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Improving the sensitivity of the IBR-gE ELISA for testing IBR marker vaccinated cows from bulk milk

Verbesserung der Sensitivität des IBR-gE-ELISAs für die Testung IBR-Markerimpfstoff-vakzinierter Kühe aus Tankmilch

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Summary

The low sensitivity of the IBR-gE ELISA compared to other diagnostic ELISA tests for IBR is a major disadvantage of IBR control programmes based on IBR marker vaccination. Therefore the IBR-gE ELISA is not generally recommended for testing pooled or bulk milk samples. The aim of this study was to determine the performance of a commercially available kit for concentrating and purifying antibodies in milk in order to improve the sensitivity of detecting IBR-gE antibody positive cows from pooled and bulk milk samples.
A single IBR-gE positive cow is likely to remain undetected in a pool of 49 negative milk samples without concentration. By contrast, the bulk milk concentration procedure improved sensitivity from 5.4% to 75.7% in a positive herd. Milk samples with a high or moderate positive signal are more likely to be detected after pool milk concentration compared to weak positive samples.
Whereas a follow up study involving a monthly testing of bulk milk samples from three marker vaccinated IBR-gE negative herds over a period of seven months yielded negative results each month, bulk milk from a herd containing < 5% IBR-gE positive cows always detected positive after concentration. Although the milk concentration procedure had no impact on specificity, it significantly enhanced the sensitivity of the detection of IBR-gE positive milk in pooled and bulk milk samples. After further evaluation this procedure could allow a cost efficient and reliable method of monitoring IBR marker-vaccinated herds for IBR-gE antibodies.

Keywords: BHV-1, IBR-gE antibody, pool milk, milk concentration

Zusammenfassung

Der IBR-gE-ELISA hat, im Vergleich mit anderen diagnostischen Tests zum Nachweis von IBR, eine geringe Sensitivität. Dies stellt einen deutlichen Nachteil bei IBR-Bekämpfungsverfahren dar, die auf Markerimpfung beruhen, weshalb dieser Test im Allgemeinen nicht für die Untersuchung von Tankmilchproben empfohlen wird. Ziel dieser Studie war es, die Eignung eines kommerziell verfügbaren Kits zur Anreicherung und Reinigung von Antikörpern aus Milchproben zur Verbesserung der Sensitivität beim Nachweis IBR-gE-positiver Kühe aus Pool- und Tankmilchproben zu prüfen.
Durch die Anreicherung von Poolmilchproben konnte die Sensitivität beim Nachweis einer IBR-gE-positiven Milch in Pools mit 49 negativen Milchproben von 5,4 % ohne Anreicherung auf 75,7 % nach Anreicherung erhöht werden. Bei deutlich positiven und mittelgradig positiven Milchproben gelingt der Nachweis im Pool sicherer als bei schwach positiven Proben.
In einer Verlaufsstudie mit monatlicher Untersuchung von Tankmilchproben von drei IBR-gE-negativen, mit einem Marker-Impfstoff vakzinierten Herden sowie einer Herde mit < 5 % IBR-gE-positiven Kühen über einen Zeitraum von sieben Monaten, wurde die positive Herde bei jedem Testintervall positiv getestet, während die IBR-gE-negativen Herden stets negativ getestet wurden. Die Anreicherung hatte keinerlei Einfluss auf die Spezifität, konnte aber die Sensitivität beim Nachweis IBR-gE-positiver Tiere in Pool- und Tankmilch deutlich verbessern. Die hier vorgestellte Methode könnte nach weitergehender Evaluierung eine kosten-

günstige und zuverlässige Überwachung von IBR-markergeimpften Milchviehbeständen auf IBR-gE-Antikörper ermöglichen.

Schlüsselwörter: BHV-1, IBR-gE-Antikörper, Poolmilch, Milchkonzentrierung

Introduction

Bovine herpesvirus 1 (BHV-1) causes infections with various symptoms in cattle, infections of the respiratory tract being known as Infectious Bovine Rhinotracheitis (IBR). The virus is spread worldwide and shows high prevalence in many countries. However, Denmark, Austria, the Bolzano province in Italy, Sweden, Finland, Switzerland and two Bavarian regions are approved BHV-1-free (Probst and Kubitza, 2010). The BHV-1-eradication programmes in Germany, the Czech Republic and two provinces in northeast Italy were approved by EU-authorities in Commission Decision 2008/233/EC dated 17. March 2008.

Livestock disease eradication programmes are often based on "test and cull" strategies. Approaches using marker vaccines combined with diagnostic tests which differentiate infected from vaccinated animals (DIVA) have been used successfully for instance to eradicate Aujeszky´s disease in various countries (Müller et al., 2011; Hahn et al., 2010; Müller et al., 2003; MacDiarmid et al., 2000; Commission Decision 2010/434/EU dated 6. August 2010).

A glycoprotein E-(gE) negative strain of BHV-1 has been reported to be a safe, efficacious IBR-vaccine (Kaashoek et al., 1994). Van Oirshot et al. (1997) described an ELISA for the differentiation of cattle naturally infected with BHV-1 and vaccinated cattle based on this BHV-1gE-negative strain. Wellenberg and collegues (1998a) reported a commercial IBR-gE ELISA with a relative sensitivity of 96% when testing individual milk samples compared to the results for serum samples. They also described the use of an IBR-gE ELISA to detect antibodies in 88.4% of bulk milk samples originating from BHV-1 positive herds (Wellenberg et al., 1998b).

Kramps et al. (2004) reported the evaluation of tests for antibodies to BHV-1 in national reference laboratories in Europe during an EU ring trial. In this report the IBR-gE ELISA scored a sensitivity of just 72% compared to the indirect and the IBR-gB ELISAs for serum samples (87% and 96%, respectively). The IBR-gE ELISAs showed a sensitivity of only 58% versus 98% for the indirect ELISA and 81% for the IBR-gB blocking ELISA for individual milk samples.

In conclusion the IBR-gB ELISAs had the highest sensitivity, detection limit and repeatability for testing serum samples. The indirect ELISAs and the IBR-gE ELISAs clearly performed worse than the virus neutralization tests and the IBR-gB ELISAs. In contrast with the results of testing serum samples, the indirect ELISAs performed best in correctly scoring milk samples (Kramps et al., 2004).

Similar conclusions were reported from a German BHV-1 ring trial where the IBR-gE and the IBR-gB blocking ELISAs showed a low sensitivity for BHV-1 antibodies , even in individual milk samples (Reichelt et al., 2005). However, indirect ELISAs optimized for use with bulk milk samples of up to 50 individual cows can reliably indicate the BHV-1 status of these animals (OIE 2010). There is only one commercially available IBR-gE ELISA, however it is not recommended by the German BHV-1 Reference Laboratory for testing milk or bulk milk samples due to its low sensitivity.

While an IBR eradication programme based on IBR marker vaccine and serological surveillance with the IBR-gE ELISA is initially more economic than "test and cull", the picture is different in the later stages of such programme. In Germany, currently 87.7% of dairy- and sucking cow herds are BHV-1-free or BHV-1-gE-free. Since the IBR-gE ELISA is not recommended in Germany for milk samples because of its insufficient sensitivity, specificity and reproducibility (Beer et al., 2003), IBR-gE marker vaccinated herds are tested on individual blood samples (Höreth-Böntgen et al., 2009), which results in high costs. In 2009 3.81 million blood and individual milk samples were tested in Germany (Höreth-Böntgen et al., 2009). The authors also noted that cost-effective bulk milk testing was only performed on non-vaccinated herds, with a total of 286 469 bulk milk samples tested during 2009 in Germany.

Bulk milk sample testing is a non-invasive method which does not cause stress for the animals. Moreover, milk samples are taken routinely during milk quality assurance programmes, providing a cost-effective way of monitoring herd status.

Bulk milk concentration enhances the sensitivity of the IBR-gB and IBR-gE ELISA testing of bulk milk samples (Engemann et al., 2009) and also helps clarify the diagnosis from individual milk samples. CATTLETYPE® Milk Prep (Labor Diagnostik GmbH Leipzig, Germany) is a commercially available kit for the concentration and purification of antibodies from individual, pooled and bulk milk samples.

The purpose of this study was to evaluate the ability of CATTLETYPE® Milk Prep (Labor Diagnostik GmbH Leipzig, Germany) to enhance the sensitivity of the IBR-gE ELISA and its suitability for use in a state veterinary diagnostic laboratory. A reliable, cost-effective method of monitoring IBR marker vaccinated IBR-gE negative herds is crucial. Therefore, one key aspect of this study was the detection of one IBR-gE positive milk sample pooled with 49 negative milk samples. This would enable for example the reintroduction of an IBR-gE positive cow into a negative herd to be detected with the IBR-gE ELISA.

Material and Methods

Milk samples

13 milk samples from the 2004 EU ring trial were tested individually and in pools. Pools were made up of one milk sample from the EU ring trial together with 49 negative milk samples from Bavaria. Pools were tested before and after milk concentration. A pool size of 50 milk samples is commonly used in Germany for moni-

TABLE 1: *BHV1 status of milk samples from the 2004 EU ring trial according to the ring trial coordinator*

Sample no.	Sample value
#101	positive positive field sample, infected animal
#102	weak positive positive field sample, infected animal
#106	weak positive positive field sample, infected animal
#110	milk from infected cow, 1:2 dilution
#117	milk from infected cow, 1:4 dilution
#120	milk from infected cow, 1:8 dilution
#118	milk from infected cow, 1:16 dilution
#122	milk from infected cow, 1:32 dilution
#119	milk from infected cow, 1:64 dilution
#103	gE negative, gB positive hyper-vaccinated (IBR-gE-negative DIVA vaccine)
#104	negative negative field sample
#107	negative negative field sample
#109	negative negative field sample

toring IBR, enzootic bovine leukosis and bovine brucellosis. The BHV-1 status of those samples (as reported by the ring trial coordinator) is described in Table 1.

37 milk samples from cows with a history of being IBR-gE positive belonging to an IBR marker vaccinated but recently BHV-1 re-infected herd of 250 cows were tested as individual milk samples, and as pools (each positive sample together with 49 negative milk samples from Bavaria) both before and after milk concentration.

In addition four IBR marker vaccinated herds were tested monthly for IBR-gE antibodies using concentrated milk pools of 50 milk samples in duplicates over a period of seven months from November 2009 to May 2010. Individual blood samples at the beginning and the end of the study were used to ascertain the BHV-1-gE status. As shown in Table 2, two herds had been negative for IBR-gE for many years (herds A and C). Biannual blood testing of herd B showed that all animals were negative in 2009 while herd D had less than 5% IBR-gE positive cows remaining. The results of testing bulk milk were compared to herd history and serum results obtained biannually.

Herd A was routinely tested only once a year because of its long term BHV-1-free status. Herd C (BHV-1-free since 2008) was tested twice yearly by choice. Herds B and D, which were working towards a BHV-1-free status, were routinely tested twice yearly.

Milk concentration and ELISA testing

Milk samples were pooled manually or robotically using a Genesis liquid handling robot (Tecan GmbH, Germany).

Milk concentration from pooled and bulk milk samples was performed using CATTLETYPE® Milk Prep (Labor Diagnostik GmbH Leipzig, Germany) according to the manufacturer's instructions. To summarize, casein from 5 ml native milk (protocol for individual milk) and 50 ml pooled milk (protocol for pooled milk) was precipitated and a matrix was added to the milk whey. After incubation of two hours the matrix was washed and 200 µl of concentrated milk antibodies were extracted.

TABLE 2: *Herd size and BHV1-status of four herds, used for the field study*

Herd	Herd size	Herd status
Herd A	241 cattle, including 106 cows	BHV-1 negative since March 2007
Herd B	1049 cattle, including 514 cows	all cows IBR-gE negative since 2009
Herd C	327 cattle, including 153 cows	BHV-1 negative since April 2007
Herd D	861 cattle, including 426 cows	final stage of IBR eradication, < 5% IBR-gE positive cows

Milk, pool milk samples and the extract of the concentrated pool milk samples were tested using HerdChek® IBR gE Ab Test (IDEXX GmbH, Germany) according to the manufacturer's instructions for milk samples with one exception. Whereas the manufacturer classified S/N-(sample/negative control) ratios > 0.8 as negative and ≤ 0.8 as positive, for this study the status of the results was classified as follows: S/N-ratio > 0.8 negative, 0.5–0.8 weak positive, 0.2 to < 0.5 positive and < 0.2 strong positive.

Determination of IgG in milk and milk concentrates

Immunoglobulin G (IgG) in pool milk samples and concentrates of pool milk samples were determined using the Bovine IgG ELISA Kit (Bethyl Laboratories Inc., USA) following the manufacturer´s instructions. The IgG concentrations of the samples were calculated on the basis of standard solutions, measured in parallel with the samples. Finally the concentration factor (CF) was calculated.

Results

The concentration effect of the CATTLETYPE® Milk Prep Kit (Labor Diagnostik GmbH Leipzig, Germany) was determined by measuring the IgG concentration exemplarily in six milk pool samples of 50 milks before and after concentration and calculating the concentration factor (Tab. 3). These pool milk samples showed factors of 11.0 up to 21.0.

To demonstrate the concentration effect for the detection of IBRgE-antibodies in pool milk samples (one positive milk in 49 negative milks), the pool milk samples and their extracts were tested with the IBR-gE ELISA. The positive milk samples I and II showed strong positive, samples III and IV positive and sample V a weak positive S/N-ratio in the IBR-gE ELISA when tested as individual milk (Tab. 3). As shown in Table 3, out of the five pool milk samples, only the pool containing the strong positive milk II was found to be weak positive in the IBR-gE ELISA. The milk pools with milk I, III, IV and V and the negative pool milk sample tested negative. After concentration, the pools containing milk I, II, III and IV revealed strong positive, positive and weak positive results, while the negative pool tested negative. The pool milk sample containing the weak positive milk V tested negative after concentration.

Testing the 2004 EU ring trial milk samples individually and as pool milk samples as well as pool milk concentrates resulted in the following findings: The gE-positive sample #101 tested positive both as an individual milk sample and as pool milk concentrate, but negative as non-concentrated pool milk. The weak positive sample #106 was found to be borderline as an individual milk sample but negative in both the pool milk sample and pool milk concentrate. The weak positive sample #102 showed negative in all three tests. From the dilution series, only sample #110 (the 1:2 dilution) was detected as an individual sample, but not as

TABLE 3: *Determination of IgG concentration in milk pool samples (positive pools with one positive milk in 49 negative milks, and a negative pool of 50 negative milk samples) before and after concentration (Milk I and II = strong positive milk samples, Milk III and IV = positive milk samples, Milk V = weak positive milk sample). The concentration factor (CF) was calculated. Detection of specific IBR-gE antibodies in the positive individual milk samples, the milk pool samples (S/N-[sample/negative control] ratios > 0.8 are negative; 0.5–0.8 weak positive, 0.2–0.5 positive and < 0.2 strong positive) and the extract of the milk pool samples*

	Sample	IgG concentration			IBR-gE ELISA					
		Pool milk	Extract	CF	Individual milk		Pool milk		Extract of pool milk	
		IgG [mg/ml]	IgG [mg/ml]		S/N	Result	S/N	Result	S/N	Result
BHV-1-positive pool	pool with Milk I	0,618	12,540	20,3	0,110	**strong positive**	0,820	negative	0,167	**strong positive**
	pool with Milk II	0,640	13,450	21,0	0,078	**strong positive**	0,691	**weak positive**	0,149	**strong positive**
	pool with Milk III	0,622	9,325	15,0	0,341	**positive**	0,892	negative	0,478	**positive**
	pool with Milk IV	0,674	7,400	11,0	0,410	**positive**	0,898	negative	0,565	**weak positive**
	pool with Milk V	0,625	10,700	17,1	0,733	**weak positive**	0,884	negative	0,814	negative
BHV-1-negative pool	pool of 50 BHV-1-neg. milks	0,603	10,800	17,9			0,935	negative	0,953	negative

a pool milk sample or a pool milk sample concentrate. The hyper-vaccinated sample and the three negative samples detected negative as individual milk samples as well as pooled milk and pool milk concentrates. Compared to gE-negative individual milk, the milk pools and pool milk concentrates showed lower S/N-ratios (Fig. 1).

Two of 37 IBR-gE antibody positive milk samples of the IBR marker vaccinated but recently BHV-1 reinfected herd tested positive in a pool of 50 milk samples (5.4%). After pool milk concentration, 28 of the 37 pool milk concentrates scored positive (75.7%). Pools with one strong positive milk sample were more likely to be detected. Individual milk samples which were weakly positive for IBR-gE antibodies with S/N-ratios of 0.5 or higher yielded mostly negative results after pool milk concentration.

Individual milk samples of the IBR-gE positive cows showed S/N-ratios of 0.07 to 0.74, mean 0.31 (Fig. 2).

Blood testing of all cows of the three IBR-gE negative field study herds (A, B, C) verified the gE-negative status of herds A, B, C and confirmed the prevalence of IBR-gE positive cows of less than 5% in herd D.

Milk pool concentrates of herds A, B, and C scored negative at all seven sampling intervals.

Positive pool milk concentrates were found in herd D in each monthly testing from November 2009 to April 2010. Positive pools contained an average of three positive samples (minimum one, maximum seven). From January 2010 to April 2010, this herd was also tested for IBR-gE without concentration of the milk pools yielding negative results. Based on the results of blood testing in March 2010, IBR-gE-positive cows were removed from this herd in April 2010. Concentrated pool milk sample testing in May 2010 always yielded negative results for IBR-gE antibodies.

The results of the three negative herds and one positive herd tested monthly as pool milk samples are shown in Figures 3A and 3B.

Positive milk pools of 50 milk samples from herd D contained between one and five positive milk samples. The S/N values for positive pool concentrates and individual milk samples in these pools determined in the December 2009 are shown in Figure 4.

Discussion

The purpose of disease eradication programmes based on the DIVA strategy is to eliminate infected animals while protecting non-infected animals from infection by using marker vaccination. Although this DIVA approach has been deployed very successfully to eradicate Aujeszky´s disease in several countries, it has some major drawbacks regarding BHV-1-eradication. Compared to pigs, bovines have a much longer life-span. Therefore immunity after vaccination and reliable diagnostic results are required for up to ten years. The IBR-gE ELISA has low sensitivity among the diagnostic tools for BHV-1. This has an impact on serum testing and even more so on testing milk (Kramps et al., 2004) and bulk milk samples.

This study confirmed the low sensitivity of the IBR-gE ELISA for testing bulk milk. Testing the 2004 European ring trial samples and IBR-gE-positive milk samples from an infected herd demonstrated that the IBR-gE ELISA could not detect any of the ring trial samples and only 5.4% positive field milk samples when tested in pools consisting of one positive and 49 negative milk samples.

Although the frequent testing of bulk milk samples for IBR-gE without concentration may be a convenient tool for prevalence studies, a transition from negative to positive is unlikely to be detected until 10–15% of the

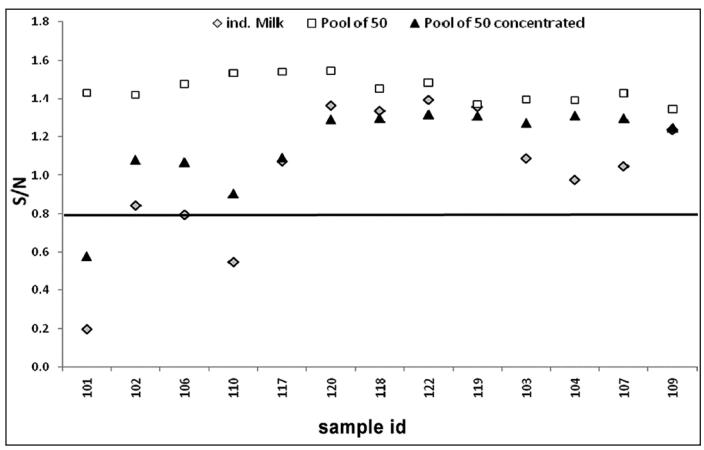

FIGURE 1: *Test with the 2004 EU ring trial samples. The samples were tested as individual milk samples (◊), as milk pool samples (□) comprising one ring trial sample and 49 negative milk samples and as milk pool concentrates (▲). The cut-off for the S/N-ratio (sample/negative control-ratio) is 0.8. Samples with a ratio ≤ 0.8 are positive, > 0.8 are negative. The positive results are subdivided into weak positive (S/N-ratio 0.5–0.8), positive (S/N-ratio 0.2–0.5) and strong positive (S/N-ratio < 0.2).*

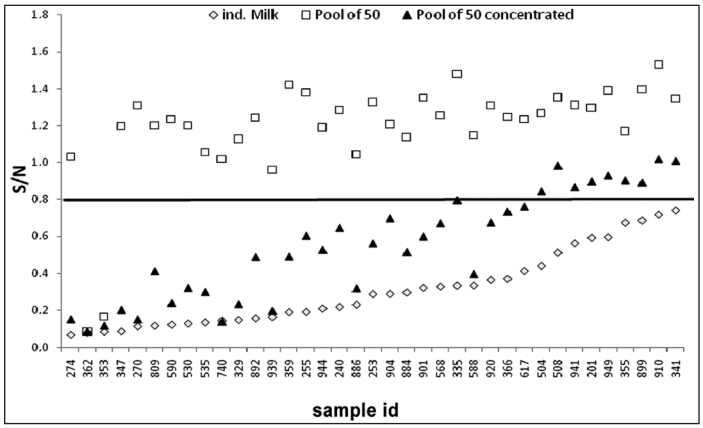

FIGURE 2: *Test with IBR-gE antibody positive milk samples from a marker vaccinated herd with recent BHV1 reinfection. The milk samples were tested as individual milk samples (◊), as milk pool samples (□) comprising one IBR-gE positive sample and 49 negative milk samples from Bavaria and as milk pool concentrates (▲). The cut-off for the S/N-ratio (sample/negative control-ratio) is 0.8. Samples with a ratio ≤ 0.8 are positive, > 0.8 are negative. The positive results are subdivided into weak positive (S/N-ratio 0.5–0.8), positive (S/N-ratio 0.2–0.5) and strong positive (S/N-ratio < 0.2).*

animals in a herd become seropositive (Wellenberg et al., 1998b). Given that IBR marker vaccination protects such herds, seroconversion of 10–15% of the milking cows is rare, and reinfection may remain undetected for a long time. Furthermore IBR-gE-positive cows from apparently uninfected herds may be sold to other herds, thus maintaining and spreading infection.

Accordingly in the German mandatory IBR eradication programme, marker vaccinated herds are not allowed to be tested using bulk milk. Instead, annual blood sampling and testing are required. Those farmers who successfully eradicate IBR-gE-positive cattle cannot switch to cost-efficient bulk milk testing as long as they continue to have marker-vaccinated cattle in their herd.

Forschner and Bünger (1986) described a method for the purification of milk and bulk milk samples. In 2009 LDL developed a new procedure for concentrating and purifying antibodies from milk and bulk milk samples. Compared to the method of Forschner and Bünger the CATTLETYPE® Milk Prep Kit (Labor Diagnostik GmbH Leipzig, Germany) is more efficient, well standardized and easier to perform. The determination of IgG in pool milk samples before and after concentration indicated concentration factors from eleven to 21.

Comparing the S/N-ratios of positive pool milk samples in the IBR-gE ELISA, all pool milk samples (with the exception of one pool with one strong positive milk) were found negative with S/N-ratios marginally above the cut-off of 0.8, indicating that there might be at least one positive milk in the pool milk sample. After extraction those pool milk samples with strong positive milks tested positive. Those with positive milks tested positive and weak positive. And the pool milk sample with the weak positive milk tested negative. The S/N-ratios of the IBR-gE ELISA with individual positive milk samples were comparable to those signals of extracts of milk pools containing such positive milk samples.

These findings show that the pool milk concentration might help to identify serologically strong positive and positive cows when testing bulk milk. To overcome the problem of failing to detect weak positive milk samples in concentrated pool milk samples, reducing the pool size from 50 to 20 milks or establishing regular testing (e. g. monthly) might improve matters, although further investigation is required.

The concentration procedure for pooled milk samples using the CATTLETYPE® Milk Prep Kit (Labor Diagnostik GmbH Leipzig, Germany) resulted in one IBR-gE positive ring trial sample being detected in a total pool of 50 milk samples. 75.7% of the field milk samples were positive for IBR-gE antibody after concentration in pools of a total of 50 milk samples. Testing pooled milk without concentration resulted in none of the ring trial samples and only 5.4% of the positive field milk samples being detected. The weak positive milk samples

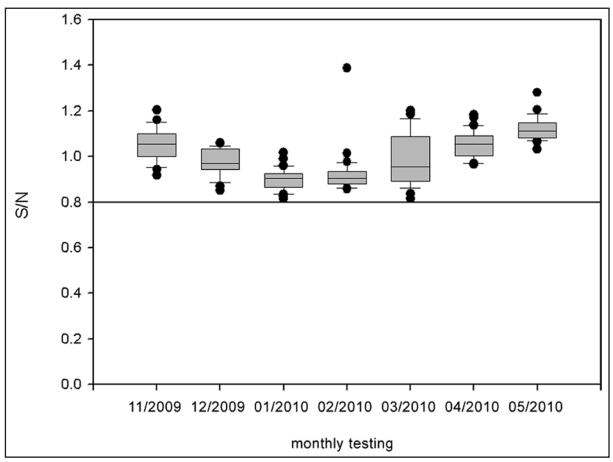

FIGURE 3A: *Distribution of S/N values of pool milk concentrates from three IBR-gE negative herds. The cut-off for the S/N-ratio (sample/negative control-ratio) is 0.8. Samples with a ratio ≤ 0.8 are positive, > 0.8 are negative. The positive results are subdivided into weak positive (S/N-ratio 0.5–0.8), positive (S/N-ratio 0.2–0.5) and strong positive (S/N-ratio < 0.2).*

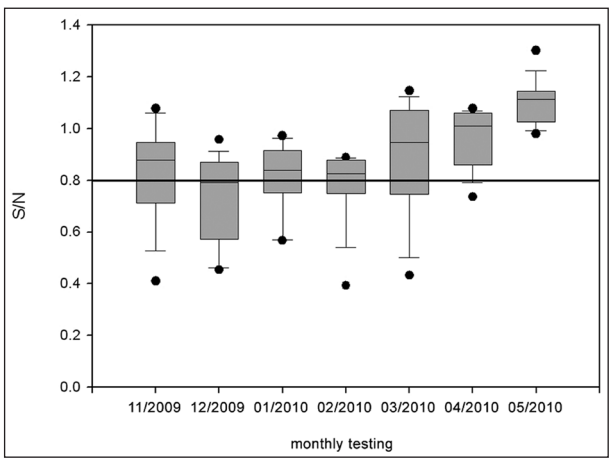

FIGURE 3B: *Distribution of S/N values of pool milk concentrates from one IBR-gE positive herd. The cut-off for the S/N-ratio (sample/negative control-ratio) is 0.8. Samples with a ratio ≤ 0.8 are positive, > 0.8 are negative. The positive results are subdivided into weak positive (S/N-ratio 0.5–0.8), positive (S/N-ratio 0.2–0.5) and strong positive (S/N-ratio < 0.2).*

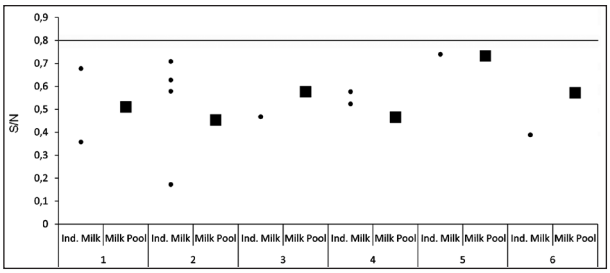

FIGURE 4: *Distribution of S/N values of the positive milk pools and individual milk samples in those pools from the IBR-gE positive herd from monthly testing. The data in this example are from December 2009. For each of the six pools the S/N values of each positive individual milk sample and also the S/N values of the same positive milk samples in a milk pool of 50 milk samples are shown. The cut-off for the S/N-ratio (sample/negative control-ratio) is 0.8. Samples with a ratio ≤ 0.8 are positive, > 0.8 are negative. The positive results are subdivided into weak positive (S/N-ratio 0.5–0.8), positive (S/N-ratio 0.2–0.5) and strong positive (S/N-ratio < 0.2).*

and dilutions of a positive milk sample were still overlooked in a pool together with 49 negative milk samples after concentration.

Individual milk samples with a strong positive or positive antibody level are likely to be detected in pools of 50 milk samples after concentration. Weak positive milk samples may remain undiscovered in pools even after concentration. In the negative ring trial and negative field samples tested, bulk milk sample concentration showed no negative impact on specificity.

Similar results were reported recently from Switzerland where milk concentration increased the specificity of an indirect Bluetongue antibody ELISA in both bulk and single milk samples (Chaignat et al., 2010).

In this study, the monthly testing of pool milk of 50 samples in a herd with a prevalence of < 5% IBR-gE positive cows yielded positive pools after concentration in all tests until the remaining positive cows where removed from the herd. Concentration and purification of bulk milk samples using CATTLETYPE® Milk Prep Kit (Labor Diagnostik GmbH Leipzig, Germany) might be a useful tool to overcome the poor sensitivity of the IBR-gE ELISA on bulk milk and allow the reliable yet cost-effective monitoring of IBR-gE antibodies in IBR marker vaccinated herds.

This was the first study testing the ability of the CATTLETYPE® Milk Prep Kit (Labor Diagnostik GmbH Leipzig, Germany) to enhance the sensitivity of the IBR-gE ELISA and it´s suitability in routine testing of field milk samples in a state veterinary diagnostic laboratory. Further investigations with more clearly defined positive and negative samples are necessary to evaluate the reproducibility of these results.

Conflict of interest

Carsten Schroeder, Nicole Bürger, Claudia Engemann, Eva V. Knoop and Jörg Gabert are employed by Labor Diagnostik GmbH Leipzig, Germany, the developer of the CATTLETYPE® Milk Prep Kit.

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