COMPARISON OF DIFFERENT ELISA METHODS FOR THE DETECTION OF ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS (FMDV) TYPE O

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Abstract

Diagnostic value of ELISA methods - the liquid-phase blocking ELISA (LPBE), solid phase competition ELISA (SPCE) and Ceditest FMDV type O - for the detection and quantification of FMDV type O antibodies was tested. These methods were compared using a panel of sera collected from negative (non-infected), experimentally infected, and vaccinated cattle as well as the panels of the FAO/OIE international reference sera for the purposes of international trade. The solid-phase ELISA methods (SPCE and Ceditest) had a better specificity than the LPBE (99.1 – 99.3% and 92.2%, respectively). A relative sensitivity of SPCE, Ceditest ELISA and LPBE was very high and achieved more than 99%. Variation in results obtained by triplicate testing of individual sera indicates a higher reproducibility of the ELISA than of the virus neutralization test (VNT). The OIE cut-off reference sera for FMDV antibody assays were inconclusive or negative in all the used techniques. The results of our studies showed that the solid-phase ELISA methods are more suitable than the LPBE and VNT ones for a large scale serological testing.

Key words: cattle, FMD, serology, ELISA, diagnostic value.

Foot-and-mouth disease (FMD) is a highly contagious and devastating disease of all cloven-hoofed animals. There is an obligation towards countries wishing to be recognized as free from FMD, within the terms of the ‘OIE Code’ (4), to carry out surveillance of the disease. In countries where the vaccination against FMD is prohibited this is readily done by measuring antibodies to the capsid, structural proteins of the virus, as the presence of the antibody structural proteins is the evidence of previous exposure to FMDV. As a rule, the large-scale serosurveillance of cattle for the presence of FMDV antibodies has been applied in the majority of the European countries (3). In Poland such screening studies have been carried out since 1991 within the national FMD serosurveillance program.

The internationally accepted tests for FMD serology are methods recommended by the Office International des Epizooties (OIE). Actually the reference method (“gold standard”) is virus neutralization test (VNT) (7). However, this assay is laborious, time consuming, variable in the results due to its biological nature and must be performed in high security conditions. The other prescribed test is the liquid-phase blocking ELISA (LPBE) developed by Hamblin et al. (9, 10) and adopted by a large number of laboratories in Europe (16). In the Department of Foot-and-Mouth Disease in Zduńska Wola this technique has been routinely used since 1993 (13, 19). The LPBE replaced the VNT for routine serological screening because it is quicker, more reproducible and at least as sensitive as the VNT (19). However, the LPBE due to problems with the false-positive results is generally criticized, particularly for large-scale screening studies, e.g. export/import purposes (8). Recently, some solid-phase ELISA methods for the detection of antibodies against FMDV have been developed and validated (6, 11, 17). These methods offer an improvement for FMDV antibody detection and were introduced in some FMD laboratories for routine serological screening studies. By the fifth month of the UK 2002 FMD epidemic, the Pirbright Laboratory (UK) using the solid-phase competition ELISA (SPCE) for type O had tested more than 500 000 samples of sera (1).

The aim of these studies was to compare the diagnostic value of different ELISA methods for the detection of antibodies against FMDV type O.

Material and Methods

Sera. The following sera were examined:
a) negative sera: a total of 680 samples of sera from Poland which originated from healthy cattle neither vaccinated nor exposed to FMD virus as
confirmed by the VNT results. These sera were tested within the national FMD serosurveillance program.

b) positive sera: 232 "post-vaccination" sera of cattle from the premises around Zduńska Wola repeatedly vaccinated with trivalent anti-FMD vaccine during 1985-1989. A total of 23 convalescent sera from animals infected with FMDV type O were supplied by the World Reference Laboratory for FMD at Pirbright, U.K.

c) international reference sera: sera generated for the FAO Collaborative Study Phases XIII (15) and XVI (14). The last panel comprised OIE reference sera to FMDV strain O;Manisa – a strong positive, weak positive, and cut-off serum.

Virus neutralization test (VNT). The assay was performed in a flat-bottomed microtitre plate with BHK-21 cells, according to the method described by Golding et al. (7). Antibody titres were expressed as the reciprocal of the final dilution of serum in the virus-serum mixture which was capable of neutralizing an estimated 100 TCID₅₀ of virus in the method of Kärber (12). VNT titres of less than or equal to 11 (log 1.04) were considered negative, titres between 16 (log 1.20) and 32 (log 1.50) inconclusive, and titres greater than or equal to 45 (log 1.65) were considered positive.

ELISA methods. The LPBE was performed according to the method described by Hamblin et al. (9). Antibody titres for FMDV type O were expressed as the final dilution of test serum giving 50% of the mean OD₅₂₀ value recorded in the virus control wells where test serum was absent. Titres greater than 40 (log 1.60) were considered positive.

The SPCE was carried out according to the method recommended for the purposes of the FAO Collaborative Study Phase XVII (19), which is a modified version of original method described by Mackay et al. (17), with crude tissue culture antigen instead of the purified antigen and positive cut-off percentage of inhibition (PI) values - 60%.

Moreover, the presence of antibodies against FMDV type O was examined using the commercially available Ceditest FMDV type O Cedi-Diagnostics B.V, the Netherlands. The test was carried out according to the manufacturer’s protocol. Sera were considered positive if the colour development was inhibited by ≥ 50% when compared with the standard negative reference serum corrected for the background signal by subtracting the OD of the high positive reference serum.

Comparative specificity of ELISA methods was estimated using negative sera originated from healthy cattle neither vaccinated nor exposed to FMD virus (Table 2).

In 680 VNT negative sera tested in LPBE, 53 were considered positive or doubtful (92.2%). In comparison, the SPCE for type O antibodies had a specificity of 99.1 % when testing 428 cattle from the same population of animals. The specificity of Ceditest was 99.3% (out of 420 tested negative sera, 417 were negative).

Twenty-three sera from cattle infected with FMDV type O₁ and 65 sera from cattle vaccinated with trivalent FMD vaccine were examined in triplicate test by VNT and ELISA methods (Table 3).

The overall data shows that 85% of the serum titres recorded by VNT and 96-97% recorded by ELISA methods were within two-fold dilution step. The pooled estimate of the standard deviation (S.D.) for reciprocal log₁₀ titres of individual sera was 0.168 and 0.106 for VNT and LPBE, respectively. S.D. of SPCE and Ceditest for the percentage of inhibition of the tested sera was less than 5.

Sera from FAO Standardization Exercise (Phases XIII and XVI) were examined by the ELISA methods and VNT. In the Phase XIII Exercise, panel of 4 reference sera for FMDV strain O₁ BFS 1860 was tested (Table 4).

In all the applied assays the reference sera were positive with the exception of RS-4 (threshold serum) which was borderline positive in LPBE (log₁₀ 1.60), inconclusive in VNT (log₁₀ 1.47), and negative in SPCE and Ceditest methods.

In the Phase XVI Exercise, a panel of sera referred as the OIE reference sera, consisted a strong positive (RS–SP), weak positive (RS-WP), and a cut-off serum (RS-CO) made from dilutions of a strong positive bovine serum raised against O;Manisa (Table 5).

The strong positive and weak positive reference sera were positive in all the used techniques. However, the cut-off sera were inconclusive in VNT and negative in all the ELISA methods (titre LPBE < 1.65, PI of SPCE < 60% and PI of Ceditest < 50%).

Discussion

Countries such as Poland, which are recognized as being free from foot-and-mouth disease gain enormous economic advantage from their ability to trade freely in livestock and animal products. An important component securing and maintaining FMD-free status is the ability to detect animals which had contact with FMD virus either through infection or vaccination. This is usually done by measuring antibodies to the capsid, structural proteins of the FMDV. A serological test for FMDV antibody detection should be rapid, simple and reliable.
Table 1  
Comparative sensitivity of ELISA methods

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of sera examined</th>
<th>No. of positive sera</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid-phase blocking ELISA</td>
<td>221</td>
<td>219</td>
<td>99.1</td>
</tr>
<tr>
<td>Solid-phase competition ELISA</td>
<td>181</td>
<td>180</td>
<td>99.4</td>
</tr>
<tr>
<td>Ceditest FMDV type O ELISA</td>
<td>122</td>
<td>121</td>
<td>99.2</td>
</tr>
</tbody>
</table>

Table 2  
Comparative specificity of ELISA methods

<table>
<thead>
<tr>
<th>Test</th>
<th>No. of sera examined</th>
<th>No. of negative sera</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid-phase blocking ELISA</td>
<td>680</td>
<td>627</td>
<td>92.2</td>
</tr>
<tr>
<td>Solid-phase competition ELISA</td>
<td>428</td>
<td>424</td>
<td>99.1</td>
</tr>
<tr>
<td>Ceditest FMDV type O ELISA</td>
<td>420</td>
<td>417</td>
<td>99.3</td>
</tr>
</tbody>
</table>

Table 3  
Variation recorded in triplicate tests assessing FMDV antibodies in individual sera using VNT and ELISA methods

<table>
<thead>
<tr>
<th>FOLD VARIATION</th>
<th>VNT&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LPBE&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SPCE&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Ceditest&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2</td>
<td>85</td>
<td>96</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> – VNT, virus neutralization test
<sup>b</sup> – LPBE, liquid-phase blocking ELISA
<sup>c</sup> – SPCE, solid-phase competition ELISA
<sup>d</sup> – Ceditest FMDV type O ELISA

Table 4  
Reference sera (RS) from the FAO Phase XIII Standardization Exercise were examined by the ELISA methods and VNT

<table>
<thead>
<tr>
<th>METHOD</th>
<th>RS 1 (strong positive)</th>
<th>RS 2 (positive)</th>
<th>RS 3 (weak positive)</th>
<th>RS 4 (cut-off)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPBE</td>
<td>3.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.97</td>
<td>1.89</td>
<td>1.60</td>
</tr>
<tr>
<td>SPCE</td>
<td>90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81</td>
<td>62</td>
<td>30</td>
</tr>
<tr>
<td>Ceditest</td>
<td>95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74</td>
<td>53</td>
<td>34</td>
</tr>
<tr>
<td>VNT</td>
<td>2.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.52</td>
<td>1.62</td>
<td>1.47</td>
</tr>
</tbody>
</table>

<sup>a</sup> – logarithmic value
<sup>b</sup> - percentage of inhibition (PI)

Table 5  
Reference sera (RS) from the FAO Phase XVI Standardization Exercise examined by the ELISA methods and VNT

<table>
<thead>
<tr>
<th>METHODS</th>
<th>RS-SP (strong positive)</th>
<th>RS-WP (weak positive)</th>
<th>RS-CO (cut-off)</th>
<th>RS-NEG (negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPBE</td>
<td>2.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.16</td>
<td>1.46</td>
<td>1.28</td>
</tr>
<tr>
<td>SPCE</td>
<td>90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Ceditest</td>
<td>91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>VNT</td>
<td>2.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58</td>
<td>1.20</td>
<td>0.85</td>
</tr>
</tbody>
</table>

<sup>a</sup> – logarithmic value
<sup>b</sup> - percentage of inhibition (PI)
The aim of this study was to determine the diagnostic value of different variants of ELISA: liquid-phase blocking ELISA, solid-phase competition ELISA and Ceditest FMDV type O, the modified version of the solid-phase blocking ELISA (SPBE) developed by Chenard et al. (6). The solid phase ELISA methods were validated and extended for routine use only for serotype O. According to the recommendation of the FAO, the serological tests should be based on O\textsubscript{1},Manisa strain (2). That is why our experiments were performed with this strain.

The greatest problem in carrying out the serological tests to certify animals as free from FMDV antibodies prior to international trade is the problem of false-positive reactors, i.e. animals which react positively for antibody to one or more serotypes of FMDV but never had contact with the virus through either infection or vaccination. Currently, the LPBE is used widely as an initial screening test but it produces a high rate of unspecific reactions - up to 4\% in normal non-vaccinated cattle (18) and can be as high as 18\% in those which are stressed (8). Our results confirmed lower specificity of this assay as compared to the solid phase ELISA. The sensitivity of LPBE determined by testing VNT positive sera was almost equivalent to that of the solid-phase ELISA. The variation in the antibody titres which were recorded in triplicate tests using sera from individual animals was quite similar by using ELISA methods and lower than that received by VNT. The higher reproducibility of LPBE than VNT was earlier found by others (5, 9, 10) as well as in our laboratory (19). Recently, Mackay et al. (17) showed that in terms of reproducibility, LPBE and SPCE generally had lower coefficient of variation than the VNT. The coefficient of variation of the sera in the SPCE was similar to but slightly higher than that in the LPBE. Chenard et al. (6) also found that the OIE reference sera showed very consistent results when tested in duplicate six times over a period of 21 months using SPBE test kits from three consecutive production lots.

The panels of international reference sera recognized as the FAO and OIE standards for the purposes of international trade were tested in different FMD diagnostic methods and the results were compared. As expected, consistent results were obtained for strong positive sera. However, the cut-off reference sera were inconclusive or negative in all the applied tests. It indicates that these sera are too weak to be used as the reference standards. The results obtained in the Netherlands during the mass serology campaign also confirmed that the cut-off defined by OIE reference serum is too low (6). They suggest that the cut-off levels for serology should be based on the results of a large number of tests performed on infected and non-infected animals in the target population. On the meeting of the staffs of EU reference laboratories for FMD in Brussels (26 March 2001), it was agreed that the cut-off level for type O of FMDV, as defined by the OIE reference serum, could be raised (Aldo Dekker, personal information). According to the proposition of the participants of FAO Collaborative Exercise Phase XVII, the definitions for the weak and cut-off sera should be corrected and explicitly related to the purpose for which the testing is performed. It was suggested that the weak positive serum should represent a minimum standard for the detection in any test used for herd-based serosurveillance. The cut-off should represent a minimum standard for detection in any test used for individual animal certification. The weak and cut-off positive sera should be stronger, so that the weak positives were consistently positive by VNT, and the cut-off sera were consistently inconclusive positive (i.e. not negative) (20).

The results of our studies show that the solid-phase ELISA methods are easier to use, quicker and more stable. Due to their high specificity, sensitivity and low variation in results, the methods are more suitable than the LPBE and VNT for large scale testing. It can be expected that these methods will soon be worldwide adopted and replace the LPBE for the use in monitoring and eradication programmes as well as import/export testing in support of international trade.

References

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