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# ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETECTION OF BVD VIRUS ANTIGEN IN BUFFY COATS OF MUCOSAL DISEASE AFFECTED CATTLE.

By

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## SUMMARY

Enzyme linked immunosorbent assay (ELISA) in comparison with tissue culture method was used for detection of bovine viral diarrhea virus (BVDV) antigen in 70 out 152 buffy coats from mucosal disease affected cattle which were submitted to cattle clinic in Justus Liebig University, Giessen, Germany from 1994 to 1996. ELISA and tissue culture methods were performed in the Institute of Animal Hygiene and Infectious Diseases, Justus Liebig University, Giessen, Germany. The results showed that, 58 (82.9%) and 61 (87.1) of buffy coats were positive in cell culture and direct ELISA respectively. All samples which were positive in cell culture were also positive in ELISA. Three cattle with chronic mucosal disease were positive with direct ELISA, but negative with cell culture method. In conclusion, diagnosis of mucosal disease by isolation and detection of BVD virus using tissue culture method is a time consuming technique. Direct ELISA is a sensitive, rapid, but expensive method for the detection of BVD virus antigen in comparison to the cell culture technique.

Key words: Bovine viral diarrhea virus (Bvdv) - Enzyme linked immunosorbent assay (ELISA) - Mucosal disease (MD) - Tissue culture.

## INTRODUCTION

BVD virus is the causative agent of bovine viral diarrhea and mucosal disease (Juntti et al., 1987). BVD virus is the pathogen of cattle that cause abortion and congenital disorders following exposure of early gestation - pregnant females and causes fatal gastrointestinal disease in immunologically tolerant animals infected in utero (Haines et al., 1992).

BVD virus infection remains a bewildering enigma for the practitioner and researcher. The maniestations of BVD virus infection are complex

and diverse, ranging from apparently healthy persistently infected (PI) animals to classical mucosal disese, and from subtile intrauterine growth retardation to overt congenital defects in the foetus (Perdrizet et al., 1987). BVD virus is a ubiquitous pathogen of cattle that presents a confusing array of diseases to the clinician and is an annoying burden for diagnostic laboratories. Non-cytopathic and cytopathic BVD viruses causes several clinical forms of disease and exacerbate disease processes associated with other infectious agents (Bolin, 1990). Although BVDV strains differ in biotype and in virulence, all BVD viruses are related antigenically, and separate viral serogroups have not been defined (Bolin, 1995).

Gottschalk et al. (1992) used an antigen capture enzyme immunoassay (EIA) for detection of BVD viral antigen in peripheral blood lymphocytes of cattle for the screening of 241 animals. They showed that the antigen capture EIA detected virus in all samples from which virus was isolated by standard cell culture methods. The availability of an antigen capture EIA would greatly facilitate the direct identification of viraemic animals in terms of less labour and higher speed.

BVDV infection should be considered in the investigation of severe acute outbreaks of enteritis in cattle. In contrast to mucosal disease, isolation of virus from blood may be difficult (David et al., 1993). Because of the existence of the noncytopathic biotype, the detection of the virus may be problematic (Dubovi, 1990). Therefore, it was the aim of this study to introduce and standardize one of the recent and rapid diagnostic laboratory techniques such as direct enzyme linked immunnosorbent assay (ELISA) and evaluation of its validity beside the conventional used technique for the isolation and detection of BVD virus by cell culture method.

## MATERIAL AND METHODS

### Clinical examination

From April 1994 to March 1996, all clinically mucosal disease suspected cattle which were submitted to cattle clinic in Justus Liebig University, Giessen, Germany were examined clinically. Clinical suspicion of mucosal disease depended on acute catarrhal enteritis specially that with erosive lesions in the buccal cavity and interdigital space, and/or bronchopneumonia in young calves and adult cattle. Clinical symptoms of acute and chronic mucosal disease in different age groups of these cattle were discussed in details by Abd El-Rahim (1996); Abd El-Rahim and Grunder (1996).

### Isolation of BVD virus on tissue culture:

SDTA - blood samples and sterile nasal swabs were taken for the isolation of BVDV. Cytopathic BVD virus was isolated on Foetal calf lung (FCL) cell culture and identified by immunoflurescent technique. Noncytopathic BVD virus was isolated on FCL cell culture and identified by interferenc (Shirai, et al., 1984) and immunofluorescent technique (Afshar, et al., 1991).

**Enzyme linked immunosorbent assay (ELISA):**

Direct enzyme linked immunosorbent assay (ELISA) was used for detection of BVD virus antigen in leukocytic fractions. 5ml of haemolytic buffer (HLB) were added to 5 ml EDTA-Blood sample in pointed sterile tube and left for haemolysis (at least 10 minutes). The sample was centrifuged at 150 xg (1000 rpm) for 10 minutes and the supernatant was discarded. The pellet was resuspended in 2 ml of HLB and recentrifuged at 150 xg for 10 minutes. The supernatant was discraded and the pellet was resuspended in 0.3 ml of leukocytic lysis buffer (LLB) and shacked gently for 1 hour using microshaker.

Direct microplate ELISA was used for the detection of BVDV antigen in leukocytic fraction according to Chekit BVD-virus ELISA test (Bommeli Laboratory, Bern, Switzerland) as following:

**1- Sample distribution:**

On the Cheki BVD-virus microplate, 0.1 ml of washing solution was pipetted to each well. The tested and control (positive and negative control) samles were distributed in 0.1 ml/well and incubated overnight at 2-8°C in humid chamber.

**2- Washing of the Chekit BVD-virus microplate:**

The samples were poured off and the plate was washed with washing buffer (0.3 ml/well) for 3 minutes. The washing solution was removed and the plate was tap dried. The microplate-washing process was repeated 2 times.

**3-Chekit-anti-BVDV-peroxidase-conjugate:**

The Chekit-anti-BVDV-peroxidase-conjugate was diluted 1:200 and 0.2 ml were dispensed to each well. The microplate was incubated at room temperature for 1 hour in humid chamber.

**4- Washing of the Chekit-BVD-virus microplate:**

The Chekit-BVD-virus microplate was washed 3 times with the washing solution (as above).

**5- Distribution of Chekit-chromogen and reading the results:**

0.2 ml/well of Chekit-chromogen (25°C) was dispensed. The microplate was incubated for 15-30 minutes at the room temperature and shacked for homogenization of the colour. The microplates were read in an automatic ELISA reader at 405 nm and the results expressed as optical density (OD). The reaction was stopped by addition of 0.05 ml stopping solution/well.

**6- Interpretation of the results:**

$$\text{Sample \%} = \frac{\text{ODS - ODneg}}{\text{ODpos - ODneg}} \times 100$$

OD = optical density

Isolation and detection of BVD virus on tissue culture and by enzyme linked immunosorbent assay (ELISA) were done in the Virological Laboratory of the Institute of Hygiene and Infectious diseases, Justus Liebig University, Giessen, Germany.

## RESULTS

According to the history, severity of clinical signs and course of the disease, 117 cattle showed the acute form of mucosal disease while 35 cattle were chronically affected (Fig. 1). In case of acute mucosal disease, the main clinical signs were inappetence, fever, profuse watery diarrhea, erosive stomatitis, and erosions in the nasal orifices and in some cases in the interdegital space. Chronically mucosal disease affected cattle showed a change in the appetite, intermittent diarrhea, erosive lesions in the buccal cavity and in few cases in the interdegital space. Some cases had signs of bronchopneumonia. Most animals became clinically ll between 4-24 months of age.

### Isolation and detection of BVD virus antigen:

The results of detection of BVD virus antigen using direct ELISA in comparison with virus isolation by cell culture method in buffy coats of 70 out of 152 mucosal disease affected cattle were presented in teable (1). From this table it is clear that 58 (82.9%) and 61 (87.1%) of buffy coats were positive in cell culture and direct ELISA respecitively. All samples which were positive in cell culture were also positive in ELISA. There animals with chronic mucosal disease were positive with direct ELISA while negative with cell culture method.

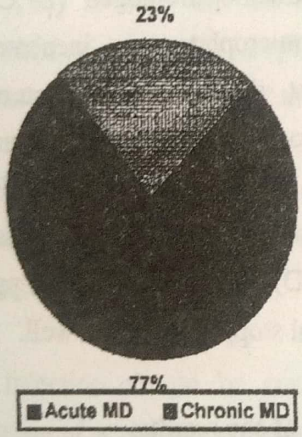


Fig 1: Percentage of cattle with acute and chronic mucosal disease.

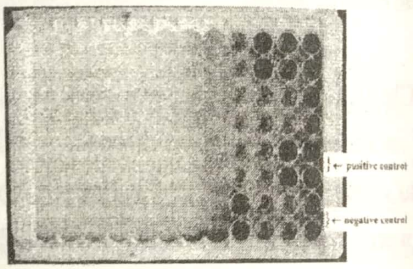

Fig. (2): Shows detection of BVD virus antigen in the buffy coat from mucosal disease affected cattle using enzyme linked immunosorbent assay was used for

Table 1: The results of direct ELISA used for detection of BVDV antigen in 70 buffy

| Age | No. of tested animals | Cell cul- | | ELIS | |
|---|---|---|---|---|---|
| | | +ve | % | +ve | % |
| < 4 months | 5 | 4 | 80 | 4 | 80 |
| 4-24 months | 57 | 48 | 84.2 | 50 | 87.7 |
| > 24 months | 8 | 6 | 75 | 7 | 87.5 |
| | 70 | 58 | 82.9 | 61 | 87.1 |

## DISCUSSION

Clinical mucosal disease occurs only in cattle which were infected with a ncp bvd virus in early gestation and were born with persistent viral infection and specific immunotolerance (littlejohns and walker, 1985; alenius et al., 1986; radostits and littlejohns, 1988; shimizu et al., 1989). Mucosal disease breaks out when a persistent bvdv-infected animal at any age is infected by a homologous cytopathogenic bvd virus (Straub, 1994), or as result of spontaneous mutation of the persistent ncp-bvdv into a cp biotype (Donis, 1988; Bolin, 1990; Wilhlmsen et al., 1991; Cortese, 1992; Meyers et al., 1991; Meyers et al., 1992; Trautz et al., (1994).

Bvd virus is capable of producing a wide variety of disease syndromes in cattle of different age. Depending on the severity of clinical signs and course of the disease, 117 (77%) out of 152 virologically positive cattle showed the acute form of mucosal disease with severe clinical symptoms and short course of the disease. While 35 (23%) animals had the chreonic form of the disease showed less sever clinical manifestations with long course of the disease. The antigenic homology of noncytopathic persistent virus and cytopathic virus is probably the most important factor in the pathogenesis of mucosal disease (Bolin et al., 1985; Brownile et al., 1987; Schimizu et al., 1989; Westenbrink et al., 1989; Schimizu 1990; Nakajima et al., 1993). Acute mucosal disease arises when the superinfecting cp-bvdv is closely homologous to the persisting ncp-bvdv (Brownlie et al., 1986; ames and baker 1990; moennig et al., 1990). Chronic md may occur as a result of a superinfection of persistently viraemic animals with cytopathogenic isolates that have partial antigenic homology with the persisting noncytopathogenic virus (Brownlie 1990). Some acute cases of md do not lead to death within the expected time of several days and become chronically ill (Radostits and Littlejohns, 1988).

The definitive diagnosis of clinical mucosal disease is dependent upon isolation of bvd virus from nasal swabs, blood or tissue samples (Grunder, 1986, HaineS et al., 1992) . In this study , 58 (82.9%) out of 70 clinically mucosal disease (MD) suspected cattle were virologically

positive by cell culture technique. An antigen capture enzyme immunoassay (EIA) was used by Gottschalk et al. (1992) for the detection of BVD viral antigen in peripheral blood lymphocytes of cattle. We also used a direct enzyme linked immunosorbent assay (ELISA) for the detection of BVD viral antigen in leukocytic fractions. Our results indicated that direct ELISA detected BVD viral antigen in all leukocytic fractions from which BVD virus was isolated by cell culture method. Three buffy coat samples from three MD-affected cattle were positive in ELISA, while negative by cell culture method. This indicated that detection of BVD virus antigen using direct ELISA is more sensitive than isolation and detection of the virus by using the cell culture method especially in the samples obtained from chronic MD affected cattle.

We noticed that the isolation and detection of BVD virus using tissue culture method is a time consuming technique. HAINES et al., (1992) mentioned that the isolation of BVD virus on tissue culture may require several days or even weeks and that the test may be insensitive due to it being dependent upon the presnec eof infectious virus in the submitted specimens. Our study concluded that, direct ELISA is sensitive, rapid test for the detection of BVD viral antigen especially in large field scales. However it was more expensive than the tissue culture method.

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