Development of a Competitive Chemiluminescence Immunoassay Using a Monoclonal Antibody Recognizing 3B of Foot-and-mouth Disease Virus for Differentiating Infected From Vaccinated Animals

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Methodology

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Abstract

Background: Foot-and-mouth disease (FMD) is a devastating animal disease. Differentiation of infected from vaccinated animals (DIVA) is very important for confirming suspected cases, evaluating the prevalence of infection, certifying animals for trade and controlling the disease.

Methods: In this study, a competitive chemiluminescence immunoassay (3B-cCLIA) for DIVA was developed for the rapid detection of antibodies against non-structural proteins (NSPs) in different species of livestock animals using monoclonal antibody 9E2 as a competitive antibody that recognizes NSP 3B.

Results: The cut-off (50%), diagnostic sensitivity (97.20%, 95.71%, and 96.15%) and diagnostic specificity (99.51%, 99.43%, and 98.36) of the assay were estimated by testing a panel of known background sera from swine, cattle and sheep. The accuracy rate of 3B-cCLIA was further validated followed by its comparison with two commercial diagnostic kits. The early diagnostic performance showed that antibodies to NSPs occurred later (about 1–2 days) than antibodies to structural proteins. Furthermore, NSP antibodies present in animals vaccinated multiple times (false-positive), especially in cattle and sheep, were confirmed, and the false-positive rate increased with the number of vaccinations.

Conclusions: These results indicated that 3B-cCLIA is suitable to rapidly detect antibodies against FMDV NSP 3B in a wide range of species for DIVA.

Background

Foot-and-mouth disease (FMD) is a highly contagious and economically damaging viral disease that affects cloven-hoofed animals. FMD virus (FMDV) possesses a positive-sense, and single-stranded RNA that codes for four structural proteins (SPs: VP4, VP2, VP3 and VP1) and ten non-structural proteins (NSPs: L, 2A, 2B, 2C, 3A, 3B, 3C, 3D, 3AB and 3ABC) [1–3]. The FMDV exists in form of seven serotypes (A, O, C, Asia 1, SAT 1, SAT 2, and SAT 3) and serotype O and A are currently prevalent in China [4, 5].

To date, slaughtering infected and contacted animals together with prohibiting the import of animal and animal products from FMD endemic countries have been adopted to prevent the disease in FMD-free nations. Considering the economic costs, a vaccination policy was adopted for control and eradication of the disease in endemic countries [6, 7]. However, vaccination with inactivated vaccines raises other issues, such as the differentiation of FMDV-infected from vaccinated animals (DIVA) and the production of carrier animals to shed the virus [6, 7]. Therefore, detecting antibodies against NSPs has been one of the widely preferred and applied diagnostic methods for DIVA and helps in identifying the subclinical infections, evaluate the prevalence of infection and control the disease [8] because a series of purifying techniques remove the majority of NSPs from the inactivated vaccine during production [3, 9].

There are two main diagnostic types of detecting antibodies against NSPs for DIVA. One is indirect ELISA based on recombinant NSPs, peptides or epitopes [1, 8, 10–15]. Species-specific conjugates need be used in indirect ELISA, which make simultaneous examination of sera from different species difficult [16]. Another is block or competitive ELISA using polyclonal antibodies or monoclonal antibodies (mAbs) [6, 9, 16–20]. Compared with indirect ELISA, block or competitive ELISA can be used to detect each species that is susceptible to FMD. Most commercial ELISAs are currently block ELISA, such as 3ABC monoclonal antibody blocking ELISA (3ABC-bELISA, LVRI, China) and PrioCHECK FMDV NSP ELISA.

In previous study, the mAb 9E2 recognizing NSP 3B that can be used to develop the diagnostic method for DIVA has been preliminary verified. However, mAb 2G5 recognizing NSP 3A is not suitable for use alone in establishing a diagnostic method because of the mutation and deletion of the epitope identified by mAb 2G5 [3]. In the study, we used mAb 9E2 as the detection antibody to develop a competitive chemiluminescence immunoassay (termed 3B-cCLIA) for the rapid detection of NSP antibodies. To decrease the false positives in cattle and sheep vaccinated multiple times, we attempted to use two mAbs recognizing NSP 3A and 3B to develop 3A + 3B-cCLIA.

Materials And Methods

Serum samples

Serum samples from naïve animals: Serum samples from clinically healthy and unvaccinated animals, including 310, 175, and 61 samples from swine, cattle, and sheep, respectively, were collected and tested using liquid-phase blocking ELISA of FMDV O (O-LPBE) and A-LPBE (presented negative results; titer, < 1:4). The diagnostic specificity (Dsp) and cut-off value were calculated using these serum samples (Table S1).

Serum samples from infected animals: Collection of 107 serum samples was carried out from swine infected with FMDV A/GDMM/2013 or O/Mya98 at 7–25 days post infection (dpi) in the Animal Biological Safety Level 3 (ABSL-3) Laboratory at Lanzhou Veterinary Research Institute (Lanzhou, China); 70 serum samples were collected from cattle infected with FMDV (A/GDMM/2013 or O/Mya98) at 8–20 dpi; 52 serum samples were collected from sheep with clinical symptoms in the field and tested as NSP-positive using two commercial diagnostic kits (3ABC-bELISA and PrioCHECK FMDV NSP ELISA). The diagnostic sensitivity (Dsn) and cut-off value were calculated using these samples. In addition, a total of 32 sera collected from four unvaccinated control swine experimentally challenged with FMDV O/Mya98 at 0 dpi and 2–8 dpi were used for comparing the early diagnostic performance of 3B-cCLIA and two commercial diagnostic kits and identify seroconversion to FMDV SP and NSP (Table S1).

Serum samples from vaccinated animals: One hundred serum samples were collected at 21 days post vaccination (dpv) from swine vaccinated with FMDV O univalent multiple-epitope recombinant vaccine developed by our research group. The Dsp and cut-off value were calculated using these samples. In addition, a collection of 120 serum samples was carried out from sows vaccinated 3–15 times with commercial O/A divalent inactivated vaccines. Similarly, a collection of 129 serum samples was carried out from dairy cows vaccinated with commercial O/A divalent inactivated vaccines every four months for a total of 2, 5 or 10 vaccinations. Similarly, collection of seventy-seven serum samples was carried out from 15 sheep vaccinated 1–3 times with laboratory-made FMDV A/AF72, O/Mya98/BY/2010, or Asia 1/JS05 univalent inactivated vaccine. These samples were employed for evaluating the diagnostic performances of 3B-cCLIA and two commercial diagnostic kits and verified the false-positive phenomenon with animals vaccinated multiple times (Table S1).

Serum samples collected in the field: 173 field sera collected from swine suspected of FMDV infection during 2010–2018. These sera were used for comparing the coincidence rates between 3B-cCLIA and two commercial diagnostic kits (Table S1).

Serum samples from other virus-infected swine: In this study, one serum sample from classical swine fever virus (CSFV)-infected swine, one from senecavirus A (SVA)-infected swine, one from porcine parvovirus (PPV)-infected swine, one from porcine reproductive and respiratory syndrome virus (PRRSV)-infected swine, and two from porcine circovirus type 2 (PCV2)-infected swine were investigated (Table S1).

Control sera: The serum sample derived from swine infected with FMDV O/Mya98 at 25 dpi served as the standard positive serum (P51). The percentage inhibition (PI) of the serum in the 3ABC-bELISA and PrioCHECK FMDV NSP ELISA was 92% and 93%, respectively. The standard negative serum sample (P734) was taken from clinically healthy swine who had not been immunized against FMD. The serum was tested with O-LPBE and A-LPBE (produced negative result; titer, < 1:4), 3ABC-bELISA (produced negative result; PI = 1%), and PrioCHECK FMDV NSP ELISA (produced negative result; PI = -7%).

Antigen and antibodies

The 3ABC coding region of FMDV A/GDMM/2013 mutated at positions 46 aa and 163 aa was cloned into the pProEXHTB plasmid. The expression and purification of the recombinant 3ABC protein has been described [13].

The mAbs against the 3ABC protein, designated 2G5 and 9E2, were obtained at our laboratory, and their minimally identified epitopes are “⁹²EYIEKA⁹⁷”, which is located in 3A, and “²³EGPYAGPLE³¹”, which is located in 3B [3]. MAbs 2G5 and 9E2 were largely produced by injecting hybridomas into the peritoneal cavities of BALB/c mice and purified by affinity protein G column chromatography. Then, the mAbs 2G5 and 9E2 were conjugated with horseradish peroxidase (HRP). The polyclonal antibody was obtained by inoculating rabbits with the purified 3ABC protein.

Development of competitive CLIA using mAb against NSP 3B

Checkerboard titration was used to optimize the conditions of 3B-cCLIA. The coating of mAb 2G5 onto 96-well white plates (Costar) was carried out at 1, 0.5, 0.25, and 0.125 µg/mL concentrations in a 100-µL volume followed by overnight incubation at 4 °C. After washing, purified 3ABC protein was diluted to 0.5, 0.25, and 0.125 µg/mL in PBST (PBS containing 0.05% Tween-20) and added to each well, and the plate was incubated for 1 h at 37 °C. After three PBST washes, each well received 200 µL of blocking buffer, followed by incubation at 37 °C for 2 h. Then, the serial dilution of standard positive serum (P51) and standard negative serum (P734) was carried out with dilution buffer (10% equine serum, 1% casein in PBST) at 1:2.5–1:20 dilutions, and 50 µL of the serum was transferred to each well. Simultaneously, each well was added 50 µL of HRP-conjugated mAb 9E2 (9E2-HRP) at concentrations of 0.08, 0.04, 0.02, 0.01, 0.005, and 0.0025 µg/mL followed by incubation of the plate at room temperature. After being washed five times, chemiluminescence (CL) substrate (KEY-BIO, Beijing, China) including 50 µL of solution A (luminol and luminous enhancer) and 50 µL of solution B (peroxide solution) were added. The signals of CL were measured with Varioskan lux (Thermo Scientific, USA) after 5 min. The determination of the optimum mAb 2G5 concentration, 3ABC

protein concentration, serum dilution, 9E2-HRP concentration, and incubation time was carried out based on the ratios of CL values of standard negative serum to standard positive serum (N/P).

3B-cCLIA was carried out in optimal conditions. The following formula was used to calculate the PI:

$$PI\% = (1 - CL_s / CL_n) \times 100\%,$$

Where mean CL values of the standard negative serum control are represented by CL_n while the CL values of the test samples are represented by CL_s .

A mean $CL_n \geq 6,000,000$ and mean PI of the standard positive control $> 80\%$ indicate assay validity.

Similarly, 3A+3B-cCLIA was developed, and detailed information is presented in the Additional file 1.

Cut-off value, Dsn, and Dsp

The cut-off value of 3B-cCLIA was determined by testing 875 serum samples from different origins. NSP-negative sera from swine ($n = 410$), cattle ($n = 175$), and sheep ($n = 61$) were used to estimate the Dsp of each species, and NSP-positive sera from swine ($n = 107$), cattle ($n = 70$), and sheep ($n = 52$) were used to estimate the Dsn of each species using MedCalc software.

Comparison of accuracy rates and diagnostic performances

The accuracy rates of two commercial diagnostic kits were also evaluated using the abovementioned 875 NSP-negative and positive serum samples. The diagnostic performances of 3B-cCLIA, 3A+3B-cCLIA and two commercial diagnostic kits were compared by testing 430 serum samples, including 120, 60, and 77 samples from vaccinated swine, cattle, and sheep, respectively, and 173 samples from the field. In addition, serum samples from four swine challenged with FMDV O/Mya98 collected at different times were utilized to evaluate and compare the early diagnostic performance of the four assays.

Estimation of the repeatability and stability of 3B-cCLIA

To calculate the intra- and inter-batch repeatability performances, three replicates of seven serum samples with varying PI levels were evaluated on various days using plates coated in the same and different batches. To determine the shelf life of the coated plates, they were vacuum-packed and stored at 37°C for 15 days or at 4°C for 1 year after blocking.

Results

Optimization of 3B-cCLIA

The reaction conditions were optimized using checkerboard titration for obtaining high ratios of N/P. In 3B-cCLIA, the coating concentrations of mAb 2G5 and 3ABC protein were fixed at $0.25\text{ }\mu\text{g/mL}$, serum dilution was 1:2.5, concentration of 9E2-HRP was $0.005\text{--}0.02\text{ }\mu\text{g/mL}$ ($0.01\text{ }\mu\text{g/mL}$), and reaction time was 10 min.

Determination of cut-off value, Dsn, and Dsp of 3B-cCLIA

The cut-off values of 3B-cCLIA were determined using known background sera from swine (NSP-negative sera, $n = 410$; NSP-positive sera, $n = 107$), cattle (NSP-negative sera, $n = 175$; NSP-positive sera, $n = 70$) and sheep (NSP-negative sera, $n = 61$; NSP-positive sera, $n = 52$) by analysing the interactive dot diagram and receiver operating characteristic (ROC) (Fig. 1 and Table 1). According to the analysis of ROC curve, when the cut-off of 3B-cCLIA was 50%, the Dsn was 97.20% ($104/107$), 95.71% ($67/70$), and 96.15% ($50/52$), and Dsp was 99.51% ($408/410$), 99.43% ($174/175$), and 98.36% ($60/61$) in swine, cattle, and sheep, respectively. Furthermore, cross-reaction evaluation of 3B-cCLIA with sera from different virus-infected swine (CSFV, SVA, PPV, PRRSV, and PCV2) revealed no cross-reaction ($PI < 20\%$).

Table 1

Comparison of 3B-cCLIA with 3A + 3B-cCLIA, 3ABC-bELISA and PrioCHECK FMDV NSP ELISA for the detection of NSP antibodies in sera from naïve, vaccinated and infected animals

Sample source	Total No. samples	3B-cCLIA			3A + 3B-cCLIA			3ABC-bELISA			PrioCHECK® NSP		
		p ^a	N ^b	Accuracy rate	p ^a	N ^b	Accuracy rate	p ^a	N ^b	Accuracy rate	p ^a	N ^b	Accuracy rate
Naïve swine	310	1	309	99.68%	1	309	99.68%	2	308	99.35%	0	310	100.00%
Vaccinated swine with multiple-epitope recombinant vaccine	100	1	99	99.00%	1	99	99.00%	1	99	99.00%	0	100	100.00%
Infected swine	107	104	3	97.20%	105	2	98.13%	102	5	95.33%	91	16	85.05%
Naïve bovine	175	1	174	99.43%	1	174	99.43%	2	173	98.86%	0	175	100.00%
Infected bovine	70	67	3	95.71%	67	3	95.71%	66	4	94.29%	63	7	90.00%
Naïve sheep	61	1	60	98.36%	1	60	98.36%	2	59	96.72%	0	61	100.00%
Infected sheep	52	50	2	96.15%	50	2	96.15%	51	1	98.08%	48	4	92.31%
Vaccinated sows with commercial O/A divalent vaccine (3–15 times)	120	1	119	99.17%	1	119	99.17%	3	117	97.50%	0	120	100.00%
Vaccinated dairy cows with commercial O/A divalent vaccine (2–10 times)	129	12	117	90.70%	13	116	89.92%	19	110	85.27%	15	114	88.37%
Vaccinated sheep with O, A or Asia 1 univalent inactivated vaccine (1–3 times)	77	5	72	93.51%	4	73	94.81%	6	71	92.21%	2	75	97.40%
Swine in the field	173	13	160		14 (13)	159 (159)	99.42% ^c	15 (11)	158 (156)	96.53% ^c	9 (7)	164 (158)	95.37% ^c
^a Positive, ^b Negative													
^c The coincidence rate of PI value of 3B-cCLIA with those of 3A + 3B-cCLIA, 3ABC-bELISA, and PrioCHECK FMDV NSP ELISA in testing field sera collected from swine was 99.42% (172/173), 96.53% (167/173), and 95.37% (165/173), respectively.													

Accuracy rates and diagnostic performances

These serum samples (n = 875) were also examined using two commercial diagnostic kits to evaluate and compare the test methods' accuracy (Table 1). The accuracy rate of 3B-cCLIA (97.20%, 95.71%, and 96.15%) was almost equivalent to that of 3ABC-bELISA (95.33%, 94.29%, and 98.08%) and better than that of PrioCHECK FMDV NSP ELISA (85.05%, 90.00%, and 92.31%) for serum samples from infected animals. For the naïve serum samples, the accuracy rate of 3B-cCLIA (99.51%, 99.43%, and 98.36%) was slightly higher than that of 3ABC-bELISA (99.27%, 98.86%, and 96.72%) and lower than that of PrioCHECK FMDV NSP ELISA (100%).

The accuracy rates of 3B-cCLIA and PrioCHECK FMDV NSP ELISA in testing serum samples from sows inoculated 3–15 times were almost equal to those in testing negative swine sera, and the accuracy rate of 3ABC-bELISA was slightly lower (Table 1). However, the accuracy rates of the three assays in testing these sera from dairy cows inoculated 2–10 times were significantly lower than those in testing naïve cattle sera (namely, false-positive rate increase). Similarly, the accuracy rates in testing sera from sheep vaccinated 1–3 times with lab-made FMDV A, O, or Asia 1 univalent inactivated vaccine were lower than those in testing naïve sheep sera (Table 1). Furthermore, false positives increased with the number of vaccinations in cattle and sheep (Table 2). To address this problem, a competitive CLIA (3A + 3B-cCLIA) using 3ABC polyclonal antibody as the capture antibody to capture purified 3ABC protein and two mAbs (2G5-HRP and 9E2-HRP) as the detection antibody was developed to simultaneously detect antibodies against 3A and 3B (Fig. S1). However, the accuracy rate of 3A + 3B-cCLIA in testing sera from cattle and sheep vaccinated multiple times remained lower than that in testing naïve sera (Table 1).

Table 2
Antibody to FMDV NSP in sera from cattle and sheep collected following repeated vaccination

Sample source	Total No. samples	3B-cCLIA			3A + 3B-cCLIA			3ABC-bELISA			PrioCHECK® NSP		
		P ^a	N ^b	False-positive rate	P ^a	N ^b	False-positive rate	P ^a	N ^b	False-positive rate	P ^a	N ^b	False-positive rate
Vaccinated dairy cows with commercial O/A divalent vaccine (2 times)	30	1	29	3.33%	1	29	3.33%	1	29	3.33%	1	29	3.33%
Vaccinated dairy cows with commercial O/A divalent vaccine (5 times)	33	2	31	6.06*	2	31	6.06*	3	30	9.09%	3	30	9.09%
Vaccinated dairy cows with commercial O/A divalent vaccine (10 times)	66	9	57	13.64%	10	56	15.15%	15	51	22.73%	11	55	16.67%
Vaccinated sheep with O, A or Asia 1 univalent inactivated vaccine (1 time)	29	0	29	0%	1	28	3.45%	0	29	0%	0	29	0%
Vaccinated sheep with O, A or Asia 1 univalent inactivated vaccine (2 times)	30	2	28	6.67%	1	29	3.33%	1	29	3.33%	0	30	0%
Vaccinated sheep with O, A or Asia 1 univalent inactivated vaccine (3 times)	18	3	15	16.67%	2	16	11.11%	5	13	27.78%	2	16	11.11%
^a Positive, ^b Negative													

In addition, serum samples from 173 swine in the field were tested, and the results showed that the coincidence rates of 3B-cCLIA with 3A + 3B-cCLIA, 3ABC-bELISA, and PrioCHECK FMDV NSP ELISA were 99.42%, 96.53%, and 95.37%, respectively (Table 1).

Comparison of early diagnostic performance

Seroconversion to NSP and SP was studied in four unvaccinated control swine post-challenge using O-LPBE and ID Screen® FMD Type O Competition to detect the SP antibody and using 3B-cCLIA, 3A + 3B-cCLIA, 3ABC-bELISA, and PrioCHECK FMDV NSP ELISA to detect the NSP antibodies. Antibodies against SPs were first detected at 5 dpi, and all the sera examined were positive at 7 dpi (Fig. 2a). Antibodies against NSPs were detected early at 7 dpi and identified as positive in all swine at 8 dpi except for the PrioCHECK FMDV NSP ELISA (Fig. 2b). Therefore, seroconversion to NSP occurred approximately 1–2 days later than that to SP.

Repeatability and stability of 3B-cCLIA

The assay was highly repeatable, as determined by four positive and three negative serum samples tested on different days on same and different batch plates (Fig. 3).

The standard negative serum (P734) and standard positive serum (P51) were examined while using 3B-cCLIA plates stored at 37°C for 15 days or at 4°C for 1 year. The CL values of P734 were > 6,000,000, and the PI value of P51 was > 80%. The findings showed that 3B-cCLIA

plates may be preserved for up to 15 days at 37°C and up to 1 year at 4°C.

Discussion

DIVA tests are important for serological surveillance by providing evidence of vaccinated herds infected with or freedom from FMD [1, 3, 17]. To avoid omitting animals infected with different serotype isolates, identifying conserved mAbs is important in developing a diagnostic method for DIVA. The epitope “²³EGPYAGPLE³¹” identified by mAb 9E2 is located in 3B and is well conserved among different serotypes of FMDV, which has been verified in a previous study [3]. Therefore, in this study, a competitive CLIA (3B-cCLIA) using mAb 2G5 as the capture antibody to capture purified 3ABC protein and 9E2-HRP as the detection antibody was developed for DIVA. The cut-off PI value of 3B-cCLIA was determined to be 50% by testing panels of serum samples from different origins. Based on the cut-off PI of 50%, Dsn (97.20%, 95.71%, and 96.15%) and Dsp (99.51%, 99.43%, and 98.36) were determined in naïve and infected swine, cattle and sheep, respectively (Fig. 1). The PrioCHECK FMDV NSP ELISA had good Dsp (accuracy rate 100%) in testing naïve sera but had low Dsn in testing infected sera when compared with 3B-cCLIA and 3ABC-bELISA.

Although most NSPs are removed from the inactivated vaccine, residual NSPs are still present in the inactivated vaccine [3] and can induce the corresponding antibodies after repeated vaccination, especially in cattle [11, 12, 21–23], which will interfere with the DIVA test based on detecting NSP antibodies and affect the serological surveillance and evaluation of the infection status [19]. This phenomenon was also confirmed in this study, in which dairy cows vaccinated multiple times with the traditional inactivated vaccine produced a high false-positive rate using 3B-cCLIA and two commercial diagnostic kits (Tables 1 and 2). In addition, the false-positive rate in sheep vaccinated three times also increased (Tables 1 and 2). However, the false positives in sows vaccinated multiple times were almost equal to those in negative sera (Tables 1 and 2), as found in previous reports [12, 18]; this may be due to different immune systems in different species. False positives increased with the number of vaccinations. To address this problem, we attempted to use two mAbs recognizing NSP 3A and 3B to develop 3A + 3B-cCLIA. In theory, only when the two antibodies were simultaneously present in serum, the serum was considered to be positive, thereby using the assay to try to decrease the false-positive rate in vaccinated animals. However, the results showed that 3A + 3B-cCLIA could not solve this false-positive problem (Tables 1 and 2).

Sera from animals vaccinated multiple times are usually weak positive. Therefore, it is a strategy to increase the cut-off (compromise true sensitivity) to decrease the false-positive rates in testing vaccinated animals. Another approach is to establish the correlation between residual NSPs in the inactivated vaccines with different rounds of purification and the NSP antibody in different species that were inoculated different times with the corresponding inactivated vaccine, thereby conforming to a threshold value of residual NSPs in the inactivated vaccine to guide vaccine manufacturers to improve vaccine purity. In addition, the development and use of new FMD vaccines that do not contain NSP components, such as marker vaccines [24, 25], virus-like particle VLP vaccines [26], multiple-epitope recombinant vaccines [27], and synthetic peptide vaccines, could eliminate this barrier.

In this study, seroconversion to NSP occurred later than that to SP, which is similar to previous reports [11, 21]. The seroconversion time of the PrioCHECK FMDV NSP ELISA was delayed compared with that of the other three methods, which explains the lower Dsn of the PrioCHECK FMDV NSP ELISA in detecting sera from infected animals.

Conclusion

A competitive CLIA that could rapidly detect antibodies against FMDV NSP 3B was developed in this study. 3ABC-bELISA and PrioCHECK FMDV NSP ELISA use the blocking ELISA format and need to be washed twice in process, but 3B-cCLIA uses the competitive format, only needs to be washed once and requires 15 min to obtain results. In addition, the early diagnostic performance, repeatability, and stability of 3B-cCLIA were also evaluated. These results indicated that 3B-cCLIA can be a promising tool for large-scale serological surveys in vaccinated areas for DIVA.

Abbreviations

FMD: Foot-and-mouth disease; FMDV: Foot-and-mouth disease virus; DIVA: Differentiation of infected from vaccinated animals; CLIA: Chemiluminescence immunoassay; NSPs: Non-structural proteins; SPs: Structural proteins; ELISA: Enzyme-linked immunosorbent assay; mAbs: Monoclonal antibodies; O-LPBE: Liquid-phase blocking ELISA of FMDV O; A-LPBE: Liquid-phase blocking ELISA of FMDV A; Dsp: Diagnostic specificity; Dsn: Diagnostic sensitivity; dpv: Days post vaccination; dpi: days post infection; CSFV: Classical swine fever virus; SVA: Senecavirus A; PPV: Porcine parvovirus; PRRSV: Porcine reproductive and respiratory syndrome virus; PCV2: Porcine circovirus type 2; PI: Percentage inhibition; HRP: Horseradish peroxidase; N/P: The ratios of CL values of standard negative serum to standard positive serum; ROC: Receiver operating characteristic.

Declarations

Ethics approval and consent to participate: All procedures involving animals were approved by the Animal Ethics Committee of LVRI, Chinese Academy of Agricultural Sciences and implemented in accordance with the animal care and ethics guidelines.

Consent for publication: Not applicable.

Availability of data and materials: All data associated with this study are included in the paper and its supplementary material.

Competing interests: The authors declare that they have no conflicting interests.

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Authors' contributions: WL, JS, HC conceived and designed the study. WL, GZ, SY, JL, ZG, SG, HY conducted experiments. WL and GZ analysed the data. WL wrote the manuscript. All authors read and approved the manuscript.

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Figures

Figure 1

Receiver operating characteristic (ROC) analysis for the determination of the cut-off value of 3B-cCLIA. (a, c, e) Interactive dot diagram of 3B-cCLIA in testing sera from swine, cattle, and sheep. 0, negative serum samples (n = 410, 175, and 61); 1, positive serum samples (n = 107, 70, and 52). (b, d, and f) Each point on the ROC curve represents a sensitivity-specificity pair in testing the sera from swine, cattle, and sheep.

Early production of antibodies against FMDV SPs and NSPs in sera from experimentally challenged swine. (a) Thirty-two serum samples from four unvaccinated control swine after challenge with FMDV O/Mya98 were collected at 0 dpi and 2–8 dpi and tested using O-LPBE, ID Screen® FMD Type O Competition to detect SP antibodies. The dashed lines – – – – and represent the cut-off values of O-LPBE and ID Screen® FMD Type O Competition, respectively. (b) Thirty-two serum samples were tested using 3B-cCLIA, 3A+3B-cCLIA, and two commercial diagnostic kits (3ABC-bELISA and PrioCHECK FMDV NSP ELISA) to detect NSP antibodies. The dashed line (– – – –) represents the cut-off value of 3B-cCLIA, 3ABC-bELISA, and PrioCHECK FMDV NSP ELISA, and the dashed line (.....) represents the cut-off value of 3A+3B-cCLIA. The bar represents the range of values obtained by detecting sera from four swine at the given time.

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