Journal of the Hellenic Veterinary Medical Society

Comparison of two ELISA methods for detection of antibodies against Footand- Mouth Disease virus of cattle breeds in Turkey

Ö. B. INCE, R. KALKAN, S. ÇAKIR

To cite this article:

Research article
Ερευνητικό άρθρο

Comparison of two ELISA methods for detection of antibodies against Foot-and-Mouth Disease virus of cattle breeds in Turkey

Ince Ö.B.[1] Kalkan R.[2], Çakır S.[3]

[1]*Agriculture and Rural Development Support Institution, 03100, Afyonkarahisar, Turkey*
[2]*Institute for Foot and Mouth Disease , 06100 Ankara, Turkey,*
[3]*University of Abant İzzet Baysal, Department of Poultry Science, Faculty of Agriculture and Natural Sciences, Bolu, Turkey*

ABSTRACT. The study was conducted using two ELISA methods - the liquid phase blocking ELISA (LPBE) and solid phase competition ELISA (SPCE) for the detection of foot-and-mouth disease virus (FMDV) serotype A- and O-specific antibodies of different cattle breeds in Turkey. These methods were compared in 426 cattle previously vaccinated with oil-adjuvanted bivalent vaccine as well as in sera from 40 cattle with no history of foot-and-mouth disease infection or vaccination. The results were found that SPCE had a better specificity (serotype A; 100% and serotype O; 97.50%) than LPBE (serotype A 95.00% and serotype O 92.50%). Sensitivity of SPCE had also better values (serotype A; 99.30% and serotype O; 98.59%) than LPBE (serotype A; 97.89% and serotype O; 96.48%). The results of the present study showed that the SPCE method is more reliable than LPBE.

Keywords: Foot-and-mouth disease, liquid-phase blocking ELISA, solid-phase competition ELISA

INTRODUCTION

*F*oot-and-mouth disease (FMD) is a highly contagious and economically devastating disease of cloven-hoofed animals holding a wide host spectrum, such as cattle, pigs, sheep, goats, buffalo, deer, antelope and wild pigs and can severely constrain international trade of animals and animal products. Seven distinct serotypes of foot-and-mouth disease virus (FMDV) have been recognized, which can be further divided into a number of subtypes (Lina et al., 2011). Specifically, FMDV serotypes O, A, C, Southern African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1, with indistinguishable clinical effects exist. Recovery from infection, or protective vaccination, with one serotype will not protect against subsequent infection with another. Moreover, within a serotype a wide range of strains may occur, some of which may be sufficiently divergent to reduce the efficacy of existing vaccines (Habiela et al., 2010; Neeta et al., 2011).

FMDV has a wide host range, an ability to infect in small doses, a rapid rate of replication, a high level of viral excretion and multiple modes of transmis-

Corresponding author: Ö.B. Ince, Agriculture and Rural Development Support Institution, 03100 Afyonkarahisar, Turkey,
E-mail address: incebaris@gmail.com, omerbaris.ince@tkdk.gov.tr

sion, including airborne transmission. These features make FMD a difficult and expensive disease to control and eradicate, and one that is much feared by farmers, veterinarians and those associated with livestock production. Countries which are disease-free take great precautions to ensure that the virus does not enter. FMD is a major constraint to international trade in livestock and animal products. The situation is further complicated by the fact that after the acute stage of infection FMDV may cause a prolonged, symptomless, persistent infection in ruminants (Van Bekkum *et al.,* 1959b; Knight and Rushton, 2013). The carrier state can occur in convalescent animals or in vaccinated animals exposed to live virus. Recognition of the carrier state and the risk of viral transmission by carrier animals have a major impact on the design of control and eradication strategies for FMD (Batista *et al.,* 2010; Valdazo *et al.,* 2012).

At the beginning of the 21st century the protocol for production of inactivated FMDV vaccines allows the use of serological tests that can differentiate infected from vaccinated animals, formulation of vaccines that include multiple serotypes and adjuvants (Doel, 2003; Fmd-Disconvac, 2013). Using oil-adjuvanted vaccines has been shown to induce higher levels of antibodies than aluminium hydroxide gel-saponin adjuvanted vaccines (McKercher *et al.,* 1977). The OIE-prescribed tests for serological diagnosis of FMD are the virus neutralisation test (VNT) (Golding *et al.,* 1976), and the LPBE (Hamblin *et al.,* 1986). LPBE has since been adopted by a large number of laboratories worldwide (Mackay *et al.,* 1994; 1998) to replace VNT for routine screening, because it is quicker, with reproducible results that correlatewell with those obtained from VNT and is not affected by the biological variability that is inherent in VNT.However, it has several drawbacks, including lack of stability of inactivated antigens and false positive reactions occurring at a rate of 4% up to 18% (Clavijo *et al.,* 2004).The aim of this study was to compare the performance of the ELISA methods used for the detection of antibodies against the structural proteins of FMDV serotypes A and O.

MATERIALS AND METHODS
Sera

A total of 426 cattle of variousTurkish breeds with no history of FMD. Cattle were vaccinated with oil-adjuvant bivalent vaccine (O_1 Manisa and A_{22} Iraq FMDV strains) formulated in a double oil emulsion adjuvant. The same batches of the vaccine were used in all animals. Serum samples were collected at day 28 after vaccination. Negative sera from cattle (40) with no history of infection or vaccination with FMDV were provided by Institute for Foot and Mouth Disease, Turkey.

Test reagents

International reference sera for FMDV serotype A and serotype O, rabbit anti-FMDV sera, and guinea pig antiserum were obtained from the Institute for Animal Health, Pirbright Laboratory, UK. Antigens of the serotype A and serotype O and horseradish peroxidase conjugated rabbit anti-guinea pig immunoglobulin were obtained from the Institute for Foot–and-Mouth Disease, Turkey.

Liquid-phase blocking ELISA

LPBE was performed according to the method described by Hamblin *et al.,* 1986. Antibody titres for FMDV type O and A were expressed as the final dilution of test serum giving 50% of the mean optical density. Each well was read at 492 nm using a spectrophotometer, and percentage of inhibition (PI) values was calculated. The sera were considered positive at PI ≥ 50% of the value recorded in the virus control wells where test control serum was absent virus. Titres greater than 50 ($\log_2$) were considered positive.

Solid-phase competition ELISA

The assay is based on the competition between serotype specific guinea pig anti-FMDV antiserum and antibodies present in the test serum and is described (OIE, 2000). The SPCE was carried out according to the method recommended for the purposes of the FAO, which is a modified version of original method described by Mackay *et al.,* OIE, 2000 with crude tissue culture antigen instead of the purified antigen and

positive cut-off percentage of inhibition (PI) value 60%. Sera giving PI values equal to or greater than 60% were considered positive (Paiba, 2004).

Statistic Evaluation

The collected data were entered and stored into Microsoft Excel spread sheet 2010. All data were entered into a dataset using R. All statistical analyses were performed using R version 3.1.2 (R Development Core Team, 2014). All data were screened for errors. When data mismatches were detected, data were re-checked to determine the source of the mismatch and where possible this was corrected. The relative specificity and sensitivity of the applied LPBE were calculated using standard methods and were expressed as percentages (Jacobson 1998; OIE 2000).

RESULTS

The specificity of LPBE was evaluated with sera collected from the 40 cattle that had never been infected or vaccinated with FMDV. A total of 40 sera were tested for antibodies to serotype A, 2 out of 40 sera (95.00%); serotype O, 3 out of 40 sera (92.00%) gave positive results (Table 1).

Regarding the SPCE specificity, the 40 sera were tested against serotype A, indicating a specificity of 100%. Regarding serotype O, 97.50% gave positive results. Thus, the specificity of LPBE and SPCE was over 92.50%, 97.50% respectively (Table 1).

When testing by the LPBE, 417 out of 426 sera were positive serotype A-specific antibodies (97.89%) (95% CI ; 96.03-99.03%) Regarding serotype O-specific antibodies, 96.48%(95% CI ; 94.26 -98.02 %) gave positive result (411/426). Out of 426 samples of sera, 423 were seropositive for serotype A (99.30%) (95% CI ; 97.96 -99.85%) ; For serotype O, 420 out of 426 animals were positive(98.59%) (95% CI ; 96.96-99.48%) in SPCE (Table 2).

Reference sera were examined by LPBE and SPCE for screening assays. Reference sera, consisted a strong positive, weak positive, and a cut-off serum made from dilutions of a strong positive bovine serum raised against A_{22} Iraq and O_1 Manisa. The overall data shows that in LPBE, the strong positive (PI: 85–96%) and weak positive (PI: 54–73%) reference sera were found to be positive. The negative results were 0–30% PI. In SPCE, reference sera produced results within the expected range for the negative (PI: 0–30%), weak positive (PI: 54–74%), and strong positive (PI: 83–98%) samples. LPBE and SPCE scored all negative references as negative. Reference sera showed consistent results when tested by LPBE and SPCE. PIs for all negative controls were below the cut-off for each serotype (cut-off of 50% PI and 60% PI for LPBE and SPCE, respectively) (Table 3).

DISCUSSION

Developing countries , which are recognized as FMD-free gain enormous economic advantage from their ability to trade freely livestock and animal products. An important component securing and maintaining FMD-free status is the ability to detect animals which had contact with FMDV either via infection or vaccination. This is usually done by detecting antibodies against structural proteins of FMDV. FMD is endemic in the Anatolian region of Turkey; however, the Thrace region of Turkey has not had a FMD case since 2001. Most FMD outbreaks were attributed to FMDV serotypes are serotypes O or A. In Turkey, control strategies for FMD are based on vaccination, quarantine, and control of animal movements (Bulut and Aktas 2006; Klein *et al.,* 2006). Postvaccination serosurveillance is an important indicator for the evaluation of preventive vaccination programs (Şevik and Öztürk 2013).

LPBE has been applied as a routine screening method for FMDV in many laboratories. (Hamblin *at al.,*1986; OIE, 2012). However, it has several drawbacks, including lack of stability of inactivated antigens and false positive reactions occurring at a rate of 4% up to 18% (Hass, 1994; Clavijo *et al.,* 2004). For these reasons, SPCE has been developed for the detection of antibodies against FMDV. One of the advantages of SPCE is its highly purified and adequately stable 146S preparations of virus used as antigen (Mackay *et al.,* 2001).

The aim of this study was to determine the diagnostic value of different of ELISAs: a) liquidphase blocking ELISA, and b) solid-phase competition ELISA, the modified version of the solid-phase blocking ELISA (SPBE) developed by Chenard *et al.,* (2003). LPBE and SPCE were compared for their specificity and sensitivity. It was determined that specificities of LPBE for serotype A and serotype O were lower than those achieved with SPCE. Serotype A and O LPBE gave a specificity of 95.00% and 92.50%, respectively, at a cut-off of 50 PI. A cut-off value of 60 PI was used for serotypes A and O SPCE, which gave a specificity of 100% and 97.50%, respectively. Mackay *et al.* (2001) and Niedbalski (2004), Şevik and Öztürk 2013) also obtained similar results, and they reported that specificity of SPCE was considerably higher than that of LPBE. In another study, Paiba *et al.,* (2004) reported that specificity of SPCE for serotype O at a cut-off point of 60 PI was 99.44% for cattle sera, 99.50% for sheep sera, and 100% for pig sera. The sensitivity of SPCE determined by testing of positive sera was slightly higher than that of LPBE. Similar results were obtained in other studies (Mackay *et al.,* 2001; Brocchi *et al.,* 2004; Li *et al.,* 2012). Martinez and Quintero 1997) reported that sensitivity of LPBE for serotype O_1, Cruzeiro was 96%. Brocchi *et al.,* (2004) found that diagnostic sensitivity of SPCE was 99.7%. Niedbalski (2004) reported that sensitivity of LPBE and SPCE was 99.1% and 99.4%, Şevik and Öztürk (2013) reported sensitivity 98.89% and 97.22%, respectively.

International reference sera recognized as the FAO and OIE standards for the purposes of international trade were tested in different FMD diagnostic methods. The results were compared. As expected, consistent results were obtained for strong positive sera. Weak positive sera showed very consistent results when tested using LPBE and SPCE. It has been suggested that weak positive reference serum is the minimum standard for the serologic assays used for herd-based serosurveillance (Jacobson 1998; Niedbalski 2004; Jaworski *et al.,* 2011). In the present study, the highest titers obtained by SPCE for samples of serotypes A and O were higher than those measured by LPBE. These results suggested that the SPCE can detect lower amounts of FMDV serotype A and serotype O specific antibodies than the LPBE.

CONCLUSION

This study has demonstrated that the SPCE methods are easier to use, quicker and more stable than LPBE SPCE is more suitable than LPBE for the evaluation of vaccination programs, Due to their high specificity, sensitivity and low variation in results, the SPCE methods are more suitable than the LPBE.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGMENTS

Table 1. Specificity of ELISA Methods

FMDV	Methods	No. of sera	No. of positive sera	Specificity (%)	Confidence Interval (CI) 95%
A_{22} Iraq	Liquid phase blocking ELISA	40	38	95.00	83.08 -99.39
	Solid phase competition ELISA	40	40	100.00	91.19 -100.00
O_1 Manisa	Liquid phase blocking ELISA	40	37	92.50	79.61 -98.43
	Solid phase competition ELISA	40	39	97.50	86.84 -99.94

Table 2. Sensitivity of ELISA Methods

Cattle Breeds	FMDV	Methods	No. of sera	No. of positive sera	Sensitivity (%)	95%CI
Holstein Friesian hybrid	A_{22} Iraq	LPBE	90	88	97.77	92.20 -99.73
		SPBE	90	89	98.88	93.96 -99.97
	O_1 Manisa	LPBE	90	87	96.66	90.57 -99.31
		SPBE	90	89	98.88	93.96 -99.97
Brown swiss hybrid	A_{22} Iraq	LPBE	70	69	98.57	92.30 -99.96
		SPBE	70	70	100	94.87-100.00
	O_1 Manisa	LPBE	70	68	97.14	90.06 -99.65
		SPBE	70	69	98.57	92.30 -99.96
Jersey hybrid	A_{22} Iraq	LPBE	70	68	97.14	90.06 -99.65
		SPBE	70	69	98.57	92.30 -99.96
	O_1 Manisa	LPBE	70	67	95.71	87.98 -99.11
		SPBE	70	69	98.57	92.30 -99.96
Local Eastern Anatolian Red	A_{22} Iraq	LPBE	66	64	96.96	89.48 -99.63
		SPBE	66	65	98.48	91.84 -99.96
	O_1 Manisa	LPBE	66	63	95.45	87.29 -99.05
		SPBE	66	65	98.48	91.84 -99.96
Southeast Anatolian Red	A_{22} Iraq	LPBE	70	69	98.57	92.30 -99.96
		SPBE	70	70	100	94.87 -100.00
	O_1 Manisa	LPBE	70	68	97.14	90.06 -99.65
		SPBE	70	69	98.57	92.30 -99.96
Local Graybreed	A_{22} Iraq	LPBE	60	59	98.33	91.06 -99.96
		SPBE	60	60	100	94.04 -100.00
	O_1 Manisa	LPBE	60	58	96.66	88.47 -99.59
		SPBE	60	59	98.33	91.06 -99.96
Total	A_{22} Iraq	LPBE	426	417	97.89	96.03-99.03
		SPBE	426	423	99.30	97.96 -99.85
	O_1 Manisa	LPBE	426	411	96.48	94.26 -98.02
		SPBE	426	420	98.59	96.96 -99.48

Table 3. Reference sera examined by the ELISA methods

Methods	FMDV	strong positive	weak positive	cut-off	negative
LPBE	A_{22} Iraq	85[a]	63	30	10
	O_1 Manisa	91	60	30	11
SPCE	A_{22} Iraq	88	64	30	11
	O_1 Manisa	95	63	29	13
	[a]Results are expresses as the percentage inhibition (PI)				

REFERENCES

Batista A, Quattrocchi V, Olivera V (2010) Adjuvant effect of Cliptox on the protective immune response induced by an inactivated vaccine against foot and mouth disease virus in mice. Vaccine, 28:6361-6

Brocchi E, Grazioli S, Yadin H, Simone F (2004) Validation of a solid phase competitive ELISA (SPBE) based on the use a single neutralising monoclonal antibody for the measurement of antibodies to FMDV type Asia 1. In: Session of the Research Group Standing Technical Committee of the European Commission for the Control of Foot and Mouth Disease. Crete, Greece: EuFMD, pp: 288–297.

Bulut N and Aktas S (2006) Turkey report to the EUFMD Commission, FMD in Turkey. In: Report of the 73rd Session of the EUFMD Executive Committee. Rome: FAO, pp: 23–27.

Chenard G, Miedema K, Moonen P, Schrijver RS, Dekker A (2003) A solid-phase blocking ELISA for detection of type O foot-and-mouth disease virus antibodies suitable for mass serology. J. Virol. Methods, 197:89-98.

Clavijo A, Wright P, Kitching P (2004) Developments in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease. Vet J, 167: 9–22.

Doel TR (2003) FMD vaccines. Virus Research 91(1):81–99.

FMD-Disconvac. (2013) Assessment and improvement of heterologous protection by FMD vaccines. Work package 3. Veterinary and Agrochemical Research Centre, Brussels, Belgium, pp:3-5

Golding SM, Hedger RS and Talbot P (1976) Research in Veterinary Science 20: 142-147.

Haas B (1994) Application of the FMD liquid-phase blocking sandwich ELISA. Problems encountered in import/export serology and possible solutions. In: Session of the Research Group of the European Commission for the Control of Foot and Mouth Disease. Vienna, Austria: EuFMD pp: 124–127.

Hamblin C, Barnett ITR, Hedger RS (1986) A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. I. Development and method ELISA. J Immunol Methods 93: 115–121.

Habiela M, Ferris NP, Hutchings GH, Wadsworth J, Reid SM, Madi M, Ebert K, Sumption KJ, Knowles NJ, King DP, Paton DJ (2010) Molecular characterization of foot-and-mouth disease viruses collected from Sudan. Transbound. Emerg. Dis. 57:305–314

Jacobson RH (1998) Validation of serological assays for diagnosis of infectious diseases. Rev Sci Tech Off Int Epiz, 17: 469–486.

Jaworski JP, Compaired D, Trotta M, Perez M, Trono K, Fondevila N (2011) Validation of an r3AB1-FMDV-NSP ELISA to distinguish between cattle infected and vaccinated with foot-and-mouth disease virus. J Virol Methods,178: 191-200.

Klein J, Parlak U, Ozyoruk F, Christensen LS (2006) The molecular epidemiology of foot-and-mouth disease virus serotypes A and O from 1998 to 2004 in Turkey. BMC Vet Res, 4: 2–35.

Knight-Jones TJD, Rushton J (2013) The economic impacts of foot and mouth disease– what are they, how big are they and where do they occur? Prev Vet Med; 112(3–4):161–73.

Li Y, Swabey KG, Gibson D, Keel PJ, Hamblin P, Wilsden G, Corteyn M, Ferris NP (2012) Evaluation of the solid phase competition ELISA for detecting antibodies against the six foot-and-mouth disease virus non-O serotypes. Journal of Virological Methods 183 (2), pp. 125-131.

Li-na M, Jie Z, Hao-tai C, Jian-hua Z, Yao-zhong D and Yong-sheng L (2011) An overview on ELISA techniques for FMD. Virology Journal 8:419

Mackay DKJ, Rendle T and Armstrong RM (1994) Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Vienna, 19-22 September, 1994,pp: 128-145.

Mackay DKJ, Rendle T and Kitching RP (1998) Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, Aldershot, UK, 14-18 September, 1998, pp:148-157.

Mackay DKJ, Bulut AN, Rendle T, Davidson F, Ferris NP (2001) A solid-phase competition ELISA for measuring antibody to foot and- mouth disease virus. J Virol Methods, 97: 33–48.

Martinez CS and Quintero M (1997). The use of a liquid phase blocking ELISA kit for detection of antibodies against foot-and-mouth disease virus in Colombia. In: Proceedings of the Use of ELISA for Epidemiology and Control of Foot-and-Mouth Disease and Bovine Brucellosis in Latin America. Vienna, Austria, pp:13-17

McKercher PD, Graves JH (1977) A review of the current status of oil adjuvants in foot-and-mouth disease vaccines. Dev Biol Standard 35: 107–112.

Neeta L, Rajib D, Ak S, Tilling T, Awachat V and Saxena V (2011) A Brief Review on Diagnosis of Foot-and-Mouth Disease of Livestock: Conventional to Molecular Tools Veterinary Medicine International Volume 2011, Article ID 905768, doi:10.4061/2011/905768

Niedbalski W, Fitzner A, Paprocka G, Kęsy A (1994) Application of liquid-phase blocking sandwich ELISA (LPBE) for the detection of antibodies against foot-and mouth disease virus. Bull. Vet. Inst. Pulawy, 38: 105-114.

OIE (2000) Manual of Standards for Diagnostic Tests and Vaccines. 4th ed. Paris, France: OIE, pp:1-8

OIE (2012) Chapter 2.1.5. Foot and mouth disease. In Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France.

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.05_FMD.pdf.

OIE (2012) Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.6th ed. Paris, France:OIE, pp:1-12

Paiba GA, Anderson J, Paton DJ, Soldan AW, Alexandersen S, Corteyn M, Wilsden G, Hamblin P, MacKay DK, Donaldson AI (2004) Validation of a foot-and-mouth disease antibody screening solid-phase competition ELISA Journal of Virological Methods 115: 145–158.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online: http://www.R-project.org, 2014

Şevik M and Öztürk FF (2013) Comparative evaluation of liquid-phase blocking ELISA and solid-phase competition ELISA methods for the detection of antibodies to the structural proteins of foot-and mouth disease types O and A viruses. Turk J Vet Anim Sci, 37:523-528

Valdazo-Gonzalez B, Polihronova L, Alexandrov T, Normann P, Knowles NJ, Hammond JM (2012) Reconstruction of the transmission history of RNA virus outbreaks using full genome sequences: foot-and-mouth disease virus in Bulgaria in 2011. PLoS ONE, 7(11):e49650

Van Bekkum JG, Frenkel HS, Frederiks HHJ and Frenkel S (1959b) Observations on the carrier state of cattle exposed to foot-and-mouth disease virus. Tijdschriet voor Diergeneeskunde, 84:1159–1164.