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CLINICAL AND EPIDEMIOLOGICAL AND SEROLOGICAL STUDIES ON BOVINE VIRAL DIARRHEA (BVD) AND INFECTIOUS BOVINE RHINOTRACHITIS (IBR) IN SOME DAIRY FARMS IN BEHERA GOVERNORATE.

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ABSTRACT

This study was done on 1134 cattle present in five private dairy cattle farms at Behera governorate. 823 animals were apparently healthy while 311 ones showed one or more of the following signs, fever , bloody diarrhea , dehydration ,cough , nasal discharges , oral lesions and/or bilateral corneal opacity. Serum samples were collected from all animals, also 148 Buffy coat samples, 160 nasal swabs and 163 fecal swabs were collected from diseased animals. These samples were taken at autumn and winter seasons. From 1134 animals, 828 animals were vaccinated against BVD and IBR while 306 animals were not vaccinated.

All serum samples (1134) were subjected to serum neutralization test (SNT), (47.7 %, and 42.1%) of non vaccinated animals and (63%, 48.7%) of vaccinated animals showed antibodies against BVDV and IBR, respectively.

BVDV was isolated from (24.1%, 14.3%, and 11.7%) and (21%, 25.3%, 16.3%) of Buffy coat samples, nasal swabs and fecal swabs from non vaccinated and vaccinated animals respectively.

IBR was isolated from (17.2%, 9%, 51%) and (21%, 24%, 13.9%) of Buffy coat samples, nasal swabs and fecal swabs from non vaccinated and vaccinated animals respectively.

From the obtained results we can conclude that bovine viral diarrhea and infectious bovine rhinotracheitis viruses are prevalent in many dairy cattle farms in Behera governorate. Also vaccination of late pregnant animals only is not sufficient to prevent persistent BVDV infection and vaccination against BVD and IBR viruses shall be done before conception to prevent the occurrence of persistent BVD infection and it can be repeated before parturition to protect the newly born calves from infection.

Key words: Bovine viral diarrhea, Infectious bovine rhinotracheitis, Behera.

INTRODUCTION

Bovine viral diarrhea virus (BVDV) and bovine herpes virus type-1 (BHV-1) are well known important pathogens of cattle that give rise to substantial economic losses due to reproductive failures and increased calf mortality as well as enteric and respiratory disease. These pathogens have a world wide distribution and tend to be endemic in most populations although national and regional variations occur ***(Houe, 1999)*.**

Bovine herpesvirus-1 (BHV-1) causes a wide variety of syndromes, rhinotracheitis, vulvovaginitis, abortion, conjunctivitis, encephalitis and generalized systemic infections. More over, BHV-1 infections may be complicated with secondary bacterial infections. Following infection latency of the virus is established. As a result of stressful condition the latent virus may be reactivated and shed at irregular intervals. Animal once infected must

therefore regarded as life long potential shedders of BHV-1. Vaccination of animals does not uniformly prevent the establishment of latency. Herd to herd transmission of BHV-1 most commonly occurs by introduction of infected animals into non infected herds ***(Kramps et al.,1994)*** .

BVD virus is associated with a variety of disease manifestation, causing a world wide economic losses and a considerable threat to the live stock industry specially calves industry ***(Baker, 1996)*** .

BVD virus can be transmitted via semen of persistently infected as well as acutely infected bulls . The infection rate can be 100% in the case of persistent infected (PI) bulls (artificial insemination) ***(Meyling and Jensen, 1988)*** although virus titers in acutely infected bulls usually low **(*Kirkland et al., 1991)***.

The overall aim of this study is to investigate the prevalence of BVDV and IBR infections in some dairy farms in Behera governorate and to determine efficacy of vaccination programs.

3-MATERIAL AND METHODS

3.1. Materials:

3.1.1. Animals:

1134 cross-bred cattle aged from 2 months to 4 years belong to five private dairy farms in different localities in Behera governorate were used in this study. 823 animals were apparently healthy and 311 cattle showed one or more of the following clinical signs, fever, nasal discharges , cough , rales , erosions and ulceration in oral mucosa as well as diarrhea which some times was bloody and accompanied with dehydration, Conjunctivitis and corneal opacity.

Two localities (Abo-Elmatamir and Wady El-natron farms) in which no vaccination programs against BVDV or IBRV was done. Other localities (Badr, El-noparea and Damanhour cities) used 2 doses with 30 days interval of Cattle Master-4 vaccine (inactivated BVDV, genetically modified live BHV1, live attenuated PI-3 and BRSV viruses) to late pregnant cows at 7^{th} and 8^{th} month of pregnancy according to manufacturer (Pfizer, USA).

3.1.2. Samples :

Table (1) Number of samples collected from infected and apparently healthy cattle from different localities in Behera governorate

	Number of animals	Types of samples			
		Serum samples	Buffy coat samples	Nasal swabs	Fecal swabs
Non vaccinated animals	306	306	29	77	77
Vaccinated animals	828	828	119	83	86
Total	1134	1134	148	160	163

3.1.3.Tissue culture:

Madin Darby Bovine Kidney (MDBK) cell line ***(Marcus and Moll, 1968)*** was used for virus isolation and serum neutralization test (SNT) .

Supplied by the Department of Virology, Animal Health Research Institute, Agricultural Research Center, Cairo, Egypt.

3.1.4.Viruses :

a. Bovine viral diarrhoea virus (BVDV): NADL strain of BVDV (10^6 $TCID_{50}$/ml).

b. Infectious bovine rhinotracheitis virus (IBRV) : local strain of IBRV (Abu Hammad strain) ($10^{6.5}$ $TCID_{50}$/ml) .

These viruses were obtained from the Department of Virology, Animal Health Research Institute, Agricultural Research Center, Cairo, Egypt .

3.1.5. Hyperimmune serum:

Hyperimmune serum contains specific antibodies for BVDV (Sanofi , France) and IBRV (IDEXX Laboratories B. V. , Switzerland) , it was used on indirect fluorescent antibody technique .

3.1.6. Anti-bovine immunoglobulin conjugated with fluorescein isothiocynate (FITC) :

It was used for detection of BVD and IBR viral antigen on tissue culture cells after adding of hyperimmune serum , it was obtained from Ames, Iowa Laboratries , USA .

3. 2. Methods:

3.2.1.Clinical examinations:

Clinical examination had been carried out on 1134 cattle aged from two months to four years from five private dairy farms in Behera governorate ; in Abo-Elmatamir, Badr , El-noparea , Wady El-natron and Damanhour cities.

3.2.2. Viral isolation from nasal swabs, fecal swabs and Buffy coat samples on tissue culture (MDBK cells) :

- The viral isolation was carried out according to ***Hanaa (1995)*** **and** ***Otto et al.(1996)*** .

3.2.3. Indirect fluorescent antibody technique (IFAT) for detection of BVD and IBR viral antigens :

It had been carried out according to the method of ***Potgieter and Aldridge (1977)***.

3.2.4.Titration of BVD and IBR viruses for serum neutralization test:

The end point $TCID_{50}$ / 1 ml was calculated and expressed as Log_{10} $TCID_{50}$ ***Reed and Muench (1938)*** method .

- The infectious bovine rhinotracheitis virus (IBRV)has an infectivity titer ($10^{6.5}$ $TCID_{50}$ /ml) .

- The bovine viral diarrhea virus (BVDV) has an infectivity titer (10^6 $TCID_{50}$ /ml) .

3.2.7. Serum neutralization test (SNT) for detection of antibodies of BVD and IBR viruses in serum samples :

- It has been carried out according to ***Fulton et al. (1995)*** .

4- RESULTS and DISCUSSION

Bovine viral diarrhea and infectious bovine rhinotracheitis viruses are major causes of economic losses at dairy cattle farms . Concurrent infection with bovine viral diarrhea and infectious bovine rhinotracheitis viruses usually results in immunosuppression, respiratory manifestations and diarrhea leading to high morbidities and mortalities among dairy cattle in Egypt ***(Ali et al., 2003)***.

The present study was performed to determine the prevalence of BVD and IBR viruses among some dairy farms in Behera

governorate and evaluation of vaccination programs.

As shown in table (2), The clinical investigation of the animals subjected to this study revealed that , the most prominent clinical symptoms were fever (53.1 % from the diseased cattle), diarrhea and dehydration (46.9%) and cough with nasal discharges (33.4 %) . oral lesions (ulceration and erosions at oral mucosa) appeared at (29.9 %) from the diseased cattle while the less prominent clinical symptom was bilateral corneal opacity (11.9 %). These results agreed with ***(Hafez , 1973 ; Baz , 1982 ; Drew et al., 1987 ; Belknap , 1993 Brodersen and Kelling, 1998 ; and Ali et al., 2003)*** who reported similar symptoms associated with BHV-1 and BVDV infection .

Table (2) Clinical findings in dairy cattle farms in different localities in Behera governorate

	No of diseased animals	Fever (39.5-41.5 ^{o}C)		Diarrhea & dehydration		Cough & nasal discharges		Oral lesions salivation &		Corneal opacity & conjunctivitis	
		No	%	No.	%	No.	%	No.	%	No.	%
Non vaccinated animals	106	39	36.8	64	60.4	24	22.6	50	47.1	24	22.6
Vaccinated animals	205	126	61.5	82	40	80	39	43	21	13	6.3
Total	311	165	53.1	146	46.9	104	33.4	93	29.9	37	11.9

These clinical symptoms appeared after introduction of new semen straws to El-noparea farm, so we suggested that semen straws might be the source of infection as mentioned by ***Meyling and Jensen(1988)*** . At Badr farm , the symptoms appeared after introduction of newly purchased infected cattle, from which the two viruses had been isolated later so we suggested that newly purchased cattle may play an important role in the transmission of the two viruses, these results support the results obtained by **(*Rola et al. 2005)*** who found that introduction of newly purchased animals into the herd initiate the onset of an outbreak caused by BHV-1 . ***(Kampa 2006)*** reported that introduction of dually infected cattle and artificial insemination are obvious and important risk factors.

The prevalence of antibodies against BVD and IBR viruses was investigated in all animals included in this study using serum neutralization test (SNT) .

Antibody tests indicate exposure and are useful in assessing the status of groups of animals or a whole herd prior to, or as a part of a disease control program . The viral neutralization test is the most common serologic method used for determining levels of BVDV antibodies and it has been accepted as the reference method for BVDV tests ***(Edwards , 1990)***

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Table (3) Screening of serum samples by serum neutralization test (SNT)

	No of tested samples	BVDV positive samples		BHV1 positive samples	
		No	%	No	%
Non vaccinated animals	306	146	47.7	129	42.1
Vaccinated animals	828	522	63	403	48.7
Total	1134	668	58.9	532	46.9

As shown on table (3), The prevalence of BVDV seropositive animals was (58.9 %) which is lower than the result obtained by ***Ghirotti et al. (1991)*** who found (76.2 %) of animals were BVD seopositive and the results is close to the result obtained by ***Houe et al. (1995)*** who reported that prevalence of BVDV seropositive animals is usually between 60 and 80% with the proportion of persistent infected animals being around 1 to 2 % with some difference between regions and countries which may related to difference in cattle densities , housing, vaccination and management systems as well as animal trading activities . The result obtained from the present study is close to the result obtained by ***Yavru et al. (2005)*** which was (53.9 %) . Presence of antibody in the serum of the animal indicates vaccination or previous exposure to the infection.

BVDV seronegative animals may had not been exposed to infection or persistently infected. ***Johnson and Muscoplat (1973)*** and ***Coria and McClurkin (1978)*** reported that persistent infected animals can not make antibodies for BVDV.

As shown on table (3), The prevalence of IBR antibodies among all serum samples was (46.9 %) which was lower than that obtained by ***Zhou et al. (1988)*** who found(75.2 %) of animals were IBR seropositive and the results is higher than that obtained by ***Chinchkar et al.(2002)*** who found (31%) of animals were IBR seropositive. IBRV seropositive animals may be vaccinated or previously exposed to infection. ***York (1968)*** reported that detection of neutralizing antibodies for IBR indicates previous exposure to infection while ***Ackermann et al. (1982)*** supposed that IBR seropositive animals should be considered as latently infected.

63% of vaccinated cattle and 47.7% of non vaccinated animals were BVDV seropositive, these results are in accordance with that obtained by ***Anita and Anita (2006)***. These results indicate the importance of vaccination for seroconversion and subsequently protection against BVDV infection but these vaccines shall be done before conception to prevent the infection of dams and subsequent calves which may leads to persistent infection if occurred before the day 110 of pregnancy ***(Brownlie,1991)*** .

48.7 % of vaccinated cattle , were seropositive for IBR , this result is lower than that obtained by ***Lazic et al. (1998)*** which was 69 % . while 42.2% of non-vaccinated cattle were IBR seropositive, this result is higher than that obtained by ***Hastink et al. (1994)*** which was 11.7 % and lower than that obtained by ***Solis-Calderon (2003)*** which was 54.4% .

As shown on table (4), the prevalence of BVDV infection was 18.5% which is lower than 34.8% obtained by ***Selim and Elazhary (1983)*** and higher than 4.7% obtained by ***Kabongo and Van Vuuren (2004)*** and lower than 31.66% obtained by ***Abd El Hafeiz et al. (2005)***.

Table (4) Results of isolation and identification of BVDV by indirect fluorescent antibody technique (IFAT) on tissue culture

	Buffy coat samples		Nasal swabs		Fecal swabs		Total	
	Positive samples	%	Positive samples	%	Positive samples	%	No.	%
Non vaccinated animals	7/29	24.1	11/77	14.3	9/77	11.7	27 / 183	14.8
Vaccinated animals	25 / 119	21	21 /83	25.3	14 / 86	16.3	60 / 288	20.8
Total	32 / 148	21.6	32 / 160	20	23 /163	14.1	87/471	18.5

Fluorescent antibody technique was applied on tissue culture which indicate that infection was mainly due to cytopathic strain although ***Brock (1995)*** reported that most commonly BVDV isolates obtained from field cases are non cytopathic in cell culture .

The highest percentage of BVD viral isolates was obtained from Buffy coat samples which was (21.6%) while the lowest percentage was obtained from fecal swabs (14.1 %) which may return to the viraemic condition of most of the animals from which the samples were taken ***(Roeder and Drew, 1984*)** .

As shown in table (5), the highest percentage of IBR viral isolates was obtained from Buffy coat samples which was (20.3%) while the lowest percentage was obtained from fecal swabs (9.8 %) which also may return to the viraemic condition of animals from which samples were taken . As shown in the results , all farms included in this study were infected with BVD and IBR viruses . Mixed infection may be occurred due to the immunosuppressive effect of BVDV and subsequent activation of latent infection of IBR. ***Malmquist (1968)*** reported that BVDV make depletion to lymphocytes which leads to infection with other viral or bacterial diseases, also ***Boden (1991)*** reported that immunosuppression caused by BVDV may reactivate latent infection of IBRV. This mixed infection may be due to the similar factors which might be of importance for the risk of introduction of both these infections to dairy herds ***(Paton et al.,1998)*** .

Table (5) Results of isolation and identification of BHV1 by indirect fluorescent antibody technique (IFAT) on tissue culture

	Buffy coat samples		Nasal swabs		Fecal swabs		Total	
	Positive samples	%	Positive samples	%	Positive samples	%	No.	%
Non vaccinated animals	5/29	17.2	7 / 77	9	4 / 77	5.1	23 / 183	12.6
Vaccinated animals	25 / 119	21	20 / 83	24	12 / 86	13.9	95 / 288	33
Total	30 / 148	20.3	27 /160	16.9	16 /163	9.8	128/471	27.2

As shown on table (4 and 5), vaccinated and non vaccinated farms had been infected with BVD and IBR viruses in which there is no significant difference in the rates of infection between them . The presence of infection with BVDV among vaccinated cattle may be due to antigenic variation between BVDV strains , presence of persistent infected animals which can not make antibodies for BVDV ***(Coria and McClurkin, 1978)*** or due to failure of vaccination as the animals were vaccinated before parturition not before conception so that fetal infection in early pregnancy stage may occur leading to persistent infection ***(Brownlie, 1991)*** .

The presence of infection with IBR among vaccinated farms may return to failure of vaccination as live attenuated and killed vaccines of BHV-1 do not prevent latent infection with wild types of virus **(*Soulebot et al., 1981 and Pastoret et al., 1982*)**

Also strict hygienic measures shall be done including examination of newly purchased animals before introduction to the herd for the presence of antibodies as well as BVD and IBR viral antigens, IBR seropositive animals should be considered as latently infected ***(Ackermann et al., 1982)*** while BVD seronegative animals should be suspected as persistently infected with bovine viral diarrhea virus unless proved negative for antigen detection . Also semen straws should be examined for the presence of the two viruses before its use in artificial insemination process.

From the present study we can conclude that, bovine viral diarrhea and infectious bovine rhinotracheitis viruses are prevalent in many dairy cattle farms in Behera governorate. Introduction of newly purchased animals into the farm should be considered as one of the most important sources of infection as they may be latently infected for IBRV or persistent infected with BVDV, then symptoms appear few days later due to the immunosuppressive effect of transportation. Vaccination against BVD and IBR viruses shall be done before conception to prevent the incidence of BVDV persistent infection and it can be repeated before parturition to protect the newly born calves from infection.

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