



Investigation of The Association Between Bovine Viral Diarrhea Virus and *Neospora caninum* as a Cause of Abortion in Cattle

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ABSTRACT

This study aimed to investigate the complementary association between Bovine Viral Diarrhea Virus (BVDV) and *Neospora caninum* (NC) in the induction of abortion in cattle. The study was carried out on 116 serum samples collected from 116 aborted cows in different Egyptian localities. All blood samples were immediately transported to the diagnostic laboratory. Serum was harvested after centrifugation at 1500×g for 10 min. All sera were divided equally into two microtubes and stored at -20 °C until laboratory testing. Double antibody sandwich immunoenzymatic assay (DS.ELISA) is used for bovine viral diarrhea virus (BVDV) p80/p125 antigen detection and Anti-*Neospora caninum* (NC) antibodies were detected using an indirect ELISA. Our results cleared that the incidences of BVDV and NC among examined cow's serum samples were 66 (56.9 %), where the incidences of BVDV and NC were 31 (26.72 %) and 35 (30.17 %), respectively. There were 18 (27.3 %) samples that showed mixed infection BVDV and NC incidences among examined cattle serum samples. The study concluded that the prevalence of BVDV and NC are high and widely spread in cattle farms in Egypt. There is a pronounced association between BVDV and NC and concurrent infection in cattle farms in Egypt. So we should coordinate surveillance and control programs on abortifacient pathogens (BVDV and NC) should be developed and implemented, which would reduce the losses associated with these diseases in cattle farms.

Keywords: Abortion, Bovine viral diarrhea virus (BVDV), Cattle, ELISA, *Neospora caninum* (NC).

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INTRODUCTION

Abortion is critical to the dairy sector because it hinders heifers and cows' scheduled lactation and replacement. It also leads to culling, which has a negative impact on herd genetics, resulting in lower farm revenue. Furthermore, abortion storms can be psychologically devastating to the farmer. Many diseases cause cattle abortion as Campylobacteriosis, Leptospirosis, and Brucellosis (Carpenter *et al.*, 2006 and Olmo *et al.*, 2018). However, there is a scarcity of research on other additional diseases such as bovine viral diarrhea (BVD) and neosporosis as well as their interrelationships as causes of abortion. Nonetheless, these play a significant influence in reproductive wastage globally (Okumu, 2014 and Okumu *et al.*, 2019).

Bovine viral diarrhea is considered one of cattle's most important viral diseases worldwide (Yitagesu *et al.*, 2021). This is a Pestivirus in the family Flaviviridae closely related to the border disease virus of sheep and the classical swine fever virus of pigs (OIE, 2018). The virus has a wide distribution, and certain animals may have subclinical infections (Lindberg and Houe, 2005).

Bovine viral diarrhea virus is disseminated by direct contact with saliva, feces, semen, urine, tears and milk of infected cattle, as well as in utero infection of fetuses (Radostits *et al.*, 1994 and Okumu *et al.*, 2019), Early embryonic death, fetal death, and mummification are all possible outcomes of infection in naive pregnant cows and heifers (Okumu *et al.*, 2019 and Yitagesu *et al.*, 2021).

In South American dairy cattle herds, bovine viral diarrhea virus seropositivity rates of up to 70%-80% have been reported (Stahl *et al.*, 2006). Prevalence rates in European and American herds have been reported to be between 21-98% in unvaccinated cattle herds (Waldner, 2005). In Africa, the disease has been found in a range of domestic and wild ruminants (Kabongo and Van Vuuren, 2004).

Neospora caninum (NC) is found all over the world and is one of the most frequent parasitic causes of abortion in cattle (Dubey and Schares 2011). The definitive hosts are domestic and wild canids (Gondim, 2006), and NC can infect a wide range of intermediate hosts, including bovine. Ingestion of food and/or water contaminated with oocytes shed in dog feces leads to cattle or congenital infection (Pan *et al.*, 2004 and Olum *et al.*, 2020). NC is considered one of the main causes of cattle abortion.

Worldwide (Paradies *et al.*, 2007; Silva *et al.*, 2007). *Neospora caninum* causes abortions in calves as early as 3 months of pregnancy; however, they are most common around 5 and 6 months. One of the obvious evidence of infection of cattle is foetal resorption, autolysis, mummification, and stillbirth; however, some calves are born alive with neuromuscular problems and others appear healthy with persistently infected (Dubey and Schares, 2006).

Congenital/vertical transmission of neosporosis from seropositive dams to their offspring is prevalent, and abortion is common in future pregnancies (Dubey *et al.*, 2007). Reported risk factors for bovine abortions due to *Neospora caninum* include geographical location, exposure to dogs, and being pregnant heifers (Dubey and Schares, 2006; Koiwai *et al.*, 2006).

Neospora caninum is an intracellular protozoan parasite that is found globally and is a serious economic concern in the cattle sector. According to Fereig *et al.* (2016), *N. caninum* can result in abortion storms and a high culling rate. Also, there are currently no data on the prevalence of neosporosis in humans or animals in Southern Egypt. Concurrent infection with the Bovine Viral Diarrhea Virus (BVDV) has also been suggested as a contributing factor to *N. caninum*-induced miscarriage in cattle in recent research in the Philippines (Konnai *et al.*, 2008 and Okumu *et al.*, 2019). NC and BVDV are essential because they induce reproductive losses and have the propensity to cause persistent infections (Chernick *et al.*, 2018).

Neospora caninum and bovine viral diarrhea virus have been reported as major causes of bovine abortion worldwide (Olum *et al.*, 2020). There has been a hypothesis that both diseases have a synergism

in inducing abortion, while there's no evidence to back this up (Konnai *et al.*, 2008). This study aimed to investigate the association between BVDV and *Neospora caninum* infection as causes of abortion in cattle.

MATERIALS AND METHODS

1. Animals:

One hundred sixteen aborted cattle from 15 small to medium-sized cattle farms in different localities (Giza, El Fayoum and Beni Suef governorates). All the farms were vaccinated only with routine vaccination. The animals have a history of abortion. The abortion percentage in these farms ranged between 6 to 14%. The aborted animals were from 3 to 8 years old. Most aborted cases were in the first trimester and the rest were in the second trimester.

2. Sampling:

One hundred sixteen serum samples were collected from 116 aborted cattle. Blood samples were collected by jugular venipuncture followed by centrifugation at 1500 ×g for 10 min. All sera were divided equally into two microtubes and stored at -20 °C until testing in the laboratory.

3. *Neospora Caninum* Indirect ELISA:

All 116 Serum samples were analyzed for anti-*Neospora* antibodies were detected using an indirect ELISA ID. Vet kit (France). The manufacture instructions were followed when using kits. Read in an ELISA microplate reader at a wavelength of 450 nm. For each sample, calculate the S/P percentage (S/P %): The sample (OD_{sample}) is divided by the mean positive control value (OD_{PC}) multiplied by 100.

$$S/P\% = \frac{OD_{\text{sample}} - OD_{\text{NC}}}{OD_{\text{PC}} - OD_{\text{NC}}} \times 100$$

So if

Samples with an S/P% was ≤ 40% meant Negative while, if the sample was 40 % < S/P % < 50%, that told Doubtfully and if the sample was S/P % ≥ 50%, this meant positive.

4. BVDV Sandwich ELISA:

All 116 sera samples (50µl) were submitted to BVDV antigen ELISA (ELISA BVDV-Ag) using (INGEZIM BVD DAS) kits. All procedures were performed according to manufacturing instructions. Briefly, ELISAs are carried out in solid-phase microplates. Wells are coated with anti-BVDV antibodies. Samples to be tested (serum) and controls are added to microwells. Anti-p80/p125 Biotin

conjugated monoclonal antibodies (MAbs; conj.1) is added and subsequently, Streptavidin – peroxidase conjugate (conj.2) is added to each well. After washing, the substrate solution (TMB) is added and the optical density (OD) is read at 450 nm. The tests can be considered valid if the ratios OD of the positive control/OD negative control > 10.

Results interpretation:

Cut off = OD of positive control x 0.1
 Samples with an OD higher than the cut-off value plus 15% must be considered as positive.
 Samples with OD lower than the cut-off value minus 15% must be considered as negative.
 Samples with OD values between both values (of positive and negative samples) are considered as doubtful. S/P ratio was ≥0.2

5. Statistical analysis:

The statistical analysis was carried out using the Chi-square test to examine the incidences of BVD, NC and Both BVD with NC among examined cattle serum samples using SPSS PC+ -Version 20.

RESULTS

The result showed that 66 of examined animals (N=116) were positive to BVDV and/or NC 66 (56.9%), where the incidences of BVDV and NC were 31(26.72%) and 35(30.17%), respectively. There were 18 (27.3%) samples that showed concurrent infection BVDV & NC incidences among positive cow serum samples as shown in Fig (1).

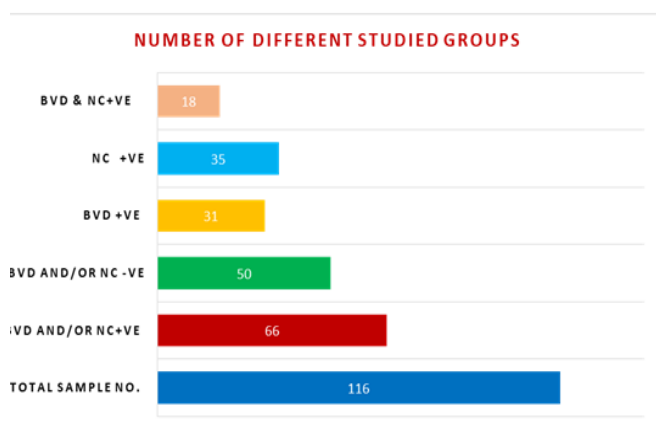


Fig. 1: Total number of different diagnosed sample groups.

1. Incidence of bovine viral diarrhea virus:

Results cleared that, the incidences of BVDV among examined cattle serum samples was 31(26.72 %) and was (47%) among +ve samples as in table (1).

Table 1: Incidences of bovine viral diarrhea among examined and positive samples using double sandwich ELISA.

	N	Positive	
		Numbers	%
BVDV	Examined samples N=116	31	26.72
	Positive samples N=66	31	47.00

2. Incidences of Neospora Caninum:

Result cleared that, the incidences of NC among examined cattle serum samples was 35 (30.17 %) while it was (53%) among +ve samples as seen in table (2).

Table 2: Incidences of Neospra caninum among examined and positive samples using indirect ELISA test.

	N	Positive	
		Number	Percent
NC	Examined samples N=116	35	30.17
	Positive samples N=66	35	53.00

3. Incidences of concurrent infection of Bovine viral diarrhea virus and Neospora caninum among positive samples.

Results cleared that, the concurrent incidences of BVDV with NC among examined cattle serum BVDV and/or NC positive cases was 18 (27.3%), and the concurrent infection percentage was (15.52%) among total examined samples, as observed in table (3) and Fig.2.

Table 3: Incidences of concurrent infection among positive and total examined samples using ELISA test.

	N	Positive	
		Number	Percent
Concurrent infection, BVDV with NC	Examined samples N=116	18	15.52
	Positive samples N=66	18	27.3

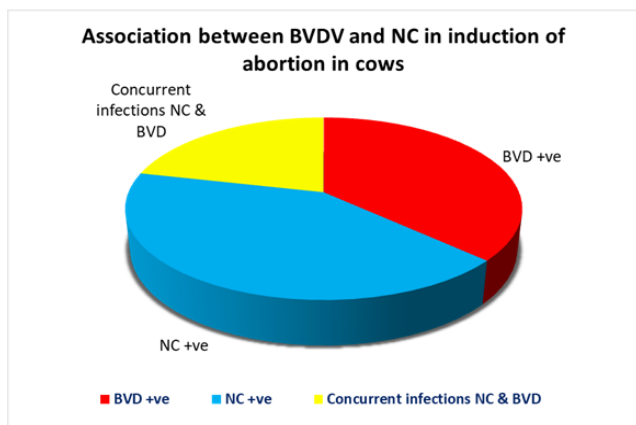


Fig. 2: Incidences of bovine viral diarrhea virus, *Neospora caninum* and concurrent infections (BVDV and NC) among examined positive samples.

4. Examination of the prevalence of BVD, NC, and concurrent infections in the serum samples of the cattle examined using Chi square test.

All the percentages related to the total number of samples (116) showed a high but non-significant relationship between BVDV and NC (Table 4) .

Table 4: Statistical examination of the incidences of BVD, NC, and concurrent BVD and NC among studied cattle serum samples.

NC	BVDV		Total
	Negative	Positive	
Negative	32(27.59%)	31(26.72%)	63(54.31%)
Positive	35(30.17%)	18(15.51%)	53(45.69%)
Total	67(57.75%)	49(42.24%)	116
Chi-square test	2.74ns		
P-value	0.098		

DISCUSSION

The detection of Bovine Viral Diarrhea in the present study was done using a double-antibody sandwich immunoenzymatic assay that detects BVDV p80/p125 antigen. As high conserved immunogenic antigen in cytopathic (p80) or noncytopathic (p125) biotypes, it is well detected in PI animals' blood sera. Because of that, this test has formed the basis for successful eradication policies in many countries as a more rapid screening test and generating information on the infectious status. Also, Ag-ELISA showed a high sensitivity for detecting BVDV infected calves (Cornish *et al.*, 2005).

Our results cleared that, the incidences of BVDV among examined cattle serum samples was 31(26.72

%). It was (47%) among positive samples Table (1); these results were agreed with those of (El-Bagoury *et al.*, 2012), where they reported that the seroprevalence of BVD was 40% and 23% for cattle and buffaloes, respectively. Herd biosecurity strategies like disinfection, double fencing, and keeping a closed herd have been reported to diminish the prevalence of BVDV infection. (Kampa *et al.*, 2004; Gates *et al.*, 2013). BVDV infection of pregnant cows and heifers has been recorded as a cause of abortion and other reproductive abnormalities, as well as increased age at first calving and decreased ovarian function in affected herds, which could explain the widespread BVD infections and associated economic losses. (Kabongo and Van Vuuren, 2004; Heuer *et al.*, 2007). Bovine Viral Diarrhea Virus has also been reported to be fetopathogenic in cattle, thus leading to early embryonic death, repeat breeder syndrome, and abortion (Yang *et al.*, 2012 and Yitagesu *et al.*, 2021).

It was proved that all animals which have antibodies to *Neospora* spp are diagnosed as persistently infected (Bachofen *et al.*, 2010) While the detection of BVDV virion or viral antigens indicates both acute infection (transiently infected animals - TI) and persistent infection (persistently infected animals - PI) (Olum *et al.*, 2020). Moreover, animals that are both serologically positive for *Neospora* spp. and positive for BVDV antigen in viral isolation or ELISA-Ag tests are thought to be co-infected by the two agents (Dubey *et al.* 2007). The coexistence of viruses and parasites should be linked to a number of epidemiological parameters, the most important of which is BVDV-induced immunosuppression (Melo *et al.* 2004).

Our results showed that the incidence of NC among examined cattle serum samples was 35 (30.17 %). In comparison, it was (53%) among positive samples Table (2), these results were agreed with that recorded by (Dubey *et al.*, 1998) where the prevalence of *N. caninum* antibodies were 68% and 20.43% in buffalo and cattle, respectively in the delta region in Egypt. Our results are higher than that reported by (Fereig *et al.*, 2016), where they revealed that 18.9% of examined cattle were positive for *N. caninum*. Also, NC prevalence was high compared to other studies that had reported prevalence ranging from 1.9%-39.7% (Konnai *et al.*, 2008; Asmare *et al.*, 2013). This may be due to the low level of knowledge by farmers and animal health providers on abortion caused by animal health. Thus, the high prevalence of NC in this study may be associated with the low level of knowledge on this disease and the subsequent lack of a control program (Sarrazin *et al.*, 2013), in addition to the role of stray dogs in Egyptian cattle farms.

On other prospects, our results agreed with the results of (Fereig *et al.*, 2016), where they reported a higher incidence of infection on small cattle farms compared with large farms; the incidences were 50.2% versus 37.8%, respectively. They observed that cows in Sohag had a considerably greater prevalence than that in Qena, with a seroprevalence of 46.1% compared with 31.6%, respectively. Pathogenesis of NC leads to abortions and fetal losses by inducing placental damage or causes the release of maternal prostaglandins that in turn cause luteolysis and abortion (Dubey *et al.*, 2007 and Olum *et al.*, 2020). The high seroprevalence recorded in this study indicates a high incidence of NC infections in cattle. These necessitates the application of more effective strategies for combating these types of infections on farms in Egypt.

Our results observed that, the concurrent incidences of BVDV with NC among examined cows serum samples was 18 (27.3%) Table (3), this gives a clear illustration about the association between BVDV and *Neospora caninum*, that play role or may be involved in the induction of abortion in cattle where the percentages of concurrent infection were (15.52%) among total examined samples. The statistical examination of BVD, NC, and concurrent BVD and NC incidences among studied cattle serum samples showed a high but non-significant relationship between BVDV and NC, Table (4). These results agreed with (Quinn *et al.*, 2004 and Lassen *et al.*, 2012). They found a non-significant correlation between BVDV infection and *Neospora* spp. On the contrary, (Duong *et al.*, 2008) found a significant association between *N. caninum* and BVDV in small cattle farms. The high incidence rates of the NC and BVD was observed in their study (12.9%); the most common cause of fetal loss was *Neospora caninum* (29.0%), followed by mixed NC and BVDV infections of NC and BVDV (12.9%), BVDV (9.9%).

In addition, the 102 dairy cattle seropositive to *Neospora caninum*, 85 (83.3%) were also seropositive to BVDV (Konnai *et al.*, 2008; Okumu, 2014 and Okumu *et al.*, 2019) reported similar findings in cattle herds with abortion; however, the biological impact of such coinfections requires further investigation. The concurrent infections had been thought to be due to the immunosuppressive effects of BVDV, increasing the chances of fetal infection by NC in pregnant cattle, thus increasing the chances of abortion. Previous studies have reported the consequences of concurrent NC and BVDV infections in cattle-producing abortion (Dubey and Schares, 2006; Yang *et al.*, 2012). BVDV and *N. caninum* coinfection is reported in other studies and suggested that it should be related to higher rates of reproductive losses and, consequently, economic losses (Alves, *et al.*, 2020).

The high incidence of BVDV 31 (26.72%) and the high abortions coupled with NC 35 (30.17%) in addition to BVD & NC 18 (27.3%) concurrent infection, Fig (1&2) may be due to BVDV lead to immunosuppression that enhances subsequent infection by latent or other agents and contributes to the severity of infection (Yitagesu *et al.*, 2021). NC might be a secondary cause of abortion and also may exacerbate abortion caused by other agents (Asmare *et al.*, 2013).

The high level of seropositivity in this study may have been due to the lack of adequate control measures such as vaccination, screening and isolation of new introductions and improved biosecurity for these two diseases; this was probably due to lack of awareness of the presence of the conditions in dairy cattle in Egypt and consequently low levels of knowledge by the animal health industry players on the impact of these diseases (Asmare *et al.*, 2013 and Fereig *et al.*, 2016).

CONCLUSION

This study concluded that, the prevalence of BVDV and NC are high and widely spread in a cattle farm in Egypt. There is a high non-significant association between BVDV and NC and the concurrent infection present in more than a quarter of positive cases. So we should coordinate surveillance and a comprehensive policy on the control BVD and NC should be developed and implemented by the government with the involvement of all stakeholders in the dairy industry; laboratories should offer diagnostic tests for BVDV and NC to help farmers determine their roles in controlling abortions on their farms.

Declaration of Conflicting Interests

The authors revealed that there is no potential conflicts of interest.

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