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A study on the role of Bovine Viral Diarrhea Virus (BVDV) and C. perffringens as causative agents of cattle diarrhea in Giza Governorate

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Abstract
In this study, a total of 190 samples (60 feacal swabes, 15 buffy coat, 15 nasal swabes, 50 milk and 50 serum samples) were collected from diarrhoeic cattle, from some farms in Giza Governorate (8-18 months old). They were examined for prevalence of Bovine Viral Diarrhea Virus (BVDV) and C.perfringens. The results of BVDV antigen detection using Agar gel immunodiffusion test (AGPT) revealed a number (9 and 5) positive samples from the fecal swabs and buffy coat respectively. Isolation of the virus from the collected samples firstly done by inoculation onto Median Darby Bovine kidney cells (MDBK) for one passage, then detection of the viral antigen by the indirect immunoperoxidase technique (IPI). The results showed that (14, 8 and 2) samples were positive from fecal swabs, buffy coat and nasal swabs respectively with showing intracytoplasmic red brown colour in the infected cells. BVDV isolation was completed for the positive samples with both (AGPT and IPI) by applying second and third passage on MDBK cell culture then its identification with the IPI technique. The percentage of agreement between (AGPT and IPI) was 88.9%. Percentage of sensitivity of the AGPT test in comparison with IPI was 58.3% while Percentage of specificity was 100%. Results of detection of antibodies againt BVDV using blocking ELISA, revealed number of (33 and 34) positive samples in the serum and milk samples respectively. C.perfringens was isolated from 35 fecal and 15 milk samples by a percentage (58.3 and 30%) respectively. Typing of C.perfringens isolates revealed that type A was more prevalent than type D in a percentage (65.7 and 20%) and (60 and 26.7%) for fecal and milk samples respectively .Antibiogram sensitivity of C.perfringens isolates to different antibiotics was studied. Statitical analysis of the results and the correlation between BVDV and C.perfringens in causing cattle diarrhea were recorded.

Key words: *(BVDV) isolation and identification , C. perffringens isolation, Typing*

Introduction

Diarrhea syndrome is one of the most common causes of serious economic losses, as it may lead to calf mortality, weight loss or even late growth. It has complex etiological agents (infective agents, environmental factors, nutritional factors and immunological factor). The infective agents may

Bovine Viral Diarrhea (BVD) is affecting calves during the first year of life, but reinfection can occur at any age **(OIE, 2008).** It is an important respiratory, gastrointestinal, ocular, and reproductive tract disease complex of dairy and beef cattle. Calves infected with BVD in utero may be aborted, born malformed or born apparently normal, and calves infected as neonates, may has diarrhea, respiratory or neurologic signs. Calves infected from 60 to 120 days of gestation may become persistently infected (PI) animals. Such PI animals are often clinically normal, may survive to adulthood, shed large amounts of BVD virus from body secretions throughout their lives **(Erol and Rustem, 2011).**

BVDV is a member of the genus Pestivirus, a group of small-enveloped RNA viruses in the family Flaviviridae. It is an enveloped virus with single-stranded RNA .Two genotypes of BVDV (BVDV-1 and BVDV-2) have been recognised based on serological and genetic relatedness and they can exhibit two different biotypes namely, non-cytopathic (ncp) and cytopathic (cp), according to their lytic effect in infected cells **(Yakup *et al.,* 2011).**

The main source of BVDV infection is the (PI) animals that shed virus in the environment, but other infected species can also transmit it by direct contact. Transmission by indirect contact may happen through the use of contaminated equipment or insemination with BVDV infected semen **(Fulton *et al.,* 2005)**.

BVDV has immunosuppressive effect, mainly due to the strong affinity of the virus for immunocompetent cells, which may be destroyed or functionally impaired, so predisposes the animal to other diseases caused by viral or bacterial pathogens **(Potgieter 1995 and Bagge, 2012).**

In Egypt BVDV-MD complex was considered as endemic disease before 1960 **(Baz, 1992). Hafez, (1972)** isolated BVDV for first time from calf suffering from pneumoenteritis syndrome and the virus designated as IMAN-7912.The virus was also isolated from cattle have the same syndrome by **(Baz, 1975).** BVD disease becomes widely distributed in Africa, and the Middle East including Egypt **(Hassanein, 2003).**

BVD is a disease of great importance to dairy and beef producers. The broad natures of the disease, transmission, and the lack of treatment, have made BVD a global pandemic, and need accurate diagnosis *(***William, 2012).** Numerous test strategies are used around the world for BVDV diagnosis, including viral antigen detection ELISAs, virus isolation, virus identification by IP, IFR, and IHC. Detection of virus specific antibodies by using different serological tests as virus neutralization test and enzyme linked immunosorbent assays are important ways for the virus diagnosis **(OIE, 2008 and David *et al.,* 2012).**

An attempt has been made to gather current relevant clinical informations related to the etiology of calf entritis especially that of bacterial origin. Different clostridia species cause intestinal disorders and enterotoxaemia in various animals' species particularly young calves. Enterotoxaemia development is peracute in most cases and the animal can die suddenly while grazing in the pasture, therefore treatment is possible only in very rare cases and the use of the immune-prophylactic measures is preferable to combact the disease (**Songer, 1996).** Strains of type A are the widest spread in intestine of worm blooded animals had been isolated from calves with tympany and intestinal ulceration **(Collec *et al.,* 1996).**

C. perfringens is a gram positive anaerobic spore forming bacterium, classified into types A, B, C and D. This bacterial species can produce up to 12 different toxins, four of which (alpha, beta, epsilone and iota) are responsible for the tissue lesions and the death of hosts. C. perfringens is an ubiquitous bacterium, infection occurs via food, water, animal litter or soil **(Mona and Mervat, 2006).**

Aim of this work

- Investigation and throw light on the association of viral (BVDV) and bacterial (C. perfringens) infective agents in diarrhoeic cattle.
- The use of the immunoperoxidase test as a sensitive technique to identify BVD viral antigen in the isolates.
- Estimate the prevalence of BVDV antibodies in the milk and serum of cattle as it is important in BVD eradication and the immune status evaluation.

Materials and Methods.

Samples:

A total of 190 samples were collected from diarrhoeic cattle (8-18 months old), from some cattle farms, in Giza Governorates.These samples included:

Swabes:

60 fecal and 15 nasal swabes **(Table 1),** were collected and prepared according to (**OIE,**

2008) for BVDV isolation**.**

Blood samples:

15 Buffy coats for BVDV virus isolation and 50 serum samples for BVDV antibodies detection from diarrhoeic cattle **(Table 1)**. The samples were prepared according to **(Abdel-Azeem *et al.*, 2013).**

Milk samples:

50 milk samples (20 samples from mastitic cattle), centrifuged for 10 minutes at 3000 xg at 4°C in cooling centrifug, according to **(Niskanen *et al* 1991), s**kimed milk was collected**.**

BVDV antiserum:

BVDV polyclonal antiserum, obtained from (National Veterinary Service Laboratory (NVSL) Ames, Iowa, USA.), kindly supplied from the ELISA Research Unit and Viral Strains Bank, Animal Health Research Institute (AHRI), Agriculture Research Center.

BVDV (NADL strain):

It was obtained from Veterinary Serum and Vaccine Research Institute, Abbassia. It was used for applying AGPT and IPI.

Anti-bovine immunoperoxidase conjugate:

Anti-bovine immunoperoxidase conjugate, supplied by (ELISA Research Unit and Viral Strains Bank), Animal Health Research Institute. It was used for applying IPI for detection of BVDV.

Cell culture:

Median Darby Bovine kidney cells (MDBK) free from non-cytopathic strains of BVDV obtained from virology department Animal Health Research Institute (AHRI), grown in Eagles MEM supplemented with 10% fetal calf serum and used for applying virus isolation **(Clark *et al.*, 1984).**

Table (1). Different number of samples collected from cattle at Giza Governorate.

Samples / Governorate	Number of samples					Total
	Swabes		Buffy coats	Seum samples	Milk samples	
	Fecal	Nasal				
Giza	60	15	15	50	50	190

A-Virological examination.

Detection of BVDV antigen using Immunodiffusion technique.

Agar gel diffusion technique is used to detect BVDV antigen in the Fecal, nasal swabes and buffy coats, according to **(Hosny *et al.*, 1996).**

Virus isolation and immunoperoxidase identification.

BVDV isolation and immunoperoxidase identification was done according to **(Katz, *et al.*, 1987),** by inoculation of the prepared fecal, nasal swabs and buffy coat onto Madin-Darby Bovine Kidney (MDBK) cell culture in 24 wells tissue culture plates**.** The plates were incubated at 37c^{o} for five days with daily microscopic observations. After 5 days the culture were fixed with a fixation buffer (PBS, pH 7.6 containing 36% acetone), then in 0.5% H_2O_2 for detection of BVDV antigen by indirect immunoperoxidase staining (IPI). The cells were washed with PBS (pH 7.6) and reacted sequentially with anti-BVDV polyclonal antiserum diluted to 1:100 in binding buffer (PBS, pH 7.6 containing 0.02% Tween-20 and 3% NaCl) for 1h at room temperature. MDBK cells were washed with PBS (pH 7.6), reacted to ati- bovine peroxidase conjugate, diluted in binding buffer to 1:1,000 for 30 min at room temperature and washed with PBS (pH 7.6). Finally, freshly prepared substrate solution (diaminobenzidine solution) was added to cells. A red-brown stain appeared within 5 to 15 min in the cytoplasm of infected cells. A lack of staining in infected cells was considered to indicate a negative result.

After the first passage in MDBK cells, all samples with positive reaction to IPI and AGPT

were inoculated for the second and third passage in MDBK cells with applying the IPI test isolate identification.

Detection of BVDV antibodies in the milk and serum samples by ELISA technique.

A commercial ELISA kit **(Cypress Diagnostics)** for detection of antibodies against BVDV in serum, plasma and milk in cattle. This assay is a blocking ELISA. 50 serum and 50 milk samples were tested by this kit according to the instructions. The plates are ready coated with monoclonal antibodies against BVDV. The test is depending on two monoclonal antibodies-one is coated in the plate while the other is in the conjugate and both of them against NS-3 (P80) of BVDV. When we put the samples (contain antibodies against BVDV), it will form antigen-antibody complex, which will washed away through washing step, so the conjugate will found nothing to catch and the positive sample give no colour with the substrate. In the negative sample there are no antibodies against BVDV so the antigen will form Ag-Ab complex and the conjugate will catch them giveing colour with the substrate **(Talebkhan *et al.,* 2008).**

The reading was done at 450 nm and the percentage of inhibition (PI) is calculated as follow:

PI of sample =100 - (corrected OD of tested sample/corrected OD of max) ×100

Validation of the test:

PI of positive serum sample must be ≥50% while the negative one <50%.

PI of positive milk sample must be ≥30% while the negative one <30%.

Statistical analysis

The percentage of agreement, sensitivity, specificity and Chi-square value were calculated according to **Bailey (1995).**

B-Bacteriological examination.

Isolation and identification of C. perfringens.

Each swab from fecal and milk samples was inoculated into tubes of freshly prepared cooked meat broth and incubated anaerobically at 37 ºC for 48 hours. A loopful from each tube was streaked onto neomycin sulfate (200 µg/ml) blood agar plates. All plates are examined after anaerobic incubation at 37 ºC for 24 hours. The suspected growing surface colonies were purified, reinoculated into cooked meat broth for further identification which based on cultural, morphological and biochemical chrachter, according to (**Koneman *et al.,* 1992).**

Typing of C. perfringens.

Purified C. perfringens isolates were typed using the intradermal inoculation test in albino guinea pigs according to **(Stern and Batty, 1975 and Oakaly and Warrack, 1953).**

Antibiotic and sensitivity tests.

The sensitivity of C.perfringens to different antibiotics was done by the disc diffusion method, according to **(Stern and Batty, 1975 and Baily and Scott 1990).**

Correlation between BVDV and C. perfringens in the examined samples.

The correlation BVDV and C. perfringens in the tested samples was studied in detail.

Results.

A-Virological Examination:

Detection of BVDVantigen using Agar Gel Immuno-diffusion technique:

The results of BVDV antigen detection using (AGPT**)** revealed a number of (9, and 5) positive sample from the fecal swabs and buffy coat respectively **(Table 2).**

Virus isolation and detection by the immunoperoxidase technique:

Detection of BVDV antigen in the first MDBK cell culture passage by IPI technique revealed a number of (14, 8 and 2) positive samples from the fecal swabs, buffy coat and nasal swabs respectively **(Table 2)**. Red-brown stain appeared within 5 to 15 minutes in the cytoplasm of infected cells**.**

BVDV isolation after the second and third MDBK cell culture passages, revealed a number of (2 and 3) positive isolates from the fecal swabs and buffy coat respectively. Identification of the isolates by the IPI technique revealed intracytoplasmic red brown stain **(figure 1a and b).**

Table (2). Results of BVDV antigen detection by AGPT and IPI technique.

Governorate	Samples		BVDVantigen detection by AGPT		BVDVantigen detection in by IPI	
	Kind	Number	Number of positive	Number of negative	Number of positive	Number of negative
El-Giza	Fecal swabes	60	9	51	14	36
	Buffy coates	15	5	10	8	7
	Nasal swabes	15	-	15	2	13
	Total	90	14	70	24	66

Table (3). The Percentage of Agreement between AGPT and IPI techniques for detection of BVDV antigen in the collected fecal, nasal swabs and buffy coat.

Test	Positive	Negative	Total	Agreement	
AGPT	14 (A)	76 (B)	90 (A+B)	+ve 14	-ve 66
IPI	24 (C)	66 (D)	90 (C+D)		
Total	38 (A+C)	142 (B+D)	180 (N)`	٨٠	

Percentage of agreement =Total No. of agreement / Total No. of examined samples × 100
Percentage of agreement = 80 / 90 × 100 =88.9%
χ^2 =N {(C×B) - (A×D)} / (A+C) × (B+D) × (A+B) ×(C+D) = 3.3

Table (4). The Percentage of Sensitivity and Specificity of AGPT in Comparison to IPI technique.

AGPT	IPI		
	Positive	Negative	Total
Positive	14 (A)	00 (B)	14
Negative	10 (C)	66 (D)	76
Total	24(A+C)	66(B+D)	90

Percentage of sensitivity = A/ A+C × 100 = 14/24× 100= 58.3%
Percentage of specificity = D/ B+D × 100 = 66/0+66=66/66 × 100 = 100%

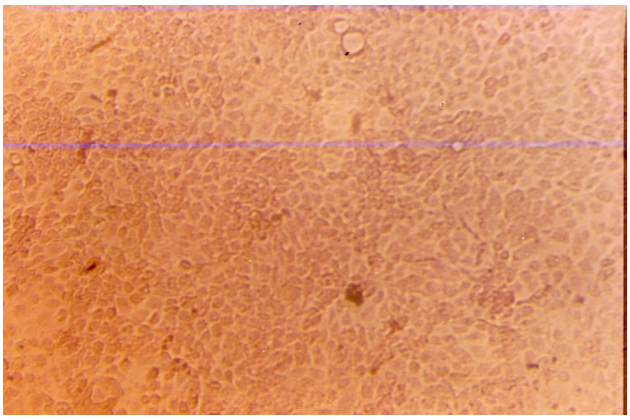

Figure (1a). Non infected MDBK cells stained by immunoperoxidase (400X).

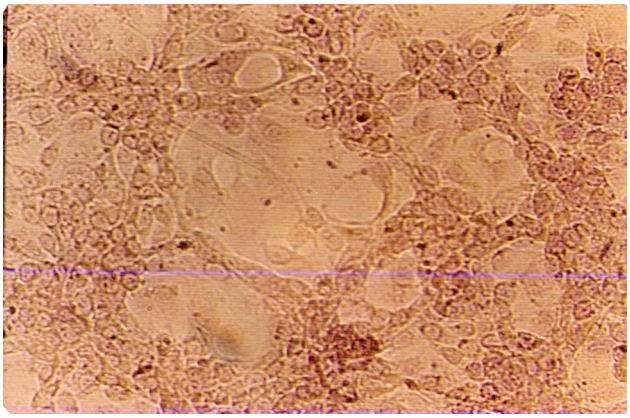

Figure (1b). Infected MDBK cells stained by immunoperoxidase, showing red brown intracytoplasmic colour (400X)

Detection of BVDV antibodies in the serum and milk samples using ELISA technique.
The results of BVDV antibodies detection in the collected serum and milk samples, from El-Giza Governorate by using Blocking ELISA, revealed a number of (33 and 34) positive samples with a percentage (66 and 68) respectively (**Table 5).**

Table (5). Detection of BVDV antibodies in the serum and milk samples using ELISA techniques.

Governorate	Type of sample					
El-Giza	Serum			Milk		
	Number of +ve	Number of -ve	% of +ve	Number of +ve	Number of -ve	% of +ve
	33	17	66	34	16	68
Total	٥٠			٥٠		

B-Bacteriological examination.
Out of 60 fecal and 50 milk samples (20 mastitic cattle), 35 (58.3%) and 15 (30%) were positive for C.perfringens **(table 6).**
A study on the typing of C.perfringens toxins by the use of neutralization test in mice and dermonecrotic reaction of guinea pigs , investigated and reveled that type A was much prevalent (65.7 and 20 %) than type D (60 and 26.7) in the fecal and milk C.perfringens isolates respectively **(table 7).**

Table (6). Prevalence of C.perfringens in fecal and milk samples.

No. and sourse of examined samples	Bacteriological examination			
	Positive cases for C.perfringens		Negative case for C.perfringens	
60 fecal samples	No.	%	No.	%
	35	58.3%	25	41.7%
50 milk samples	15	30%	35	70%

Table (7). Typing of C.perfringens isolates recovered from fecal and milk samples.

No. of C.perfringens isolates	Toxigenic isolates				Non toxigenic isolates	
	Type A		Type D			
	No.	%	No.	%	No.	%
isolates from fecal samples	23	65.7	7	20	5	14.3
solates from milk samples	9	60	4	26.7	2	13.3

Table (8). antibiogram studies of antibiotic sensitivity tests for C.perfringens from milk and fecal samples.

Antimicrobial discs	concentration	C.perfringens isolates (50)		% of sensitivity
		sensitive	resistant	
Penicillin G	10 µg	46	4	92
Clindamycin	15 µg	46	4	92
Lincomycin	15 µg	43	7	86
Tylosine	16 µg	42	8	84
Cephaletidine	30 µg	9	41	18
Erythromycine	15 µg	7	43	14
Neomycine sulphate	30 µg	-	50	0
Streptomycine	10 µg	3	47	٦
Tetracycline	30 µg	7	43	14

The % is calculated according to the total no. of C.perfringens isolates 35 +15= 50

Discussion

Diarrhea syndrome is one of the most important syndromes in cattle as It has a great etiological complexity. Diarrhea is a major form of gastroenteritis caused by differet bacterial and viral agent. This syndrome cause loss of animal health and death may occur due to enter toxicity of causative agents **(Mona and Mervat, 2006).**

BVD continues to be a disease of great importance to dairy and beef producers. The broad natures of the disease, transmission, and lack of treatment have made BVD a global pandemic, and one of the most significant cattle diseases in the world. A rapid, cheap, accurate and sensitive diagnostic tool for BVDV and its antibodies is very important to prevent, control and eradicate the persistently infected animals (**Bahgat, 2006 and William, 2012).**

Bovine Viral Diarrhea Virus (BVDV) infections are seen in all ages and breeds of cattle worldwide .The Persistently infected (PI) and acute infected animals continuously shed the virus in the milk, urine, tears, saliva, semen, fetuses, placentae, reproductive tract discharge, and in some cases feces **(Mais *et al.,* 2010 and Khawlah and Saleem, 2012)**.

Many studies applied the agar gel immunodiffusion technique for diagnosis of BVDV in cattle **(Bahgat, 2006).** In the current study the results of BVDV antigen detection using (AGPT**)** revealed a number of (9 and 5) positive sample from the fecal swabs and buffy coat respectively **(Table 2).** This result agreed with the studies reported by **(Bolin *et al.,* 1985 and Hanel, 1993**), which resulted in few positive numbers by using this technique. This can be explained on that the immunodiffusion technique is a less sensitive as it requires high titer of antibodies or antigen for detecting the precipitin line, also the samples may be need ultracentrifugation for more virus concentration **(Hopkinson *et al,* 1979).**

Detection of infected cattle and removal of the PI calf are the keys to control and eradicate BVDV. Cattle can be tested for both the presence of virus and its antibodies to detect their exposure to BVDV infection **(Herdsure protocol, 2012**). Detection of BVDV in animals is based on virus isolation and identification using immunoperoxidase and immunofluorescence assays in blood and serum samples **(Gaolatlhe, 2003).** Immunoperoxidase (IP) test is a standard method to identify BVDV PI animals and used in the BVDV control programmes (**Jaruwan *et al,* 2007)**

In the present study the results of BVDV detection in the first MDBK cell culture passage, using IPI test resulted in (14, 8 and 2) positive samples from fecal swabs, buffy coat and nasal sawbs respectively **(Table 2)**. BVDV isolation by inoculation of the positive (AGPT and IPI) samples for second and third MDBK cell culture passages, revealed a number of (2, 3) positive isolates from the fecal swabs and buffy coat respectively. Identification of the isolates by the IPI technique revealed intracytoplasmic red brown stain **(figure 1a and b).** This result agreed with the study of **(Aly *et al.,* 2003)** which recorded an outbreak of acute BVDV infection in three cattle herds in Egypt, with clinical manifestations of of enteritis by isolation of ncp BVDV biotype from the nasal swabs and buffy coats using the cell culture and IP identificationn method. Another studies stated that, the most useful sample for BVD virus isolation is the blood and the highest sensitivity can be obtained by culture from leukocytes in the buffy coat, then its IP identification **(Aykut, 2002 and Bagge, 2012).**

The percentage of agreement between AGPT and IPI tests was (88.9%) **(Table, 3)**. The percentage of sensitivity of AGPT in comparison with IPI technique was 58.3%, while the percentage of specificity was 100% **(Table, 4).** Significant difference between the two tests was detected through calculation of the chi-square (3.3). IPI proved to be more sensitive and specific technique for detection of BVDV antigen than AGPT technique which was less sensitive. This result agreed with **(Bagge, 2012).**

While blood samples are usually obtained for antibody detection, milk could be a useful as its collection is non invasive and easier than blood sampling. Milk antibody testing provides farmers an inexpensive method for investigations and monitoring the presence of common endemic infections in the herd (**Niskanen *et al.,* 1991).** ELISA for antibody detection is a fast inexpensive and simple method for screening large number of test samples or pooled samples as bulk tank milk samples (**Zadnik *et al.,* 2001).** Regarding to the results of BVDV antibodies detection in the collected serum and milk samples by using Blocking ELISA, a number of (33 and 34) positive samples in a percentage (66 and 68) were recorded respectively (**Table 5).** These results agreed with the studies of (**Alvarez *et al.,* 2012 and Adel Abdel-Azeem *et al.,* 2013),** which detect the antibodies against BVDV in bulk milk and in serum samples. Moreover, **(Wenzhi *et al.,* 2009)** use blocking ELISA for the detection of (BVDV) antibodies in cattle serum and bulk milk with high reproducibility, specificity and sensitivity. Also, **Mervat**, **(1999),** found no significant difference between serum and milk results in its study.

Enteritis is considered a major cause of economic loss. The infectious agents responsible for enteritis and enterotoxaemia are clostridium species, especially C.perfringens. Results in **(table 6),** shows that the number of positive isolates of C. perfringens from the fecal samples was 35, while that from milk of mastitic cattle was 15 by a rate of (58.3 and 30%) respectively. These results are in general agreed with that found by **(Songer 1996 and Mona and Mervat, 2006)** which stated that, C. perfringens may be the most widely distributed pathogenic bacteria and is certainly the most important cause of clostridial entric disease of domestic animals.

As shown in **(table, 7),** typing of C.perfringens isolates revealed an incidence of type A (65.7 and 20 %) which is higher than type D (60 and 26.7) in the fecal and milk samples respectively. The non toxigenic isolates were (14.3and 13.3%) for the fecal and milk samples respectively. These results are nearly similar to those obtained by **(Sumathi *et al.,* 2008).** Also studies by **(Ginter *et al.,* 1995 and Mona and Mervat, 2006)**, identified mixed culture of C.perfringens type A and type D. Farther more these results agreed with the study reported by **(Khan and Khan, 2006)** who attempted to investigate the percentage of toxigenic C.perfringens in the fecal and milk sample in diarrhoeic cattle.

Antibiogram study to detect susceptibility of C.perfringens isolates (39) revealed that Penicillin G and Clindamycin are effective against these isolates than the other amtibiotics **(table, 8)**. This result agreed with the study reported by **(Francisco *et al.,* 1972)** which obtained nearly similar results and concluded that Penicillin G was superior to other used antibiotics for treatment of diarretic cattle, on the other hand, (**Durda, *et al.,* 2011** and **Hala *et al.,* 2012),** found that Cephaletidine has low effect in vitro as antibiotic susceptibility against C.perfringens isolated from diarrehic cattle**.**

Regarding to the correlation between BVDV and C. perfringens infectious agents, as they were isolated from the collected samples from diarrhoeic cattle, **it** can be explained as, during acute or persistent infected cattle with BVDV, viral replication occurs in a variety of cell types located in the alimentary canal, nervous system, respiratory tract, and immune system. Although many cell types are permissive for viral replication, BVDV has a predilection for cells of the immune system (thymocytes, lymphocytes, monocytes, macrophages, and dendritic cells). Viral replication in lymphoid cells alter the immune function by the destroyed immune cells or become functionally impaired, causing immune supression which enhance severity of the disease of BVD or resulted in mixed infections of BVDV with other pathogens virus or bacteria **(Steven, 2002 and William, 2012).** Another study by **(Mona and Mervat, 2006)**, reported that an enteropathogenic (BVDV, Rota V., C. perfringens and

E.coli) were isolated from diarrhoeic calves.

Conclusion

- Concurrent infection with BVDV is considered as one of the important causes of enteritis and its immune suppressive effect can lead to mixed infection with other pathogen with subsequent high morbidities and mortalities among young calves, so preventive and control measures for BVD viral disease in cattle herds should be all over the country.
- Herd screening for detecting BVDV infection and PI cattle befor vaccination is an important step to avoid vaccine failure as it has immune suppressive effect.
- C.perfringens plays a serious role in causing diarrhea through proliferation in the intestine and production of several exotoxins as alpha, beta, epsilon and iota which can cause sudden death of the calves.
- The immunoperoxidase technique is one of the most sensitive and accurate laboratory tests for the diagnosis of BVD viral infections,
- Testing milk samples for BVDV antibodies could be used for monitoring BVD as its results nearly similar to that of serum samples and milk collection is easier than blood.

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