

An Update on Current Concepts of The Epidemiology of Bovine Pestivirus Infection in Cattle

Kafi M.

Department of Clinical Sciences, Veterinary School,
Shiraz University, Shiraz, 71345, Iran.

Summary

Bovine pestivirus is a worldwide pathogen of the cattle populations. Over 90% of pestivirus infections remain inapparent. Pestivirus infection is usually considered a disease of the alimentary tract but increasing evidence indicates involvement of the reproductive tract with significant effects on the reproductive performance of cattle. A majority of reproductive loss is associated with the non-cytopathic MCP biotype of the virus. The present review focuses on the current knowledge concerning the ways in which this pathogen may spread within the cattle population.

Key words: *Flaviviridae*, Cattle, Pestivirus

Introduction

Conventionally, pestivirus was considered to be a genus of the family *Togaviridae*. In light of new findings on the genetic composition of pestiviruses they have been recategorised as a genus of the family *Flaviviridae* (Horzinek 1991). The *Flaviviridae* have similar molecular biology to the family *Togaviridae* (Potgieter 1992). The genus pestivirus includes the antigenically related bovine viral diarrhoea virus (BVDV), hog cholera virus and Border disease virus. BVDV and Border disease virus have a much higher degree of antigen variation than the hog cholera virus (Baker 1987). BVDV infection is responsible for a variety of clinical syndromes including subclinical infection, immunosuppression, immunotolerance, mucosal disease syndrome, congenital defects and a variety of reproductive losses due to perinatal mortality, stillbirth, foetal mummification, abortion, embryonic death and "repeat breeding"

(Brownlie 1991). Because of this diversity of syndromes it is more appropriate to use the term "bovine pestivirus infection" rather than BVDV infection, the latter describing only one particular syndrome caused by pestivirus infection.

Distribution of pestivirus in body tissues

Lymphoid and certain epithelial tissues such as mucosa of the alimentary tract are the most common predilection sites of the virus in cattle (Bielefeldt, 1987). It is thought that the initial site of replication is the oronasal mucosa, particularly the palatine tonsil (Brownlie 1991). The systemic spread of the virus could occur either from free virus in serum or via circulating white blood cells (Tyler *et al.* 1965). Experimental infection of cattle with pestivirus was shown to decrease the B and T-cell lymphocytes population (Bolin *et al.* 1985a) and cause a neutrophil dysfunction (Roth *et al.* 1981). Taking this finding together with the role of inflammatory cells in the ovulatory process (Espesey and Lipner 1994) it may explain the ovulatory failure observed in Friesian heifers that were experimentally infected with pestivirus 9 days prior to insemination (Kafi *et al.* 1997).

The central nervous system of the developing bovine foetus is another important target organ of bovine pestivirus. The virus has been shown to persist in the central nervous system of immunotolerant calves born with persistent infections (Fernandez *et al.* 1989, Brownlie 1991). Results of a German study indicate that bovine pestivirus has a strong affinity for neurons (Wohrmann *et al.* 1992) confirming previous reports of Cutlip *et al.* (1980). Further, it was shown that predilection sites of the virus in the central nervous system are the cerebral cortex and hippocampus. Interestingly, virus antigens were found in morphologically normal cells of the anterior pituitary (Wohrmann *et al.* 1992). In an earlier report, virus antigens had been detected by immunohistochemistry, in the granular cells of the anterior pituitary of a persistently infected (PI) bull (Barlow *et al.* 1986).

Testicular (Kirkland *et al.* 1991) and ovarian tissues of persistently (Booth *et al.* 1995) and transiently (Kafi *et al.* 1997, McGowan and Kirkland, unpublished data) infected cattle are other sites of replication of pestivirus. Pestivirus has been frequently isolated from the follicular fluid. A series of recent *in vitro* experiments did not show any adverse effect of pestivirus on the process of bovine oocyte maturation (Kafi 1996). Whether these apparently normal matured oocytes that were exposed to pestivirus could grow to the blastocyst stage and result in a normal calf was not examined.

Prevalence of bovine pestivirus infection

In an early serological study, Mirshamsi *et al.* (1970) demonstrated 16 to 69% of cattle tested by a serum neutralisation test were serologically positive for bovine

pestivirus. Later in a study conducted by Sedigi-Nezhad (1996) the virus was isolated from northern, western and central parts of Iran. The prevalence of bovine pestivirus infection in different countries is shown in Table 1.

Table 1: The prevalence of bovine pestivirus infection in different countries.

Country	Number tested	Test used	%Antibody positive	References
Australia	5129	VNT	60.6	St. George et al. (1967)
Iran	NA	VNT	16-69	Mirshamsy et al. (1970)
New Zealand	922	VNT	34.0	Durham & Faulkner (1975)
Britain	1593	VNT	62.2	Harkness et al. (1978)
Egypt	NA	VNT	33.4	El-Dobeigy et al. (1983)
U.S.A.	3157	VNT	89.0	Bolin et al. (1985b)
Argentina	1494	VNT	37.0	Rweyemamu et al. (1990)
Chile	525	VNT	77.0	Rweyemamu et al. (1990)
Norway	1133	VNT	18.5	Loken et al. (1991)

NA = not available VNT = virus neutralisation test

The unintentional introduction of a PI animal to a herd may occur with subsequent transmission of the virus by close contact between seronegative (susceptible) animals and the PI animal. The absence of PI animals in a herd has been considered the reason for the occurrence of serologically negative herds (Blood and Radostits 1989). Before about 125 days of gestation in cattle, the foetal immunological responses are not well developed. Therefore, if the foetus becomes infected with a nCP biotype of pestivirus in this particular period of gestation, it is unable to respond to the infection and produce serum neutralising antibodies, and is thus likely to be born PI with the virus. Immunotolerance and persistent viraemia are two permanent features observed in these animals. PI calves may appear clinically healthy or illthrift. Premature birth and undersized calves may also be seen as a consequence of infection at this time (Bolin 1990). PI calves may have a mortality rate of up 50 per cent in their first two

(Bolin 1990). PI calves may have a mortality rate of up to 50 per cent in their first two years of age (Duffel and Harkness 1987). The majority of deaths are due to the development of mucosal disease. PI cattle are important reservoirs of pestivirus and they appear to be efficient transmitters of the virus, shedding large amounts of virus over a long period of time (Duffel and Harkness, 1985). This could be the major mechanism maintaining pestivirus in cattle populations (Roeder and Harkness 1986). The prevalence of PI animals in the cattle population is relatively low (1-2 per cent) (Meyling 1984). Howard *et al.* (1990) reported a prevalence of 0.78 per cent (12 out of 1538) PI bulls in 4 artificial insemination AI centres in the USA. A sex-related prevalence of PI (in males) animals has been proposed (Littlejohns and Horner 1990) but this finding requires further investigation.

Transmission and source of infection

Based on available information, the possible ways of transmission and the sources of infection are discussed below:

Contact with PI animals

A significant number of PI carrier animals are clinically healthy. These animals can survive for years, and at the same time they may breed successfully. Those cases which have varying degrees of abnormality in growth rate or in general appearance may be discarded, or die before puberty (Meyling *et al.* 1990) and therefore may not contribute significantly to the spread of the virus. However, keeping the unrecognised apparently normal PI carrier animals in the herd is a major hazard to groups of susceptible animals. The progeny of these animals may in turn be apparently healthy but persistently infected (Straver *et al.* 1983).

Another possible route of introducing pestivirus into a herd is the purchasing of pregnant heifers that are carrying an infected foetus (Meyling *et al.* 1990). This highlights the significant role of national or international cattle trading as a pathway for the spread of pestivirus. The virus is most likely to be isolated from any secretion or excretion of a PI carrier animal. The faeces is not usually a common source of the virus even when there is severe damage to the gut (Brownlie *et al.* 1987). On the other hand, ocular and nasal discharges, saliva, urine, milk and semen are common sources of the virus (Straver *et al.* 1983; Radostits and Littlejohns 1988). The principal routes of infection are inhalation or ingestion of these materials (Afshar and Eaglesome, 1990). It was reported (McGowan *et al.* 1993) that 50 to 63 per cent of seronegative animals became infected following a 24 hour period of close contact with two PI

animals in a small concrete yard. However, the rate of transmission may depend on the strain of the virus, stocking rate, management system (Pasture or a feedlot type system), and also the occurrence of natural activities such as oestrus and calving which encourage contact between cattle.

Contact with transiently infected animals

Those animals which undergo an acute postnatal infection with pestivirus experience a transient viraemia and the virus may be isolated from most secretions from days 4 to 10 after exposure. The virus has been recovered until day 19 after exposure (Brownlie *et al.* 1987). In these cases, which are referred to as transient infections, the amount of virus shed in the body discharges is lower than that from PI cattle. No transmission of the virus to susceptible animals was observed in spite of close contact between non-infected and transiently infected animals (Meyling and Jensen 1988). Also, animals experimentally infected by parenteral injection of an CP strain of pestivirus did not act as a source of infection for other animals that were in contact with them (Pritchard 1963). In contrast, transmission can occur when the transiently infected animals return to oestrus after artificial insemination and the virus is shed in uterocervical discharges. However, the evidence of spread of infection from transiently infected animals suggests that it is not an efficient mode of transmission. The mean time from exposure to seroconversion has been reported to be about 13 days (McGowan *et al.* 1993). It is believed that the antibody production provide the animal with lifelong immunity.

Use of semen from PI or transiently infected bulls

The semen produced by PI bulls can be an important mode of virus transmission. These bulls may shed the virus in semen over a long period of time (Stober, 1984). The immediate consequence of using this semen in a susceptible herd may be a decrease in conception rate (Kirkland *et al.* 1994). On the contrary, satisfactory conception rates have been achieved with semen from PI bulls (Meyling and Jensen 1988, Wentink *et al.* 1989). Kirkland *et al.* (1997) found that the use of semen from a transiently infected bull has the potential to infect susceptible cattle. They reported a low initial incidence of infection after AI (3 out of 60) but observed a secondary cycle of infection apparently associated with an originally infected animal expelling an infected conceptus and uterine fluids approximately 21 days after AI.

Transfer of infected embryos

Foetal calf serum (FCS) which is normally utilised in embryo transfer (ET) procedures and embryo research has been reported to be contaminated with pestivirus (Abraham 1993). Improper handling and poor sanitation of embryos could result in spread of the virus. A high frequency of persistent infections among ET calves have been reported (Anderson *et al.* 1988). The contamination of oestrus cow serum (Zurovac *et al.* 1994) and/or FCS (Kafi, 1996) with pestivirus antibody has been observed during studies on the effect of pestivirus on bovine *in vitro* derived embryos. It is also possible that bovine serum albumin as the serum component of *in vitro* fertilisation media or synthetic oviductal fluid contains immunoglobulin against pestivirus. Neither standard washing nor trypsin treatment was effective for removal of bovine pestivirus from zona pellucidae intact *in vitro* derived embryos following *in vitro* exposure to the virus (Trachte *et al.* 1997).

Transmission through the use of live vaccines

Contamination of FCS with pestivirus is frequently observed in cell culture work and this could be the cause of pestivirus contamination of biological products, such as vaccines and pharmaceuticals (Radostits and Littlejohns 1988).

Vaccination of susceptible cows, pregnant between 51 and 190 days of gestation, with a commercial modified live bovine pestivirus resulted in transplacental infection (Liess *et al.* 1984). The outcomes of vaccination were reported to be abortion, congenital abnormalities of the CNS, perinatal deaths, body growth retardation and persistent viral infection (Liess *et al.* 1984, Trautwein *et al.* 1986). Transmission has also been reported following the use of other live vaccines contaminated with pestivirus (Lohr *et al.* 1983).

Transmission via fomites and flies

The role of pestivirus contaminated-hypodermic needles in producing infection in susceptible cattle was recently demonstrated (Gunn, 1993). Also, the use of shared cattle nose tongs was shown to be a way of transmitting the virus amongst susceptible animals (Gunn 1993). Lang-Ree *et al.* (1994) investigated the possibility of the transmission of bovine pestivirus from a PI animal to other susceptible heifers via use of a shared glove used for rectal palpation. Bovine pestivirus was isolated from five of eight heifers that were palpated per rectum in this experiment. Further, all eight heifers produced neutralising antibodies against bovine pestivirus within 13 days. Under experimental conditions, biting flies (such as *Haematopota pluvialis*) and

non-biting flies (*Hydrotaea irritans*, *Musca autumnalis*) were unable to transmit pestivirus to cattle and sheep (Tarry *et al.* 1991, Gunn 1993). Further studies are required to clarify whether flies can infect animals by feeding around their eyes or on abrasions of the body surface.

Transmission through contact with other species of animals

Pestivirus infections occur in a large number of different species of animals (Nettleton 1990). Experimentally, other animals such as sheep, goats, buffalo, deer and pigs have become infected with bovine pestivirus (Stewart 1980, Nettleton 1990). It is believed that interspecies transmission may occur (Harkness and Roeder 1988). The transmission of Border disease virus from PI sheep to cattle has been reported (Barlow *et al.* 1980) and also the foetopathogenicity of border disease virus in cattle has been described (Gibbons *et al.* 1974). The mechanisms involved in the dissemination of the virus among these different species is worthy of investigation.

Conclusions

There is now overwhelming evidence that reproductive losses are the main consequence of pestivirus infection in cattle. Reproductive and lymphoid tissues are two main predilection sites of the virus. The non-cytopathic biotype is the type that causes majority of pestivirus infections and thus the greatest economic impact. PI animals are the primary source of infection in cattle populations. Gametes, serum and somatic cells are potential sources for introduction of bovine pestivirus into bovine *in vitro* embryo production systems. Recent findings emphasize the significance of (IVF) and embryo transfer activities as potential means of pestivirus transmission among cattle populations.

Acknowledgements

The author would like to thank Dr. M. McGowan and M. Young for their critical review of the manuscript. This work was financially supported by Shiraz University, Iran and Australian Dairy Research Development and Cooperation.

References

Abraham A. (1993). Bovine viral diarrhoea virus contamination of commercial bovine foetal serum. *Israeli Journal of Veterinary Medicine*. 48:120-122.

- Afshar A, Eaglesome MD.(1990). Viruses associated with bovine semen. *Veterinary Bulletin.* 60:93-109.
- Anderson JB, Pedersen H, Ronsholt L. (1988). Embryo transfer in farm animals. *Review Scientifique et. technique Office Int. Des Epizooties.* 8:38-42.
- Baker JC. (1987). Bovine Viral Diarrhoea: A review *JA, Vet. Medical Association.* 190:1449-1458.
- Barlow RM, Vantsis JT, Gardiner AC, Rennie JC, Herring JA, Scott FMM. (1980). Mechanisms of natural transmission of border disease. *J. Comparative. Pathology.* 90, 57-65.
- Barlow RM, Nettleton PF, Gardiner AC, Greig A, Campbell H, Bonn JM.(1986). Persistent bovine viral diarrhoea virus infection in a bull. *The Veterinary Record.* 118, 321-324.
- Bielefeldt O, Ronsholt L, Bloch B. (1987). Demonstration of BVDV in peripheral blood mono-nuclear cells of persistently infected, clinically normal cattle *J General Virology.* 68, 1971-1982.
- Brownlie J, Clark MC, Howard CJ, Pocock DH. (1987). Pathogenesis and epidemiology of bovine virus diarrhoea virus infection of cattle. *Annals Research of Veterinary.* 18, 157-166.
- Brownlie J. (1991). The pathways for bovine virus diarrhoea virus biotypes in the pathogenesis of disease. *Archive of Virology. Suppl.* 3, 79-96.
- Bolin SR, McClurkin AW, Coria MF. (1985a). Effects of bovine viral diarrhoea virus on the percentages and absolute numbers of circulating B and T lymphocytes in cattle. *American Journal of Veterinary Research.* 46, 884-886.
- Bolin SR, McClurkin AW, Coria MF. (1985b). Frequency of persistent bovine viral diarrhoea virus infection in selected cattle herds. *Am. J. of Veterinary Research.* 46, 2385-2387.
- Bolin SR. (1990). The current understanding about the pathogenesis and clinical forms of BVD. *Veterinary Medicine.* October, 1124-1132.
- Booth PJ, Stevens DA, Collins ME, Brownlie J. (1995). Detection of bovine viral diarrhoea virus antigen and RNA in oviduct and granulosa cells of persistently infected cattle. *J. Reproduction and Fertility.* 105, 17-24.
- Blood DC, Radostits OM. (1989). *Veterinary Medicine.* 7th ed. pp. 846. Bailliere Tindal. UK.

- Cutlip RC, McClurkin AW, Coria MF. (1980). Lesions in clinically healthy cattle persistently infected with the virus of BVD; Glomerulonephritis and Encephalitis. *American J. of Veterinary Research.* 41, 1938-1941.
- Durham PJK, Forbes-Faulkner JC. (1975). The prevalence of antibodies to bovine virus diarrhoea and infectious bovine rhinotracheitis viruses in diagnostic sera from New Zealand cattle. *New Zealand Veterinary J.* 23, 226-229.
- Duffell SJ, Harkness TW. (1985). Bovine virus diarrhoea - Mucosal disease infection in cattle. *The Veterinary Record.* 117, 240-245.
- Duffell SJ, Harkness JW. (1987). The bovine virus diarrhoea mucosal disease complex. *Veterinary Annual.* 27, 91-97.
- El-Dobeigy AI, Baz TI, Taha MM, Saber MS. (1983). Incidence of bovine viral diarrhoea, infectious bovine rhinotracheitis and parainfluenza-3 viruses in Egypt. *Agricultural Research Reviews.* 61, 89-106.
- Espey LL, Lipner H. (1994). Ovulation. In: *The physiology of Reproduction.* ed. by Knobilo E, Neill JD, 2nd ed. pp. 754-760. New York. Raven Press.
- Fernandez A, Hewicker M, Trautwein G, Pohlens J, Liess B. (1989). Viral antigen distribution in the central nervous system of cattle persistently infected with bovine viral diarrhoea virus. *Veterinary Pathology.* 26, 26-32.
- Gibbons D, Winkler C, Shaw I, Terlecki S, Richardson C, Done J. (1974). Pathogenicity of the Border disease agent for the bovine foetus. *British Veterinary J.* 130, 357-360
- Gunn HM. (1993). Role of fomites and flies in the transmission of bovine viral diarrhoea virus. *The Veterinary Record.* 132, 584-585.
- Harkness JW, Sands JJ, Richards MS. (1978). Serological studies of mucosal disease virus in England and Wales. *Research in Veterinary Science.* 24,98-103.
- Harkness JW, Roeder PL. (1988). The comparative biology of classical swine fever. In: *Classical swine fever and related viral infections.* ed. by B. Liess., PP. 233-288. Martinus Nijhoff publishers. The Hague. The Netherlands.
- Horzinek MC. (1991). Pestiviruses-Taxonomic perspectives. *Archieve of Virology.* (suppl3), 1-5. Howard TH, Bean B, Hillman R, Monke DR. (1990). Surveillance for persistent bovine viral diarrhoea virus infection in four artificial insemination centers. *J. American Veterinary Medical Association.* 196, 1951-1955.
- Kafi M, McGowan MR, Kirkl PD, Jillella D. (1997). The effect of bovine pestivirus infection on the superovulatory response of Friesian heifers. *Theriogenology.* 48, 985-996.

- Kafi M. (1996). Studies on the Pathogenesis of reproductive failure associated with bovine pestivirus around the time of AI in cattle. Ph.D Studies. The Uni. of Queensland. QLD, Australia.
- Kirkland PD, Richards SG, Rothwell JT, Stanley DF. (1991). Replication of bovine viral diarrhoea virus in the bovine reproductive tract and excretion of virus in semen during acute and chronic infections. *The Veterinary Record*. 128, 587-590.
- Kirkland PD, Richards SG, Moyle A. (1994). The outcome of widespread use of semen from a bull persistently infected with pestivirus. *The Veterinary Record*. 135, 527-529.
- Kirkland PD, McGowan MR, Mackintosh S, Moyle A. (1997). Insemination of cattle with semen from bulls transiently infected with pestivirus. *The Veterinary Record*. 140, 124-127.
- Lang-Ree JR, Vatn T, Kommisrud E, Loken T. (1994). Transmission of bovine viral diarrhoea virus by rectal examination. *The Veterinary Record*. 22,412-413.
- Lies B, Orban S, Frey H R, Trautwein G, Wiefel A, Bindow H. (1984). Studies on transplacental transmissibility of a bovine virus diarrhoea (BVD) vaccine in cattle. *Zentbl. Veterinary Medicine*. B. 31, 669-681.
- Littlejohns IR, Horner GW. (1990). Incidence, epidemiology and control of bovine pestivirus infections and disease in Australia and NewZealand. *Review Scientifique et. Technique Office International Epizooties*. 9, 195-205.
- Lohr HC, Everman JF, Ward AC. (1983). Investigation of dams and their offspring inoculated with a vaccine contaminated by bovine viral diarrhoea virus. *Veterinary Medicine and Small Animals Clininic*. 78, 1263-1266.
- Loken T, Krogsrud J, Larsen IL. (1991). Pestivirus infections in Norway. A Serological study in cattle, sheep and pigs. *Acta Veterinary Scandinavia*. 32, 27-34.
- McGowan MR, Kirkland PD, Richards SG, Littlejohns IR. (1993). Increased reproductive losses in cattle infected with bovine pestivirus around the time of insemination. *The Veterinary Record*. 133, 39-43.
- Meyling A. (1984). Detection of BVD virus in viraemic cattle by an indirect immunoperoxidase technique. In: *Recent Advances in virus diagnosis*. ed. by McNulty MS, Macferran JB. pp.37-46. Boston,¹Martinus Nijhoff publishers.
- Meling A, Jensen AM. (1988). Transmission of bovine virus diarrhoea virus (BVDV) by artificial insemination (AI) with semen from a persistently infected bull. *Veterinary Microbiology*. 17, 97-105.

- Meyling A, Houe H, Jensen AM. (1990). Epidemiology of bovine virus diarrhoea virus. *Review Scientifique et. technique office International Des Epizooties.* 9, 75-93.
- Mirshamsy H, Shafyi A, Bahrami S. (1970). The occurrence of bovine virus diarrhoea/Mucosal Disease in Iran. *Archives of Razi Institute.* 22, 197-201.
- Nettleton PF. (1990). Pestivirus infections in ruminants other than cattle. *Review Scientifique et. technique office International Des Epizooties.* 9, 131-150.
- Potgieter LND. (1992). Bovine viral diarrhoea. In *Veterinary Diagnostic Virology.* ed. by A.E. Castro and W. Heuschele. pp. 88-92. Mosby year book. Missouri. USA.
- Pritchard WR. (1963). The bovine viral diarrhoea-mucosal disease complex. In: *Advances in Veterinary Science.* 8, 1-47.
- Rdostits OM, Littlejohns IR. (1988). New concepts in the pathogenesis, diagnosis, and control of diseases caused by the bovine viral diarrhoea virus. *Canadian Veterinary J.* 29, 513-528.
- Roeder PL, Harkness JW. (1986). BVD virus infection: prospects for control. *The Veterinary Record.* 118, 143-147.
- Roth JA, Kaeberle ML, Griffith RW. (1981): Effects of BVDV on bovine polymorphonuclear leukocyte function. *American Journal of veterinary research.* 42, 240-250.
- Rweyemamu MM, Fernandes AA, Espinosa AM, Schudel AA, Lager, IA, Mueller SBK. (1990). Incidence, epidemiology and control of bovine virus diarrhoea virus in South America. *Review Scientifique et. technique office International Des Epizooties.* 9, 207-214.
- Sedigi-Nezhad S. (1996). A survey on BVD-MD in Iran (in persian). *Research & Reconstruction.* 30, spring, 128-131.
- St. George WA, Parsonson IM, French EL. (1967). A serological surveys of *Australian Veterinary J.* 43, 549-557.
- Stewart WC, Miller LD, Kresse JI, Synder ML. (1980). Bovine viral diarrhoea infection in pregnant swine. *A.J. of Vet. Research.* 41, 459-462.
- Stober M. (1984). Current knowledge of the BVD syndrome of cattle. agent, immune response, course and spread. *Bovine Practitioner.* 19, 49-60.
- Straver PJ, Journee DLH, Binkhorst GJ. (1983). Neurological disorders, virus persistence and hypomyelination in calves due to intrauterine infection with bovine virus diarrhoea. *Veterinary Quaternary.* 5, 145-155.

- Tarry DW, Bernal L, Edwards S. (1991). Transmission of bovine virus diarrhoea virus by blood feeding flies. *The Veterinary Record*. 128, 82-84.
- Trachte EA, Stringfellow DA, Riddell KP, Galik PK, Riddell MG, Wright JC. (1997). Washing and trypsin treatment of IVF bovine embryos exposed to bovine viral diarrhoea virus. *Theriogenology*. 47, 383.
- Trautwein G, Hewicker H, Liess B, Orban S, Grunert E. (1986). Studies on transrectal transmissibility of a bovine virus diarrhoea (BVD) vaccine virus in cattle. *J. of Veterinary Medicine*. B. 33, 260-268.
- Tyler DE, Ramsey FK. (1965). Comparative pathologic, immunologic and clinical responses produced by selected agents of the bovine mucosal disease virus diarrhoea complex. *American Journal of veterinary research*. 26, 903-913.
- Wentink GH, Remmen JL, van Exsel ACA. (1989). Pregnancy rate of heifers bred by immunotolerant bull persistently infected with bovine viral diarrhoea virus. *Veterinary Quaternary*. 11, 171-174.
- Wohrmann T, Hewicker-Trautwein M, Fernandez A, Moennig V, Liess B, and Trautwein G. (1992). Distribution of bovine viral diarrhoea antigens in the central nervous system of cattle with various congenital manifestations. *J. Veterinary Medicine*. B. 39, 599-609.
- Zurovac OV, Stringfellow DA, Brock KV, Riddell MG, Wright JC. (1994). Noncytopathic bovine viral diarrhoea virus in a system for in vitro production of bovine embryos. *Theriogenology*. 41: 841-453.