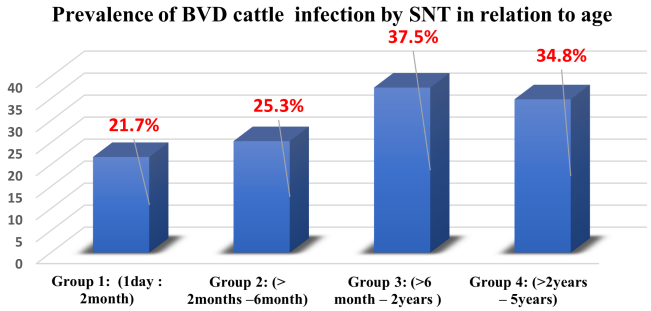

Figure 1: Prevalence of BVD cattle infection by SNT in relation to age

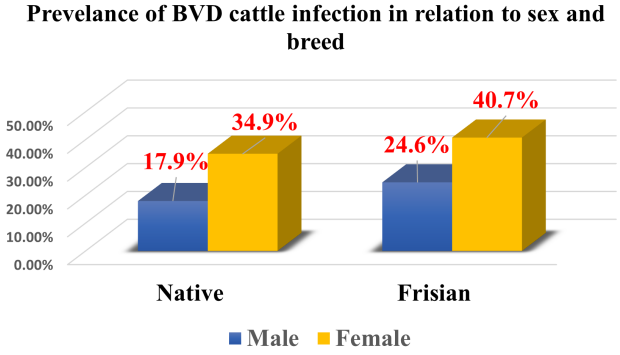

Figure 2: Prevalence of cattle BVD in relation to sex and breed

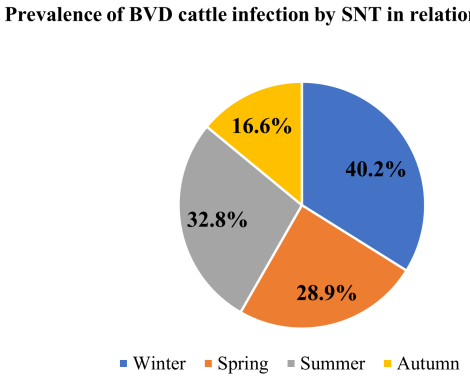

Figure 3: Prevalence of BVD cattle infection by SNT in relation to Season

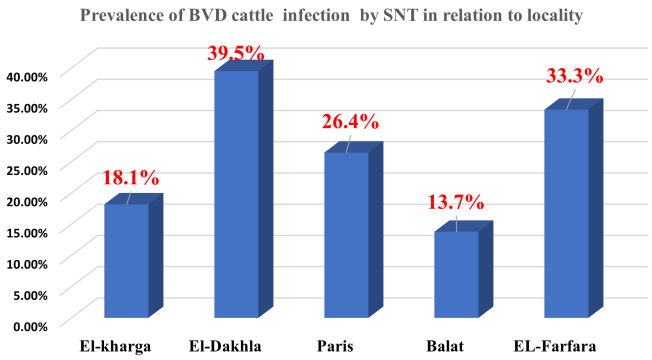

Figure 4: Prevalence of BVD cattle infection by SNT in relation to locality

4. Discussion

Infection with bovine viral diarrhea virus (BVDV) occurs globally and is imposing large direct and indirect significant economic losses on the beef and dairy industries. The most observed clinical signs in the study were (respiratory manifestation, diarrhea, growth retardation, oral lesion, congenital anomalies and abortion) the signs obtained are similar to the signs observed by [20, 11, 21] Knowledge of the epidemiological situation and estimating the level of BVDV infection through serodiagnosis in cattle herds of New Valley province (no previous studies present) will aid in establishment of control strategy. This agreed with the studies which stated that serological examination of serum samples for detection of BVDV antibodies is a useful tool for BVDV herd screening and also for monitoring of BVDV free herd status [5]. In this serological survey the results revealed that 83 cattle out of 260 (31.9%) were detected as seropositive (positive for BVDV antibodies) which is in agreement with previously reported prevalence by [22] who recorded (32.7%) of the herds cattle were seropositive to BVDV. And in harmony with [23] who showed that 27 samples (23.7%) were positive for BVDV antibodies from total samples 114. These results were in agreement with study by [24] for detection of antibodies against BVDV (Seropositivity rates were 26 %). The previous findings disagree with the findings of [25] who found that the seropositivity rate was > 90% for BVDV-specific antibody and those reported by [26, 27] where they reported that the prevalence of BVD antibodies (62%) and (68.33%) respectively This variation in seroprevalence

Table 3: Prevalence of BVD cattle infection by SNT in relation to sex and breed

Sex / Breed	Male			Female			Total		
	Total No.	positive	%	Total No.	positive	%	Total No.	positive	%
Native	39	7	17.94	83	29	34.93	122	36	29.50
Frisian	57	14	24.56	81	33	40.74	138	47	34.05
Total	96	21	21.87	164	62	37.80	260	83	31.92

The obtained Chi2 value (0.432) showed insignificant p. value ($\alpha \geqslant 0.05$) which means that prevalence of infection and breed are two independent factors

(a) cattle with oral ulcer **(b)** cattle with oral lesion **(c)** Growth Retardation in 9-month male calf

(d) cattle suffering form diarrhea **(e)** Diarrhea **(f)** Congenital anomalies in calf

Figure 5: clinical symptom in cattle

Table 4: Prevalence of BVD cattle infection by SNT in relation to age

Age group	SNT result		
	No. of examined	Positive	
		NO.	%
Group 1: 1day: 2month	23	5	21.73
Group 2: (>2months: 6month	75	19	25.33
Group 3: (>6 month – 2years)	96	36	37.50
Group 4: (>2years – 5years)	66	23	34.84
Total	**260**	**83**	**30.95**

The obtained Chi2 value (0.237) showed insignificant p. value ($\alpha \geqslant 0.05$) which means that prevalence of infection and age of cattle are two independent factors

Table 5: Prevalence of BVD cattle infection by SNT in relation to Season

Season	SNT result		
	No. of examined	Positive	
		NO.	%
Winter	82	33	40.24
Spring	69	20	28.98
Summer	73	24	32.87
Autumn	36	6	16.66
Total	**260**	**83**	**30.95%**

The obtained Chi2 value (0.079) showed insignificant p. value ($\alpha \geqslant 0.05$) which means that prevalence of infection and season are two independent factors

in different countries may be due to difference in cattle population age, cattle density, herd size, housing systems, bio-security and managemental practices, which in general could be important risk factors in transmission and persistence of BVDV [28] The seroprevalence of BVDV for antibodies was higher in in cattle aged >6M to 2 year, this is consistent with the previously reported studies [29, 22] , where a comparatively high BVDV seroprevalence in old aged herds has been described. The increase in seroprevalence with increasing age may be due to the higher exposure to the virus, and that BVDV antibodies in most cases are lifelong [29]. also, according to our findings female cattle (37.80%) had a greater infection rate than male (21.87%) and infection was higher in Frisian bread (34.05%) than native bread (29.50%). our results are going in harmony with those obtained by [30] who reported the infection rate of BVD virus was higher in female (34%)

Table 6: Prevalence of BVD cattle infection by SNT in relation to locality

Locality	SNT Result		
	No. of examined	Positive	
		NO.	%
El-kharga	22	4	18.18
El-Dakhla	124	49	39.51
Paris	34	9	26.47
Balat	29	4	13.79
EL-Farfara	51	17	33.33
Total	**260**	**83**	**30.95%**

The obtained Chi2 value (0.03) showed high significant p. value ($\alpha \leqslant 0.05$) which means that prevalence of infection and locality are two correlated factors.

than male 18% Concerning to season, the highest prevalence of infection was (40.24%) in Winter and the lowest rate was (16.66%) in Autumn, this disagree with the findings of [21]. who found that the higher percentage was higher in summer.

Conflict of interests

The authors declared that no conflict of interest exists.

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