A. Ü. Vet. Fak. Derg.
34 (3): 555-561, 1987

IMPACT OF INTRAUTERINE BOVINE VIRAL DIARRHOEA (BVD) VIRUS INFECTION IN CATTLE*

B. Liess[1], **H. R. Frey**[1], **S. Orban**[1], **G. Trautwein**[1]

Sığırlarda intrauterin bovine viral diare (BVD) virusunun etkisi

Özet: *Fetusda immun yanıta yol açmayan ve transplasental BVD virusunun transmisyonuna neden olan aşılamaya kadarki en kısa gebelik süresi, bu çalışmada* 123 *gün olarak belirlenmiştir. Elde edilen bilgiler, gebeliğin* 90–120. *günlerinde, dişilerin BVD virus ile aşılandıklarında fetusda immun yanıtın meydana gelebileceğini ortaya koymaktadır. Bu gebelik periodu içinde, canlı virusla yapılan aşılamalar,* 5 *buzağıda merkezi sinir sistemi semptomları ve* 2 *sinde de persistent BVD virus infeksiyonu, ayrıca, muhtemelen aşı virusuna bağlı olan* 2 *abortus da meydana gelmiştir.*

Gebeliğin erken döneminde ayni aşı ile aşılanan gebelerden doğan 10 *konjenital infekte buzağıda sinirsel bozukluklar gözlenemedi. Bu duruma göre, sinirsel semptomların başlaması için en duyarlı periodun, fetal gelişiminin* 90. *gününden sonra başladığı ve gebeliğin* 120. *gününe kadar BVD aşı virusu ile maternal temas sonu meydana geldiği anlaşılmıştır. Diğer bir ifade ile, bu periodda, nötralizan antikorların oluşma yeteneği de kazanılır.*

Summary: *The shortest period of gestation up to vaccination resulting in transplacental transmission of BVD virus without immune response of the fetus was, in the present study,* 123 *days. From the data obtained it appears reasonable to estimate* 90 *to* 120 *days of gestation as the period when vaccination with BVD live virus with BVD live virus infection of dams can be expected to initiate an immune reaction of the fetus. It was within this gestational period that live virus vaccination furnished five calves suffering from CNS symptoms and two calves with persistent BVD virus infection in addition to two abortions possibly related to the vaccine virus infection.*

---

* This subject was presented at the Turkish Veterinary Medical First Science Congress, Ankara, 23–25 September 1987.

1 Tierärtzliche Hochschule, Hannover, Germany.

Neurological disorders were not observed in ten congenitally infected calves derived from dams which received the same vaccine at earlier stage of gestation. Thus it appears that the vulnerable phase for the development of neurological symptoms commences later than 90 *days of fetal development and results from maternal contacts with the BVD vaccine virus until about* 120 *days of gestation; that means precisely in the period when the ability to form neutralizing antibodies is acquired.*

Since suspicion was first raised of transplacental transmission of bovine virus diarrhoea (BVD) virus (Bürki and Germann, 1964; Romvary, 1965) and immune tolerance suggested as cause of mucosal disease (Malmquist, 1968), persistent BVD virus infections have been proven as being prerequisite for late onset disease (Liess, 1973; Liess et al., 1974; Coria and McClurkin, 1978; Roeder, 1984).

Little is known about the incidence of persistent infections and their impact possibly unnoticed because of untypical clinical symptoms and lesions or even their complete absence. Relevant information upon these questions require inverstigations not only into clinically suspect cases of BVD virus infections but more so, without bias, prospectively in unselected groups of cattle in order to get a rough estimate on the proportion of clinically normal animals shedding BVD virus constantly.

Cattle available for such investigations were categorized according to their status of BVD virus infection clinically and virologically (Table 1).

Table 1. Categorization of cattle serving as source for the detection of animals with transient or permanent BVD viraemia

Category	Status of BVD Virus Infections
A	breeding cattle in early pregnancy clinically unsuspect of BVD
B	cattle with various clinical symptoms suspect of BVD
C	hitherto undetected cattle persistently infected in herds with clinically suspect cases of BVD
D	persistent infections following maternal BVD live virus vaccination (51 to 118 days of pregnancy)

The results of cultural isolation of BVD virus from blood leucocytes for the determination of frequency and types of viraemia (transient or persistent) are summarized in Table 2.

Amongst 235 viraemic animals found in one of the four categories A – D, 19 were proven to be permanently viraemic indicating per-

Table 2. Persistent BVD virus infections in BVD viraemic cattle detected in unsuspect (A), suspect (B, C) and experimental groups of animals (D)*

Status of BVD infections	Number of cattle in category			
	A	B	C	D
unknown pool of animals	2317	837	539	48**
viraemic	22 (0.9 %)	185 (22 %)	16 (3 %)	12 (25 %)
permanently viraemic:				
proven—	1	2	11	5
folllowed up	1	2	11	3

* Categories according to Table 1

** pregnant cattle inoculated with BVD live virus vaccine between days 51 – 259 of gestation

sistent BVD infections. There might have been more animals persistently infected which for technical reasons did not allow to be retested after a significant time elapse as it was possible for 17 of those 19 mentioned. The latter were followed up for observation periods one month or longer as indicated in table 3.

Table 3. Time elapse between detection of persistently BVD virus infected cattle and sacrifice when moribund

Time elapse (months)	Occurence of clinical symptoms after detection of BVD virus persistence in Category*			
	A	B	C	D
1 – 6	1	2	1	–
7 – 12	–	–	5	–
13 – 24	–	–	–**	3

* Categories according to Table 1

** Five animals survived this time inspite of heavy growth retardation and unthriftiness, so that the owner was prepared to sell them

All but one animal developed clinical symptoms and 11 were sacrificed when moribund. At necropsy typical gross lesions consisted of enteritis, bronchopneumonia and thymus atrophy (Table 4). Five animals belonging to category C of animals showed more or less growth retardation or unthriftiness.

The only persistently BVD virus infected animal which did not show any overt clinical symptoms was pregnant when detected. It belonged to category A of animals and was considered by experts worth exporting. After slaughtering, various organs including placentomes

Table 4. Clinical symptoms and lesions observed in persistently BVD virus infected cattle

Age when sacrificed (months)	Diarrhoea or enteritis	Bronchopneumonia	Thymus atrophy	No symptoms or lesions
1 – 6	–	1	1	–
7 – 12	2	–	–	–
13 – 24	1	3	3	1

and the unopend uterus were made available for further examination. BVD viral antigen and BVD virus were readily detectable in both the mother and the fetus (estimated age 4 months). One milliliter of about one liter of amnioallantoic fluid was measured to contain $10^{3.5}$ $TCID_{50}$ of BVD virus.

Further to the impacts of intrauterine infections mentioned, BVD viral antigen was demonstrated by immunofluorescence in the central nervous system of 7 out of 11 persistently infected animals necropsied. The viral antigen was localized in the cytoplasm on neurons predominantly of the cerebral cortex.

Perinatal death, untrhiftiness, CNS symptoms or late onset diseases might lead to suspicion of BVD not earlier than several months after introduction of BVD virus in a hitherto "clean" herd of cattle (or sheep). These consequences of infection are certainly part of the impact which was clearly evidenced in the categories B, C and D of animals.

Category B represented a group of animals selected with clinical symptoms of BVD. 185 were viraemic and of those hospitalized in the clinic 81 % died or were moribund when sacrified.

In category C suspicion of BVD virus infection came up in cattle herds with either multiple cases of abortion or CNS syndromes. Only by combined serology and virus isolation procedures, individuals with persistent BVD virus infection otherwise unrecognized were detected. Thorough inquiries in those herds revealed previous problems not specifically attributed to BVD virus infection. However, of those 11 persistently infected animals only a three months old calf showed evidence of diarrhoea while the others at an age between 7 and 24 months showed no clinical signs of disease whatsowever, except unthriftiness in some cases growth retardation compared with peers. This latter sign seemed to be accepted by the owners and mainly attributed to inheritance. During further observation of the persistently infected

animals in this category (C), clinical symptoms appeared within one to two years after BVD virus isolation from buffy coats was first successful.

A true prospective study was possible in category D of animals with some of those persistently infected being followed up for considerable time.

After a commercial live attenuated virus vaccine was shown to contain BVD virus readily transmissible to fetuses as judged from their immune responses, the same vaccine was applied routinely in two breeding herds to animals early in pregnancy. The vaccinees were monitored up to the term of delivery and some of the calves, if not sold up to 2 years of age. 50 cows and heifers 51 to 259 days pregnant allowed evaluation since they proved to be seronegative (neutralizing serum dilution $< 1/5$). After calving colostral samples of all seroconverted animals showed neutralizing antibodies to BVD virus.

Apart from five abortions registered following vaccination of cows pregnant 123 to 259 days, 23 healthy calves were born in series in this group of dams. All the calves from which precolostral blood samples were obtained (14/23) showed antibody titres up to 1/240. In none of these calves including those that had suckled before blood collection could BVD virus be detected by cultural isolation from the buffy coats.

Of 21 dams vaccinated before the 120th day of gestation, 13 delivered apparantly healthy offspring (group I of animals) while the calves of the other animals showed more or less severe clinical symptoms with or without perinatal death (group II).

In group I of animals nine calves proved to be viraemic on the day of delivery including three for which precolostral blood samples were made available which had no neutralizing activity. All of te viraemic calves were derived from dams vaccinated on day 118 of gestation or earlier.

Group II of calves consisted of eight animals which either died on the day after delivery or showed various degrees of CNS symptoms. The latter animals were derived from dams vaccinated between the 90 th and 113 th day of gestation. From all of these calves only postcolostral blood samples were available which had neutralizing titres. In no case was BVD virus isolated from the buffy coats or from organ tissues collected at autopsy. But in one case was BVD virus isolated

from cerebrospinal fluid which at the same time had no detectable neutralizing antibody.

Twelve of a total of 21 calves belonging to either group I or II showed viraemia at birth. Viraemia persisted in those four calves which survived for at least 21 days post partum.

Thereafter, these animals showed growth retardation with only one animal remaining healthy in spite of permanent viraemia as demonstrated regularly during the following two years. This bull was always shedding BVD virus by various routes including semen (Kleine Büning et al., 1985).

The results briefly described add new arguments to the discussion on whether and how to use and to test BVD live virus vaccines so that no congenital infection with perinatal death, persistent infection, CNS symptoms, growth retardation or late onset of disease occurs. BVD virus infections of bovine fetuses (as in Border Disease of sheep and possibly goats) in the immune incompetent phase of ontogeny bears an important impact as to the epidemiology as well as to the disease pattern. The present study allowed to plot the various outcomes of transplacental stages of the dams when first contacted with BVD virus (Liess, 1985).

References

Bürki, F. and E. Germann (1964). *Letale Pneumoenteritiedn bei Kalbern verursacht durch den Erreger der bovinen Virus-Diarrhoe. Berliner Münch.* Tierärztl. Wschr. 16, 324–326.

Romvary, I. (1965). *Incidence of virus diarrhoea among newborn calves.* Acta Vet. Acad. Scient. Hung. 15, 341–347.

Malmquist, W.A. (1968). *Bovine viral diarrhea-mucosal disease: etiology, pathogenesis and applied immunity.* J. Am. Vet. Assoc. 152, 763–768.

Liess, B. (1973). *Pathogenese virusbedingter Darminfektionen der Tiere.* Dtsch. Tierärztl. Wschr. 80, 360–364.

Liess, B. (1985). *Bedeutung der Immuntoleranz für die Pathogenese der bovinen Virusdiarrhoe (BDV).* Berl. Münch. Tierärztl. Wschr. 98, 420–423.

Liess, B., H.-R. Frey, H. Kittsteiner, G. Baumann and W. Neumann (1974). *Beobachtungen und Untersuchungen über die "Mucosal Disease" des Rindes – einer immunbiologisch erklärbaren Spätform der BVD-Virusinfektion mit Kriterien einer "slow virus infection"?.* Dtsch. Tierärztl. Wschr. 81, 477–500.

Coria, M.F. and A.W. McClurkin (1978). *Specific immune tolerance in an apparanetly healthy bull persistently infected with bovine diarrhea virus.* J. Am. Vet. Med. Assoc. 1172, 449-451.

Roeder, P.L. (1984). *Bovine mucosal disease – persistent viral infection.* Proc. VIIth Intern. Sympos. Wotld Assoc. Vet. Microbiologists, Immunologists, Specialistes in Infectious Dis. of Animals.

Kleine Büning, M., H. Bader, B. Liess, G. Trautwein, S. Orban and W. Peters (1985). *Persistente BVD-Virusinfektion beim Bullen – Virusausscheidung und Spermaqualitat.* Zuchthyg. 20, 113.