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Prevalence of Bovine Viral Diarrhea Virus Persistency in 12 Holstein Cattle Dairy Herds of Charlottetown, Canada

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Abstract.- The prevalence of bovine viral diarrhea virus (BVDV) persistency was examined in 12 Holstein dairy herds in Charlottetown, Canada, from Feb. 2009 to Oct. 2009. A total of 469 serum and ear notch biopsy pairs were collected. Depending upon the age and vaccination status, the sampled animals were divided into three groups, A, B1 and B2. Group A consisted of 146 animals originating from 4 herds (≤ 6 month of age, FN1, FN2, FN3, FN4), group B1 comprised 267 animals representing 5 vaccinated herds against BVDV (≥ 6 months of age, FV1, FV2, FV3, FV4, FV5), whereas, 56 samples were taken from 3 BVDV non-vaccinated herds of group B2 (≥ 6 months of age, FN5, FN6, FN7). All the animals were pre-screened through serum neutralization test (SNT), whereas, all ear notch biopsies of group A (n =146) and only of those animals of group B1 (n = 15) and B2 (n = 37) which had SN titer ≤ 1:64 were processed to confirm the BVDV persistent infection. Only five animals were found positive originating from group A (n = 3) and B1 (n = 2) during first sampling. Upon follow up testing, two animals from each of group A and B1 remained positive confirming persistent infection (PI). A prevalence of 0.85% and 2.03% was observed in all the collected and pre screened animals, respectively. The results obtained in this study, demonstrates that the prevalence of PI animals in dairy herds of Prince Edward Island is similar to that, reported from other surveys carried out in other countries. This prevalence PI animals with BVDV in Holstein dairy herds of Charlottetown, Canada, reveal the necessity of further studying BVDV infection in the area.

Key Words: Prevalence, Bovine Viral Diarrhea Virus, Persistent infection, Holstein dairy cattle.

INTRODUCTION

Bovine viral diarrhoea virus (BVDV) is a major viral pathogen (Gunn *et al.,* 2005) associated with reproductive, respiratory and gastrointestinal diseases of cattle (Houe, 1999). The BVDV is a heterogenous group of viruses belong to genus pestivirus of family flaviviridae (Becher *et al.,* 2003). It has worldwide distribution and exits as a cytopathogenic (CP) and noncytopathogenic (NCP) biotype, based on the presence or absence of visible cytopathic effects in the cell culture (Dubovi, 1990). Postnatal BVDV infection is generally considered of little importance, however, it shows severe consequences in case of transplacental infection. These includes early embryonic death, mummification, still birth, abortion, congenital ocular, neural defects of fetus and most importantly birth of persistently infected (PI) calves (Fray *et al*., 2000; Straub, 2001; Ackermann and Engels, 2006). Fetuses infected with non-cytopathogenic biotype of BVDV between 30 to 120 days of gestation develop immune tolerance and will be persistently infected (PI). Such calves have increased susceptibility to other diseases, and frequently succumb to mucosal disease (McClurkin *et al.,* 1984; Sandvik, 2005).

As PI animals have very high and persistent viremia, therefore, they serve as primary reservoir of BVDV and disseminate infection by shedding the virus through body excrements like nasal discharge, saliva, semen, urine, faeces, tears and milk (Brock *et al*., 1991). PI animals usually have a very high and persistent viremia (Brock *et al.,* 1998). It has already proved that under experimental conditions, PI animals transmit infection approximately to 60% susceptible animals within 24 h (Littlejohns, 1985; McGowan *et al.,* 1993).

The estimation of economic losses due to persistent infection depends upon the initial herd immunity, pregnancy status of the animal at the time of infection and the virulence of the infecting virus strain. It may vary in individual herd outbreaks from

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a few hundred to several thousand dollars (Houe, 1999). Determination of prevalence of persistently infected animals with BVDV indicates the number of fetal infection during early gestation. In non-vaccinated herds carrying antibody against BVDV have been used to determine the presence or absence of PI cattle. In case of vaccinated herds, distribution of antibody carriers require further elucidation. Epidemiological surveys revealed a 0.5 to 2% prevalence of BVDV persistency in the cattle population in different countries of the world (Taylor *et al*., 1995; Brock, 2003; Peterhans *et al.,* 2003).

There is paucity of information regarding prevalence of BVDV persistency in Charlottetown, Prince Edward Island, Canada. Therefore, present study was conducted to determine the prevalence of PI animals among Holstein dairy cattle herds of Charlottetown.

MATERIALS AND METHODS

The study comprised of 9 months period from February 2009 to October 2009. A total of 469 animals were sampled from randomly selected 12 Holstein dairy cattle farms (names were coded to maintain owner confidentiality). Both serum and ear notch biopsy were taken from each animal and transported to the Research Virology Laboratory, Atlantic Veterinary College, University of Prince Edward Island, Canada for further processing. Depending upon the age and vaccination status, the sampled animals were divided into three groups, A, B1 and B2. Group A consisted of 146 animals originating from 04 herds (≤ 6 month of age, FN1, FN2, FN3, FN4), group B1 comprised 267 animals representing 5 vaccinated herds against BVDV (≥ 6 months of age, FV1, FV2, FV3, FV4, FV5), whereas, 56 samples were taken from 03 BVDV non-vaccinated herds of group B2 (≥6 months of age, FN5, FN6, FN7).

Complete history (age, breed of the animals, total number of animals on a farm, pregnancy status, and previous disease if any) was recorded. The groups were made due to initial screening test (serum neutralization test-SNT), the reason being, that normally, PI animals older than 6 months of age, were accompanied by absence of specific anti-BVDV antibodies due to immune tolerance (McClurkin *et al.,* 1984). Nevertheless animals below 6 months of age might had passive antibodies in the course of persistency, if the mother passed the virus to fetus in the course of transient infection and was not herself persistently infected, so they could not be pre-screened by serology. Hence, all the ear notch biopsies of group A and only those animals of group B1 and B2 which had SN titre less than or equal to 1:64 were processed to confirm the BVDV persistent infection through antigen capture ELISA. Furthermore, the animals tested positive were re-sampled after 30 days of first round of testing to differentiate transient and persistently infected animals.

Serum neutralization test (SNT)

To determine the antibody status of herds against BVDV, serum samples were subjected to Serum neutralization test as described by Deregt *et al.* (1992).

Antigen capture ELISA (AC-ELISA)

For the detection of BVDV, all ear notch biopsies of group A, 15 of group B1 and 37 of group B2 were subjected to antigen-capture ELISA, using commercial antigen detection test kit (HerdChek IDEXX, USA), as per manufacturer recommendations. The kit detected the highly stable, Erns (gp48) virus protein, which was secreted extracellularly during virus replication. (Saliki *et al.,* 2000). The assay was validated as per manufacturer instructions and presence and absence of BVDV was determined by sample to positive (S/P) ratio for each sample. The S/P ratio < 0.2 was taken as negative and > 0.39 is considered as positive. A S/P ratio between 0.2 to 0.39 was considered as suspected. The suspected samples were tested twice along with positive and negative control, first with the standard working detector reagent and then with modified working detector reagent. For suspected samples, the S/P ratio > 0.2 was taken as positive while < 0.2 as negative.

RESULTS AND DISCUSSION

The SNT based serological survey showed a seroprevalence of 100 percent and 64.28 percent in vaccinated (group B1) and non-vaccinated herd

(group B2) respectively (Tables I, II). The seroprevalence observed in the non-vaccinated study herds differs from previously reported 87 percent to 98 percent in non-vaccinated herds (with one or more PI animals), has been reported (Waldner and Campbell, 2005; Seki *et al.,* 2006; Houe *et al.,* 2006). The reason of comparatively low seropositive animals in the non-vaccinated herds seems to be due to absence of PI in the study herds. Previously Stahl *et al.* (2008) reported a high seropositivity in herds with PI animals than in herds without PI animals, even if the herds had been vaccinated with a inactivated vaccine.

Irrespective of the vaccination status, higher seroconversion (93.8%) was observed among older animals as compared to youngers (91.78%) (Table III). This is consistent with the previously reported studies (Mockeliu *et al.,* 2004; Garoussi *et al.,* 2008), where a comparatively high BVDV seroprevalence in old aged herds has been described. The increase in seroprevalence with increasing age may be due to the higher exposure to the virus, and that BVDV antibodies in most cases are life long (Mockeliu *et al.,* 2004).

An overall seroprevalence of > 90% was recorded in the test herds which is in agreement with previously reported prevalence (70% to 100%) in various studies worldwide (Edwards *et al*., 1987; Reinhardt *et al.,* 1990; Houe and Meyling, 1991; Niskanen *et al.,* 1991; Niskanen, 1993; Obando *et al.,* 1999). Nevertheless, the seroprevalence slightly varies with the findings reported in USA, Canada**,** Germany, England, Kenya, New Zealand, Australia, Colombia and Argentina, in which 50-90% of the tested animals were found seropositive (Tayler and Rampton, 1968; Harkness *et al.,* 1978; Edwards *et al.,* 1987; Houe and Meyling, 1991). This variation in seroprevalence in different countries may be due to difference in cattle population age, cattle density, herd size, housing systems, bio-security and managemental practices, which in general could be important risk factors in transmission and persistence of BVDV (Wittum *et al.,* 2001; Sthal *et al.,* 2007). The high seroprevalence observed in the studied herds demonstrates that the BVDV situation in Charlottetown, Prince Edward Island is critical. The presence of high number of seropositive animals was an indirect indication of the presence of one or more PI animals in the test herds.

It has been well documented previously that PI animals are immunotolerant to the infecting strain, but if exposed to a heterologous strain, they may develop low level of antibody (1:2-1:4) (Fulton *et al.*, 2003b). Thus, the samples from animals showing low antibody titres were selected together with antibody negative samples for the detection of PI animals. On the basis of SNT, 15 ear notch biopsies of group B1, 37 of group B2 and all 146 of group A were selected.

For the detection of BVDV, antigen capture ELISA was applied using ear notch biopsies as this assay may yield false negative results, if antibodies are present in the sample. This must be considered when testing blood based diagnosis in young animals that might have the maternal antibodies (Zimmer *et al.*, 2004). Moreover, antigen capture ELISA using ear skin tissues has been shown reliable and independent of the presence of colostral antibodies, thus overcoming the young age related problem (Hill *et al.,* 2007). Therefore, in this study, ear notch biopsies were selected for confirmation of BVDV. Three ear notch biopsies originating from each of group A (FN1, FN2 and FN4) and B1 (FV1) were tested positive with S/P ratios of 0.704, 0.822, 0 895 0.921 and 0.395 respectively, in the first round of testing (Table IV). Before follow up sampling of positive animals, one positive calf of FN4 herd died due to respiratory disease before collection of the second samples. Therefore, four animals were possible to be resampled and tested again to discriminate transient and persistent infection. In the second round of testing, all the positive animals remain positive with S/P ratios of 0.661, 0.615, 0.406 and 0.457, respectively (Table IV). The variation in S/P ratios between first and second rounds of testing may be due to the fluctuating nature of viremia in these animals. This finding is in agreement with that of Brock *et al.* (1998), who revealed that viremia can transiently diminish, specifically when a PI animal is superinfected by a heterologous BVDV strain, resulting in the production of antibodies that cross-react with the strain of the PI animal. In such cases, the viremia will be diminished until the antibody titre decreases. This temporary interruption of viremia might result in reduced virus shedding

Table I.- Anti-BVDV-serum neutralizing antibody titres in vaccinated herds.

Farm ID	Herd size	Age (months)	Sign	SNT(Log_2) range						Seroprevalence (%)
				<1:2		1:2 to 1:64		1:128 to 1:2048		
				n	%	n	%	n	%	
Vaccinated farm 1	42	24-36	Abortion	0.0	0.0	09	21.2	33	78.57	
Vaccinated farm 2	45	24-36	Abortion	0.0	0.0	0.0	0.00	45	100	
Vaccinated farm 3	70	24-36	Abortion	0.0	0.0	0.0	0.00	70	100	100
Vaccinated farm 4	54	12-36	Nil	0.0	0.0	0.0	0.00	54	100	
Vaccinated farm 5	56	18-36	Nil	0.0	0.0	06	10.71	50	89.28	

Table II.- Anti-BVDV-serum neutralizing antibody titres in non-vaccinated herds.

Farm ID	Herd size	Age (months)	Sign	SNT(Log_2) range						Seroprevalence (%)
				<1:2		1:2 to 1:64		1:128 to 1:2048		
				n	%	n	%	n	%	
Vaccinated farm 1	45	1-6	Nil	0.0	0.0	16	35.6	29	64.4	
Vaccinated farm 2	29	1-6	Nil	10	34.48	3	10.34	16	55.17	
Vaccinated farm 3	40	1-6	Nil	02	05	12	30	26	65	91.78
Vaccinated farm 4	32	1-6	Nil	0	0.0	1	3.12	31	96.87	
Vaccinated farm 5	11	12-18	Nil	02	18.18	4	36.36	05	45.5	
Vaccinated farm 6	36	24-36	Nil	17	47.22	12	33.33	7	19.4	64.28
Vaccinated farm 7	09	6-12	Nil	1	11.1	01	11.1	07	77.8	

Table III.- Group wise distribution of anti-BVDV-serum neutralizing antibody titre.

Group	Total animal	SNT(Log_2) Range			Overall seropositivity
		<1:2	1:2-1:64	1:128-1:2048	
A	146	8.82	23.52	69.86	91.78 %
B1, B2	323	6.19	9.90	83.9	93.8%

Table IV.- Antigen-capture ELISA on ear notch biopsies.

Group	No. of animals	Positive Sample ID		S/P Ratio		No. of PI animals	Persistency (%)
		1st testing	2nd testing	1st testing	2nd testing		
A	146	615	Done	0.704	0.661		
		736	Done	0.822	0.615	2/145	1.37
		080891	Nd	0.895	Nd		
B1	15	529	Done	0.921	0.406	2/15	13.3
		7621	Done	0.395	0.457		
B2	37	Nil	Nil	Nil	Nil	0/37	0.00

leading to PI animals to escape from detection if the amount of virus is lower than the detection level at the moment of sampling.

In this study no discrepancy in terms of detecting BVDV, between first and second round of testing has been observed and no acutely infected animal was detected by AC-ELISA, which is in agreement with the finding of Hilbe *et al.* (2007). However, there are some studies that have revealed detection of BVDV infection at acute stage using

tissue samples by AC-ELISA (Cornish *et al.,* 2005). This disagreement might be due to the fact, that acutely infected animals do not have a significant amount of antigen in the skin tissue in the vast majority of cases (Saliki and Dubovi, 2004). Based on the results obtained during this study, it can be concluded that AC-ELISA using ear notch biopsies could be used for the determination of PI status of animals.

Table V.- Prevalence of BVDV persistency (in percentage) in all collected and tested samples.

Group	Vac. history	Tests	No. of PI animal	PI. prevalence (age)
Collected samples				
A	Non-Vac	145	2	1.37
B1	Vac	267	2	0.74
B2	Non-Vac	56	Nil	0.00
A+B	Vac+ non-Vac	468	4	0.85
Tested samples				
A	Non-Vac	145	2	1.37
B1	Vac	15	2	13.3
B2	Non-Vac	37	Nil	0.00
A+B	Vac+ non-Vac	197	4	2.03

The data of one positive calf died before follow up sampling was excluded, therefore, 0.85% and 2.03% prevalence of persistency was observed in remaining 468 sampled and 197 tested animals, respectively (Table V). The results obtained in this study, demonstrates that the prevalence of PI animals in dairy herds of Prince Edward Island is similar to that, reported from other surveys carried out in other countries (Reinhardt *et al.,* 1990; Houe and Meyling, 1991; Houe *et al.,* 1994; Houe, 1995; Frey *et al.,* 1996; Braun *et al.,* 1997; Vega *et al.,* 1997). However, the prevalence observed in this study deviate from those reported in previous studies by Seyyal *et al. (*2002) and Taylor *et al. (*1995). Again, this could be due to the some differences between regions and countries related to management systems, as well as animal trading activities (Nuotio *et al.,* 1999). This prevalence PI animals with BVDV in Holstein dairy herds of Charlottetown, Canada, reveal the necessity of further studying BVDV infection in the area.

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