Identification of Foot-and-Mouth Disease Virus-Specific Linear B-Cell Epitopes To Differentiate between Infected and Vaccinated Cattle

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Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals. For several years, vaccination of animals, which had proven to be successful for the eradication of the disease, has been forbidden in the United States and the European Community because of the difficulty of differentiating between vaccinated and infected animals. In this study, detailed investigations of the bovine humoral immune response against FMD virus (FMDV) were performed with the aim of identifying viral epitopes recognized specifically by sera derived from FMDV-infected animals. The use of overlapping 15-mer synthetic peptides, covering the whole open reading frame of FMDV strain O₁K in a peptide enzyme-linked immunosorbent assay, allowed the identification of 12 FMDV strain O₁K-specific linear B-cell epitopes. Six of these linear B-cell epitopes, located in the nonstructural proteins, were used in further assays to compare the reactivities of sera from vaccinated and infected cattle. Antibodies recognizing these peptides could be detected only in sera derived from infected cattle. In further experiments, the reactivity of the six peptides with sera from animals infected with different strains of FMDV was tested, and strain-independent infection-specific epitopes were identified. Thus, these results clearly demonstrate the ability of a simple peptide-based assay to discriminate between infected and conventionally FMD-vaccinated animals.

Foot-and-mouth disease (FMD) is a highly contagious viral disease of ruminants and swine that is responsible for large economic losses due to both the disease itself and the restrictions imposed on animal products and trade. Conventional vaccines against the virus exist, but for several years, vaccination of animals, which had proven to be successful for the eradication of the disease, has been forbidden in the United States and the European Community because of the difficulty of differentiating between vaccinated and previously infected animals.

For discrimination between FMDV-infected and -vaccinated animals, several approaches have been undertaken during the last decade. Because all conventional FMDV vaccines consist of purified inactivated viral particles, it should be impossible to detect antibodies against nonstructural proteins in vaccinated animals. Nonstructural proteins should exist only after infection of cells with live virus, and antibodies specific for these nonstructural proteins should only arise after infection.

In 1966, a viral infection-associated antigen was found (2), which was later identified as the nonstructural protein 3D (11). However, quite early it was recognized that antibodies specific for this antigen could also be detected in sera of animals that had been vaccinated several times with an inactivated virus vaccine (1, 3, 14, 16). Therefore, the use of this antigen as a possible marker for the differentiation of vaccinated and in-

fectured animals was questioned. In further investigations, other nonstructural proteins were examined for their ability to elicit an antibody response in infected and vaccinated animals (4, 7–10, 15, 19). Antibodies specific for the bacterially expressed 3ABC fusion protein seemed to be especially suitable for the differentiation of vaccinated from infected animals (4, 8, 15, 19). However, this test system has different disadvantages: e.g., difficulty in identifying virus shedding “carriers” (20) and differentiating between often-vaccinated and infected animals (8).

Analysis of results was also impeded by antibodies specific for antigens derived from the expression systems that were used for the synthesis of the proteins: e.g., proteins from *Escherichia coli* when a bacterial expression system was used (9, 19) or proteins from insects when the baculovirus system was used (9). In these cases, false-positive results occurred.

Also the number of epitopes found on such a long recombinant protein could result in unspecific reactions, because of cross-reactivities with antibodies to other picornaviruses (10).

To overcome these problems, synthetic peptides containing B-cell epitopes of the virus could be used as shown by Shen and coworkers (18). They synthesized synthetic peptides of different lengths from the proteins 2C and 3ABC of FMDV strain A12 for the differentiation of FMDV-vaccinated and -infected animals of different species and identified as a good candidate a 57-amino-acid (aa) peptide that practically contained the amino acid sequences of the three 3B proteins (18). With this peptide, it was possible to differentiate sera of guinea pigs, cattle, and swine infected with different FMDV serotypes from sera of vaccinated animals. Because of the cost and difficulties of synthesizing such a long 57-mer synthetic peptide, it would

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FIG. 1. Identification of FMDV-specific linear B-cell epitopes in cattle. For the identification of FMDV-specific linear B-cell epitopes, the reactivity of a serum derived from an FMDV-infected animal (black columns) was compared with the reactivity of its preimmune serum in a peptide ELISA (white columns) with 442 peptides encoded by the open reading frame of FMDV O₁K. The reactivity of 16 positively identified peptides with this animal is demonstrated. P and N represent control peptides as described in Materials and Methods. All tests were performed with at least two replicates, and the standard deviation was <10% in all groups.

be desirable to identify shorter peptides to achieve the same goal.

Therefore we used 14- and 15-mer synthetic peptides, which overlapped in 10 aa, for detailed investigations of the bovine humoral immune response against FMDV. The final aim was to identify with synthetic peptides linear viral B-cell epitopes that are recognized specifically by sera derived from FMDV-infected animals and which can be mimicked by small synthetic peptides.

MATERIALS AND METHODS

Sera. Sera of FMDV-infected and -vaccinated cattle were derived from experiments performed at the Federal Research Centre for Virus Diseases of Animals, Tübingen, Germany. For the vaccination of the animals, conventional inactivated virus vaccines were used. The virus subtypes used for vaccination and challenge infections are indicated below.

Peptides. Overlapping peptide amides (14 or 15 aa in length, overlapping each other by 10 aa) were synthesized based on the sequence of FMDV strain O₁K (5).

Peptides were prepared by fully automated solid-phase peptide synthesis with 9-fluorenylmethoxycarbonyl/*tert*-butyl (Fmoc/tBu) chemistry and Rink amide 4-methylbenzhydrylamine polystyrene resins. A sevenfold molar excess of single Fmoc-L-amino acids and an optimized diisopropylcarbodiimide/1-hydroxybenzotriazole method were used for the coupling steps. The peptides were cleaved from the resins, and the side chain was deprotected with trifluoroacetic acid-phenol-ethanedithiol-thioanisole-water and precipitated at -20°C by addition of cold diethylether. The precipitates were washed twice by sonication with diethylether and were lyophilized from *tert*-butyl alcohol. The identity of the peptides was confirmed by electrospray mass spectrometry, and the purity was higher than 80% as determined by high-performance liquid chromatography. The peptides were stored at -70°C in a stock solution of 10 $\mu\text{g}/\mu\text{l}$ in dimethyl sulfoxide.

Peptide ELISA. The peptide enzyme-linked immunosorbent assay (ELISA) for the detection of peptide-specific antibodies in the respective sera was performed as follows. The stock solution (0.1 μl per well) of the indicated peptide was diluted in 50 μl of H₂O and dried on an ELISA plate (Nunc Immunoplate

Maxisorb; Nunc, Wiesbaden, Germany). To reduce nonspecific binding of the sera, the free binding sites on the plates were blocked for 2 h with 3% (wt/vol) bovine serum albumin (BSA) in phosphate-buffered saline (PBS). After each incubation step, the plates were washed three times with PBS-Tween (0.1% [vol/vol] Tween 20). Before the substrate was added, five washing steps were performed. Sera as well as all conjugates used in the experiments were diluted in PBS-0.5% BSA. Unless indicated otherwise, all sera were used in a dilution of 1:100. The coated and pretreated plates were then incubated with the respective sera (50 μl per well) for 1 h at 37°C and thereafter with horseradish peroxidase-labeled goat anti-bovine immunoglobulin (H+L) (Southern Biotechnology Associates, Birmingham, Ala. [dilution, 1:2,500]) for an additional hour at 37°C . The existence of peptide-specific antibodies in the sera was detected in a color reaction by adding 50 μl of substrate per well. *ortho*-Phenylenediamine (OPD) diluted in citrate buffer (4.67 g of citrate, 9.15 g of Na₂HPO₄, 0.5 g of OPD per liter of H₂O) served as a substrate. The enzymatic reaction of the horseradish peroxidase with the substrate was performed in the dark for about 10 min. The reaction was stopped by adding 100 μl of 2 M H₂SO₄. The intensity of the resulting color was analyzed at 492 nm in an ELISA reader (Titertek, Helsinki, Finland).

A 32-mer peptide (TVYNGECRYNRNAVPNLRGDLQVLAQKVARTL) from the 1D region of FMDV served as a positive peptide control (13), and a 24-mer peptide (N5501; SQRQKKVTFDRQQVQDDHYRDVLR) from the RNA-dependent RNA polymerase region of hepatitis C virus served as a negative control.

A synthetic peptide was identified as bearing a linear FMDV-specific B-cell epitope when the reactivity of the serum, derived from the infected animal in the peptide ELISA, was at least twice the optical density (OD) of the serum before infection. Negative 15-mer peptides from the O₁K sequence, identified in previous experiments, served as additional controls. Normally, peptides from the 3D nonstructural protein regions, where no linear B-cell epitopes could be identified, were used as additional negative controls. The following 15-mer 3D-peptides were randomly selected out of the negative peptide fraction and included in the assays: NKDPRLNEGVLDEV, PEVEAALKLMEKREY, and IGSVGCNPVDVGWQR. The mean value of the reactivity of the respective sera against at least two replicates of each of the three peptides was calculated and used as the denominator for the calculation of the OD index. The standard deviation was determined in all experiments and was <10%.

FIG. 2. Localization of the linear FMDV-specific B-cell epitopes. The genome organization of the open reading frame of FMDV is presented together with the viral proteins and their distribution in structural and nonstructural proteins. The location of the synthetic peptides identified as B-cell epitopes in Fig. 1 is shown as gray lines in the respective areas of the viral proteins. The internal numbers of the peptides are indicated.

RESULTS

Identification of bovine FMDV-specific linear B-cell epitopes. For the identification of bovine FMDV-specific linear B-cell epitopes, sera of cattle infected with FMDV strain O₁K were examined for antibodies that recognize synthetic peptides in the peptide ELISA described in Materials and Methods. The 442 FMDV-specific 14- to 15-mer synthetic peptides, which overlapped in 10 aa, were synthesized on the basis of the amino acid

sequence of FMDV O₁K (5). Nonspecific interactions of the sera with the synthetic peptides were excluded by testing the sera of the same animals obtained prior to infection. Peptide P, a 32-mer peptide representing the loop region of FMDV O₁K with a B-cell epitope described previously (12), served as a positive peptide control, and peptide N, a 24-mer peptide from the polymerase of hepatitis C virus, served as a negative control.

Figure 1 (gray columns) shows an example of the reactivity

FIG. 3. Induction of FMDV peptide-specific antibodies during an FMDV infection. Sera from an FMDV O₁K-infected cow (no. 2422) were acquired prior to the infection (black columns), 4 days p.i. (dark gray columns), 15 days p.i. (gray columns), and 30 days p.i. (white columns). All sera were analyzed with the peptide ELISA for antibodies to the respective peptides indicated in Materials and Methods. To standardize the system and to enable a comparison of the respective sera, three control peptides derived from the FMDV nonstructural protein 3D (see Materials and Methods) were included to determine a peptide-independent background staining. All tests were performed with at least two replicates, and the standard deviation was <10%. In correlation with the control peptides, the OD index was calculated as described in the text. The OD index for the control peptides was 1.

of a serum against the identified peptides. The serum was obtained from a cow 3 weeks postinfection (p.i.). The reactivity of a control serum derived from the same animal prior to infection is indicated by white columns.

Out of the 442 synthetic peptides representing the whole polypeptide of FMDV O₁K, 16 peptides showed reactivity with the sera of infected animals, but not with the preimmune sera (Fig. 1). Figure 2 shows the localization of the peptides identified as linear B-cell epitopes (indicated by gray bars), their respective numbers, and their distribution among the viral proteins 1D, 2B, 2C, 3A, 3B, and 3D. Most of the identified linear B-cell epitopes are located within the nonstructural proteins. Clusters of B-cell epitopes could be identified at the N-terminal end of protein 2C with the corresponding peptides 93, 100, and 101 as well as on the C-terminal end of protein 3A (peptides 410, 412, and 417).

Also interesting is the high density of linear B-cell epitopes on proteins 3B1, 3B2, and 3B3, represented by peptides 434, 436, 437, 438, and 440. The three 3B proteins are 23 or 24 aa long and have high amino acid sequence homology. Therefore, four of the peptides tested (434, 436, 437, and 438) contain linear B-cell epitopes with the common amino acid motif QKPLK. The amino acid sequences of the identified peptides containing linear B-cell epitopes are presented in the lower part of Fig. 2. The QKPLK motif of the 3B peptides is highlighted in boldface.

Induction of peptide-specific antibodies during an infection.

So far, all experiments had been performed with sera from FMDV-infected cattle obtained approximately 3 weeks after infection. In these animals, an antigen-specific immune response with considerable levels of virus-specific antibodies had enough time to develop. If antibodies to viral structures will be involved in the detection of infected animals, several important questions must be answered. When is the onset of the antigen-specific B-cell response? Also, when is the earliest time point for the detection of peptide-specific antibodies? Therefore, an experiment to determine the time kinetics of the peptide-specific antibody response was set up. An animal was infected with FMDV strain O₁K, and serum samples were taken 4, 15, and

30 days p.i. Figure 3 shows the reactivity of the sera with the respective peptides. For a better comparison, the data were presented in OD indices, which were calculated by the quotient of the ODs of the respective sera with the presented peptide and the mean OD of three nonspecific peptides (FMDV 3D nonstructural protein region [see Materials and Methods]). As shown in Fig. 3, no peptide-specific antibodies could be detected prior to infection (black columns) and at an early time point after infection (dark gray columns, 4 days p.i.). After 15 days, a weak antibody response was detectable against several peptides (light gray columns) representing the structural (peptide 266) and nonstructural protein regions (peptides 4, 93, 410, and 412). One interesting finding was the high reactivity against peptides derived from proteins 3B1, 3B2, and 3B3 (peptides 433, 434, 436, and 437). The intensity of the reaction against the single peptides had only marginally altered 2 weeks later (white columns).

Comparison of immunogenic sequences derived from different FMDV strains. It is known that, in contrast to the structural proteins, the amino acid sequences of nonstructural proteins of FMDV are highly conserved within different subtypes and serotypes. Figure 4 shows sequence alignments for six of the peptides identified as linear B-cell epitopes. The RGD amino acid motif of the structural protein 1D is known as a highly conserved region within FMDV. Peptide 266 enclosed this region and showed that, even for this peptide, there was only 60% homology at the amino acid level, which is close to that of the O₁K-related FMDV subtype O₁Geshure (O₁G). For other, more distantly related viruses (including A strains (A₁₀Argentina [A₁₀A]), C strains (C₃Argentina [C₃A]), and SAT strains (SAT2 Kenya [SAT2K]), even less homology was found.

A comparison of the amino acid sequences of peptides derived from the nonstructural protein regions revealed in all cases at least 80% homology at the amino acid level. Mostly only 1- or 2-aa exchanges were demonstrated based on the O₁K sequence. Only SAT2K showed for peptide 437 an exchange of 3 aa. These exchanges also affected the QKPLK

peptide 266 (protein 1D)		
O ₁ K	Y N R N A V P N L R G D L Q V	
O ₁ G	G E G H A V	60%
A ₁₀ A	S . A S D S . R S S	33%
C ₃ A	S . . A G V R A H	33%
SAT2K	T . K T T A I R E	40%
peptide 93 (protein 2C)		
O ₁ K	L K A R D I N D I F A I L K N	
O ₁ G		100%
A ₁₀ A		100%
C ₃ A		100%
SAT2K		100%
Peptides 100/101 (protein 2C)		
O ₁ K	S E E K F V T M T D L V P G I L E K Q R	
O ₁ G	A	93%
A ₁₀ A		100%
C ₃ A		100%
SAT2K		100%
peptide 410 (protein 3A)		
O ₁ K	N E Y I E K A N I T T D D K	
O ₁ G		100%
A ₁₀ A		100%
C ₃ A		100%
SAT2K	D G	87%
peptide 433 (protein 3B)		
O ₁ K	G P Y A G P L E R Q K P L K	
O ₁ G		100%
A ₁₀ A		100%
C ₃ A		100%
SAT2K	A M	87%
peptide 437 (protein 3B)		
O ₁ K	P M E R Q K P L K V K A K A	
O ₁ G		100%
A ₁₀ A		100%
C ₃ A	L	93%
SAT2K	Q A R	80%

FIG. 4. Comparison of the immunogenic sequences derived from different FMDV strains. The sequences of six synthetic peptides containing linear B-cell epitopes are shown for different FMDV serotypes and subtypes. The sequence of subtype O₁K was used as a basis for the other sequences. Sequence homologies are indicated as white fields; changes between the types and subtypes are marked by the respective amino acids. The percentages on the right side summarize the homology of the respective subtypes to O₁K.

motif, formerly mentioned as a potential B-cell epitope, which is modified in SAT2K to QQPLK.

Differentiation between FMDV-infected and -vaccinated cattle. To investigate the reactivity of the peptides representing nonstructural protein regions with sera from animals infected with various FMDV serotypes and subtypes, sera from animals infected with different O strains (O₁K, O₁Lausanne [O₁L], and O₁Syria [O₁S]) were analyzed in a peptide ELISA using peptides 93, 100, 101 derived from the 2C region; peptide 410 from the 3A region; and the two 3B1-3-related peptides, 433 and 437.

As shown in Fig. 5 all sera from the O₁-infected animals showed a clear reactivity with peptide 433 from the 3B1 region. In addition, for peptides 437 and 410, reactive antibodies could be found in several animals. The differences in the detection of these antibodies are due to individual differences in the animals, because single animals of all three groups (infected with O₁K, O₁L, and O₁S) were able to elicit antibodies recognizing these peptides. Antibodies reactive with the peptides 100, 101, and 93 could only be detected in O₁K- and/or O₁L-infected animals.

Sera from animals vaccinated with a commercially available conventional inactivated-whole-virus vaccine (Bayer, Le-

		peptide no.						
	cattle no.	93	100	101	410	433	437	
infected	425	+	+	+	+	+	+	O ₁ K
	59578		+			+	+	
	69064				+	+	+	
	2422					+	+	
	SP98	+			+	+	+	O ₁ L
	SP97	+			+	+	+	
	SQ0	+				+		
	SP96					+		
	SP99					+		
	SQ1					+		
vaccinated	296				+	+	+	O ₁ S
	285					+	+	
	773							O ₁ M
	778							
	780							
	787							
	793							

FIG. 5. Reactivity of sera from FMDV-infected and -vaccinated animals with synthetic peptides derived from the homologous FMDV strain. Shown is a summary of data from a peptide ELISA performed with sera from FMDV O strain-infected and vaccinated animals. Positive reactions (+) are defined by twofold OD compared with reactivity with control peptides.

verkusen, Germany) did not show any reactivity with the peptides used in the assays. Because sera from O₁K-vaccinated animals were not available, sera from cattle vaccinated with the closely related strain O₁Manisa (O₁M) were used. The results of these assays demonstrate that in contrast to the infected animals, sera from vaccinated animals do not recognize any of the tested peptides from the nonstructural protein regions at least 3 weeks after vaccination (Fig. 5).

As shown earlier, the amino acid sequences of the peptides used for this assay, are highly conserved within different serotypes. Therefore, in a further experiment, the assay was used to detect peptide-specific antibodies in sera of animals infected with other serotypes of FMDV. These data are summarized in Fig. 6. Sera from animals infected with Asia1 Shamir (Fig. 6a, cattle 460, 10, and 290) showed a strong reactivity with the synthetic peptides from proteins 410, 433, and 437. In contrast, cattle vaccinated with a subtype-specific conventional vaccine (Bayer) did not contain antibodies recognizing the peptides. A similar result was found when sera from A₅Bernbeuren-infected animals were used (Fig. 6b). Eleven of 13 sera recognized peptide 437; 13 of 13 sera showed a clear reactivity with peptide 433. Again, for this serotype, no peptide-specific antibodies could be detected in animals revaccinated up to five times.

Similar results could be found in cattle infected or vaccinated with FMDV serotype SAT1. The sera of all three animals showed antibodies to either peptide 433 or 437, and two of them had antibodies to peptide 410. In none of the vaccinated animals could antibodies to the peptides be detected.

Taken together, these results demonstrate for the first time

a)

	cattle no.	peptide no.					
		93	100	101	410	433	437
infected	460				+		+
	10				+	+	+
	290	+			+	+	+
vaccinated	1132						
	1133						
	1134						
	1138						
	1139						

b)

	cattle no.	peptide no.					
		93	100	101	410	433	437
infected	748					+	+
	749	+				+	
	726					+	
	725				+	+	+
	727		+			+	+
	729	+			+	+	+
	755	+			+	+	+
	744					+	+
	735	+			+	+	+
	756	+			+	+	+
	746			+		+	+
	754	+		+	+	+	+
	741	+		+	+	+	+
vaccinated	701						
	703						
	707						
	708						
	714						
	724						

c)

	cattle no.	peptide no.					
		93	100	101	410	433	437
infected	38				+	+	
	743				+	+	
	296						+
vaccinated	38						
	159						
	663						
	743						
	296						

FIG. 6. Reactivity of sera from FMDV-infected and -vaccinated animals with synthetic peptides. Shown is a summary of data about reactivity of sera in the peptide-based ELISA. Sera were derived from cattle infected or vaccinated with FMDV serotype Asia1 (a), serotype

the possibility of clearly discriminating between FMDV-infected and -vaccinated animals by determining antibodies to two 15-mer synthetic peptides.

DISCUSSION

Because of the tremendous loss of animals, the last outbreak of FMDV in Great Britain in 2001 reopened the discussion about vaccination policies in various countries. The main factor against effective vaccination with the existing vaccines is that discrimination between vaccinated and infected cattle doesn't seem to be possible.

For several years, considerations have been made about how to differentiate FMDV-vaccinated and -infected animals. Because all conventional FMDV vaccines consist of purified capsid particles, one theoretical approach might be to detect antibodies against nonstructural proteins in FMDV-infected animals.

In our experiments, several peptides derived from the nonstructural protein region that represent linear B-cell epitopes in FMDV-infected cattle could be identified. These epitopes enable effective discrimination between FMDV-infected and -vaccinated animals.

An interesting role was played by peptides 410 (protein 3A), 433, and 437 (protein 3B), which were recognized by sera of all infected animals, independent of the strain with which the animals had been infected. In all experiments, none of the sera of vaccinated animals contained antibodies directed against these peptides. Therefore, these peptides could be used as the basis for a fast and simple assay to differentiate between infected and vaccinated animals.

As mentioned for recombinant proteins expressed in *E. coli* or the baculovirus system (9, 19), no reactivity of noninfected or vaccinated animals could be observed. Furthermore, animals vaccinated five times did not show any reactivity to the peptides.

Our results confirm data for a peptide-based ELISA described by Shen et al. in 1999 (18). However, in contrast to the 57-aa peptide they used for the detection of infection-dependent antibodies, our 15- or 14-mer peptides are easier to synthesize and are much cheaper for the production of commercially available diagnostic systems. Further experiments with shorter peptides based on the sequences of the respective peptides and the definition of a shorter core motif are in progress. Eventually the 5-aa peptide QKPLK, representing the common sequence found in peptides 433, 434, 436, 437, and 438, will be enough for effective recognition and differentiation between infected and conventionally vaccinated cattle.

In the comparison between 15- and 57-aa peptides, one should keep in mind that the ELISA with the 57-aa peptide was able to differentiate sera of guinea pigs, cattle, and swine infected with different FMDV serotypes from sera of vaccinated animals. So far, the 15-mer approach using 3B peptides 433 and 437 has been tested only with cattle. However, further

A (b), or serotype SAT1 (c). Their reactivity was tested with the heterologous peptides derived from FMDV strain O₁K. Positive reactions (+) are defined by twofold OD compared with reactivity with control peptides.

experiments will elucidate the reactivity of sera from other species and will surely give results about their FMDV-specific B-cell epitopes.

Another important point is the influence of several vaccinations on the production of antibodies to the nonstructural protein peptides. The sera of the vaccinated animals we used were derived from animals that were maximally vaccinated five times (FMDV strain A₅Bernbeuren). Whether sera of animals that were vaccinated considerably more often contain antibodies to the 15-mer peptides is not clear, but the chance seems to be very low.

Another important question is what happens when a vaccinated animal is infected? In preliminary experiments based on only five animals, we showed with the peptide ELISA that these animals, which had been vaccinated two to five times prior to infection, had antibodies to the 3B peptides 3 weeks after challenge infection. That means that "carrier" animals, which can be generated by vaccination and consecutive infection, might be detected in the peptide-based ELISA. These animals without FMDV symptoms are a continuous threat for nonvaccinated animal populations. A sensitive method for detection of these animals is highly important for FMDV diagnosis, and whether these animals carry the virus should be determined early on. Detailed analyses of carrier animals and their antibody repertoire will be the subjects of further studies.

When evaluating the peptide ELISA, additional individual differences in the reactivity of the peptide-specific antibodies that could be detected in the serum became obvious. For only one animal were antibodies to all peptides used in the experiments (peptides 93, 100, 101, 410, 433, and 437) detected. This might depend on a different antibody titer against single peptides in the sera of individual animals. This problem could be overcome with an increase in the sensitivity of the peptide ELISA: e.g., by using biotinylated peptides coupled to streptavidin plates (17).

Sensitivity might also play a role when a peptide-based ELISA replaces the conventional FMDV detection systems. In contrast to the competition ELISA used in FMDV diagnostic laboratories (6), an ELISA based on peptides from the nonstructural proteins could identify only infected animals. No information would be available about former vaccinations. However, this problem can be solved by the use of synthetic peptides derived from the structural protein region (e.g., with the 32-mer peptide P from protein 1D or shorter peptides from this sequence). With this modification, the new peptide-based detection system for FMDV-specific antibodies would have clear advantages over the competition ELISA, such as the fact that the test itself does not depend on the use of previously inactivated FMDV and could be performed outside of special high-security containment.

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