Australian Journal of Basic and Applied Sciences, 5(10): 696-699, 2011
ISSN 1991-8178

Serum Antibody Detection Against Bovine Viral Diarrhea Virus (BVDV) Through Elisa Method In Sarabian Dairy Cows

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Abstract: Bovine viral diarrhoea virus (BVDV), a member of the Pestivirus genus, is an important viral pathogen of cattle that causes fatal diarrhoea syndrome, respiratory problems and reproductive failure. The objective of this study was Sera antibody detection against bovine viral diarrhea virus (BVDV) through ELISA method in sarabian dairy cows. In this study about 84 complete blood samples from apparently healthy cows were collected. Sera samples were used to detection of antibody against BVDV by special kits produced by PRIONCS Co and through ELISA method. Results showed that base on pregnancy, 59 cases (70.2%) showed negative and 25 cases (29.8%) showed positive. The diagnosis of this contamination rate indicates the vast involvement of dairy cows in the province and is itself a serious alarm for faster tackling programs against this disease in the province.

Key words: antibody, sera, bovine viral diarrhea, ELISA, sarabian dairy cows.

INTRODUCTION

Bovine viral diarrhoea virus (BVDV), a member of the Pestivirus genus, is an important viral pathogen of cattle that causes fatal diarrhoea syndrome, respiratory problems and reproductive failure. Infection during pregnancy can result in embryonic resorption, abortion, stillbirth calves, teratogenesis, or the birth of persistently infected (PI) calves, which are the principal reservoir of the virus in nature and may later develop mucosal disease, always a fatal condition (Epizooties, 2004).

Pestiviruses are able to cross species barriers and infect a wide range of hosts within the Artiodactyla order (Hamblin and Hedger, 1979). Recently, Deregt *et al.* (2005) reported BVDV infection in bisons. The phylogenetic characterization of two strains of BVDV isolated from two bisons (Bison bison bison) revealed that both clustered in BVDV-1. Although most of the reported BVDV infections refer to the Bovidae family, a few of them refer to infection in buffaloes. These studies are mostly serological (Akhtar, S., Asif, M., 1996; Sudharshana et al., 1999; Zaghawa, A., 1998). On the other hand, Becher et al. (1997) disclosed that an isolation of BVDV, originating of a pool of foetal serum of a buffalo, grouped in BVDV-1 (Becher et al., 1997). Infection with the bovine viral diarrhoea virus (BVDV) was first reported in (15. Olafson et al., 1946). In Austria, BVDV was first described to have occurred in a mountainous region in the year (Burki et al., 1972). Economic losses result from prenatal infections (Houe, H., Palfi, V., 1993), which cause abortions, infertility, malformations in calves, and the birth of persistently infected immunotolerant calves (PI) that often die of mucosal disease (MD) (Brownlie et al., 1984; Roeder and Drew, 1984). Diarrhoea and symptoms of respiratory disease are observed more frequently in infected herds (Baker, J.C., 1995). Bovine Viral Diarrhoea Virus (BVDV) is a pathogen of major concern in Europe. BVDV infection is widespread among cattle herds (Houe, H., 1999). Of considerable economic impact (11), it is characterized by abortions or congenital deformities, induces milk production losses and growth delays, and increases occurrence of other diseases. As an example, the total annual losses were estimated in Denmark to US$ 20 million in 1992 (Houe, H., 1999), while the annual incidence of acute infections was estimated to 34%. To limit BVDV spread and its consequences, control strategies are implemented in several regions in Europe (Greiser-Wilke et al., 2003; Lindberg et al., 2006). However, their efficiency is hard to evaluate or to compare to each other by field observations because no reference situations (without control) are generally available, and because observations on the long term should be performed, which is costly and sometimes unfeasible. Enzyme-linked immunosorbent assay (ELISA), also known as an enzyme immunoassay (EIA), is a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. The ELISA has been used as a diagnostic tool in medicine and plant pathology, as well as a quality-control check in various industries. In simple terms, in ELISA, an unknown amount of antigen is affixed to a surface, and then a specific antibody is applied over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal, most commonly a colour change in a chemical substrate.

Performing an ELISA involves at least one antibody with specificity for a particular antigen. The sample with an unknown amount of antigen is immobilized on a solid support (usually a polystyrene microtiter plate)

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either non-specifically (via adsorption to the surface) or specifically (via capture by another antibody specific to the same antigen, in a "sandwich" ELISA). After the antigen is immobilized, the detection antibody is added, forming a complex with the antigen. The detection antibody can be covalently linked to an enzyme, or can itself be detected by a secondary antibody that is linked to an enzyme through bioconjugation. Between each step, the plate is typically washed with a mild detergent solution to remove any proteins or antibodies that are not specifically bound. After the final wash step, the plate is developed by adding an enzymatic substrate to produce a visible signal, which indicates the quantity of antigen in the sample.

MATERIALS AND METHODS

In this study about 84 complete blood samples from apparently healthy cows were collected. These specimens were obtained from June to July 2011 and were sent to laboratory immediately. In lab, sera samples were used to detection of antibody against BVDV by special kits produced by PRIONCS Co and through ELISA method.

In lab, samples were centrifuged at 1500 round per minute for 15 minute and buffy coat achieved. In this term tried to prevention from assimilation of buffy coat with RBCS. Samples plasmaś were frozen to complementary experiments. Obtained buffy coats were distilled into 3 separate eppendorf tube and numbered. It must be note that all animals haven't any history of receiving any BVD vaccine and mucosal diseases.

RESULTS AND DISCUSSION

Comparison of trial results based on pregnancy Status:

According table 1, non-pregnant cases, 22 cases (56.4%) showed negative and 17 cases (43.6%) showed positive. In pregnant cases, 37 cases (82.2%) showed negative and 8 cases (17.8%) showed positive. By comparison of above data it has been revealed that trial outcome is significant among pregnant and non-pregnant groups (P<0.05).

Table 1: Comparison of trial results based on pregnancy Status

			Trial result		sum	χ^2	P
			Negative	Positive			
Pregnancy	Non-pregnant	Frequency	22	17	39	6.65	0.01
		%	56.4	43.6	100		
	Pregnant	Frequency	37	8	45		
		%	82.2	17.8	100		
Total		Frequency	59	25	84		
		%	70.2	29.8	100		

Comparison of trial results based on parturition Status:

Based on table 2, in cases which had not labor experience, 3 cases (20%) showed negative and 12 cases (80%) showed positive. In cases which had exactly normal pregnancy, 1 case (100%) showed positive. In cases which had normal pregnancy, 35 cases (81.4%) showed negative and 8 cases (18.6%) showed positive. In cases which had dystocia, 11 cases (84.6%) showed negative and 2 cases (15.4%) showed positive. In cases which had 3 times normal and 2 times dystocia, 1 case (100%) showed negative. In cases which had 3 times normal and 1 time dystocia, 1 case (100%) showed positive. In cases which had 2 times normal and 1 time dystocia, 6 cases (100%) showed negative. In cases which had 1 time normal and 1 time dystocia, 1 case (50%) showed negative and 1 case (50%) showed positive. In cases which had 1 time normal and 2 times dystocia, 1 case (100%) showed negative. In cases which had 9 times normal, 2 times dystocia and 1 time Stillbirths, 1 case (100%) showed negative. Based on Chi square test results it revealed that there is significant difference among groups based on parturition status (P<0.05).

Table 2: Comparison of trial results based on parturition Status

			Trial result		sum	χ^2	P
			Negative	Positive			
Parturition	Without pregnancy	Frequency	3	12	15	30.88	0.000
		%	20	80	100		
	Exactly normal	Frequency	0	1	1		
		%	0	100	100		
	Normal	Frequency	35	8	43		
		%	81.4	18.6	100		
	Dystocia	Frequency	11	2	13		
		%	84.6	15.4	100		
	3 times normal- 2 times dystocia	Frequency	1	0	1		
		%	100	0	100		
	3 times normal- 1 time dystocia	Frequency	0	1	1		
		%	0	100	100		
	2 times normal- 1 time dystocia	Frequency	6	0	6		
		%	100	0	100		
	1 time normal- 1 time dystocia	Frequency	1	1	2		
		%	50	50	100		
	1 time normal- 2 times dystocia	Frequency	1	0	1		
		%	100	0	100		
	9 times normal, 2 times dystocia and 1 time Stillbirths	Frequency	1	0	1		
		%	100	0	100		
	Total	Frequency	59	25	84		
		%	70.2	29.8	100		

Discussion:

The Bovine Viral Diarrhea Virus (BVDV) is a pestivirus, which causes two diseases: Bovine Viral Diarrhea (BVD) and Mucosal Disease (MD). Bovine Viral Diarrhea is a pathology induced by one of the two strains of the virus (cytopathogenic and non-cytopathogenic) (15). The acute form, characterized by fever and diarrhea, is transient, with high morbidity rates and low mortality rates. Adult animals can also be infected with an asymptomatic subclinical form. Mucosal Disease has low morbidity rates (1%), but high mortality rates. Most often it is characterized by ulcers at different levels of the digestive tract and diarrhea that is often hemorrhagic. Many pathologies are associated with or aggravated by the BVDV, including, among others, respiratory diseases, slow development, congenital defects, etc. Mucosal Disease occurs in calves infected during gestation (Immunotolerant Persistently Infected (I.P.I.) animals) (9). These animals were infected with a non-cytopathogenic strain by the transplacental way between the 42nd and the 120th day of gestation. This corresponds to a period when immunocompetence is being established in the foetus: foreign antigens present at this time are considered as self-antigens and no immune response is developed against them. Thus, the persistently infected animals do not produce antibodies against the strain they are infected with. Mucosal Disease is induced by mutation of the non cytopathogenic strain to a cytopathogenic strain. The main sources of infection are I.P.I. Animals, which continuously produce and shed the virus, and, in a transient manner (during 10 days), animals recently contaminated with a primary BVDV infection. Transmission of the virus can be oral nasal, conjunctival, genital or transplacental. The presence of BVDV in a herd can be detected with serological screening, which reveals the presence of animals with specific antibodies. However, it does not enable persistently infected animals to be detected. In one study by Diéguez et al., 2009 revealed that based on the serological profiles of the herds, 12 of the 101 Dairy farms were suspected of harboring an active infection at the study outset. In these farms, at least one PI animal was detected using the antigen ELISA kit although the number of PI animals identified per farm was 1–5. The age of these PI animals ranged from 1 month to 5 years. The remaining 89 farms were classified as being free of active BVDV infection according to the serological tests (free or only evidence of ancient infections). The results indicate that there was a significant relationship between the infection status (actively infected or not) of the farms and the proportion of farms with a high cumulative incidence of mortality and respiratory disorders (7). As in previous studies, relationships were observed between the infection status of

the herds and the cumulative incidence of respiratory disease. Houe, 2003 mentioned an increase of respiratory disorders from 2% to 5% in infected herds. Equally, described an increase from 1% to 10% of other pathologies that concern the health of the calves (11).

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