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Sero-Prevalence of Bovine Viral Diarrhea Virus and Bovine Herpesvirus-1 Infection in Egypt and Their Relation to Brucellosis

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Abstract: Bovine viral diarrhea virus (BVDV), bovine herpesvirus-1 (BHV-1) and brucella infection occur globally and remain agronomical burden diseases which cause great financial losses. The main purpose of this study was to investigate the sero-prevalence of BVDV and BHV-1 in locally bred cattle at rural Egypt and the relation between infection with these viruses and the incidence of brucellosis. During the 1st half of 2009, serum samples (n=409) were collected from cattle of 13 governorates in upper and delta of Egypt with a history of brucella infection. The cattle were farmed private with no vaccination program or any veterinarian supervision. Serological diagnosis of BVDV and BHV-1 by native prepared kits of enzyme linked immunosorbant assay (ELISA) and brucella diagnosis by the Rose bengal test (RBT) and serum agglutination test (SAT) were done. Results revealed that high incidence of cattle in rural Egypt are Sero-positive to BVDV and BHV-1 infection (51.84 and 58.68% respectively). There was a significant positive correlation ($r=0.48$, $P<0.05$) between BVDV and BHV-1 infection. On the other hand, there was a high incidence of brucellosis (78.24%) with high significant negative correlation between brucella infection and both BVDV and BHV-1 infection ($r=-0.76$ and -0.47, $P<0.01$ respectively). In conclusion, sero-prevalence of BVDV and BHV-1 infection is high in cattle in Egypt and control measures against them must be followed up. The correlation between viral and bacterial infection must be considered in further studies.

Key words: BVDV · BHV-1 · Brucella · Sero-prevalence

INTRODUCTION

Infections with bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BHV-1) occur globally and are imposing large direct and indirect productive losses in beef and dairy industries [1-3]. Both viruses remain agronomical burden diseases causing great financial losses [4, 5]. Also, they have immunosuppressive effects which predispose animals to infection by other pathogens [6-8].

BVDV is endemic in most cattle populations around the world [7]. In most countries, without any control program, the sero-prevalence ranging from 20 to 90% and 1- 2% of all cattle are persistently infected (PI) [9]. However, the herd and animal–level prevalence for BVDV antibodies were generally similar elsewhere [10].

Pustular vulvovaginitis and pustular balanoposthitis are clinical manifestations of BHV-1 infection that tend to remain localized in the genital tract [11]. The virus establishes lifelong latency in the trigeminal and sacral dorsal root ganglia of infected animals [12]. There is evidence that long-term persistence and reactivation also occur within germinal centers of pharyngeal tonsil [13].

Brucellosis is a zoonotic disease caused by members of the genus *Brucella*, which affects both human [14] and animals as cattle, sheep, goats, swine and dogs [15]. Bovine brucellosis is an economically important abortifaciant disease of cattle caused mainly by *brucella abortus* and *brucella melatensis* which occurs in sheep and goat and could transmitted to cattle [16].

Considering the complexity of infectious agent pathogenesis and clinical features, laboratory diagnosis plays an important role. Therefore, a sensitive and specific serological test could be a valuable tool for the diagnosis and to monitor the infection and immune status of individual animals and herds [17, 18].

The main purpose of this study was to investigate the sero-prevalence of BVDV and BHV-1 viruses in locally bred cattle in rural Egypt and the possible relation between infection with these viruses and the incidence of brucellosis.

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MATERIALS AND METHODS

Serum Samples and Animals History: This study was conducted on 13 governorates in upper and delta of Egypt. Serum samples (n=409) were collected from cattle during the 1st half of 2009 with a history of brucella infection. These cattle were farmed private with no vaccination program or any veterinarian supervision. The main clinical signs of these animals were poor healthy conditions, respiratory manifestations, poor growth and abortions.

Viruses and Cell Culture: The reference BVDV (NADL; National Animal Disease Laboratory) and BHV-1 (Colorado) strains were cultured on Madin-Darby bovine kidney (MDBK) cell culture that tested against the latent infection of BVDV and mycoplasma. The tissue culture infective dose 50 ($TCID_{50}$) for both viruses were calculated according to Reed and Muench [19] method.

Concentration and Purification of the Viral Strains: As outlined by Chu and Zee [20] and Kelling *et al.* [21] and at the viral titer $10^{5.9}$ $TCID_{50}$ for NADL strain and $10^{6.4}$ $TCID_{50}$ for Colorado strain, each viral suspension was collected, clarified by cooling centrifugation at 8000 *xg* for 30 minutes. To each 100 ml of the clarified supernatant, 2.3 g NaCl and 7 g polyethyleneglycol 6000 (PEG 6000) were added and stored at 4°C overnight. In the next day, the solution was centrifuged as above, the supernatant was discarded and the precipitate which contains the virus was suspended as 1/8 volume of TEN buffer (0.01 M Tris, 0.001 M EDTA and 0.1 M NaCl). The virus containing solution was clarified by cooling centrifugation at 5000 *xg* for 20 minutes. The supernatant was layered onto a 3 ml cushion of 15% potassium tartarate in TEN buffer. The mixture was centrifuged at 4°C, 70000 *xg* for 90 minutes and the pellet was resuspended in TEN buffer to a total volume 1/100 of the original.

Quantitation of Antigen Concentration: Total antigen concentration was estimated using "total protein liquidcolor reagent; Stanbio laboratory, Boerne, Tx, USA. As in the manufacturers' leaflet, 2 ml of total protein reagent and 20 µl of the antigen were mixed and incubated at room temperature (RT) for 10 minutes. The optical density (OD) of the mixture was read at 550 nanometer (nm). The concentration of the antigen (µg/µl) was calculated at the formula: OD of the sample/OD of the standard X concentration of standard (10 g/dl).

Indirect Enzyme Linked Immunosorbant Assay (ELISA): As the standard protocol that described briefly by Crowther [22] and according to checkerboard result, the indirect enzyme linked immunosorbant assay (ELISA) was done. Briefly, for each virus and at antigen concentration 11.5 µg per well for BHV-1 and 10 µg per well for BVDV in 100 µl coating buffer (1.59 g Na_2CO_3, 2.94 g $NaHCO_3$, pH 9.6 in 1 liter deionized water), the 96 well ELISA plates were coated and stored at 4°C overnight. In the next day and after removal the coating mixture, the wells were blocked with 200 µl per well blocking buffer (5% skimmed milk, 0.05% tween 20 in phosphate buffered saline; PBS pH 7.2) and incubated at 37°C for 2 hours. For 3 times/200 µl per well each time, the plates were washed thoroughly with the washing buffer (0.05% tween 20 in PBS pH 7.2). In the diluting buffer (0.5% skimmed milk, 0.05% tween 20 in PBS pH 7.2), the tested serum samples were diluted as 1/20 and added as duplicated as 100 µl per well and incubated at 37°C for one hour before washing as previously. After that, 100 µl per well of diluted peroxidase labeled anti-bovine IgG ($1/10^4$) in PBS pH 7.2 (Bethyl laboratories, INC, Germany) was added and incubated at 37°C for one hour. The plates were washed as previously and 100 µl per well of the substrate (0.4 mg O-phenylenediamine- OPD and one drop of 30% H_2O_2 per ml of 0.01 M citrate buffer pH 5) was added. After the color development, the reaction was stopped by 50 µl per well of 1/9 v/v sulfuric acid in water and the optical density (OD) at 492 nm wavelength was read. Positive and negative controls were included in each plate as duplicated.

Cutoff Endpoint: By divided the OD summation of the positive and negative controls on the number 4, the samples which their OD > the cutoff endpoint were considered positive while that their OD ≤ the cutoff endpoint were considered negative.

Serological Diagnosis of Brucellosis: The serum samples were tested against brucellosis using the Rose bengal test (RBT) and serum agglutination test (SAT) as recommended by Alton and Forsyth [23].

Statistical Analysis: Data on sero-prevalence of BVDV, BHV-1 and brucella infection were analyzed by Costat Computer Program, Version 3.03 copyright (1986) and compared by the least significant difference (LSD) at 1% and 5% levels of probability. Correlation coefficients were calculated to evaluate the relation between BVDV and/or BHV-1 and brucella infection.

Table 1: Sero-prevalence of BVDV, BHV-1 and brucella infection in 13 governorates in upper and delta of Egypt during the 1st half of 2009.

		BVDV		BHV-1		Brucella	
Governorates	Total	+ ve	- ve	+ ve	- ve	+ ve	- ve
El-Sharquia	15	15 (100%)	0 (0%)	15 (100%)	0 (0%)	1 (6.7%)	14 (93.3%)
Sohag	96	63 (65.6%)	33 (34.4%)	61 (63.5%)	35 (36.5%)	85 (88.5%)	11 (11%)
Domiat	12	6 (50%)	6 (50%)	6 (50%)	6 (50%)	12 (100 %)	0 (0%)
Gharbia	52	30 (57.6%)	22 (42.4%)	29 (55.7%)	23 (44.3%)	42 (80.7%)	10 (19.3%)
Behera	92	40 (43.5%)	52 (56.5%)	47 (51%)	45 (49%)	69 (75%)	23 (25%)
El-Wady Jadeed	46	18 (39.1%)	28 (60.8%)	19 (41.8%)	27 (58.7%)	39 (84.7%)	7 (15.3%)
Monufia	32	9 (28.1%)	23 (71.9%)	19 (59.3%)	13 (40.7%)	26 (81.2%)	6 (18.8%)
Beni-Suef	24	19 (9.1%)	5 (20.9%)	24 (100%)	0 (0%)	7 (29.1%)	17 (70.9%)
Kafr El-Sheikh	13	5 (38.5%)	8 (61.5%)	3 (23%)	10 (77%)	12 (92.3%)	1 (7.7%)
Elmenia	7	2 (28.5%)	5 (71.5%)	0 (0%)	7 (100%)	7 (100%)	0 (0%)
Assuit	6	1 (16.6%)	5 (83.4%)	5 (83.4%)	1 (16.6%)	6 (100%)	0 (0%)
Alexandria	11	2 (18.2%)	9 (81.8%)	9 (81.8%)	2 (18.2%)	11 (100%)	0 (0%)
6th October	3	2 (66.6%)	1 (33.4%)	3 (100%)	0 (0%)	3 (100%)	0 (0%)
Total	409	212(51.84%)	197 (48.16%)	240 (58.68%)	169 (41.32%)	320 (78.24%)	89 (21.76%)

A significant positive correlation (r=0.48, P<0.05) between BVDV and BHV-1 infection, high significant negative correlation between brucella infection and both BVDV and BHV-1 infection (r=-0.76 and -0.47, P<0.01 respectively)

RESULTS

The current results revealed that sero-prevalence of BVDV and BHV-1 infection in examined cattle raised at rural Egypt during the 1st half of 2009 averaged 51.84 and 58.68% respectively. There was a significant positive correlation (r=0.48, P<0.05) between BVDV and BHV-1 infection.

On the other hand, there was a high incidence of brucellosis (78.24%) with high significant negative correlation between brucella infection and both BVDV and BHV-1 infection (r=-0.76 and -0.47, P<0.01 respectively).

DISCUSSION

There is growing awareness of the desirability to eradicate of BVDV and BHV-1 [24, 25], although knowledge on the incidence and regional impact is lacking. No sufficient information has been available on the extent of these viruses in Egypt, so it is difficult to assess the potential economic impact on cattle production in the country. Also, there are no official control programs against these viruses and even though vaccinations do occur in some regions, no official vaccination regimen exists.

Yet, there is abundant veterinary evidence that bovine viral diarrhea (BVD) is very important from several perspectives: production, animal welfare and increased commodity price. So, there are objective reasons to give BVDV a higher priority than it is currently awarded [26]. However, during May 2005, BVD was added to the OIE list of diseases and at that time, BVD was already notifiable in seven EU countries [27]. These developments alter the pressures for control and will probably result in important changes relating to national BVD control [28].

Although attempts to eradicate BHV-1 have included the slaughter or destruction of sero-positive animals, only a small number of countries have managed to eradicate the virus. Several countries within the EU have either successfully eradicated BHV-1 (Denmark, Finland, Sweden, Austria, province of Bolzano, Italy) or implemented an EU-approved compulsory program (Germany). The arguments for and against such eradication programs, with and without the use of vaccines, have been reviewed and the need for more basic research was pointed out [29, 30].

Results of this study revealed high incidences of BVDV and BHV-1 infection in cattle at rural Egypt (51.84 and 58.68% respectively) with a significant positive correlation between BVDV and BHV-1 infection.

Both viruses have immunosupressive affects. BVDV infects macrophages and lymphocytes results in depression of phagocytosis, Fc and complement receptors expression, bactericidal activity and secretion of chemotactic factors [31, 32]. BHV-1 uses a variety of mechanisms to elude the host's immune response that interfere the immune system by infecting activated $CD4^+$ lymphocytes, suppresses major histocompatibility

complex class-I (MHC-1) results in the prevention of cytotoxic T lymphocyte (CTL) activity [33, 34].

The high incidence of brucellosis (78.24%) in this study gave a red light on the dangerous of this disease on farmer, small and large ruminant productions in Egypt. Moreover, there was a high significant negative correlation between brucella infection and both BVDV and BHV-1 infection. The brucella is characteristically able to multiply facultatively within the phagocytic cells [35]. The destruction of these phagocytic cells as a result of BVDV and/or BHV-1 infection may explain this negative correlation.

In conclusion, the prevalence of infection with BVDV and BHV-1 is high in cattle in Egypt and control measures against them must be followed up. The correlation between viral and bacterial infection must be considered in further studies.

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