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VETERINARY MICROBIOLOGY - SHORT COMMUNICATION

Frequency of bovine viral diarrhea virus (BVDV) in Argentinean bovine herds and comparison of diagnostic tests for BVDV detection in bovine serum samples: a preliminary study

Maximiliano J. Spetter[1,2] · Enrique L. Louge Uriarte[2] · Joaquín I. Armendano[3] · Ignacio Álvarez[4] · Natalia S. Norero[5] · Leonardo Storani[1,5] · Susana B. Pereyra[2] · Andrea E. Verna[1,2] · Anselmo C. Odeón[2] · Erika A. González Altamiranda[1,2]

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Abstract

Bovine viral diarrhea (BVD) is a major worldwide disease with negative economic impact on cattle production. Successful control programs of BVD require the identification and culling of persistently infected (PI) animals with bovine viral diarrhea virus (BVDV). A variety of diagnostic tests are available to detect BVDV, but no comparison has been performed among those tests in Argentina. Sera collected from 2864 cattle, belonging to 55 herds from three Argentinean provinces, were analyzed by nested RT-PCR (RT-nPCR) to detect BVDV for diagnostic purposes. Additionally, this study evaluated the agreement of the RT-nPCR along with virus isolation, antigen-capture ELISA, and real-time RT-PCR for BVDV detection in archived bovine serum samples ($n = 90$). The RT-nPCR was useful for BVDV detection in pooled and individual serum samples. BVDV was detected in 1% (29/2864) of the cattle and in 20% (11/55) of the herds. The proportion of BVDV-positive sera was not statistically different among the tests. In addition, comparisons showed high agreement levels, with the highest values between both RT-PCR protocols. The frequency of BVDV infection at individual and herd level was lower than the reported values worldwide. Since follow-up testing was not performed, the frequency of PI cattle was unknown. Also, this study demonstrated that the four diagnostic tests can be used reliably for BVDV identification in individual serum samples. Further epidemiologically designed studies that address prevalence, risk factors, and economic impact of BVDV in Argentina will be necessary to implement effective control programs.

Keywords *Pestivirus* · Argentina · Diagnosis · Pooled samples · Bovine sera

Maximiliano J. Spetter, Enrique L. Louge Uriarte and Erika A. González Altamiranda contributed equally to this work.

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✉ Enrique L. Louge Uriarte
lougeuriarte.enrique@inta.gob.ar

✉ Erika A. González Altamiranda
galtamiranda.erika@inta.gob.ar

[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290, C1033AAJ Buenos Aires, Argentina

[2] Laboratorio de Virología Veterinaria, Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible INTA CONICET, Ruta 226 km 73,5, CP 7620 Balcarce, Argentina

[3] Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Paraje Arroyo Seco s/n, CP 7000 Tandil, Argentina

[4] Laboratorio Álvarez, 25 de Mayo 139, CP 8000 Bahía Blanca, Argentina

[5] Laboratorio de Agrobiotecnología, Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible INTA CONICET, Ruta 226 km 73,5, CP 7620 Balcarce, Argentina

Introduction

Bovine viral diarrhea virus (BVDV) is a member of the genus *Pestivirus* and family *Flaviviridae* [1]. According to the genetic and antigenic features, BVDV has been classified into two main species, BVDV-1 and BVDV-2 [2], and a putative third species named BVDV-3 or Hobi-like [3]. Recently, these species were renamed as *Pestivirus A*, *B*, and *H*, respectively [4]. Independently of the species, BVDV exists as two different biotypes, non-cytopathic (ncp) and cytopathic [2]. Bovine viral diarrhea (BVD) is a major worldwide disease with negative economic impact as consequence of direct losses by reduction of productive performance as well as indirect losses through the control or eradication programs [5]. Thus, it has been added on the World Organisation for Animal Health (OIE) list of notifiable diseases [6].

Infections with BVDV result in a broad range of clinical outcomes [7]. A relevant aspect is the infection of the pregnant cow with the ncp biotype between 18 and 125 days of gestation, which may result in a persistently infected (PI) newborn calf [7]. PI animals play a key role in the transmission and maintenance of BVDV in cattle population because they excrete large amounts of the virus during their lifelong [6]. In contrast, transiently infected (TI) animals excrete the virus during a short period of time. Therefore, one of the main aspects of control programs is the identification and removal of PI animals along with vaccination and adoption of biosecurity measures [6]. Through these statements, several countries are leading successful BVD control or eradication programs at national, regional, or herd level [8]. In this regard, a recent global meta-analysis has shown a decreased of the overall prevalence of BVDV between 1980 and 2016 [8].

Laboratory diagnostic methods conducted to identify BVDV infections involve two main groups, those performed to identify the virus (antigen, nucleic acid, or the infecting virus itself) and those performed to identify viral-specific antibodies [9]. Virus isolation (VI) is considered the gold standard for BVDV identification [9, 10], but it is a time-consuming and expensive technique. Thus, it has been gradually replaced by antigen and nucleic acid detection methods in control programs [6]. The diagnostic strategies conducted to detect PI animals at large scales can be summarized by three test formats: (1) immunohistochemistry or antigen-capture ELISA (AgELISA) on individual ear notch samples; (2) AgELISA or RT-PCR on blood, buffy coat cells, serum, skin, or individual milk samples; (3) RT-PCR on pooled samples such as blood, buffy coat cells, serum, milk, or ear notch [9].

In Argentina, BVDV is endemic [11] and currently the control of the disease is based on the use of inactivated vaccines. Although several studies showed a negative impact of BVDV on reproduction, production, and economy of beef farms [12–14], the prevalence data of BVDV in Argentinean cattle population is still scarce. Moreover, the diagnostic performance of available tests for BVDV detection has not been evaluated. Based on these facts, the first aim of this study was to assess the frequency of BVDV infection both during voluntary BVD control programs or suspected clinical cases in cattle herds from important livestock regions of Argentina. The second aim was to compare different diagnostic tests for BVDV detection in archived bovine serum samples.

Material and methods

BVDV diagnosis in voluntary control programs and suspected clinical cases

Serum samples

To study the frequency of BVDV under laboratory diagnostic conditions, a total of 2864 bovine serum samples received at the Specialized Veterinary Diagnostic Service (SVDS; INTA Balcarce, Argentina) were evaluated. Sera were sampled from beef ($n = 51$) and dairy ($n = 4$) herds between November 2015 and July 2019. The samples came from herds enrolled in a voluntary BVD control program and from herds with clinical cases in which BVDV was the presumptive diagnosis. The herds were located in Buenos Aires ($n = 53$), Santa Fe ($n = 2$), and Córdoba ($n = 1$) province (Online Resource).

In Argentina, systematic or national/regional control programs are currently not implemented. The voluntary BVD program described in the present study was based on a non-systematic or herd to herd control approach [15]. The presence of BVDV-like clinical manifestations or requests from veterinarians triggered the diagnostic evaluation. Because of economic limitations, testing strategies were to prioritize the sampling of risk categories or certain groups of animals such as young cattle, bulls from artificial insemination centers, or animals introduced from other herds. Unfortunately, in most cases, the number of total animals in each herd was unknown, and the totality of animals was not sampled in any of the evaluated herds.

Equal volumes of individual serum samples (100 μl) were pooled in groups of 5 or 10 and tested for the presence of BVDV genome by a nested RT-PCR (RT-nPCR) assay described below. Individual samples from positive pools were then tested by the same RT-nPCR protocol. In clinical cases suspicious of BVDV, sera were tested individually without prior pooling. No second sample was collected in any positive case; therefore, it was not possible to distinguish between PI or TI animals.

Nested RT-PCR test for BVDV detection in serum samples

A RT-nPCR assay [16] with minor modifications [17] was performed to detect the NS5B gene of BVDV in 2864 serum

samples from voluntary control programs and suspected clinical cases. Amplification products of 360 and 604 bp were expected for BVDV-1 (*Pestivirus A*) and BVDV-2 (*Pestivirus B*), respectively. This technique has been implemented routinely in the authors' laboratory since 2015.

The detection limit of the RT-nPCR was determined with serial 10-fold dilutions of serum from a PI bovine ($10^{3.57}$ tissue culture infective doses [$TCID_{50}$]/ml) in BVDV-negative ovine serum. Also, the detection limit in pooled serum samples was determined by mixing equal volumes of the PI serum in 4, 9, 19, 49, and 99 BVDV-negative bovine sera to create pools of 5, 10, 20, 50, and 100 samples, respectively. The ovine and bovine sera tested negative for BVDV by RT-PCR using the pan-*pestivirus* primers 324–326, which target a fragment of the 5'UTR region [18], and by the RT-nPCR evaluated in the present study. The BVDV-negative bovine sera were obtained from a beef herd confirmed as BVDV-free. The detection limit, both in 10-fold dilutions and pools, was determined by performing RNA extraction and RT-nPCR in triplicate on the same day.

Comparison of diagnostic methods

Serum samples

In order to compare different diagnostic tests for BVDV detection in bovine serum samples, 90 sera (stored at − 20 °C) were used. Animals older than 6 months were selected to avoid potential interference of colostral antibodies with VI and AgELISA tests. The sera were submitted for BVDV diagnosis between 2009 and 2018 to the SVDS at INTA Balcarce, Argentina. All the samples were subjected to the diagnostic tests detailed below.

Virus isolation

Isolation of BVDV was attempted from sera according to standard procedures [14]. Briefly, 50 μl of serum was inoculated in triplicate onto confluent monolayer of MDBK cells. After four blind passages, the inoculated cell cultures were evaluated for BVDV antigen by a direct fluorescent antibody test, using a commercially available polyclonal antibody (VMRD, USA). VI was performed at the time the serum samples were collected, between 2009 and 2018.

Antigen-capture ELISA

A commercial AgELISA (INGEZIM BVD DAS, Ingenasa, Spain) was used to detect the non-structural protein p80/p125 (NS2/3) of BVDV. The assay was performed using 100 μl of serum and following the manufacturer's instructions. Optical density values were measured using a plate reader at 450 nm and the cut off value was calculated (OD of positive control × 0.1) according to the manufacturer protocol. The AgELISA method was performed during 2019.

RNA extraction

Total RNA was extracted from 100 μl of serum sample with TRIzol® reagent (Invitrogen, USA) following the manufacturer's instructions. RNA obtained was resuspended in 40 μl of RNase-free water and stored at − 80 °C. The total RNA extractions were performed during 2018 and 2019, along with the RT-nPCR assay.

Nested RT-PCR and real-time RT-PCR assays

The same RT-nPCR assay described in the voluntary control programs and in suspected clinical cases was performed during 2018 and 2019.

For real-time RT-PCR (RT-qPCR), reverse transcription was carried out using pan-*pestivirus* primers 324/326 [18], which target a fragment of the 5'UTR region. For complementary DNA (cDNA) synthesis, 4 μl of RNA sample, 0.3 μl of each primer (20 μM), and 0.4 μl of DMSO were denatured at 95 °C for 5 min. Thereafter, 2 μl of RT buffer (Promega, USA), 1 μl of dNTPs mix (10 mM each; Promega, USA), 0.12 μl (24 U) of M-MLV (Promega, USA), and 0.88 μl of RNase-free water were incubated at 37 °C for 60 min and 70 °C for 5 min. The qPCR reactions were performed as described previously [19]. Amplification and detection of the specific product (160 bp) was carried out in duplicate on an ABI 7500 cycler (Applied Biosystems, CA, USA). After amplification, melting curve analysis was performed. Samples that gave both a typical amplification curve and a melting temperature value between 80 and 82.5 °C were considered positive. Quantification cycle (Cq) values were also recorded. The RT-qPCR was performed during 2019.

Data analysis

The proportion of positive cases for each diagnostic test was analyzed by the Q Cochran and Mc Nemar test (Proc FREQ, SAS Studio v3.6, SAS Institute Inc. Cary, NC, USA). Ninety-five percent confidence intervals ($CI_{95\%}$) were calculated using the likelihood ratio method. A p value < 0.05 was considered significant.

Agreement among diagnostic tests was evaluated according to Cicchetti and Feinstein [20] and Gwet's Agreement Coefficient [21] (AC1 coefficient; "rel" package v1.3.3, R v3.5.1, R Core Team 2018, Vienna, Austria). Ninety-five percent confidence intervals ($CI_{95\%}$) were calculated by using the likelihood ratio method. Interpretation of the AC1 coefficient was performed according to McHugh [22].

Results

BVDV in voluntary control programs and suspected clinical cases

The detection limit of the RT-nPCR assay was $10^{0.57}$ $TCID_{50}$/ml in serial 10-fold dilutions of the PI serum. This technique detected BVDV in all the pool sizes evaluated, even up to one positive animal in a pool of 100 serum samples.

Bovine viral diarrhea virus was detected in 29 out of 2864 (1%) tested bovine sera, whereas 11 out of 55 (20%) herds presented at least one BVDV-positive animal (Table 1). Of the 29 positive sera, 13 were from 3-month-old calves, 13 from 6 to 18-month-old calves, and the remaining 3 sera were from cows. All the positive samples were typed as BVDV-1 (amplification product of 360 bp).

Of the 13 herds with clinicopathological findings related to BVD, 6 (46%) were positive for BVDV detection. On the other hand, 3 out of 11 (27%) herds without clinicopathological findings of BVD were positive. No information regarding clinical sings was specified in the remaining 31 herds, two BVDV-positive herds and 29 BVDV-negative herds (Online Resource).

Based on the high detection limit and the encouraging performance of the RT-nPCR for diagnostic purposes, this technique was compared along with other methods for BVDV detection.

Comparison of diagnostic methods

Of the 90 tested sera, 30 were positive by one or more of the diagnostic methods. Positive sera were comprised of 19 PI animals, 9 animals with unknown clinical condition because they were not retested to confirm TI or PI status, and 2 necropsied animals with clinicopathological findings compatible with BVD (Table 2).

The proportions of BVDV-positive samples detected by VI ($n = 23$; 25.6%, $CI_{95\%} = 17.3–35.1$), AgELISA ($n = 28$; 31.1%, $CI_{95\%} = 22.1–42.1$), RT-nPCR ($n = 26$; 28.9%, $CI_{95\%} = 20.2–38.7$), and RT-qPCR ($n = 27$; 30.0%, $CI_{95\%} = 21.1–39.9$) were not statistically different ($p = 0.09$). Discordant results between AgELISA and the RT-PCR assays were observed. Three samples positive by AgELISA were negative by the two RT-PCR methods, whereas two samples negative by AgELISA were positive by the two RT-PCR methods (Table 2).

When the diagnostic methods were compared, high percentage of positive agreement ($\%_{pos}$) and negative agreement ($\%_{neg}$) along with moderate or strong to almost perfect level of AC1 coefficient were observed (Table 3). Values below 90% were recorded for $\%_{pos}$ and AC1 in the comparisons of VI vs. AgELISA, VI vs. RT-qPCR, and AgELISA vs. RT-nPCR (Table 3).

Discussion

To assess the frequency of BVDV detection in the main livestock production regions in Argentina, an optimized RT-nPCR in the authors' laboratory was used. The detection limit of the RT-nPCR in serum samples ($10^{0.57}$ $TCID_{50}$/ml) was similar to those reported by Gilbert et al. [16]. This detection limit is enough to detect PI animals, which usually have a viral titer ranging from $10^{1.6}$ to 10^{7} $TCID_{50}$/ml [23]. The high analytical sensitivity of the RT-PCR methods has allowed pooling of samples for PI animals screening with the aim to reduce costs and time lab consuming [6, 9]. In this study, the RT-nPCR was able to detect a single BVDV-positive serum in pools of up to 100 samples. Despite this high sensitivity, pool size would depend on several factors such as the prevalence of the disease, herd size, and age of the animals [24].

The optimized RT-nPCR was used to detect BVDV in pooled and individual serum samples in Argentinean bovine herds. The herds were located in Buenos Aires, Santa Fe, and Córdoba provinces, which represent a high proportion (35.5%, 11.3%, and 8.9% respectively) of the national cattle stock [25]. Previous reports have described BVDV outbreaks in Argentina [12–14], but no studies have estimated the frequency of BVDV in a representative number of cattle herds. Although the current investigation was not conceived as an epidemiological study, the obtained results are a relevant contribution to the limited knowledge of BVDV prevalence in Argentina. Based on the results found herein, the rate of BVDV viremic animals is around 1%. Worldwide, the prevalence ranges from 3.1 to 5.82% for viremic animals and from 0.59 to 0.97% for PI ones [8].

In both virus infections conditions, PI and TI, animals are viremic; therefore, it is necessary to do a second test 3–

Table 1 Pooled and individual bovine serum samples positive to BVDV detection by nested RT-PCR in Argentinean cattle herds

	Sera tested	Pools tested	Positive pools	Positive sera	Herds tested	Positive herds
Pooled samples	2791	412	10	16	22	2
Individual samples	73	NA	NA	13	33	9
Total	2864	412	10 (2.4%)	29 (1%)	55	11 (20%)

Nested RT-PCR (Gilbert et al., 1999) [16]. *NA*, not applicable

Table 2 Summary of results from 30 bovine serum samples positive for BVDV by virus isolation, antigen-capture ELISA, nested RT-PCR, and real-time RT-PCR

Serum	Diagnostic tests							Clinical status
	VI[a]		AgELISA[b]	RT-nPCR[c]		RT-qPCR[d]		
	Result	Biotype		Result	Species	Result	Cq	
1	+	ncp	+	+	1	+	26.9	PI
2	+	ncp	+	+	1	+	25.3	PI
3	+	ncp	+	+	1	+	29.6	PI
4	+	ncp	+	+	1	+	27.5	PI
5	+	ncp	+	+	1	+	26.4	PI
6	+	ncp	+	+	1	+	31.8	necropsy[e]
7	+	ncp	+	+	1	+	26.7	PI
8	+	ncp	+	+	1	+	26.4	PI
9	+	ncp	+	+	1	+	28.8	PI
10	+	ncp	+	+	1	+	26.1	PI
11	+	ncp	+	+	1	+	23.4	unknown[f]
12	+	ncp	+	+	1	+	21.4	unknown
13	+	ncp	+	+	1	+	24.2	necropsy
14	+	ncp	+	+	1	+	25.9	PI
15	+	ncp	+	+	1	+	26.4	PI
16	+	ncp	+	+	1	+	27.7	PI
17	+	ncp	+	+	1	+	24.6	PI
18	+	ncp	+	+	1	+	29.6	PI
19	+	ncp	+	+	1	+	29.3	PI
20	+	ncp	+	+	1	+	27.9	PI
21	+	ncp	+	+	1	+	29.1	PI
22	+	ncp	+	+	1	+	23.2	PI
23	+	ncp	–	+	1	+	25.7	unknown
24	-	NA	+	+	1	+	24.0	unknown
25	-	NA	–	+	1	+	33.4	unknown
26	-	NA	+	+	1	+	25.9	PI
27	-	NA	+	–	NA	–	NA	unknown
28	-	NA	+	–	NA	–	NA	unknown
29	-	NA	+	–	NA	–	NA	unknown
30	-	NA	+	–	NA	+	34.6	unknown

[a] Virus isolation (Odeón et al., 2003) [14]

[b] Antigen-capture ELISA, INGEZIM BVD DAS, Ingenasa, Spain

[c] Nested RT-PCR (Gilbert et al., 1999) [16]

[d] Real-time RT-PCR (Mari et al., 2016) [19]

[e] Animal with clinicopathological findings compatible with bovine viral diarrhea-mucosal disease

[f] Not retested to confirm PI status

ncp, non-cytopathic; *Cq*, quantification cycle value; *PI*, persistent infected animal; *NA*, not applicable

4 weeks later to confirm the status of the infection [26]. Since in the current study follow-up testing was not performed in any of the positive cases, it was not possible to determine the frequency of PI cattle. Resample was not possible because some farmers were opposed to taking the risk of keeping a potential PI in the herd until the second sampling; consequently, the animals were culled after the first positive result. This situation is not unusual, and it has also been described previously [27]. Moreover, the proposal to perform the second test on positive cattle for free of charge was not successful in motivating farmers and veterinarians. Because the period in which BVDV can be detected is comparatively short in TI animals, it is probable that most of the BVDV-positive sera in the present study were from PI bovine [10, 24]. In this

Table 3 Agreement among virus isolation, antigen-capture ELISA, nested RT-PCR, and real-time RT-PCR for BVDV detection in 90 bovine serum samples

Diagnostic tests	Percentage agreement[e]		Gwet's Agreement Coefficient[f]		
	Negative	Positive	AC1	$CI_{95\%}$	Level of agreement[g]
VI[a] vs. AgELISA[b]	94.6	86.3	0.87	0.77–0.98	Moderate to almost perfect
VI vs. RT-nPCR[c]	97.7	93.9	0.94	0.88–1.00	Strong to almost perfect
VI vs. RT-qPCR[d]	96.2	89.8	0.93	0.85–1.00	Strong to almost perfect
AgELISA vs. RT-nPCR	95.2	88.9	0.88	0.79–0.98	Moderate to almost perfect
AgELISA vs. RT-qPCR	96.0	90.9	0.90	0.82–0.99	Strong to almost perfect
RT-nPCR vs. RT-qPCR	99.2	98.1	0.98	0.94–1.00	Strong to almost perfect

[a] Virus isolation (Odeón et al., 2003) [14]

[b] Antigen-capture ELISA, INGEZIM BVD DAS, Ingenasa, Spain

[c] Nested RT-PCR (Gilbert et al., 1999) [16]

[d] Real-time RT-PCR (Mari et al., 2016) [19]

[e] Estimated according to Cicchetti and Feinstein (1990) [20]

[f] Estimated according to Gwet (2008) [21]

[g] Interpretation according to McHugh (2012) [22]

regard, studies have reported that 63% to 92% of the positive cattle were confirmed as PI [28, 29], and 90% of the BVDV-positive pools contained at least one PI animal [26].

As expected, in the current study, the detection of BVDV was higher in younger cattle. Identification of PI cattle occurs less frequently at adult age because the lifetime of those animals is usually limited [30]. However, three BVDV infected cows (10%; 3/29) were identified, which was in accordance with a previous report [30]. Thus, it is important to test both young and old cattle in BVDV control programs.

The proportion of BVDV-positive herds (20%) in the current study was consistent with the herd prevalence estimated in a recent global meta-analysis [8]. Also, BVDV was detected in approximately 50% of the herd suspicious of BVDV infection. This finding is supported by the analysis of Scharnböck et al. [8], who have reported a higher prevalence of viremic (49.2%) and PI (44.5%) cattle in herds with compatible clinical signs in contrast to those with no clinical signs (viremic: 8.9%; PI: 29.3%). According to the results of the present study, most of the herds had no BVDV-positive animals. However, when a BVDV infection is identified, more than one positive cattle are usually present in the herd. The identification of several PI cattle in a single herd has been previously reported [13, 28, 29]. Since in the current study whole herds were not sampled, it is likely that all infected cattle were not detected. Moreover, it is possible that in some cases cattle have died because of clinical BVD or mucosal disease before sampling. Consequently, the prevalence of viremic cattle reported herein may be underestimated.

All the strains identified were typed as BVDV-1 according to the primers designed by Gilbert et al. [16]. Previous studies described that around 90% of the strains in Argentina were BVDV-1 [31–34]. Although type 2 was not identified in the tested samples, the primers used here were able to detect this BVDV type in Argentinean field isolates [34]. In this regard, false-negative results for BVDV-2 identification are not expected.

Several diagnostic methods are available for BVDV detection, mostly used to identify PI animals [6, 10]. The performance of those methods has been well documented in the bibliography. However, in Argentina, such data are not yet available. Thus, the current study was also aimed to compare the RT-nPCR along with different diagnostic tests available in Argentina for BVDV detection in bovine serum samples.

It is worth noting that VI was performed at the moment of the reception of the serum for diagnosis (between 2009 and 2018), while the other tests were performed some time latter (between 2018 and 2019). Therefore, viral RNA and/or antigen degradation or the presence of inhibitors in the samples cannot be excluded; consequently, lower performance of the AgELISA or the two RT-PCR assays compared to VI might be expected. However, the results of the present study showed that these three methods gave positive results in more samples than VI, although no statistical differences were observed. Moreover, these findings imply that BVDV antigen and RNA may remain stable for at least 9 years in serum samples conserved at − 20 °C.

In the present study, $\%_{pos}$ and $\%_{neg}$ was calculated among tests. The high observed values of $\%_{neg}$ mean that the specificity of the tests is comparable, thus presenting the same ability to identify BVDV-negative serum samples [20]. High values of $\%_{pos}$ were also observed, but slightly lower than $\%_{neg}$, probably attributed to the lower number of positive samples detected by VI. However, the proportion of positive sera identified by VI was higher than expected [35]. This observation may be explained by the fact that this method

was performed at the time of samples reception. Also, several of the serum samples were from PI animals (Table 2), which may present viral titers 10^2–10^3 $TCID_{50}$/ml higher than in serum from TI animals [36].

The $\%_{pos}$ values around 90%, which were observed in the comparisons of AgELISA and the two RT-PCR, are related to the discrepant results between them. Those discordant results may be attributed to the different analytes that are detected by each method and their persistence under long-term storage conditions. Since the sera were from animals older than 6 months, interference by BVDV specific colostral antibodies in the two AgELISA-negative samples is not expected. Moreover, one of the two serum samples was positive by VI. On the other hand, the comparison between the two RT-PCR assays showed the higher agreement levels. This result demonstrated that these molecular-based methods, targeting two different conserved regions of the BVDV genome (5'UTR and NS5B), were able to detect the virus with the same performance. Based on this, the RT-qPCR may be useful for BVDV detection in pooled samples as previously reported [24, 27].

Despite the aspects discussed above, the high AC1 values demonstrated that any of the evaluated methods can be selected for BVDV detection in individual serum samples. Since each test has advantages, disadvantages, and applicability according to the diagnostic situation, some considerations should be made when selecting one of these techniques [35]. For instance, colostrum-derived antibodies in serum samples may produce false-negative results when using VI or AgELISA, whereas RT-PCR performance is not affected by this interference [9]. In the current study, the animals were older than 6 months, thus interference by colostral antibodies may be not expected. Another consideration is that diagnostic methods in this study were compared on individual serum, but no comparison was performed on pooled samples. RT-PCR tests are considered more suitable than AgELISA for identification of BVDV in pooled samples [10, 27].

Programs aimed at controlling or eradicating BVDV should be designed based on the epidemiologic conditions of the geographical region. When screening for BVDV in Argentinean herds, a testing strategy may be addressed as follows; first, define the risk categories from the herd; second, test the selected animals by RT-nPCR or RT-qPCR using pooled samples, and finally test individual serum from positive pools either by VI, AgELISA, or RT-PCR assays. It is important to highlight that in the present study the diagnostic tests were compared using local BVDV isolates. Variation in the prevalence of BVDV species/subtypes by geographic regions should be taken into consideration in the design or implementation of diagnostic methods [2].

In conclusion, the RT-nPCR showed high analytical sensitivity when used either on individual and pooled serum samples and proved to be a useful tool for BVDV detection under the epidemiologic conditions of Argentina. The frequency of BVDV at individual and herd level was lower than that reported worldwide between 1980 and 2016. Unfortunately, better estimates of BVDV prevalence in Argentina are not available and additional studies are necessary in this regard, as well as on risk factors and economic impact of the disease. Regarding diagnostic tests comparison, the results of this study demonstrated that the four tests assayed have a high agreement for BVDV detection in individual bovine serum samples, particularly between the two RT-PCR protocols. Therefore, the choice of a diagnostic test will depend on several criteria such as animal age, clinical presentation, sample size, cost, simplicity, and laboratory infrastructure.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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