A NEW DIAGNOSTIC SCREENING METHOD USING EAR TAG SAMPLES TO DETECT BVD VIRUS INFECTION

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INTRODUCTION

Bovine Virus Diarrhea Virus (BVDV) is one of the most important viral pathogens of cattle causing considerable economic losses worldwide. Up to 1 % of the cattle population is BVDV positive. The Pestivirus (family Flaviviridae) provokes a variety of clinical symptoms that range from inapparent infection to a fatal lethal disease called Mucosal Disease (MD). BVDV infection during pregnancy may result in infection of the foetus depending on the development stage. Calves that survive infection during the first trimester of gestation are immunotolerant to the restrictet virus and born with a persistant and lifelong infection. These persistently infected (PI) animals are viraemic, antibody negative and constantly shed virus. Here we present a new screening method using TypiFix™ ear tag samples (AGROBIOGEN GmbH) in combination with a BVDV-Antigen/Serum ELISA (IDEXX Laboratories), which allows to test large numbers of cattle and to identify particularly PI animals, the primary reservoir of BVD-Virus.

MATERIAL AND METHODS

I. Sample collection with the TypiFix™-sampling system (AGROBIOGEN GmbH, Germany): TypiFix-system is an easy-handling tissue collection system that includes storage of the sample, transcription of the sample ID into the data base and error-free sample processing in the lab. Using the TypiFix ear tag system, the application of the ear-tag and the entire sampling procedure work simultaneously. No cooling or refrigeration during transport was done. Mummification of samples in the sample containers allowed storage at 4 °C, room temperatures or even higher temperatures (37°C) for one ore more weeks (storage assay). Figure 1 illustrates the application of the TypiFix-system.

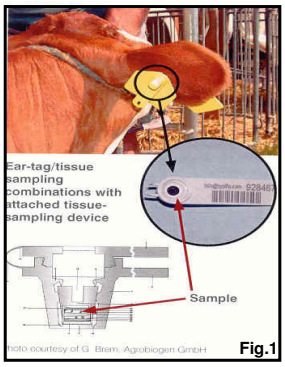

Fig.1

RESULTS

1. Comparison with reference techniques

The following two tables describe the results obtained for BVDV diagnosis of 84 ear tag and blood samples from Austria. The number of right and false positive or negative results is shown in Tab.1a. The contradictory result regarding one sample* in Tab.1a was due to the fact, that this animal had an acute infection. Test performance of the TypiFix™ /E^{rns}Antigen-ELISA system is indicated in Tab.1b (Sensitivity: A / A+C; Specifity: B / B+D).

Ear notch samples			Blood		
E^{rns}-Antigen ELISA	positive	negative	E^{rns}-Antigen ELISA	positive	negative
positive	54	0	positive	53	1*
negative	0	30	negative	0	30

Tab.1a *Transient infection

E^{rns}-Antigen ELISA performance	Ear notch Samples N	Results observed	Results IDEXX Lab.**
Sensitivity:	54	100%	100%
Specifity:	30	100%	100%

**C.Schroeder, IDEXX Laboratories, Westbrook, Main, USA Tab.1b

2. Large Scale BVDV-Screening

Between September 2004 and February 2005, 787 field samples[1)] of cattle were analysed. In 27 cases (= 3,4 % of the total sample number) BVDV protein could clearly be detected in E^{rns}-Antigen ELISA. Only 0,6 % of the results must be reanalysed using a second TypiFix sample (Fig.2).

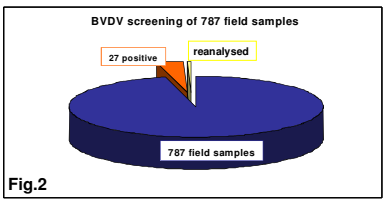

Fig.2

3. Storage Assay

For storage Assay 20 TypiFix-collectors with mummified ear tag samples of one PI animal were stored at 4 °C or 37 °C for 1 to 5 weeks and subsequently analysed in E^{rns}-Antigen-ELISA (Fig.3). Three tissue samples of the same PI animal were tested initially, and served as reference (0 weeks, not shown). Cut-off level of the assay was OD450nm = 0,3. *(notice also AVID 2004, Kühne S. et al.)*

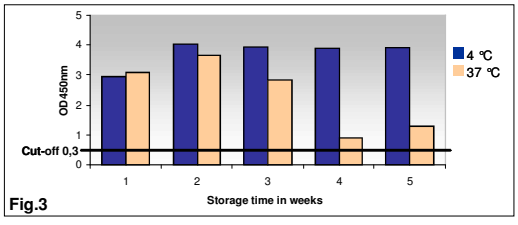

Fig.3

II. BVDV detection with HerdCheck E^{rns}-Antigen/Serum-ELISA Plus (IDEXX Laboratories, USA): The ear tag samples were extracted from TypiFix-system and transferred into 2ml reaction tubes with 120 µl soaking dilution buffer (IDEXX). After over night-incubation of the samples at 4 °C, 50 µl dilution buffer of each sample was applied in the above mentioned ELISA according to manufacturers instructions.

III. Blood samples test protocols and origin of the samples: Reference blood samples were analysed by the Department of Veterinary Medicine in Austria (Bommeli Checkit®-BVD-Virus-III enzyme immunoassay (EIA); E^{rns}-Glycoprotein detection) and by the Department of Microbiology, LMU Munich in Germany (WB103/105[anti-NS2/3]-immunolabeled leucocytes in Fluorescence Activated Cell Sorter (FACS)-analyses). Tissue samples of PI calves were provided by Dr. Wolf, Department of Microbiology, Munich (Germany) and by Dr. Götz / Dr. Ludwig, Department of Veterinary Medicine, Bavaria (Germany). Field samples[1)] of cattle were provided by Dr. Götz / Dr. Ludwig and Mag. Oettl, Department of Veterinary Medicine, Innsbruck / Tirol (Austria).

3. Size effect

TypiFix ear tag samples have a size of 2-3 mm (~10 mg). To determine the sensitivity of the E^{rns}-Antigen ELISA (IDEXX Laboratories), tissue samples were divided into two, four, eight or sixteen sections. Undivided samples of PI-animal served as positive control. Interestingly, even 1/16 of the original tissue size gives a significant positive results in the ELISA test (data not shown).

5. Diagnostic gap of PI calves

The effectiveness of this BVDV-screening system during the *diagnostic gap* (Palfi et al., 1993) was evaluated with 57 ear tag tissue samples from 11 different neonatal PI-calves. Samples were removed from day 0 to 101 *post partem* and subsequently analysed in E^{rns}-Antigen-ELISA (IDEXX Lab.; Dr. G. Wolf). All PI-animals could be clearly identified and the positive signals were many times over the cut-of level of OD450nm = 0,3. Fig.4 shows the results.

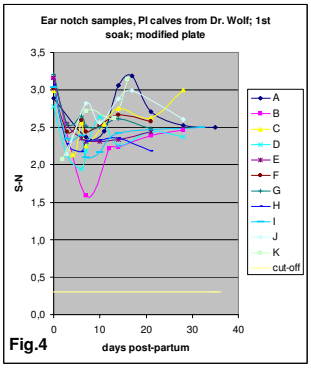

Fig.4

6. Genomic DNA purification after ELISA application

For 137 samples tested we could show, that isolation of genomic DNA with the nexttec (Leverkusen) *DNA purification system* is even possible after over-night incubation in soaking dilution buffer (IDEXX). Quantity (18-83 ng/µl) and quality of genomic DNA was sufficient for downstream applications like PCR or traceability tests.

DISCUSSION and CONCLUSION

Early detection of PI calves is economically and strategically advantageous. Fascinatingly, TypiFix/ E^{rns}-ELISA diagnostic system meets all requests. Here we could show that there is an excellent agreement between BVDV detection with the reference method and with ear tag samples of the TypiFix™-system. In large scale screening only 0,6 % of the results had to be verified and no ELISA test was invalid. Size effect studies demonstrate that large amounts of E^{rns}-antigen in ear tag samples are probable (Njaa et al., 2000; Shin et al., 2001; Grooms et al., 2002) and that there is no interference with maternal BVDV-antibodies in colostral phase. The results obtained in storage assays ensure that detection of BVDV-antigen is nearly independent of storage time and surrounding temperature. All these data the possibility of DNA isolation and identification qualifies the TypiFix™/ E^{rns}-Antigen ELISA BVDV-detection system as a powerful diagnostic tool to screen whole cattle populations.

REFERENCES

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