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Prevalence of Infectious Bovine Rhinotracheitis and Bovine Viral Diarrhoea Viruses in Female Buffaloes with Reproductive Disorders and Parasitic Infections

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Abstract: Out of 360 female buffaloes, examined in Lower Egypt during a period of two years (2004-2006), 171 (47.5%) were suffering from reproductive disorders and 216 (60.0%) were infected with internal parasites. The main recorded reproductive disorders were ovarian inactivity (27.5%), endometritis (8.6%), delayed puberty (8.3%), mastitis (5.8%), retained placenta (2.2%), repeat breeding (1.1%) and abortions (1.1%). The main recorded parasitic infections were coccidiosis (42.5%), ascariasis (20.8%), trichostrongylosis (6.9%) and fasciolosis (4.7%). Prevalence of antibodies to Infectious Bovine Rhinotracheitis (IBR) in sera using indirect Enzyme Linked Immunosorbent Assay (ELISA) revealed 43.3%, meanwhile, detection of Bovine Viral Diarrhoea Virus (BVDV) in blood of the examined buffaloes revealed 12.2% using direct-ELISA. Detection of antibodies against IBR in milk samples using milk-ELISA gave good diagnostic potency (31.4%) compared with serum-ELISA (43.3%). Prevalence of IBR and BVD was extremely high in buffaloes with reproductive disorders (78.2 and 72.7%, respectively) or parasitic infections (88.5 and 84.1%, respectively) compared with those without reproductive disorders (21.8 and 27.3%, respectively) or parasitic infections (11.5 and 15.9%, respectively). The difference was statistically significant and indicated a tight connection between infections with IBR and BVD viruses on one hand and occurrence of reproductive disorders or parasitic infections on the other hand.

Key words: IBR, BVD, buffaloes, reproductive disorders, parasitic infections

INTRODUCTION

Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhoea (BVD) are viral diseases with a worldwide distribution in domestic and wild ruminants and result in severe economic losses to the dairy industry.

IBR is caused by bovine herpesvirus-1 (BHV-1), which may also cause conjunctivitis, meningoencephalitis, infectious pustular vulvovaginitis and balanoposthitis, abortions and systemic infections (Wentink *et al.*, 1993; Biuk-Rudan *et al.*, 1999). Abortions mostly occur during the third trimester of pregnancy. Infertility and shortened estrous cycles have been observed in nonpregnant cows inseminated at estrus with semen containing IBR virus. The virus causes limited necrotizing endometritis and oophoritis in both ovaries with the most severe lesions on the corpus luteum (Miller and Van Der Maaten, 1984, 1986; Miller, 1991).

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Bovine Viral Diarrhoea Virus (BVDV), which belongs to the genus *Pestivirus* of the family Flaviviridae, is a major reproductive pathogen in cattle populations throughout the world, with an incidence of infection exceeding 70% (Paton *et al.*, 1998; Houe, 1999). This virus is responsible for considerable animal suffering and economic loss (Houe, 1999). The viral infection leads to ovarian dysfunction (Grooms *et al.*, 1998; Fray *et al.*, 2000a, 2002), endometritis (Archbald *et al.*, 1973), reduced conception rate and pustular lesions on genital organs (Grahn *et al.*, 1984; Barber *et al.*, 1985) and direct damage to the embryo (Evermann and Ridpath, 2002; Grooms, 2004).

Milk has become increasingly important for detecting the presence of antibodies to wide range of bovine infections. In ruminants, immunoglobulins predominantly of the IgG class are selectively transported from the maternal circulation into the mammary secretions before parturation, to be ready for ingestion and absorption in colostrum by the suckling neonates (Husband, 1998). Unlike colostrum, most IgGs in milk were produced locally from the udder rather than transported from blood (Tizard, 2000). Milk antibody testing, therefore, is a very useful and inexpensive method for initiating disease investigations and monitoring the presence of common endemic infections in the herd. Several authors have used an ELISA to examine the relationship between milk and serum immunoglobulins for a number of bovine infections (Niskanen *et al.*, 1989; Witte *et al.*, 1989; Hoorfar and Wedderkopp, 1995; Pritchard *et al.*, 2002).

Out of the world 160 million heads of buffaloes, 3.9 million heads are found in Egypt and produce 65% of meat and milk used by local population. Despite this species has a special situation for Egyption breeders, these animals are bred under harsh socioeconomic conditions and suffering from a lot of stressful conditions, mainly reproductive disorders and parasitic infections (Ahmed, 2006). The improving of the environmental conditions and overcoming some of these problems may give these animals the opportunity to express their true good productive potentials.

Present study was designed to determine seroprevalence to IBR virus in sera and milk of the tested buffaloes using indirect-ELISA, to evaluate the diagnostic efficiency of milk-ELISA compared with serum-ELISA and to determine the prevalence of BVD virus in blood of buffalloes using direct-ELISA. Moreover, the prevalence of IBR and BVD in relation to the reproductive disorders and parasitic infections was also investigated.

MATERIALS AND METHODS

Animals
A total of 360 female buffaloes (2-5 years), reared at Lower Egypt during a period of 2 years (2004-2006), were surveyed in this study. These animals were kept in smallholder farms and fed mainly on Egyptian clover (Barseem) with inadequate amount of concentrate mixture.

History and Clinical Examination
A complete case history, owners complain, clinical examination and reproductive status were recorded for each animal. Special importance was given to examination of the udder and external genitalia. All adult female buffaloes were subjected to vaginal and rectal examinations and reproductive disorders were detected as described by Ahmed *et al.* (2006). Ovarian activity was confirmed by determination of plasma progesterone level.

Serum and Whole Blood Samples
Two blood samples were collected from each of the examined buffaloes by puncture of the jagular vein into sterile vacutainer tubes; one with anticoagulant and the other without. Blood samples without anticoagulants were centrifuged in the laboratory at 1500 x g for 20 min and sera were separated and stored at -20°C until analysed. Sera were examined for IBR virus using indirect-ELISA and screened

for brucellosis using Rose Bengal test (Alton *et al.*, 1988). Serum progesterone levels were determined by ELISA microwells test (Dima diagnostics, Germany) according to Hubl *et al.* (1982). Progesterone levels <0.002 ng mL^{-1} confirmed the occurrence of ovarian inactivity. The whole blood samples were used for preparation of leucocytes extracts, which applied for BVDV detection using direct-ELISA.

Milk Samples

A total of 105 individual milk samples were collected from lactating buffaloes in sterile plastic tubes without additives. Skim defatted milk was obtained by centrifugation of whole milk at 1500 x g at 4°C for 20 min and subsequent collection of the milk below the fat layer. The defatted milk samples were stored at -20°C until analysed by the ELISA.

Parasitological Examination

Faecal samples were collected from the rectum in polyethylene sacs for parasitological examination. Identification of parasites was performed by concentration flotation and sedimentation techniques as described by Soulsby (1982) and Thienpont *et al.* (1986).

Enzyme Linked Immunosorbent Assays
Preparation of Leucocytes Extract

In a sterile centrifuge tube, 2 mL of whole blood together with 3 mL of hemolytic buffer were added. The mixture was shaked, incubated for 15 min at room temperature until complete lyses of the red blood cells and then centrifuged for 15 min at 1000 x g. The supernatant was removed and the leucocytes pellet was diluted with 200 µL of dilution buffer, mixed and then left to settle for few minutes and the liquid supernatant phase was taken for the test (Quinn *et al.*, 1994).

Direct-ELISA for Detection of NSP2-3 and E of BVDV

Specific monoclonal antibodies directed to non Structural Protein 2-3 (NSP 2-3) and immunodominant viral protein E (E) of BVDV was supplied coated on the microplate wells by Institut Pourquier, Montpellier, France. The samples to be tested (leucocytes extracts) were co-inoculated in the wells with a buffer containing a polyclonal antibody directed specifically to BVDV. If the BVDV antigens were present in the analysed sample, a polyclonal antibody-antigen-monoclonal complex is formed, which binds to a peroxidase conjugate directed to the polyclonal antibody. After washing, the enzyme substrate (TMB) was added to the conjugate, forming a blue compound becoming yellow after blocking. The cut off was set by using positive and negative controls which must be added to each microplate. Sample to positive ratio (S/P) of 30% or more were considered positive (Murphy *et al.*, 1999).

$$S/P = 100 \ (OD \ sample-OD \ negative/OD \ positive-OD \ negative).$$

Indirect-ELISA for Detection of Antibodies Against IBR Virus

The serum and milk samples were tested for presence of antibodies against BHV-1 with a comercially available Screening/Verification blocking ELISA (HerdChek, Idexx, USA), specific for BHV-1 glycoprotein B (gB) (Kramps *et al.*, 1994). All samples were tested according to the manufacturer's instructions. Serum samples were diluted twenty-five-fold (1: 25) with sample diluent, while defatted milk samples were diluted two-fold (1: 2). Samples were dispensed first in screening plates coated only with BHV-1 gB antigen. The presence or absence of antibodies to IBR was determined by the sample to Positive (S/P) ratio to each sample. Samples with S/P ratios of 0.500 and greater were considered positive and do not require verification, while those with S/P ratios less than 0.250 were classified as negative. Samples with S/P ratios greater or equal to 0.250 but less than

0.500 were further confirmed using verification plates, which were coated with BHV-1 gB and normal host cells (NHC) antigens in alternate columns. Samples with S/NHC ratio greater than or equal to 1.80 were considered verified positive for IBR antibodies, while ratios less than 1.80 were classified as negative (Herring *et al.*, 1980; VanDonkersgoed and Babiuk, 1991; Boelaert *et al.*, 2005).

Statistical Analysis

Differences in antibody prevalence between groups with and without reproductive disorders or parasitic infections for IBR and BVD viruses were tested by the Chi-square test for tables which involve two rows against two columns (χ^2 with Yates correction) as described by Bailey (1995).

RESULTS

Incidence of Reproductive Disorders and Parasitic Infections

Out of 360 female buffaloes, examined in Lower Egypt during a period of 2 years (2004-2006), 171 (47.5%) were suffering from reproductive disorders and 216 (60.0%) were infected with internal parasites (Table 1). The main recorded reproductive disorders were ovarian inactivity with non detectable plasma progesterone level (27.5%), endometritis (8.6%), delayed puberty (8.3%), mastitis (5.8%), retained placenta (2.2%), repeat breeding (1.1%) and abortions (1.1%). However, all of these animals were negative for brucellosis. The main recorded parasitic infections were coccidiosis (42.5%), ascariasis (20.8%), trichostrongylosis (6.9%) and fasciolosis (4.7%).

Prevalence of IBR and BVD

Antibodies prevalence for IBR in sera and milk of the tested buffaloes using indirect-ELISA was 43.3 and 31.4%, respectively. Also, BVD virus was detected in 12.2% of the tested animals using direct-ELISA (Table 2).

Prevalence of IBR and BVD in Relation to Reproductive Disorders

The percentages of positive IBR and BVD were extremely significant in buffaloes with reproductive disorders (78.2 and 72.7%, respectively) compared with those without reproductive disorders (21.8 and 27.3%, respectively). The differences were statistically significant and indicated a tight connection between prevalence of IBR and BVD viruses and occurrence of reproductive disorders (Table 3).

Prevalence of IBR and BVD in Relation to Parasitic Infections

Antibodies to IBR and BVD were found in percentages of 88.5 and 84.1% in buffaloes with parasitic infections, meanwhile only 11.5 and 15.9% detected in buffaloes without parasitic infections, respectively. The difference was statistically significant and indicated a relationship between prevalence of IBR and BVD and occurrence of parasitic infections (Table 4).

Table 1: Incidence of reproductive disorders and parasitic infections in female buffaloes

Reproductive disorders	No.	%	Parasitic infections	No.	%
Ovarian inactivity	99	27.5	Coccidiosis	153	42.5
Endometritis	31	8.6			
Delayed puberty	30	8.3	Ascariasis	75	20.8
Mastitis	21	5.8			
Retained placenta	8	2.2	Trichostrongylosis	25	6.9
Repeat breeding	4	1.1			
Abortions	4	1.1	Fasciolosis	17	4.7
Total	171	47.5*	Total	216	60.0*

*From the total examined number (360 animals)

Table 2: Prevalence of IBR and BVD in Egyptian buffaloes

Disease	No. of examined animals	Positive reactors		Negative reactors	
		No.	%	No.	%
IBR (serum)	360	156	43.3	204	56.7
IBR (milk)	105	33	31.4	72	68.6
BVD (blood)	360	44	12.2	316	87.8

Table 3: Prevalence of IBR and BVD viruses in buffaloes in relation to reproductive disorders

Parameters	Buffaloes with reproductive disorders (n = 171)								Buffaloes without reproductive disorders	Chi-square test
	Total (n = 171)	Ovarian inactivity (n = 99)	Delayed puberty (n = 30)	Endometritis (n = 31)	Mastitis (n = 21)	Repeat breeding (n = 4)	Retained placenta (n = 8)	Abortions (n = 4)	(n = 189)	(χ^2)
	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	No. %	
Positive IBR (n = 156)	122 78.2	69 44.2	24 15.4	20 12.8	13 8.3	3 1.9	5 3.2	3 1.9	34 21.8	101.92**
Negative IBR (n = 204)	49 24.0	30 14.7	6 2.9	11 5.3	8 3.9	1 0.5	3 1.5	1 0.5	155 76.0	
Positive BVD (n = 44)	32 72.7	18 41.0	7 16.0	6 13.6	6 13.6	1 2.3	3 6.8	1 2.3	12 27.3	11.67*
Negative BVD (n = 316)	139 44.0	81 25.6	23 7.3	25 7.9	15 4.8	3 1.0	5 1.6	3 1.0	177 56.0	

* Significant at p<0.001, ** Significant at p<0.0001

Table 4: Prevalence of IBR and BVD viruses in buffaloes in relation to parasitic infections

Parameters	Buffaloes with parasitic infections (n = 216)										Buffaloes without parasitic infections		Chi-square test
	Total (n = 216)		Coccidiosis (n = 153)		Ascariasis (n = 75)		Trichostr-ongylosis (n = 25)		Fasciolosis (n = 17)		(n = 144)		(χ^2)
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Positive IBR (n = 156)	138	88.5	100	64.1	50	32.1	15	9.6	11	7.1	18	11.5	90.84**
Negative IBR (n = 204)	78	38.2	53	26.0	25	12.3	10	4.9	6	2.9	126	61.8	
Positive BVD (n = 44)	37	84.1	33	75.0	12	27.3	5	11.4	2	4.6	7	15.9	11.01*
Negative BVD (n = 316)	179	56.7	120	38.0	63	20.0	20	6.3	15	4.8	137	43.3	

* Significant at p<0.001, ** Significant at p<0.0001

DISCUSSION

Prevalence of antibodies to IBR virus using indirect glycoprotein B-blocking ELISA and detection of BVD virus using direct-ELISA revealed that 43.3 and 12.2% in the tested buffaloes were positive, respectively. Buffaloes in this study were not vaccinated against IBR and BVD, negative for brucellosis and showed no symptoms of disease at the time of examination. Little attempts were recorded in the available literatures concerning the prevalence of IBR and BVD in buffaloes. However, in similar cases in non vaccinated cows, antibodies to IBR virus were present in 34.9-85.8% of the examined animals, while BVD virus was present in 50.9-79.2% of the cases (Fulton and Segar, 1982; Celedon *et al.*, 1996; Biuk-Rudan *et al.*, 1999). The immunosuppressive effect of BVD virus may be a triggering factor for activation of IBR virus in latently infected animals (Biuk-Rudan *et al.*, 1999). Kramps *et al.* (1994) and de Wit *et al.* (1998) estimated the sensitivity of glycoprotein B-blocking ELISA to be 99.0%, whereas its diagnostic specificity ranged from 96.0 to 99.7%.

Detection of antibodies against IBR in individual milk samples using milk-ELISA gave good diagnostic potency (31.4%) compared with serum-ELISA (43.3%). Several authors noted a significant correlation between antibody concentrations, as detected by ELISA, in milk and sera for a number of bovine infections (Niskanen *et al.*, 1989; Witte *et al.*, 1989; Hoorfar and Wedderkopp, 1995). Milk-ELISAs performed efficiently as well as serum-ELISAs and there was no evidence that the stage of lactation had a significant effect (Prichard *et al.*, 2002). However, the relatively low prevalence of IBR by milk-ELISA in this study can largely be explained by the immunoglobulins concentration in milk which is 20 to 40 folds lower than in serum (Caffin *et al.*, 1988). Although antibody concentrations were generally lower in milk than sera, equivalent test sensitivity can be achieved by using different cut-off values or different test dilutions (Niskanen *et al.*, 1989; Witte *et al.*, 1989). Moreover, milk may actually produce fewer false-positive results than serum, since it acts as a very efficient blocker for non specific bindings (Prichard *et al.*, 2002).

The prevalence of IBR and BVD in female buffaloes with reproductive disorders was extremely high. There was a significant statistical difference in the number of IBR and BVD positive buffaloes between the group with reproductive disorders and the group without disorders, suggesting that infection with IBR and BVD viruses may have a greater influence on the occurrence of reproductive disorders in female buffaloes. Field and epidemiological studies suggested that BVDV has an adverse impact on early reproductive performance in the form of infertility, significant drop in pregnancy rate at the time of conception, early embryonic deaths and abortions (Houe *et al.*, 1993; McGowan *et al.*, 1993; Fredriksen *et al.*, 1998; Hage *et al.*, 1998; Grooms, 2006). Seroprevalence of IBR among cattle with different reproductive disorders using Ab-ELISA revealed 19.7% positive reactors. Antibodies against BHV-1 were deteced in 20, 25, 26.7, 11 and 29.7% of animals with history of abortion, retained placenta, metritis, anaestrum and repeat breeding, respectively (Rajesh *et al.*, 2003; Rola, 2003). Another study reported a significant decrease in the number of successful insemination in IBR seropositive dairy cows (Hage *et al.*, 1998). The same authors did not find an association between BHV-1 infection and mastitis in a dairy herd. Such a relation has been reported previously (Siegler *et al.*, 1984), but different BHV-1 strains may have different effects on the development of mastitis. However, Biuk-Rudan *et al.* (1999) detected antibodies to both IBR and BVD viruses in 80.8% of cows with reproductive disorders, but in only 46.8% of cows without disorders. They concluded that the interaction between IBR and BVD viruses could increase the occurrence of reproductive disorders in dairy cows.

Presence of IBR and BVD in female buffaloes was also considered in relation to other parasitic infections. In the groups of buffaloes that proved positive for IBR and BVD viruses, a statistical significant differences were observed in buffaloes with parasitic infections and those without parasitic infections. BVDV is immunosuppressive factor, which might increase susceptibility to other infectious agents and reduces the animal's resistance to other enteric pathogens (Elvander *et al.*, 1998; Fray *et al.*, 2000b; Stahl *et al.*, 2006). Concurrent infections of BVDV and other respiratory or enteric pathogens may produce a more severe disease than either pathogen alone (Potgieter *et al.*, 1984; Alenius *et al.*, 1991).

CONCLUSIONS

Results of this study clearly established that IBR and BVD were subclinically prevalent in Egyptian buffaloes. Management decisions and control strategies need the development of rapid and accurate diagnostic tools. Immunoenzymatic assays are advantageous because they are cheap, reliable and quik to perform, especially when applied to milk. Thus, ELISA may be a useful tool in large scale screening and eradication programms giving insight to the local IBR and BVD infection status. IBR and BVD viruses may be important causes of reproductive losses in buffaloes and must be considered

when investigating any reproductive problems where an infectious cause is suspected. Moreover, prevention and control of parasitic infections could be a helpful tool in reduction of IBR and BVD infections and reproductive disorders in buffaloes.

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