

Epidemiology Surveillance on Bovine Viral Diarrhea Virus and Persistently Infected Animals of Cattle and Buffaloes in Egypt

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Abstract: A total number of 1698 and 1602 animals (cattle and buffaloes) was screened for detection of BVDV infection and persistently infected (PI) cases from the Fayoum and Alexandria governorates respectively. They were investigated using commercial antigen capture enzyme-linked immunosorbent assay (ACE), immunohistochemistry (IHC), virus isolation (VI) and real time-polymerase chain reaction (RT-PCR). Blood samples were collected on anticoagulant for ACE, RT-PCR and VI. Ear notch specimens were collected for ACE and IHC. The percentage of BVDV infected cases was 6.7% and 1.6% PI in Fayoum farms and 8.4% BVDV infection and 1.4% PI in Alexandria. The majority of PI was detected in calves less than 12 months of age. A good correlation was found between RT-PCR, ACE and IHC as diagnostic tests for detection of BVDV infection but RT-PCR was the most sensitive. Epidemiological mapping of the prevalence of BVDV infection and PI in all districts of Fayoum and Alexandria governorates was presented.

Key words: Bovine Viral Diarrhea Virus • Persistent Infected • Egypt • Surveillance • Cattle • Buffaloes • Diagnostic Techniques • Epidemiology Maps

INTRODUCTION

Bovine viral diarrhea virus, with a worldwide distribution, is one of the most important pathogens of cattle [1]. Additionally, this virus can also cause infection in sheep, goats, camels, swine and some wild ruminants [2-7]. Bovine viral diarrhea virus (BVDV) is a member of the family Flaviviridae, genus pestivirus [8] that crosses the placenta in pregnant cows which are infected between days 40 and 125 of the gestation period causing a variety of disease syndromes, including reproductive losses due to abortion, stillborn calves or calves that die early in life and birth of persistently infected (PI) calves. The virus may also cause transient infection with pneumo-enteritis disease syndrome depending upon the stage of gestation (more than 5 months of pregnancy) and the type of infecting viral strains.

Several diagnostic methods are available for the detection of antigen, antibody and viral components (antigen and nucleic acids) of BVDV. These include virus isolation from blood or tissues, immunohistochemical staining of skin samples, reverse transcriptase-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) for detection of antigen or antibody [9-13]. In Egypt, BVD virus was first isolated in 1972 from calves suffering from severe enteritis [14] and since then, the disease continues to be recorded not only in cattle and buffaloes but also in other species of animals including sheep, goats and camels in several provinces of Egypt [15-22].

The majority of herd diagnosis for the detection of BVDV infection is often based indirectly on an interpretation of serological tests of animal screenings.

Although there are some reports on BVDV infection and genotyping of the isolated viruses in Egypt, still there is no appropriate data on surveillance and percentage of infection or PI because those reports are often based on selected cases. This makes it difficult to estimate the real percentage of the disease in cattle and buffaloes.

The present study was designed to investigate the epidemiological surveillance of BVDV infection and persistently infected animals of cattle and buffaloes in two governorates of Egypt (Fayoum and Alexandria) as part of our future plan for a national surveillance program throughout the country, which will aid in controlling the disease. This investigation was accomplished using virological, molecular and immunohistochemical diagnostic techniques.

MATERIALS AND METHODS

Animals and Samples: A total number of 1698 and 1602 animals (cattle and buffaloes) from Fayoum and Alexandria farms and villages was subjected for BVDV infection screening from 2011- 2012 with age of the calves ranging from 1 day to 18 months and the dams from 2- 5 years.

Blood samples were collected on EDTA as an anticoagulant for separation of Buffy coat to be used for BVDV Antigen (Ag) detection by Antigen Capture –ELISA (ACE) and real- time PCR (RT- PCR). Ear notch specimens were collected using an ear-notch puncher with half of it fixed in 10% neutral formalin for immunohistochemistry (IHC) and the other half kept in a clean sterile tube for antigen detection of BVDV by ACE. The number, species and percentage of sampling are presented in Tables (1 & 2).

BVDV Ag Detection By Antigen Capture ELISA (ACE): Antigen detection of BVDV was performed on blood (Buffy coat) or on ear notches using the commercial kits IDEXX HerdChek BVD of Ag/serum plus ELISA test or using BVDV antigen test kit/leukocytes according to the manufacture’s instruction.

Virus Isolation: All blood samples that detected positive by RT-PCR and the prepared ear notch tissues were inoculated in a Madin Darby Bovine Kidney (MDBK) Cell line as described by Cortese *et al.* [23]. The inoculated cells were observed for 7 days for any cytopathic effects (CPE). Three blind passages were applied if no CPE was detected. Indirect fluorescent antibody technique (IFAT) was applied on fixed tissue culture according to the method described by Majewska *et al.* [24] to identify the isolated virus as a cytopathic(CP) or non cytopathic (NCP) BVD virus.

Detection of Persistent Infected Animals (PI): All identified BVDV cases that were detected through the screening program of both the Fayoum and Alexandria farms were retested after 3-4 weeks from the initial screening.

BVDV Detection by Real-Time PCR (RT- PCR): Real - time PCR was used for detection of the virus on pooled 5 samples. The positive pooled samples were retested individually. RNAs were extracted from the suspected materials using the QiAmp Viral RNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. A set of primers was used for the RT-PCR reaction using forward and reverse PCR primers of BVD targeting the 5- untranslated region UTR

Table 1: Total number of investigated animals in Fayoum governorate

Species	Dams			Calves		
	Total # of cows	Total # of investigated cows	Tested%	Total # of calves	Total # of investigated calves	Tested%
Holstein	4923	1001	20.33	1324	519	39.1
Buffaloes	530	57	10.75	99	71	71.7
Native Breed	30	30	100	20	20	100
Total	5483	1088		1443	610	

Table 2: Total number of investigated animals in Alexandria governorate

Species	Dams			Calves		
	Total # of cows	Total # of investigated cows	Tested%	Total # of calves	Total # of investigated calves	Tested%
Holstein	5303	867	16.34	2113	433	20.49
Buffaloes	750	143	19.06	206	101	40.03
Native breed	35	35	100	23	23	100
Total	6153	1045		2342	557	

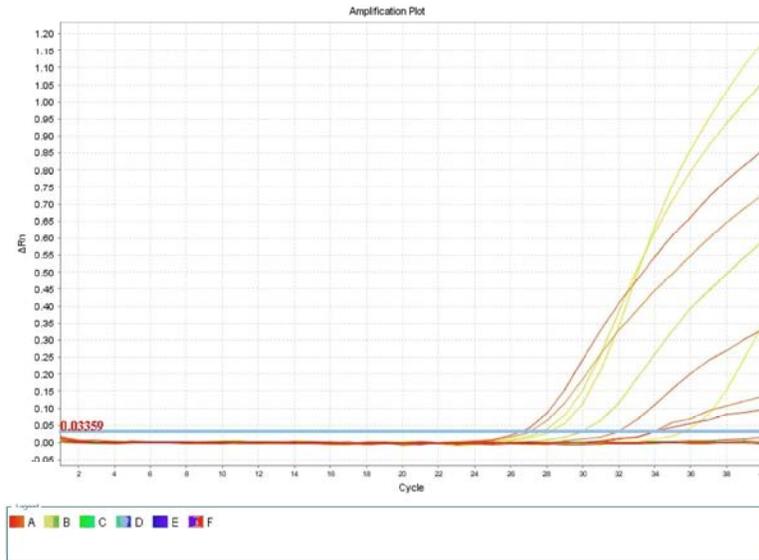


Fig. 1: Representative of the amplification plots of the RT-PCR for BVD indicating ct values for 8 samples curve from left to right (positive control 20, sample 1= 20.2, sample 2= 20.3, sample 3= 20.7. sample 4=20.8, sample 5= 33.4, sample 6, 7 & 8 and a negative control have no ct).



Fig. 2: Map of Egypt showing the location of the Fayoum and Alexandria governorates

Forward primer: 5'-GGGNAGTCGTCARTGGTTCG-3'

Probe 5'-FAM-CCAYGTGGACGAGGGCAYGC-BHQ-1-3'

Reverse primer: 5'-GTCCATGTACAGCAGAGWTTTT-3'

RT- PCR reaction was carried out using Real- time PCR kit (Quantitect Probe RT-PCR Kit, Qiagen, Germany). The thermal profile was as follows; RT- step 1 cycle at 50 C/ 30min. for reverse transcription, 1 cycle of 95 C/ 19 min. for Taq activation followed by 40 cycles of 95 C/15 sec, 55 C/ 30 sec and 72C/30 sec. The test was carried out on real-time PCR machine (Shatagene®) MXpro- 30005p.

BVDV Detection by Immunohistochemistry (IHC):

Specimens from ear notches were dehydrated, cleared, embedded in paraffin and sectioned. Two different immunohistochemical systems were used, each according to their manufacture manual using a paraffin section on coated slides (LSAB + System HRP (Dako) and super sensitive polymer- -HRP IHC detection system (Dako).

Epidemiological Mapping

Materials: Software: ESRI GIS (Geographic Information System 10).

Egypt maps from the Central Agency for Public Mobilization and Statics (CAMPAS): administrative boundaries for the governorates of Fayoum and Alexandria and for its district.

Methods: The data results of Bovine Viral Diarrhea cases and the locations of the visited farms and villages were obtained from the team of the present investigation and geographically referenced by Google earth to obtain the coordinates for the sampled farms. Subsequently, the farm sites were projected as points on the administrative map layers for the Alexandria and Fayoum governorates. Within each governorate (according to the obtained results) the proportion of antigen detection and persistent infected animals was calculated for each district a special tool was then applied for the numbers and proportion of each type of the tests applied to the samples.

Statistical Analysis: The obtained data were statistically analyzed using the Fischer Exact Probability test. Correlations between different BVDV diagnostic tests were analyzed (Tables 5 & 6) using the Pearson correlation procedure of IBM-SPSS 20 [25].

RESULTS

The percentage of positive BVDV antigen in blood and ear notches using ACE, IHC and RT-PCR are presented in Tables (3 & 4).

A total of 519 Holstein calves from Fayoum farms were tested for the presence of BVDV antigen in blood and ear notches. The result of blood testing indicated that 9.2% and 12.7% were positive by ACE and by RT-PCR respectively. Examination of ear notches revealed that 9.2% and 8% were positive by ACE and IHC respectively. Examination of 1001 Holstein cows demonstrated a lower percentage of infection by ACE, RT-PCR and IHC (3.1%, 3.3% and 2.8%). The number of examined buffaloes at Fayoum farms was 128 (71 calves and 57 dams). The calves showed a rate of 11.2% positive

BVDV antigen by both ACE and IHC and a rate of 12.7% by RT-PCR. Meanwhile the examination of the dams indicated a 1.7% positive rate (one case only) by ACE and RT-PCR. IHC didn't detect that case.

In Alexandria farms and villages 433 Holstein calves and 867 cows were tested for the presence of BVDV antigen in blood and ear notches in the same manner as in the Fayoum farms. The percentage of BVDV positive calves was 11.5%, 14% and 9% by ACE, RT-PCR and IHC respectively. Testing the cows showed BVDV positive in 3.6%, 4.7% and 3.4% by ACE, RT-PCR and IHC respectively. A total of 101 calves and 143 dams' buffaloes of the Alexandria governorate showed a positive BVDV percentage of 13.9%, 14.8% and 8.9% for calves and 6.3%, 9% and 5.6% for dams by ACE, RT-PCR and IHC respectively.

Statistical analysis of the present data showed that there is a significant difference between RT-PCR diagnostic tests compared to the other used techniques (ACE and IHC) in both governorates (Tables 3 & 4).

Table 3: BVDV antigen detection in Fayoum by various methods

Species	Number of Examined animals	Types of Test and samples					
		Positive ELISA (Buffy Coats or Ear notches)		Positive RT-PCR (Buffy Coats)		Positive IHC (Ear Notches)	
		No.	%	No.	%	No.	%
Holstein calves	519	48	9.2	66	12.7	42	8
Friesian cows	1001	32	3.1	34	3.3	28	2.8
Buffaloes Calves	71	8	11.2	9	12.7	8	11.2
Buffaloes dams	57	1	1.7	1	1.7	0	0
Native breed calves	20	2	10	4	20	2	10
Native breed cows	30	0	0	0	0	0	0
Total	1698	91	5.4 b	114	6.7 a	80	4.7 b

Insignificant difference between similar letter (a or b) using Fischer Exact Probability test.

Table 4: BVDV antigen detection in Alexandria by various methods:

Species	Number of examined animals	Types of Test and samples					
		Positive ELISA Buffy Coats or Ear notches		Positive RT-PCR (Buffy Coats)		Positive IHC (Ear Notches)	
		No.	%	No.	%	No.	%
Holstein calves	433	50	11.5	61	14	39	9
Friesian cows	867	31	3.6	41	4.7	30	3.4
Buffalo Calves	101	14	13.9	15	14.8	9	8.9
Buffalo dams	143	9	6.3	13	9	8	5.6
Native breed calves	23	2	8.7	3	13	1	4.3
Native breed	35	1	2.8	2	5.7	1	2.8
Total	1602	107	6.7 b	135	8.4 a	88	5.5 b

Insignificant difference between similar letter (a or b) using Fischer Exact Probability test.

Table 5: (Fayoum farms) Pearson Correlations

		RT-PCR	ELIZA	IHC
RT-PCR	Pearson Correlation	1	0.887**	0.829**
	Sig. (2-tailed)		0.000	0.000
	N	1698	1698	1698
ELIZA	Pearson Correlation	0.887**	1	0.934**
	Sig. (2-tailed)	0.000		0.000
	N	1698	1698	1698
IHC	Pearson Correlation	0.829**	0.934**	1
	Sig. (2-tailed)	0.000	0.000	
	N	1671	1698	1698

** Correlation is significant at the 0.01 level (2-tailed)

Table 6: (Alexandria farms) Pearson Correlation

		RT-PCR	ELIZA	IHC
RT-PCR	Pearson Correlation	1	0.882**	0.795**
	Sig. (2-tailed)		0.000	0.000
	N	1602	1602	1602
ELIZA	Pearson Correlation	0.882**	1	0.901**
	Sig. (2-tailed)	0.000		0.000
	N	1602	1602	1602
IHC	Pearson Correlation	0.795**	0.901**	1
	Sig. (2-tailed)	0.000	0.000	
	N	1602	1602	1602

** Correlation is significant at 0.01 level (2-tailed).

Table 7: Detection of PI calves after 21-30 days from the first visit of Fayoum & Alexandria investigated animals

Governorate	Total numbers of specimens	BVDV positive (RT-PCR)	
		1 st Visit Positive (Acute & PI)	2 nd Visit Positive (PI)
Fayoum	1698	114 (6.7%)	27 (1.6%)
Alexandria	1602	135 (8.4%)	23 (1.4%)

Table 8: BVDV virus isolation and identification by IFAT from blood:

Governorate	Samples	BVDV positive RT-PCR	Effect on T.C.		Positive IFAT		Total Isolates
			CPE	NCPE	CPS	NCPS	
Fayoum	Blood	114	11	103	11	29	40
Alexandria	Blood	135	11	124	11	28	39

T.C: Tissue culture IFAT: Immunofluorescence Antigen Technique

CPE: Cytopathic effect NCPE: Non-cytopathic effect

CPS: Cytopathic Strain NCPS: Non-cytopathic strain

Correlation between testing methods (Tables 5 & 6): Pearson correlation coefficients showed that the correlations between ELISA and IHC and the results of RT-PCR were significant at $P < 0.01$ (0.887 and 0.829 for Fayoum and 0.882 and 0.795 for Alexandria) and at $P < 0.01$ between ELISA and IHC (0.934 for Fayoum and 0.901 for Alexandria).

The prevalence of PI animals is demonstrated in Table (7). The RT-PCR Antigen detection test revealed that 117 out of 1698 animals in the Fayoum farms (6.8%) and 135 out of 1602 in the Alexandria farms (8.4%) were positive for BVDV antigen in the initial screening. Twenty-seven animals (1.6%) in Fayoum and 23 (1.4%) in

Alexandria remained positive after 21-30 days on the 2nd visit. The majority of PI cases were less than 12 months of age. Those calves are considered persistently infected animals. The others, which were negative for BVDV antigen after 3-4 weeks of the first visit, were transiently infected with the virus being eliminated thereafter.

Virus Isolation: As presented in Table (8), the results of BVD virus isolation from blood showed that a total of 40 BVD isolates (11 CPS and 29 NCPS) were isolated from animals of the Fayoum governorate and 35 BVD isolates (11 CPS and 28 NCPS) from animals of the Alexandria governorate.

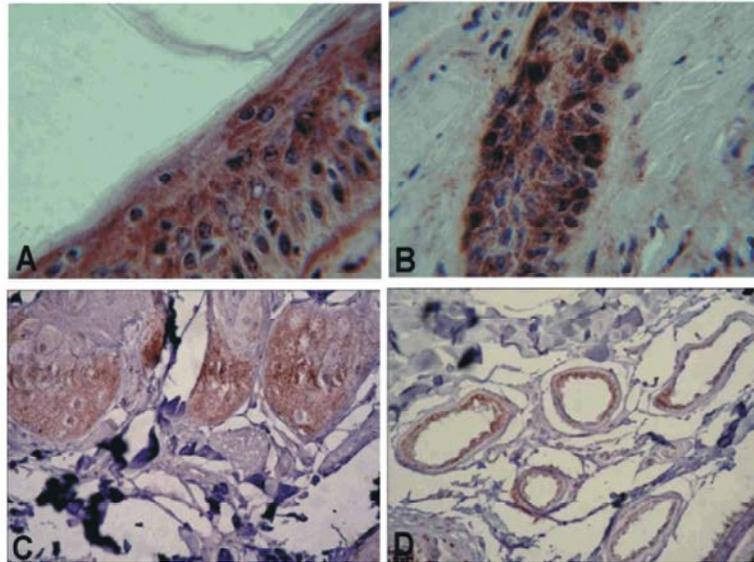


Fig. 3: Ear- notch showing specific brown immuno-staining of bovine viral diarrhea (BVD) antigen: A) within the cytoplasm of the epidermal cell layer. B) Inside the epithelial cells lining the hair follicles. C) In sebaceous glands, D) In the endothelial lining of sub-epidermal vasculature

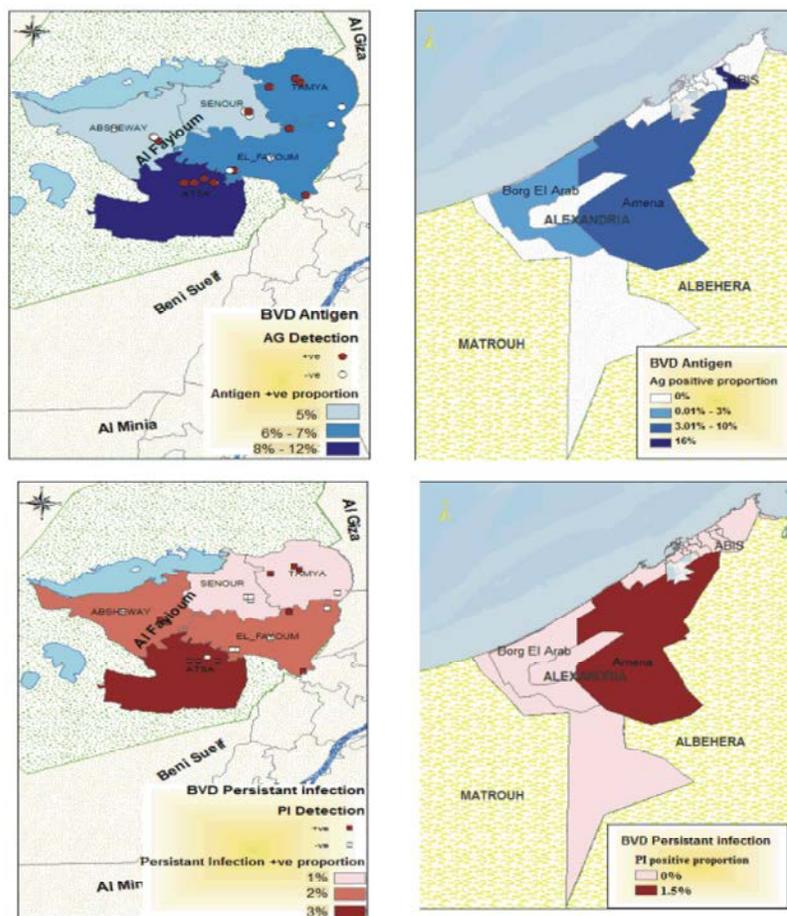


Fig. 4: Epidemiological Maps the percentage of BVDV Antigen and PI detection in Fayoum (left, upper & lower) and in Alexandria (right, upper & lower)

The results of the immunohistochemical staining technique of ear notches of all animals of Fayoum and Alexandria are presented in Tables (3 & 4). Microscopically, ear notches revealed specific immunopositive staining of the BVD viral antigen, which appeared as a golden brown granular color within the cytoplasm of the epithelial lining of the epidermal layer and that lining the hair follicles and even within the keratinocytes. Also, immunopositive staining of the BVD antigen was seen within the dendritic cells. Furthermore, specific immunoreactions were detected within the endothelial cell layer lining the sub-epidermal vasculature and within the sebaceous glands.

The Fayoum epidemiological map (Figure 4) showed that the percentage of BVDV antigen in various sectors of Fayoum varied from 5% (Absheway and senours), to 6-7% (Tamyia and EL-Fayoum) and 8-12% (Atsa) with an average of 6.8%, while the percentage of PI varied from 1% (Tamyia and senours), to 2% (Absheway and EL-Fayoum) and 3% (Atsa) with an average of 1.6%. The Alexandria epidemiological map showed that the percentage of BVDV antigen also varied from 4- 16% with an average of 8.4% in various sectors of Alexandria, while the percentage of PI averaged 1.4%.

DISCUSSION

Our investigations included screening of 2820 Friesian cattle (1868 cows and 942 calves), 372 buffaloes (200 dams and 142 calves) and 108 native breed cattle (65 cows and 43 calves) for BVDV infection in Fayoum and Alexandria farms using various antigen detection methods. Buffy coat specimens were tested using ACE and RT-PCR while ear notches were tested using ELISA and IHC.

The percentage of positive BVDV infected cases varied with the various methods and locations with an average range of 8 – 14% in Holstein calves, 8.9-14.8% in buffalo's calves and 8.7- 30% in native breed calves. However, dams of Holstein cows, buffaloes and native breeds showed a lower percentage of BVDV infection, 2.8-4.7%, 1.7-9% and 0- 5.7% respectively (Tables 3 & 4).

The results of the present work demonstrated that the RT- PCR technique is more sensitive than the ACE detection test on blood samples with BVDV antigen detected in 6.8% and 8.4% with the RT-PCR technique versus 5.4% and 6.7% by the ACE test in Fayoum and Alexandria respectively.

Meanwhile, detection of BVDV antigen in the ear notch samples using the ACE test was similar to the ACE detection test on buffy coat samples (100% compatibility).

Previous reports have recorded that the ACE test is accurate and effective for detecting BVDV- infected animals from both Buffy coat and ear notch samples [26, 27]. However in the current work, 23 and 28 cases among investigated animals were considered false negative by ACE (114 and 135 cases were detected BVDV positive by RT-PCR versus 91 and 107 by ACE) in Fayoum and Alexandria respectively. In the same manner, IHC failed to detect several cases. This result was explained by Goyal and Ridpath [13] who reported that in neonatal calves, the presence of maternal antibodies could neutralize BVDV, therefore interfering with ACE and IHC BVDV detection and demonstrating as false negative. However other authors reported a minor influence of colostrum antibodies on BVDV Ag detection from ear notch tissues or blood samples [28, 29]. Other researchers reported that IHC application on ear notch samples can detect most of PI and is not affected by the presence of maternal antibodies [11, 30-32].

Although RT- PCR is more sensitive, specific and less time-consuming than other methods for detecting BVDV especially in pooled samples, it has not been widely applied in the routine diagnosis of most animal diseases in developing countries due to the high cost of performing it. Large, pooled samples reduce the test cost but may reduce the sensitivity. In the present work, we used pooled samples of 5-specimen pools and it showed a fairly good sensitivity for BVDV antigen detection. In a study by Ridpath *et al.* [33], they recorded that pooling RT-PCR in 10- specimen pools may decrease sensitivity to the point of failure to detect 10% of positive specimens and that 100-specimen pools might result in failure to detect over 50% of PI-BVDV positive specimens. However Weinstock *et al.* [34] were able to detect the presence of viral RNA when a single viremic sample was pooled with 100 negative sera.

PI animals are considered the main source of spreading BVDV infection among herds. Therefore, control of the disease mostly relies on the accurate detection and removal of PI animals from herds [35, 36]. In the current work, all positive cases by RT-PCR (114 in Fayoum and 135 in Alexandria) were retested 3-4 weeks after the initial testing for detection of PI animals. The majority of PI animals were detected in calves less than 12 months of age at a rate of 3.4% and 2.9% in Holstein

and buffalo calves. On the other hand, Holstein cows and buffalo dams showed a lower rate of 0.5-1.7% PI cases (table 7). Similar findings were reported by Meiring *et al.* [37] who recorded that the prevalence of persistently infected cattle from samples of six large South African feedlots was found to be 2.9%. Houe [38] reported a prevalence of PI infected cattle of approximately 0.5% to 2.0% in different countries in the 1980s and 1990s. Bolin *et al.* [39] reported a 1.7% prevalence of PI in a non random population of 66 herds in the USA. On the other hand Helal *et al.* [40] reported a 7.0% prevalence of PI animals with BVDV in a dairy herd (50 cows and 40 calves) but they couldn't explain the cause of this high prevalence.

Prevalence studies have become very popular with studies focusing on dairy and beef cattle throughout the world. In a study by Wittum *et al.* [41] who reported that the presence of one or more PI animals within a cattle herd is the single most important way to maintain BVDV infection. In our study, we have found that the more percentage of PI in some districts, the higher the rate of BVDV infection. It was clear in the Fayoum map that the Atsa district has a 3.0% PI and a 8-12% BVDV positive antigen rate which was more than the Absheaway, Senours and Tamyia districts (Plate II).

Native breed cattle were not common among the farms, which we visited. 50 and 58 native breed cattle from Fayoum and Alexandria farms respectively were investigated. Future testing of more native breed cattle is needed to be able to get an estimation of BVDV infection among them.

Virus isolation of BVDV in cell cultures followed by identification of the viral isolates by IFAT is the most reliable method for the detection of BVDV infection [42, 43]. In the present work, virus isolation and IFAT were performed on blood samples collecting from animals that were positive by RT-PCR. Virus was isolated from 35% out of 114 and 29% out of 135 positive cases from Fayoum and Alexandria respectively (Table 8). The failure of virus isolation in two thirds of the positive cases which were detected by RT-PCR or ACE might be due to the presence of anti-BVDV antibodies which interfere with virus isolation from buffy coat samples as reported by Goyal and Ridpath [21]. A study by Zimmer *et al.* [44] who investigated the effect of maternal antibodies on the detection of BVDV in peripheral blood samples after intrauterine experimental infection of calves. They concluded that maternal antibodies did not affect the RT-PCR, but virus isolation and the antigen ELISA

was often false negative because of interference by maternal antibodies (the higher the maternal antibody titer, the lower the frequency of positive virus isolation).

The majority of the isolated viruses were NCPS (57 strains) from Fayoum and Alexandria versus 22 CPS. BVDV genotypes have been classified into BVDV1 strains and BVDV2 strains which exist as one of two biotypes; cytopathic and non-cytopathic [13, 45, 46]. The division into biotypes is based on the activity of the strain when propagated in tissue culture cells. Only NCPS BVDV strains have been reported to establish persistent infection. This has led to some speculation that CPS BVDV are not able to cross the placenta and thus do not infect the fetus. NCPS BVDV strains predominate over CPS BVDV in nature. The NCP biotype has a particular tropism for the gut-associated lymphoid tissues and immunohistochemical study has shown that BVDV antigen has been detected in Peyer's patches. The tissue distribution of BVDV antigen was widespread in individual cattle with all clinical forms of BVDV infection easily demonstrated in the gastrointestinal tract, lymphoid tissue, lung and eye [47].

IHC application on ear notches detected 4.7% and 5.8% positive cases versus 5.4% and 6.7% by ELISA from Fayoum and Alexandria breeding farms in the present study. The percentage of IHC - BVDV positive cases (transient or PI) was lower than those detected by RT-PCR or ELISA. This finding was in agreement of Hilbe *et al.* [48] who recorded that in BVDV acute infection in Switzerland, viral antigen could not be found in the epidermal structure of the skin and was demonstrated in PI cases by IHC of the skin. Recently, Ahmed *et al.* [49] added that both serum and ear notch biopsy can be used for the detection of PI and genotyping of BVDV. He also found out that ear notch biopsies had poor sensitivity for the detection of acute, transiently infected animals. On the contrary, Nadalian *et al.* [50] reported that IHC on ear notch samples was more effective for the detection of PI calves than ACE (blood samples). They demonstrated that RT-PCR (blood samples) detected 13 PI calves out of 552 Friesian calves and cows while ACE detected 6 cases and IHC detected 10 cases.

Statistical analysis of the Fayoum investigation data revealed a significant correlation between both techniques of ELISA and IHC with the RT-PCR technique at $P < 0.01$ (0.887 and 0.827) and also a highly significant correlation between ELISA with IHC (0.934) as presented in Table (5). Similar correlations were detected through analysis of the Alexandria data (Table 6).

Reports of previous studies on BVDV infection in cattle, buffaloes, sheep and camels in Egypt have shown that the prevalence of positive cases with BVDV antibodies was high and that BVDV was widespread in the country, causing serious economic losses [14, 17, 19, 22, 51-54].

Further work needs to be done on all governorates of Egypt to be able to get an actual estimation for the prevalence of BVDV infection and its impact on the animal wealth and to assist in a management and planning strategy for controlling the disease and eliminating persistently infected animals.

CONCLUSION

The present study estimated the prevalence of BVDV infections and persistently infected animals in two governorates of Egypt. The results provide a general picture of the incidence of the disease using various diagnostic assays, which is necessary for the planning of future screenings in all other governorates with cost effective laboratory techniques as well as for planning a national management program with collaboration from the General Veterinary Service Authority and cattle producers.

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