

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

Arfan Ahmad*¹, Masood Rabbani¹, Khushi Muhammad², Muhammad Zubair Shabbir¹, Tahir Yaqub¹, Khalid Munir³, Fariha Akhter¹ and Arnost Cepica³

¹University Diagnostic Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan

²Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

³Department of Pathology and Microbiology, Atlantic Veterinary College, UPEI, Canada

Abstract.- The prevalence of bovine viral diarrhoea 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 $\leq 1:64$ 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 Diarrhoea 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 exists 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

* Corresponding author: iffivet@hotmail.com

0030-9923/2011/0002-0255 \$ 8.00/0

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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 managerial 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 (%)
		1 st testing	2 nd testing	1 st testing	2 nd testing		
A	146	615	Done	0.704	0.661	2/145	1.37
		736	Done	0.822	0.615		
		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.

ACKNOWLEDGEMENTS

We would like to thank HEC, Pakistan for its generous support and funding to complete this research study under Ph.D. split programme. Thanks are due to the University Management of Project entitled "Establishment of dairy and poultry animals training and research centers at Ravi Campus, UVAS, Pakistan". Furthermore, thanks are also due to Dr. Toki Iwamoto and administration of AVC, UPEI, Canada for their invaluable support during execution of this research work.

REFERENCES

- ACKERMANN, M. AND ENGELS, M., 2006. Pro and contra IBR-eradication. *Vet. Microbiol.*, **113**: 293-302.
- BECHER, P., AVALOS-RAMIREZ, R., ORLICH, M., ROSALES, S.C., KONIG, M., SCHWEIZER, M., STALDER, H., SCHIRRMIEIER, H. AND THEIL, H.J., 2003. Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. *Virology*, **311**: 96-104.
- BRAUN, R., BABIUK, L. AND VAN DRUNEN LITTEL-VAN DEN HURK, S., 1997. Enhanced immune response to an intradermally delivered DNA vaccine expressing a secreted form of BHV-1 gD. *Vaccine Res.*, **6**: 151-164.
- BROCK, K.V., 2003. The persistence of bovine viral diarrhea virus. *Biologicals*, **31**: 133-135.
- BROCK, K.V., GROOMS, D.L., RIDPATH, J.F. AND BOLIN, S.E., 1998. Changes in levels of viremia in cattle persistently infected with bovine viral diarrhea virus. *J. Vet. Diagn. Investig.*, **10**: 22-26.
- BROCK, K.V., REDMAN, D.R. AND VICKERS, M.L., 1991. Quantification of bovine viral diarrhea virus in embryo transfer flush fluids collected from a persistently infected heifer. *J. Vet. Diagn. Invest.*, **3**: 99-100.
- CORNISH, T.E., VAN OLPHEN, A.L., CAVENDER, J.L., EDWARDS, J.M., JAEGER, P.T., VIEYRA, P.T. WOODARD, L.F., MILLER, D.R. AND TOOLE, D.O., 2005. Comparison of ear notch immunohistochemistry, ear notch antigen-capture ELISA, and buffy coat virus isolation for detection of calves persistently infected with bovine viral diarrhea virus. *J. Vet. Diagn. Invest.*, **17**: 110-117.
- DEREGT, D., SMITHSON, S. AND KOZUB, G.C., 1992. A short incubation serum neutralization test for bovine viral diarrhea virus. *Can. J. Vet. Res.*, **56**: 161-164.
- DUBOVI, E.J., 1990. The diagnosis of bovine viral diarrhoea infections-a laboratory review. *Vet. Med.*, **85**: 1133-

- 1139.
- EDWARDS, S., DREW, T.W. AND BUSHNELL, S.E., 1987. Prevalence of bovine virus diarrhoea viraemia. *Vet. Rec.*, **120**: 71.
- FRAY, M.D., PATON, D.J. AND ALENIUS, S., 2000. The effects of bovine viral diarrhoea virus on cattle reproduction in relation to disease control. *Anim. Reprod. Sci.*, **60-61**: 615-627
- FREY, H.R., FLEBBE, U. AND LIESS, B., 1996. Prävalenz und klinische Symptomatik Persistenter BVD-Virusinfektionen in Rinderbeständen Niedersachsens. *Prakt. Tierarzt.*, **1**: 49-52.
- FULTON, R.W., SALIKI, J.T., BURGE, L.J. AND PAYTON, M.E., 2003B. Humoral immune response and assessment of vaccine virus shedding in calves receiving modified live virus vaccines containing bovine herpesvirus-1 and bovine viral diarrhoea virus 1a. *J. Vet. Med. B. Infect. Dis. Vet. Publ. Hlth.*, **50**: 31-37.
- GAROUSI, M.T., HAGHPARAST, A. AND ESTAJEE, H., 2008. Prevalence of bovine viral diarrhoea virus antibodies in bulk tank milk of industrial dairy cattle herds in suburb of Mashhad-Iran. *Prev. Vet. Med.*, **84**: 171-176.
- GUNN, G.J., SAATKAMP, H.W., HUMPHERY, R.W. AND STOTT, A.W., 2005. Assessing economic and social pressure for the control of bovine viral diarrhoea virus. *Prev. Vet. Med.*, **72**: 149-162.
- HARKNESS, J.W., SANDS, J. AND RICHARDS, M.S., 1978. Serological studies of mucosal disease virus in England and Wales. *Res. Vet. Sci.*, **24**: 98-103.
- HILBE, M., STALDER, H., PETERHANS, E., HAESSIG, M., NUSSBAUMER, M., EGLI, C., SCHELP, C., ZLINSKY, K. AND EHRENSPERGER, F., 2007. Comparison of five diagnostic methods for detecting bovine viral diarrhoea virus infection in calves. *Vet. Diagn. Invest.*, **19**: 28-34.
- HILL, F.I., REICHEL, M.P., MCCOY, R.J. AND TISDALL, D.J., 2007. Evaluation of two commercial enzyme-linked immunosorbant assays for detection of bovine viral diarrhoea virus in serum and skin biopsies of cattle. *N. Z. Vet. J.*, **55**: 45-8.
- HOUE, H. AND MEYLING, A., 1991. Prevalence of bovine viral diarrhoea (BVD) in 19 dairy herds and estimation of incidence of infection in early pregnancy. *Prev. Vet. Med.*, **11**: 9-16.
- HOUE, H., 1995. Epidemiology of bovine viral diarrhoea virus. *Vet. Clin. North Am. Fd. Anim. Pract.*, **11**: 521-547.
- HOUE, H., 1999. Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Vet. Microbiol.*, **64**: 89-106.
- HOUE, H., LINDBERG, A. AND MOENNING, W., 2006. Test strategies in bovine viral diarrhoea virus control and eradication campaigns in Europe. *J. Vet. Diagn. Invest.*, **18**: 427-436.
- HOUE, H., LLOYD, J.W. AND BAKER, J.C., 1994. Decision-tree analysis of control strategies in Danish dairy herds with outbreaks of mucosal disease. *Prev. Vet. Med.*, **21**: 133-146.
- LITTLEJOHNS, I.R., 1985. Complications to the study of the relationship between bovine lymphocytic antigen and mucosal disease. In: *Characterization of the bovine immune system and the genes regulating expression of immunity with particular reference to their role in disease resistance* (eds. W.C. Davis, J.N. Shelton and C.W. Weems), Washington State University, Pullman, pp. 179 - 190.
- MCCLURKIN, A.W., LITLEDIKE, E.T., CUTLIP, R.C., FRANK, G.H., CORIA, M.F. AND BOLIN, S.R., 1984. Production of cattle immunotolerant to bovine viral diarrhoea virus (BVDV). *Can. J. Comp. Med.*, **48**: 156-161.
- MCGOWAN, M.R., KIRKLAND, P.D., RODWELL, B.J., KERR, D.R. AND CARROLL, C.L., 1993. A field investigation of the effects of bovine viral diarrhoea virus -infection around the time of insemination on the reproductive performance of cattle. *Theriogenology*, **39**: 443-449.
- MOCKELIŪ NIENE, V., SALOMSKAS, A. MOCKELIŪ NAS, R. AND PETKEVICIUS, S., 2004. Prevalence and epidemiological features of bovine viral diarrhoea virus infection in Lithuania. *Vet. Microbiol.*, **99**: 51-57.
- NISKANEN, R., 1993. Relationship between the levels of antibodies to bovine viral diarrhoea virus in bulk tank milk and the prevalence of cows exposed to the virus. *Vet. Rec.*, **133**: 341-344.
- NISKANEN, R., ALENIUS, S., LARSSON, B. AND JACOBSSON, S.O., 1991. Determination of level of antibodies to bovine virus diarrhoea virus (BVDV) in bulk tank milk as a tool in the diagnosis and prophylaxis of BVDV infections in dairy herds. *Arch. Virol. Suppl.*, **3**: 245-51.
- NUOTIO, L., JUVONEN, M., NEUVONEN, E., SIHVONEN, L. AND HUSU-KALLIO, J., 1999. Prevalence and geographic distribution of bovine viral diarrhoea (BVD) infection in Finland 1993-1997. *Vet. Microbiol.*, **64**: 231-235.
- OBANDO, R.C., HIDALGO, M., MERZA, M., MONTOYA, A., KLINGEBORN, B. AND MORENO-LOPEZ, J., 1999. Seroprevalence to bovine virus diarrhoea virus and other viruses of the bovine respiratory complex in Venezuela (Apure State). *Prev. Vet. Med.*, **41**: 271-278
- PETERHANS, E., JUNGI, T.W. AND SCHWEIZER, M., 2003. BVDV and innate immunity. *Biologicals*, **2**: 107-112.
- REINHARDT, G., RIEDEMANN, S., ERNST, S., AGUILAR, M., ENRIQUEZ, R. AND GALLARDO, J., 1990. Seroprevalence of bovine viral diarrhoea/mucosal disease in southern Chile. *Prev. Vet. Med.*, **10**: 73-78.
- SALIKI, J.T. AND DUBOVI, E. J., 2004. Laboratory diagnosis

- of bovine viral diarrhoea virus infections. *Vet. Clin. North Am. Fd. Anim. Pract.*, **20**: 69–83.
- SALIKI, J., HCHZERMEIER, R. AND DUBOVI, E.J., 2000. Evaluation of a new sandwich ELISA kit that uses serum for detection of cattle persistently infected with BVD virus. *Ann N.Y. Acad. Sci.*, **916**: 358–363.
- SANDVIK, T., 2005. Selection and use of laboratory diagnostic assays in BVD control programmes. *Prev. Vet. Med.*, **72**: 3–16.
- SEKI, Y., SEIMIYA, Y.M., YAEGASHI, G. AND SATO, C., 2006. Identification of herds with cattle persistently infected with bovine viral diarrhoea virus by virological evaluation of three calves. *J. Vet. Med. Sci.*, **68**: 255–258.
- SEYYAL, A.K., IBRAHIM, F., BOZKURT, H.H., LYAZ, V.G. AND KEMAL, A.K., 2002. The Prevalence of Bovine Viral Diarrhoea Virus (BVDV) infections in Cattle and Existence of Persistently Infected Cattle in the Trakya Region. *Turk. J. Vet. Anim. Sci.*, **26**: 245–248
- STAHL, K., KAMPA, J., ALENIUS, S., PERSSON, W.A., BAULE, C., AIUMLAMAI, S. AND BELAK, S., 2007. Natural infection of cattle with an atypical 'HoBi'-like pestivirus—implications for BVD control and for the safety of biological products. *Vet. Res.*, **38**: 517–23.
- STAHL, K., LINDBERG, A., RIVERA, H., ORTIZ, C. AND MORENO-LOPEZ, J., 2008. Self-clearance from BVDV infections. A frequent finding in dairy herds in an endemically infected region in Peru Bulk milk testing for antibody seroprevalences to BVDV and BHV-1 in a rural region of Peru. *Prev. Vet. Med.*, **83**: 285–296.
- STRAUB, O.C., 2001. Advances in BHV-1 (IBR) research. *Dtsch. Tierarztl. Wschr.*, **108**: 419–422.
- TAYLER, W.P. AND RAMPTON, C.S., 1968. A survey of cattle sera from Kenya and Uganda for antibodies to bovine viral diarrhoea virus. *Vet. Rec.*, **83**: 121–124.
- TAYLOR, L.F., JOYCE, V.D., EDWARD, J.D., RICHARD, J.H., JAN, V.V.D.H., CARL, S.R. AND EUGENE, D.J., 1995. The prevalence of bovine viral diarrhoea virus infection in a population of feedlot calves in western Canada. *Can. J. Vet. Res.*, **59**: 87–93.
- VEGA, S., BAYON, M.C., JIMINEZ, T., ASENSIO, A., MIRAT, F., CID, D. AND DE LA FUENTE, R., 1997. Prevalence of bovine viral diarrhoea virus in the cattle population of Comunidad de Madrid (Spain). In: *Proc. 3rd ESVV Symp Pestivirus Infections* (eds. S. Edwards, D.J. Paton, and G. Wensvoort) Lelystad, The Netherlands, 19–20 September 1996, pp. 116–119.
- WALDNER, C.L. AND CAMPBELL, J.R., 2005. Use of serologic evaluation for antibodies against bovine viral diarrhoea virus for detection of persistently infected calves in beef herds. *Am. J. Vet. Res.*, **66**: 825–34.
- WITTUM, T.E., GROTELUESCHEN, D.M., BROCK, K.V., KVASNICKA, W.G., FLOYD, J.G., KELLING, C.L. AND ODDE, K.G., 2001. Persistent bovine viral diarrhoea virus infection in US beef herds. *Prev. Vet. Med.*, **49**: 83–94.
- ZIMMER, G.M., MAANEN, C.V., DE GOEY, I., BRINKHOF, J. AND WENTINK, G.H., 2004. The effect of maternal antibodies on the detection of bovine viral diarrhoea virus in peripheral blood samples. *Vet. Microbiol.*, **100**: 145–149.

(Received 3 December 2009, revised 4 April 2010)