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Original Scientific Article

THE USEFULNESS OF TWO MOLECULAR METHODS FOR THE DETECTION OF PERSISTENTLY INFECTED CATTLE WITH BOVINE VIRAL DIARRHEA VIRUS USING ORAL SWAB SAMPLES

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Summary: Serum and oral swab samples were collected from a persistently infected cow and her calf in a two-month period to test usefulness of oral swab samples for the detection of bovine viral diarrhea virus. Nucleic acids of the virus were detected by two molecular methods: conventional gel-based RT-PCR and commercial real-time RT-PCR. The bovine viral diarrhea virus genome was detected in serum and oral swab samples on days 0, 7, 15, 22, 23, 29, 36, 37, 43, 44, 46, 51, 52, 53, and 57. The dry cotton swabs showed a reduction of diagnostic sensitivity after three days when samples were stored at room temperature (+ 21 °C), but storage of oral swab samples at + 4 °C or in a freezer (< – 15 °C) for at least 10 days had no negative impact on the detection of the virus. No reduction of diagnostic sensitivity was observed when oral swab samples were collected in tubes with a liquid virus transport medium. Oral swabs provide an easy, reliable and cost-effective sampling tool for identification of PI animals, together with RT-PCR methods. The oral swab sampling could be especially useful for screening newborn calves during testing and removing PI animals from bovine viral diarrhea virus-infected herds.

Key words: bovine viral diarrhea virus; diagnostics; oral swab; RT-PCR; real-time RT-PCR

Introduction

Bovine viral diarrhea (BVD) is a disease of cattle that reduces productivity and may increases death loss. It is caused by bovine viral diarrhea virus (BVDV), a member of the *Pestivirus* genus of the family *Flaviviridae* (1). BVDV is distributed throughout the world, with endemic areas detecting antibodies among 70%–100% of herds, while in some European countries such as Sweden, Norway, Finland and Austria, the disease has already been eradicated (2). BVDV is spread by close contact (nose-to-nose) between cattle. Virus is shed by both acutely and persistently infected (PI) animals, but levels of shedding are much higher in persistently infected cattle, which are the natural reservoir for the virus. Fetuses that become infected between 30 and 125 days of gestation and survive the infection may be born as BVDV-infected calves. The BVDV infection will persist for the life of the calf, hence the term "persistent infection", or PI. It is estimated that the incidence of PI animals is between 0.3 and

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2.6% (3, 4). PI animals are the main source of infection in infected herds and tend never to reach their productive potential and growth because of reduced fertility and increased susceptibility to other diseases (4). PI animals shed large amounts of virus in all their secretions and excretions (5). By removing PIs from the population, the source of infection is removed, and by this means, the disease can be controlled (2). Blood tests are the most frequently used method to identify BVDV in live animals. Tests can also be done on skin biopsies (taken from the ear), on milk or even on hair samples (2, 5, 6). The oral swab sampling method has become more important both in human and veterinary fields because it is less uncomfortable for the animal during sampling. It is also a simple method for farmers and can be performed without special technique or equipment (7, 8, 9, 10, 11, 12, 13). To date, only one research paper describes the successful testing of oral swab samples as an option for the detection of PI animals from BVDV-infected herds (14).

The purpose of this study was to use two RT-PCR tests for BVDV on PI cattle over a two-month period to (a) determine the usefulness of oral swab samples for the detection of BVDV in PI animals, (b) determine the sensitivity of two molecular methods for detection of BVDV from oral swab samples and (c) test the robustness of two types of swabs for oral sampling during 10 days of storage at three different temperatures.

Materials and methods

A persistently infected pregnant cow, 58 months old, was identified in a BVDV-infected herd and removed to another location where all samplings were carried out. Serum and oral swab samples were collected from the PI cow at days 0, 7, 15, 22, 29, 36, 43, 51 and 57. A clinically healthy calf was born on day 22 after the first sampling, and then serum and swab samples were also collected from the calf on days 22, 23, 29, 36, 37, 43, 44, 46, 51, 52, 53 and 57, after the first sampling in the cow. Oral swab samples containing saliva from the oral cavity of the cow and calf were collected using sterile dry cotton swabs and were immediately sent to a laboratory. The samples were homogenized in 1 ml of RPMI 1640 cell culture medium (Gibco, United Kingdom) and stored in a freezer at < – 15 °C until testing. To test the stability of viral RNA in a dry cotton swab stored at three different temperatures, 3 x 11 oral swab samples were collected on day 57 from the cow, and then 11 samples were placed for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days at room temperature (18–24 °C), 11 samples for 0 to 10 days in a refrigerator (5 ± 3 °C) and 11 samples for 0 to 10 days in a freezer (< – 15 °C). After storage at different temperatures, the oral cotton swab samples were homogenized in 1 ml of RPMI 1640 cell culture medium (Gibco, United Kingdom) and stored < – 15 °C until testing. Additional 3 x 11 oral swab samples were collected from the calf on day 57 using Virocult® tubes with liquid virus transport medium (MWE, United Kingdom) and were then stored at three different temperatures as described above.

Total RNA was extracted from 140 µl of homogenate or the liquid with Virocult® transport medium (MWE, United Kingdom) using a commercial kit for RNA extraction QIAamp® Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. One-step RT-PCR was performed on samples in one tube using primer sequences based on 5' non coding region (5'NCR), with the forward primer P 104F 5'-GCT AGC CAT GCC CTT AGT AGG ACT-3' and the reverse primer P 402R 5'-CAA CTC CAT GTG CAA TGT ACA GCA-3', which detect both genotype 1 and 2 strains of BVDV (15). The reference BVDV strain NADL was used as the positive control. Reaction mixtures without RNA served as negative controls. The reaction was performed in a total volume of 25 µl using One-Step RT-PCR® Kit (Qiagen, Germany) as follows: 15 µl of nuclease free water, 5 µl of 5x PCR buffer, 1 µl of dNTP mix (containing 10 mM of each dNTP), 0.5 µl of the stock solution with 20 µM of each primer, 1 µl of the one-step RT-PCR enzyme mix and 2 µl of the RNA template. The RT-PCR program included a reverse transcription stage at 50 °C for 30 min, followed by an initial PCR activation step at 95 °C for 15 min. This was followed by 40 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min and a final extension step at 72 °C for 10 min. The reactions were carried out on a programmable T1 thermocycler (Biometra, USA) and the RT-PCR products were visualized in 1.8% agarose gel with 0.5 µg/ml ethidium bromide, with subsequent visualization under a UV light. The size of the PCR product was compared to the 100 bp DNA ladder (Fermentas, Germany) and PCR products of about 300 bp were interpreted as positive according to

the expected DNA fragment and as negative if no specific product was visible.

Serum and oral swab samples were quantified using a commercial real-time method (RT-qPCR) TaqVet® BVDV Screening (Laboratoire Service International, France) for the detection of BVDV. After RNA extraction, RT-qPCR was performed on Mx3005P thermocycler (Stratagene, USA) using protocol according to the manufacturer's instructions, and the results were presented as a cycle threshold value for individual samples. Analysis of RT-qPCR amplification curves was performed using commercial thermal cycler system software, and an "auto baseline" was used to determine fluorescence baselines. All samples were tested in one run on the same 96-tube microplate.

The results of statistical evaluation are shown as an average ± standard deviation of the average (SD). In order to test the differences in each parameter among time sampling, t-test for two paired samples / two-tailed test and the Pearson correlation coefficient were used. For assessing agreement between two methods of clinical measurement, Bland-Altman methods were used (16). SigmaStat 3.5 (SYSTAT Software Inc.) and XLSTAT 2013 (Addinsoft 1995–2013) software was used.

Results

During the 57 days of the study, in total, 21 serum and 21 oral swab samples were collected from the two PI and tested by RT-PCR and RT-qPCR methods (Table 1, Table 2). Viral RNA was detected in all serum and oral swab samples by both methods, confirming 100% successful detection of PI animals in both types of samples (Figure 1). The detected Ct value in serum samples varied between 20.34 and 24.89 (average ± 2 x Standard Deviation: 22.88 ± 2.54), while the detected Ct value in oral swab samples was between 21.74 and 29.97 (26.26 ± 4.42); the differences between values were statistically significant ($P<0.001$). About a 3.4 lower Ct value for oral swab samples was expected compared to detected Ct value in serum samples, because during preparation of oral cotton swab samples, the dilution 1:10 of swab samples in cell culture medium was used (Figure 2). The coefficient of variation (CV) for serum samples was 5.54 and for oral swab samples, 8.40. No significant differences were observed between Ct values obtained from cow and calf (cow-serum: 23.51 ± 1.34; cow-oral swab: 26.63 ± 3.76; calf-serum: 22.40 ± 2.84; calf-oral swab: 26.16 ± 5.0) during the time of the study.

Table 1: Detection of RT-PCR product on gel electrophoresis by using RT-PCR method for the detection BVDV after storage of oral swab samples at room temperature (+ 21 °C), in refrigerator (+ 4 °C) and in freezer (< – 15 °C) for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days

	OS-C (+ 21 °C)	OS-C (+ 4 °C)	OS-C (<- 15 °C)**	OS-V (+ 21 °C)	OS-V (+ 4 °C)	OS-V (<- 15 °C)
day 0	+	+	+ **	+	+	+
day 1	+	+	+ **	+	+	+
day 2	+	+	+ **	+	+	+
day 3	+	+	+ **	+	+	+
day 4	-	+	+ **	+	+	+
day 5	-	+	+ **	+	+	+
day 6	-	+	+ **	+	+	+
day 7	-	+	+ **	+	+	+
day 8	-	+	+ **	+	+	+
day 9	-	+	+ **	+	+	+
day 10	-	+	+ **	+	+	+

*Two types of oral swabs were used: oral cotton swab samples (OS-C) and oral swab samples using Virocult® tubes with liquid virus transport medium (OS-V). The results in the third column OS-C (< -15 °C)** are for stored samples in the freezer (for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 weeks). Results are presented as positive (+) when RT-PCR product was detected and as negative (-) when RT-PCR product was not detected.

Table 2: Detection of BVDV by using RT-qPCR method after storage of oral swab samples at room temperature (+ 21 °C), in refrigerator (+ 4 °C) and in freezer (< – 15 °C) for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days

Days	OS-C (+ 21 °C)	OS-C (+ 4 °C)	OS-C (< - 15 °C)**	OS-V (+ 21 °C)	OS-V (+ 4 °C)	OS-V (< - 15 °C)
day 0	29.85	24.87	27.46**	24.92	23.91	25.16
day 1	31.55	26.69	30.08**	23.86	25.46	25.36
day 2	34.14	28.23	27.12**	30.29	25.89	26.35
day 3	38.26	27.32	30.27**	28.47	27.21	25.93
day 4	>45	26.93	33.49**	32.24	26.16	24.9
day 5	>45	27.35	32.47**	26.4	25.96	26.22
day 6	>45	31.68	32.39**	32.2	32.55	25.6
day 7	>45	31.02	31**	32.97	27.19	23.93
day 8	>45	30.01	34.32**	27.9	26.57	23.29
day 9	>45	28.79	29.21**	30.58	28.2	26.8
day 10	>45	30.42	32.75**	29.45	28.86	26.53
X	Not done	**28.48**	**30.96**	**29.03**	**27.09**	**25.46**
SD	Not done	2.10	2.38	3.04	2.25	1.10
X+2xSD	Not done	32.69	35.72	35.10	31.60	27.65
X-2xSD	Not done	24.28	26.20	22.95	22.58	23.27
CV	Not done	7.38	7.68	10.46	8.32	4.30

*Two types of oral swabs were used: oral cotton swab samples (OS-C) and oral swab samples using Virocult® tubes with liquid virus transport medium (OS-V). Results are presented as positive (with Ct value of each sample) and negative (with Ct value > 45) when Ct value was not detected. The results in the third column OS-C (< -15 °C)** are for samples stored in the freezer for 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 weeks.

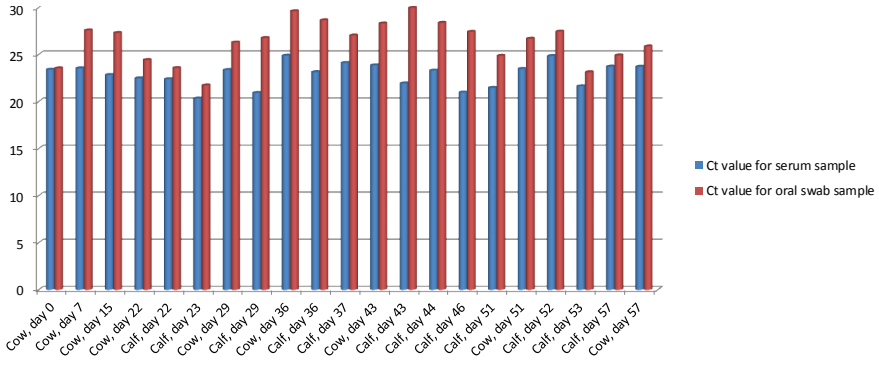

Figure 1: Detection of BVDV RNA in serum and oral cotton swab samples from PI cow and calf during 57 days of study. The results are presented with cycle threshold values (Ct) obtained by commercial real-time method for each day of sampling

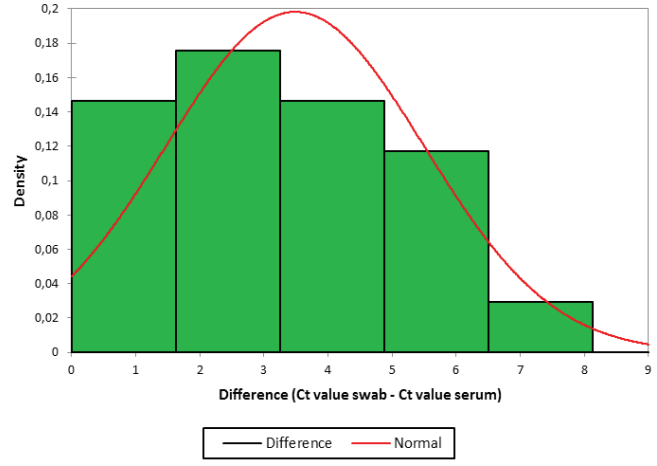

Figure 2: Plot of the difference between the Ct values obtained for 21 swabs and serum samples (average difference of Ct was 3.484)

Additional oral cotton swab and Virocult® swab sampling was used, and samples were stored at three different temperatures to test the stability of viral RNA for 10 days. When comparing the results of gel-based RT-PCR and commercial RT-qPCR, excellent matching between both methods was observed. The degradation of viral RNA in cotton oral swab samples was observed when oral cotton samples were stored at room temperature, and after four days, a negative result was detected in both methods. The degradation of viral RNA was evident through Ct values obtained by the RT-qPCR method (day 0 Ct = 29.85; day 1 Ct = 31.55; day 2 Ct = 34.14; day 3 Ct = 38.26; days 4–10 Ct = > 45). When using Virocult® swab sampling, all samples were detected positive by both methods after storage of the samples 10 days at room temperature. The storage of both types of oral swab samples for 10 days in a refrigerator (+ 4 °C) or in a freezer (< – 15 °C) had no influence on detection of BVDV in samples by RT-PCR methods. According to the results of this experiment, the storage condition of oral swab samples can influence detection of BVDV, but in general, keeping oral swab samples at + 4 °C or in a freezer (< – 15 °C) for 10 days after sampling has no impact on the sensitivity of gel-based RT-PCR and RT-qPCR. Additional testing was done on the stability of oral swab samples stored in the freezer (< – 15 °C) for 10 weeks, with detection of BVDV in all samples (average Ct = 30.96 ± 4.76 and CV 7.68; individual results are presented in Table 2). According to the uniform Ct values (Ct between 31.39 and 34.52, CV=1.69) for internal control RNA by RT-qPCR, no presence of inhibitors was detected in any of the 66 tested oral swab samples.

Discussion

The fluid collected by oral swabs is composed of saliva in the buccal cavity, produced by the salivary gland, and transudate that originates from the circulatory system; thus our results support the idea that oral samples can successfully replace some other types of samples. The general observation of this study is that the results of two molecular methods for the detection of BVDV in oral swab samples, presented equal sensitivity with comparison to results for serum samples. BVDV was detectable in the PI cow and calf for 57 days in all serum and oral swab samples tested by gel-based RT-PCR and commercial RT-qPCR. The results of the two molecular methods are comparable, despite the relatively large bias value, confirming that both molecular methods were suitable for testing oral swab samples. The structure of our study was different from previously published (14) although the data support the observation of Tajima and co-authors. According to detected Ct values in both PI animals during study, it was confirmed that the quantity of viral RNA detected by RT-qPCR in oral swab samples (saliva) was very similar to the quantity detected in serum (Ct values) collected on the same day. As shown in Figure 1, the relative quantities of viral genome variation exist from serum to serum and also from oral swab samples. Our observation in oral swab samples supports the data for detection of BVDV in nasal secretions and ear-notch samples from PI animals during a longer period (2, 5). Variations in Ct value which were observed for oral swab samples compared to serum samples are probably a result of vigorous or mild sampling of saliva in the oral cavity by different operators, but these variations were far from producing false negative results. Although only few data has been published to date regarding the usefulness of oral swab sampling for BVDV detection in PI animals, different data has also been presented for diagnostic sensitivity for other type of samples. A previous study, comparing eight types of samples, including oral swabs, collected from 40 PI animals, showed that oral swab samples were detected as BVDV positive by antigen capture enzyme-linked immunosorbent assay (ACE) in only 8% of samples (17). This observation is contrary to the results of our study and suggests a strong influence of the diagnostic method on the final results. Our study also confirmed, contrary to previous observation, that the viral load in oral fluid is very similar to that in serum. Different types of ACE are widely used in many laboratories to detect viral antigens in either serum or tissue samples, but according to previously observation ACE is not suitable for testing saliva samples. When using ACE, the target molecule is viral antigen, while when using RT-PCR, the target molecule is viral nucleic acid. The influence of the storage of samples on contamination with enzymes and inhibitors may be the reason for the low sensitivity of AEC when testing oral samples. Interestingly, in the same study, the nasal swab samples showed 100% sensitivity with AEC (17). The storage conditions during transport of any type of samples into the

laboratory, not to mention the time of transport, can vary from sample to sample and can influence the results. For this reason, two types of oral swabs were stored three different temperatures, and then oral swab samples were evaluated for 10 days to test their robustness during transport. The results of our study confirmed that storage of oral samples at room temperature (18–24 °C) reduced the sensitivity of molecular methods for BVDV detection from oral cotton swabs after four days, but when using Virocult® tubes with liquid virus transport medium for oral swab sampling, the sensitivity was not affected during the 10 days (Table 1, Table 2). The sensitivity of the two molecular methods was not reduced for samples stored at + 4 °C or at < – 15 °C for at least 10 days. In addition, all 11 samples were detected as positive during 10 weeks of storage at < – 15 °C. These data suggest that oral cotton swabs can be useful for sampling, but for this type of samples, it is recommended that they are stored at + 4 °C during transport. For oral sampling, similar to nasal swabs, tubes with liquid virus transport medium will be recommended, because this medium will also stabilize the viral RNA in samples at room temperature. In addition, when arriving in the laboratory, such samples do not need homogenization, which is practical for skipping the process of dilution of oral samples, thereby not losing the sensitivity because of that process. This would be especially important when the laboratory pools individual samples prior to extraction of RNA. The important finding in this study is that oral swab samples are useful when detection of BVDV is done by gel-based RT-PCR and RT-qPCR.

The current study revealed that oral swab samples have an equal sensitivity for the detection of PI animals compared to the standard serum samples. Oral swab sampling offers an efficient, easy, cost-effective solution for farmers, during an eradication program, to identify PI animals in infected herds. This type of sample could be especially useful for removing PI animals from an infected herd and for searching for PI animals among newborn calves for 9 months after the removal of the last PI animal. Early testing of newborn calves during the following 9 months is crucial for success, because it reduces the possibility that a newborn PI calf is the source of possible new infections (2). If serum sampling is used, this requires a call to a veterinarian for each newborn calf on the farm, but an oral swab sample can be taken and processed by an individual farmer. Oral swab samples can be a good alternative to the widely used ear-notch and serum samples. Of course, high quality identification of individual animals and careful record keeping is, in any case, very important for tracing the data.

The molecular method has been widely accepted in recent years because of its rapid turnaround time, its possibility for quantification and the fact that it enables the testing of a pool of 25–50 individual samples to reduce costs (14, 18). Although laboratory pooling of oral swab samples was not tested in our study, the observations from our study provide evidence for the possibility of the pooling of samples that is similar to pooling for serum or ear-notch testing.

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References

1. Lindenbach BD, Rice CM. Flavivirdae: the viruses and their replication. In: Knipe DM, Howley PM, eds. Fields virology. 4th ed. Philadelphia : Lippincott Williams & Wilkins, 2001: 991-1041.

2. Houe H, Lindberg A, Moennig V. Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. J Vet Diagn Invest 2006; 18: 427-36.

3. Baker JC. The clinical manifestations of bovine viral diarrhea infection. Vet Clin North Am Food Anim Pract 1995; 11: 425-45.

4. Loneragan GH, Thomson DU, Montgomery DL, et al. Prevalence, outcome and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. J Am Vet Med Assoc 2005; 226: 595-601.

5. Fulton RW, Hessman BE, Ridpath JF, et al. Multiple diagnostic tests to identify cattle with bovine viral diarrhea virus and duration of positive test results in persistently infected cattle. Can J Vet Res 2009; 73: 117-24.

6. Kennedy JA, Mortimer RG, Powers B. Reverse transcription-polymerase chain reaction on pooled samples to detect bovine viral diarrhea virus by using fresh ear-notch-sample supernatants. J Vet Diagn Invest 2006; 18: 89-93.

7. Chittick WA, Stensland WR, Prickett JR, et al. Comparison of RNA extraction and real-time reverse transcription polymerase chain reaction methods for the detection of porcine reproductive and respiratory syndrome virus in porcine oral fluid specimens. J Vet Diagn Invest 2011; 23: 248-53.

8. Corstjens PLAM, Abrams WR, Malamud D. Detecting viruses by using salivary diagnostics. J Am Dent Assoc 2012; 143: 12-8.

9. Detmer SE, Patnayak DP, Jiang Y, et al. Detection of influenza A virus in porcine oral fluid samples. J Vet Diagn Invest 2011; 23: 241-77.

10. Matteucci D, Baldinotti F, Mazzetti P, et al. Detection of feline immunodeficiency virus in saliva and plasma by cultivation and polymerase chain reaction. J Clin Microbiol 1993; 31: 494-501.

11. Prickett J, Simer R, Christopher-Hennings J, et al. Detection of porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. J Vet Diagn Invest 2008; 20: 156-63.

12. Romagosa A, Gramer M, Joo HS, et al. Sensitivity of oral fluids for detecting influenza A virus in populations of vaccinated and non-vaccinated pigs. Influenza Other Respir Viruses 2012; 6: 110-8.

13. Stenfeldt C, Lohse L, Belsham GJ. The comparative utility of oral swabs and probang samples for detection of foot-and-mouth disease virus infection in cattle and pigs. Vet Microbiol 2013; 162: 330-7.

14. Tajima M, Oshaki T, Okazawa M, et al. Availability of oral swab sample for the detection of bovine viral diarrhea virus (BVDV) gene from the cattle persistently infected with BVDV. Jpn J Vet Res 2008; 56: 3-8.

15. Barlič Maganja D, Grom J. Highly sensitive one-tube RT-PCR and microplate hybridisation assay for the detection and for the discrimination of classical swine fever virus from other pestiviruses. J Virol Meth 2001; 95: 101-10.

16. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986; 338: 307-10.

17. VanderLey B, Ridpath J, Sweiger S. Comparison of detection of bovine virus diarrhea virus antigen in various types of tissue and fluid samples collected from persistently infected cattle. J Vet Diagn Invest 2011; 23: 84-6.

18. Yan L, Zhang S, Pace L, et al. Combination of reverse transcription real-time polymerase chain reaction and antigen capture enzyme-linked immunosorbent assay for the detection of animals persistently infected with bovine viral diarrhea virus. J Vet Diagn Invest 2011; 23: 16-25.

UPORABNOST DVEH MOLEKULARNIH METOD ZA ODKRIVANJE TRAJNO OKUŽENIH ŽIVALI Z VIRUSOM BOVINE VIRUSNE DIAREJE NA VZORCIH USTNEGA BRISA

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Povzetek: Dva meseca smo pri trajno okuženi kravi z virusom bovine virusne diareje (BVD) in njenem teletu vzporedno odvzemali vzorce serumov in ustnih brisov, da bi testirali uporabnost vzorcev sline za dokazovanje prisotnosti virusa. Nukleinsko kislino virusa smo dokazovali z dvema molekularnima metodama: s klasično RT - PCR z elektroforezo v agaroznem gelu in komercialno metodo RT - PCR v realnem času. Genom virusa BVD smo dokazali v vseh vzorcih serumov in ustnih brisov, odvzetih na 0, 7. 15. 22. 23. 29. 36. 37. 43. 44. 46. 51. 52. 53. in 57. dan od začetka vzorčenja. Pri odvzemu vzorcev brisov s suho bombažno vatenko in po tridnevnem hranjenju vzorca na sobni temperaturi (+ 21 °C) smo ugotovili zmanjšanje diagnostične občutljivosti. Ko pa smo vzorce ustnih brisov hranili do 10 dni pri + 4 °C ali v zamrzovalniku na manj kot minus 15 °C, pa takšno hranjenje ni imelo negativnega vpliva na dokazovanje virusa. Znižanja diagnostične občutljivosti pri brisih pa nismo ugotovili, ko smo za odvzem vzorcev uporabili komplet komercialnega brisa, ki vsebuje transportno gojišče. Ustni bris omogoča enostavno, zanesljivo, učinkovito in cenejše vzorčenje pri identifikaciji trajno okuženih živali in zanesljivo diagnostiko, skupaj z uporabo metode RT - PCR. Uporaba vzorcev ustnih brisov bi lahko bila še posebej priročna pri pregledu novorojenih telet in odstranjevanju izločevalcev virusa iz govejih čred, ki so okužene z virusom BVD.

Ključne besede: virus bovine virusne diareje; diagnostika; ustni bris; RT-PCR, RT-PCR v realnem času